ISSN 2220-6124 (online)

World Journal of *Nephrology*

2014 Bound Volume 3 Issue 1-4: 1-323

Published by Baishideng Publishing Group Inc

WJN World Journal of Nephrology

A peer-reviewed, online, open-access journal of nephrology

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ISSN 2220-6124 (online)

World Journal of *Nephrology*

World J Nephrol 2014 February 6; 3(1): 1-15

Baishideng Publishing Group
www.wjgnet.com

World Journal of Nephrology

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AIM AND SCOPE		*World Journal of Nephrology* (*World J Nephrol*, *WJN*, online ISSN 2220-6124, DOI: 10.5527) is a peer-reviewed open access academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians. *WJN* covers topics concerning kidney development, renal regeneration, kidney tumors, therapy of renal disease, hemodialysis, peritoneal dialysis, kidney transplantation, diagnostic imaging, evidence-based medicine, epidemiology and nursing. Priority publication will be given to articles concerning diagnosis and treatment of nephrology diseases. The following aspects are covered: Clinical diagnosis, laboratory diagnosis, differential diagnosis, imaging tests, pathological diagnosis, molecular biological diagnosis, immunological diagnosis, genetic diagnosis, functional diagnostics, and physical diagnosis; and comprehensive therapy, drug therapy, surgical therapy, interventional treatment, minimally invasive therapy, and robot-assisted therapy. We encourage authors to submit their manuscripts to *WJN*. We will give priority to manuscripts that are supported by major national and international foundations and those that are of great basic and clinical significance.
INDEXING/ABSTRACTING		*World Journal of Nephrology* is now indexed in PubMed Central, PubMed, and Digital Object Identifier.
FLYLEAF	I-III	Editorial Board

NAME OF JOURNAL
World Journal of Nephrology

ISSN
ISSN 2220-6124 (online)

LAUNCH DATE
February 6, 2012

FREQUENCY
Quarterly

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http://www.wjgnet.com

PUBLISHER
Baishideng Publishing Group Co., Limited
Flat C, 23/F., Lucky Plaza,
315-321 Lockhart Road, Wan Chai,
Hong Kong, China
Fax: +852-6555-7188
Telephone: +852-3177-9906
E-mail: bpgoffice@wjgnet.com
http://www.wjgnet.com

PUBLICATION DATE
February 6, 2014

INSTRUCTIONS TO AUTHORS
Full instructions are available online at http://www.wjgnet.com/2220-6124/g_info_20100722180909.htm.

ONLINE SUBMISSION
http://www.wjgnet.com/esps/

World Journal of Nephrology

Online Submissions: http://www.wjgnet.com/esps/
bpgoffice@wjgnet.com
doi:10.5527/wjn.v3.i1.1

World J Nephrol 2014 February 6; 3(1): 1-5
ISSN 2220-6124 (online)

EDITORIAL

Frequent office visits of patients with chronic kidney disease: Is a prelude to prevention of dialysis

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Received: April 14, 2013 Revised: November 20, 2013
Accepted: January 13, 2014
Published online: February 6, 2014

Abstract

This study is an excerpt of broad-based office practice which is designed to treat patients with diabetes and hypertension, the two most common causes of chronic kidney disease (CKD), as well as CKD of unknown etiology. This model of office practice is dedicated to evaluating patients with CKD for their complete well-being; blood pressure control, fluid control and maintenance of acid-base status and hemoglobin. Frequent office visits, every four to six weeks, confer a healthy life style year after year associated with a feeling of good well-being and a positive outlook. Having gained that, such patients remain compliant to their medication and diet, and scheduled laboratory and office visits which are determinant of a dialysis-free life.

Key words: Diabetes; Hypertension; Chronic kidney disease; End stage renal disease; Preventative care; Small kidneys; Serum bicarbonate; Non-dialysis

Core tip: Diabetes and hypertension are two most common causes of chronic kidney disease (CKD). Nephrology office practice constitutes vast majority of the patients with CKD of different stages. While CKD stages 1-3 [glomerular filtration rate (GFR) (< 60 - > 30 mL/min)] produce slight or no symptoms or signs, CKD stages 4-6 (GFR < 30 - < 10 mL/min) may increase blood pressure and produce fluid electrolyte and acid-based disorders. The goal of office practice is to identify these disorders, then treat them to enable patients to live asymptomatically.

Mandal AK. Frequent office visits of patients with chronic kidney disease: Is a prelude to prevention of dialysis. *World J Nephrol* 2014; 3(1): 1-5 Available from: URL: http://www.wjgnet.com/2220-6124/full/v3/i1/1.htm DOI: http://dx.doi.org/10.5527/wjn.v3.i1.1

INTRODUCTION

Experience in direct patient care reveals that frequent office visits of patients encompassing chronic illnesses such as hypertension, diabetes, or chronic kidney disease (CKD) is a form of salutary care. This model of direct patient care is advantageous for the patients; it is educational and economical. This model of patient care is advantageous because of continuity of care which permits the patients to gain confidence in their physicians and allows them to feel comfortable in addressing their issues freely.

Similarly, continuity of care permits the physicians to identify and resolve the issues in a comfortable fashion. Other authors have reported that continuity of care has led to improved outcomes of diabetes care, delivery of preventative care and clinical satisfaction, while also decreasing the number of emergency room visits, hospitalizations, readmissions and reducing length of stay[1].

There is an interesting study which asked the question: If an outpatient repeatedly sees the same practitioner, is his care influenced? A double blind randomized trial examined the effects of outpatient health care

provider continuity on the process and outcome of the medical care for 776 men aged 55 years and older. Participants were randomized to two groups of provider care: provider discontinuity and provider continuity. During an 18 mo period, continuity group had fewer emergency admissions (20% *vs* 39%) and a shorter average length of hospital stay (15.5 d *vs* 25.5 d). The continuity group also felt that the providers were more knowledgeable, thorough and interested in patient education[2].

Giving autonomy or independence in self-care motivates patients to control their illness with prescribed medication, diet, and physical therapy uniquely in illnesses such as diabetes, hypertension or CKD. In one study, 128 patients with diabetes were tested and found to achieve significant reductions in their HbA1c values over 12 mo[3]. It is important to know that understanding of side effects of medication corresponded to compliance with any proposed regimen (87% cases *vs* 93% control: non-significant).

Despite the similarity between the two groups, 53% of cases reported that side effects of medication were explained to them, in contrast to 84% of controls. This difference is significant indicating that the side effects of therapy are often not explained to the patients[4].

Other studies have noted that a relationship exists between the way in which physicians and patients behave during an office visit and that relationship influences patients' subsequent health status. More control by the patients, less control by physicians, more negative effect expressed by both, more effective information seeking by patients, and greater overall patient conversation relative to the physician were consistently related to better control of diabetes and hypertension as measured by hemoglobin A1c and diastolic blood pressure (BP) respectively[5].

PURPOSE OF THIS COMMUNICATION

Having given that background, it is time to illustrate how chronic diseases like diabetes, hypertension or chronic kidney disease (CKD) of unknown etiology can be followed in the office setting for an indefinite period to ensure a good living for the patient. The goal of frequent office visits is to afford asymptomatic state, reduce hospitalization and prolong comfortable survival without dialysis therapy. Two patients are exemplified to that effect.

Example 1 - patient with CKD or chronic renal failure of undetermined etiology

April 2008 - first visit: A-84-year-white female referred for end stage renal disease, without history of diabetes. Patient was not aware that she was treated for hypertension and exhibited no symptoms. Physical examination revealed pulse 64/min irregular, sitting BP 140/100 mmHg, standing BP 140/90 mmHg, and chest auscultation revealed rhonchi and a questionable mass with tenderness in the left iliac fossa. Her medication included ergo/chole calciferol 2.5 mcg per oral daily, levothyroxine 75 mcg per oral daily, amlodipine 5 mg per oral daily, sodium bicarbonate 650 mg per oral three times daily, and irbesartan 75 mg per oral daily.

She brought a laboratory which was done the previous October. The findings were glucose 96 mg/dL, BUN 49 mg/dL, serum creatinine 4.3 mg/dL, estimated glomerular filtration rate (eGFR) 10 mL/min, Na^+ 143 mmol/L, K^+ 4.3 mmol/L, chloride 106 mmol/L, CO_2 25.6 mmol/L, phosphorous 4.3 mg/dL, albumin 3.9 g/dL, calcium 9.9 mg/dL hemoglobin 11.8 g/dL and hematocrit 34.9%. A urinalysis revealed protein 1+, bacteria 4+, and WBC 33/HPF. A CT scan of the abdomen done in 2001 revealed bilaterally small kidneys. Assessment was end stage renal disease. She was admitted to a local hospital for further assessment. Irbesartan was discontinued. She was treated with bicarbonate infusion and released. No dialysis was recommended. Through the years, patient has done well, remained asymptomatic but required once a year hospital admission for diarrhea and dehydration or poor appetite. Her appetite is markedly increased with initiation of megestrol. BP control was achieved with increased dose of amlodipine. Serum bicarbonate level is maintained near normal level with increased dosage of sodium bicarbonate.

Most recent visit in March of 2013, 5 years later: Symptoms: none; Appetite: good; Activity: normal; Essential medications: (1) amlodipine 10 mg per oral daily; (2) sodium bicarbonate 1300 mg per oral 4 times daily; (3) potassium chloride 20 meq per oral daily for hypokalemia. (4) appetite stimulant megestrol, 40 mg per oral daily; (5) hectoral (ergo/chole calciferol) 2.5 mcg per oral daily; (6) levothyroxine 125 mcg per oral daily; and (7) allopurinol 150 mg per oral daily. physical examination: no edema, BP 120/80 mmhg, electrocardiogram normal; Laboratory: glucose 145 mg/dL, BUN 61 mg/dL, serum creatinine 7.8 mg/dL, eGFR 5 mL/min, Na^+ 144 mmol/L, K^+ 4.3 mmol/L, chloride 109 mmol/L, CO_2 21 mmol/L, phosphorous 3.5 mg/dL, albumin 3.7 g/dL, intact parathyroid hormone 97 pg/mL; Plans: (1) continue current therapy; (2) No dialysis is recommended; and (3) Office visits are scheduled every 6 wk.

Example 2 - A-70-year-white male is a five year office follow-up for hypertension and renal function control

March 2008 - first visit: Chief complaint: breathing trouble for 2-3 years. Patient gave history of hypertension for 5 years. Significant past history includes coronary angioplasty with rupture of the coronary artery followed by coronary artery bypass graft in 1992; back surgery × 4, last one in 1995. Smoked until 1990 then quit. Significant finding on physical exam was elevated BP sitting 180/110 and 170/106 mmHg standing and pulse rate 98 per minute. A questionable bruit heard left to umbilicus. Funduscopic exam reveals arterial narrowing. Disc could not be visualized. He obtained a laboratory in March 2006 which showed hemoglobin 13.5

Table 1 Characteristics of patients with advanced chronic kidney disease

	Patient 1		Patient 2	
	First visit (2008)	Recent visit (2013)	First visit (2008)	Recent visit (2013)
Age (yr)	84	89	70	75
Symptoms	None	None	None	None
Appetite	Good	Good	Good	Good
BP (mmHg)	140/100	120/80	180/110	120/70
Edema	0	0	0	0
Bun (mg/dL)	49	61	28	35
Scr (mg/dL)	4.3	7.84	2.6	2.5
eGFR (mL/min)	10	5	26	24
CO_2 (mmol/L)	26	21	29	27
Hgb (g/dL)	12	13.7	13	12
Serum potassium (mmol/L)	4.3	4.3	4.1	4.5

eGFR: Estimated glomerular filtration rate.

g/dL, hematocrit 39.5%, BUN 21 mg/dL, serum creatinine 1.9 mg/dL, eGFR 37.6 mL/min, CO_2 27 mmol/L, and albumin 3.7 g/dL. Medication at this first office visit included amlodipine 5 mg per oral daily, furosemide 40 mg per oral daily, simvastatin 10 mg per oral daily, prednisone 10 mg per oral daily and albuterol inhalation. Laboratory done in February 2008 showed BUN 28 mg/dL, serum creatinine 2.6 mg/dL, glucose 105 mg/dL, Na 145 mmol/L, K 4.1 mmol/L, eGFR 26 mL/min. One month later in March 2008, his hemoglobin was 13.1 g/dL, hematocrit 39.3%, renin 0.6 ng/mL per hour, aldosterone 3 ng/dL. Medication was adjusted to include tenormin 50 mg per oral daily, increase amlodipine 5 mg twice daily and reduce furosemide 40 mg per oral every other day and prednisone 5 mg per oral daily. Two weeks later his BP decreased to 140/90 mmHg sitting and 130/90 mmHg standing and pulse rate was reduced to 66 beats/min. Soon thereafter his BP became normal. He records his BP at home and they are all normal of average less than 130/80 mmHg. He is followed in the office every six to seven weeks with laboratory done before each visit.

Most recent visit in February of 2013, 5 years later: Symptoms: none; Shortness of breath on exertion: none; Sitting BP: 120/70 mmHg; Current medications: atenolol 50 mg per oral daily, bumetanide 1 mg per oral Mondays and Fridays, amlodipine 10 mg per oral twice daily, digoxin 0.125 mg per oral Mondays, Wednesdays, Fridays and Sundays, sodium bicarbonate 1300 mg three times daily and sodium polysterene sulfonate (Kayexalate) in 30% sorbitol 5 g in 20 mL twice daily. Laboratory (Fasting) glucose 107 mg/dL, BUN 35 mg/dL, serum creatinine 2.48 mg/dL, eGFR 24 mL/min, sodium 142 mmol/L, potassium 4.5 mmol/L, calcium 9.8 mg/dL, phosphorous 4.3 mg/dL, intact PTH 178 pg/mL, uric acid 8 mg/dL, hemoglobin 11.6 g/dL, hematocrit 34.7%; Analysis of the life style of the two examples: Patients with advanced CKD, frequent office visits are permissive of living without dialysis. These can be viewed at a glance in Table 1.

The renal function tests including serum creatinine and eGFR in patients 1 and 2 are shown in Figures 1 and 2, respectively. In five years follow up; notably both patients are asymptomatic and living good albeit active lives. In both, BP is under perfect control and potassium control is normal. eGFR is decreased in both, more so in patient 1. The latter is probably more age related decrease rather than progression of CKD. Patient 1 is much older than patient 2. Neither is anemic by definition and required erythropoietic stimulating agent any time. Hypertension which is a most important risk factor in CKD progression is under superb control in both. In both patients, BP control is achieved with dihydropyridine calcium channel blocker (CCB) alone or a combination of CCB and beta blocker. A low dose loop diuretic and digoxin is used in patient 2 for pulmonary congestion and shortness of breath sometimes during the 5-year period. Overall, neither patient manifests symptoms or signs of fluid overload. Metabolic acidosis, hence hyperkalemia is prevented in both patients with liberal use of sodium bicarbonate and potassium exchange resin. The latter is used in sorbitol solution to avoid constipation.

COLLOQUIUM OF FREQUENT OFFICE VISIT IN CKD

Frequent office visits, for example every four to 8 wk, depending on the symptoms and laboratory findings of the previous office visit, is essential for (1) detection of unwarranted symptoms and signs at an early stage (2) maintaining the current well-being and steady state of BP control and fluid-electrolyte and acid-base status. Thus during each office visit a check list is completed of the following: (2) symptoms, (2) weight, (3) BP, (4) hemoglobin/hematocrit, (5) serum glucose: fasting and 2-h postprandial for patients with existing or new-onset diabetes, (6) renal function: BUN, serum creatinine, and eGFR, (7) electrolytes: Na^+, K^+, CO_2; and calcium, phosphorous, uric acid and albumin, (8) intact PTH, (9) arterial blood gas to determine if low CO_2 is due to metabolic acidosis or respiratory alkalosis, severity of metabolic acidosis, and PO_2 in those with suspected

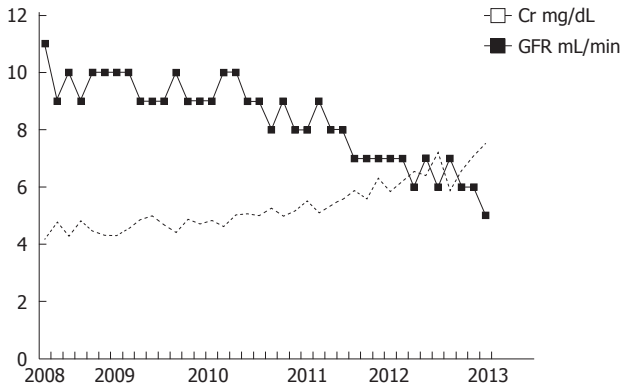

Figure 1 Serum creatinine and estimated glomerular filtration rate from patient 1 are depicted from 2008-2013. Note slow but progressive increase in creatinine (Cr) and decrease in glomerular filtration rate (GFR).

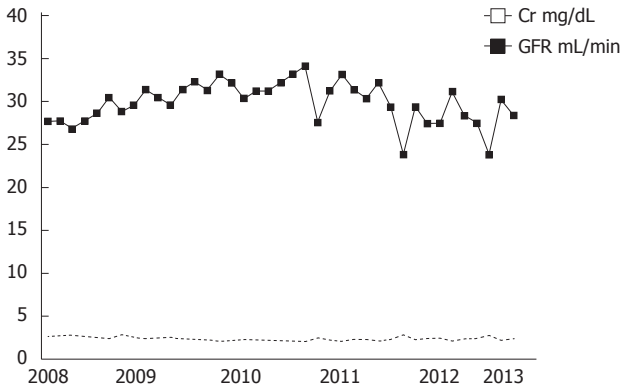

Figure 2 Elevated but essentially unchanged serum creatinin around 2.5 mg/dL yearly from 2008-2013, while estimated glomerular filtration ratevaried between 25 and 35 mL/min throughout the period from 2008-2013. Cr: Creatinine; GFR: Glomerular filtration rate.

congestive heart failure, (10) electrocardiogram in those with irregular heart rhythm, (11) review all medicines carefully. Discontinue any medicines suspected of causing renal function impairment and electrolyte imbalance.

GOALS OF FREQUENT OFFICE VISITS IN CKD

Keep BP under control. Generally BP less than 140/80 mmHg is acceptable. BP less than 130/70 mmHg is probably ideal. BP of less than 120/70 mmHg may be harmful. In order to maintain BP at the levels already mentioned, it is very important to review BP medication at every visit and adjust as required. In resistant hypertension, a diuretic is a choice to reduce sodium (salt) and water retention which is common in CKD patients. Chlorthalidone is the diuretic of choice which predictably reduces elevated BP associated with salt and water retention. The dose of chlorthalidone is 25-50 mg once daily. Hydrochlorothiazide 25-50 mg once daily can be used instead of chlorthalidone. Loop diuretic such as furosemide 40 mg per oral once or twice daily or bumetanide 1 mg per oral once or twice daily is preferable if patient has evidence of congestive heart failure such as shortness of breath on exertion, pulmonary congestion in a chest X-ray or low PO_2 in a blood gas analysis. Hyperkalemia and metabolic acidosis disorders are common and often severe, in those who are diet noncompliant, in particular consuming large meals or eating too many fruits. Prescription of angiotensin converting enzyme inhibitors or angiotensin receptor blockers by many prescribers with the obsessive idea of renal protection is a common cause of life threatening hyperkalemia ($\geqslant$ 7.5 mmol/L) and metabolic acidosis. Use of over the counter drugs commonly non-steroidal anti-inflammatory drugs for pain is also a common cause of hyperkalemia and metabolic acidosis.

Prevention of hyperkalemia is attainable but prevention of metabolic acidosis is more difficult to achieve. Since hyperkalemia is the result of transport of hydrogen ions, minimizing hydrogen build up is a key to prevention of both. Hydrogen ion build up can be minimized by controlling the source of hydrogen ion which is the food. A low protein diet (40-50 g) is beneficial in keeping BUN less than 50 mg/dL, reducing the risk of metabolic acidosis, hyperkalemia and hyperphosphatemia. A low protein diet supplies less sodium in the diet and is very useful in keeping BP under control and effective in minimizing fluid overload and CHF. However, low protein diet is non-palatable and adherence to this diet is uncommon. In addition, low protein diet is associated with malnutrition and low serum albumin which increase mortality. Thus protective effect of low protein diet cannot be relied upon; consequently therapeutic endeavor is essential. Protective effect of sodium bicarbonate therapy is documented by this author, and many other authors. Other authors have reported that sodium bicarbonate supplementation slows progression of CKD and improves nutritional status[6].

It should be noted that both patients in Table 1 are treated with sodium bicarbonate 650 mg tablet × 2, four times daily. Although CO_2 in patient 1 is lower than that of patient 2 which is due to severity of renal dysfunction in patient 1, but serum albumin level is near normal and comparable. For prevention and treatment of hyperkalemia, sodium polysterene sulfonate (SPS) (Kayexalate®) is a good therapy. SPS dispensed in 30% sorbitol is very effective in keeping potassium under control. The usual dose is 5 g in 20 mL sorbitol once or twice daily. It may cause diarrhea which of course is the mechanism to enhance potassium excretion through the bowel when renal excretion of potassium is low. Effectiveness of kayexalate is increased when dispensed in sorbitol rather than dispensed in powder form, however, in the long run sorbitol is likely to increase blood glucose level. 9-α fludrocortisone (Florinef®), a synthetic analog of aldosterone in doses of 0.1-1 mg per oral once daily (usual dose is 0.1-0.3 mg/d) is also effective in enhancing renal excretion of potassium, but it is fraught with a risk of hypertension and CHF. Serum sodium is a good index of fluid balance. Rapid decrease in serum sodium may be significant for fluid overload and CHF. Attention

must be paid in every office visit to hemoglobin level. Unchanged and normal or near normal hemoglobin (≥ 11 g/dL) is a good indirect evidence of stable renal function even if it is low. Rapid decrease of hemoglobin to less than 10 g/dL is a signal for rapidly deteriorating renal function, fluid overload, gastrointestinal bleeding or combination of any or all of these. Aspirin use should be avoided when hemoglobin is less than 11 g/dL and unstable. Phosphate control is a highly published item by the pharmaceutical industry. In the experience of the author, phosphate control has caused no problem to ensure well-being of the patients with CKD and maintain a dialysis free life.

CONCLUSION

Given the poor outcomes of many old, frail patients with multiple comorbid conditions on dialysis, there is a debate as to whether non-dialysis management as illustrated here would be more humane than dialysis management[7]. There are no studies done comparing survival or quality of life on conservative care and dialysis[8]. As described in the manuscript, conservative care should be given the highest priority in elderly people with advanced chronic renal failure. I believe that the evidence of the two patients shown here is important and should lead to a formal study.

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P- Reviewers: Duan SB, Malatino LS, Sweet S
S- Editor: Zhai HH **L- Editor**: A **E- Editor**: Liu XM

World Journal of Nephrology

Online Submissions: http://www.wjgnet.com/esps/
bpgoffice@wjgnet.com
doi:10.5527/wjn.v3.i1.6

World J Nephrol 2014 February 6; 3(1): 6-15
ISSN 2220-6124 (online)

ORIGINAL ARTICLE

Silent diabetic nephropathy

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Received: July 8, 2013 Revised: October 14, 2013
Accepted: November 1, 2013
Published online: February 6, 2014

Abstract

AIM: To examine the risk of renal events in patients with biopsy-proven diabetic nephropathy (DN) and its possible associated factors.

METHODS: Clinical and histological data of 60 patients diagnosed with diabetic nephropathy were retrospectively collected. Patients with evidence or suspicion of other nephropathies were excluded from the study. The final event was defined as renal replacement therapy (RRT) initiation or progression of chronic kidney disease (CKD), according to the KDIGO 2012 definition of a decrease in CKD category and a decrease in GFR of 25% or more.

RESULTS: A total of 45 patients with a follow-up of at least 3 mo were included. Most of the patients presented type 2 DM, with a median age of 58.3 years old. The time of evolution of DM was 9.6 ± 7.8 years, although in 13 patients, it was less than 5 years. A total of 62% of patients reached the final event in a mean period of 3.4 years (95%CI: 2.1-4.7), with 21 of them requiring dialysis. The factors that were independently associated with renal survival were estimated glomerular filtration rate (eGFR) at the time of biopsy, cardiovascular disease (CVD) history and HbA1c less than 7%. Therefore, for each 10 mL/min per 1.73 m^2 reduction in eGFR, we obtained a DN progression risk of HR = 2 (1.3-3.0) (P = 0.001); patients with CVD were at greater risk for DN progression (HR = 2.8, 1.1-7.1, P = 0.032), and CKD patients with HbA1c < 7% demonstrated greater renal risk than patients with HbA1c ⩾ 7%, with an HR of 2.9 (1.0-8.4) (P = 0.054).

CONCLUSION: A past history of CVD is a risk factor for DN progression. Levels of HbA1c less than 7% could favor an eGFR decrease in these patients.

Key words: Diabetic nephropathy; Predictors of progression; Histopathological diagnosis; Cardiovascular disease; Silent disease

Core tip: There are other forms of presentation of diabetic nephropathy (DN), in addition to progressive proteinuria, that can result in renal insufficiency. In some cases, DN is diagnosed in advanced stages, without previous suspicion of this diagnosis. The clinical course can be atypical, and the time of evolution of diabetes mellitus can be short. Not all the factors that play a role in the evolution of DN have been elucidated. Our findings suggest that in patients with chronic kidney disease secondary to DN, a previous history of cardiovascular disease and HbA1c less than 7%, are negative prognostic factors for renal function.

López-Revuelta K, Peña Galdo P, Stanescu R, Parejo L, Guerrero C, Pérez-Fernández E. Silent diabetic nephropathy. *World J Nephrol* 2014; 3(1): 6-15 Available from: URL: http://www.wjgnet.com/2220-6124/full/v3/i1/6.htm DOI: http://dx.doi.org/10.5527/wjn.v3.i1.6

INTRODUCTION

The risk factors for diabetic nephropathy (DN) include genetic predisposition[1,2], poor glycemic control[3], older age[4], male sex[5], duration of diabetes, hypertension[6] and smoking. Classically, the natural history of the disease was considered to be an evolution that began after 5-15 years after the onset of diabetes with albuminuria[7]. Albuminuria increases cardiovascular risk, but it also increases the risk of progression to proteinuria, especially in type 1 diabetes mellitus (T1DM)[8]. It is unclear what predisposes 50% of individuals with albuminuria to progress to proteinuria in a phase that lasts approximately 10 years[9]. After the development of proteinuria, 50% of patients will progress to end-stage renal disease (ESRD) in 7-10 years[10]. High risk of cardiovascular disease (CVD) further increases with deteriorating renal function. Some factors have been implicated in the increased rate of decline in kidney function, especially in type 2 diabetes mellitus (T2DM): higher baseline albuminuria; high systolic blood pressure; higher hemoglobin A1c; estimated glomerular filtration rate (eGFR); age; and coexistence of diabetic retinopathy[10,11]. However, a large inter-individual variation in the rate of decline in glomerular filtration rate (GFR) has been reported in both type 1 and type 2 DM. Recently, a nonalbuminuric renal impairment phenotype was described in T2DM, which has distinct clinical features that are not associated with HbA1c and that are correlated less strongly with retinopathy and hypertension[12]; this phenotype is associated with a higher prevalence of CVD and suggests a predominance of macroangiopathy as the underlying renal pathology, which has yet to be demonstrated. In T1DM, the development of advanced CKD relatively soon after the onset of albuminuria has been described, and this progression was not conditional to the presence of proteinuria[13]. Reduced eGFR also occurs among long-standing normoalbuminuric type 1 diabetic patients and has been associated with more advanced diabetic glomerular lesions and, probably, with increased risk of progression[14].

In patients with DN, there has also been described a nonlinear, abrupt and rapid progression pattern similar to that described by others[15] as rapid-onset end-stage renal disease, which some authors have related to inflammation and episodes of acute renal failure[16].

This spectrum of progression patterns highlights the need for the identification of risk factors for the loss of renal function early in the course of DN, especially in patients with histopathological confirmation of this diagnosis.

The aim of this study was to examine the risk of renal events in patients with biopsy-proven DN and its possible associated factors.

MATERIALS AND METHODS

We studied all the patients diagnosed with DN by renal biopsy at a Spanish center between December 1998 and December 2012, who had a minimum 3-mo follow-up after the biopsy. Of a total of 60 patients with histopathological diagnoses of DN at our center, we excluded 3 patients who had less than 6 glomeruli on biopsy, 10 patients with evidence of another associated nephropathy (2 IgA nephropathies, 1 membranous nephropathy, 1 membranoproliferative glomerulonephritis associated with HVC, 2 tubulointerstitial nephritis, 2 acute tubular necrosis, 2 with amyloidosis AA) and 2 patients with acute kidney injury: 1 with a functional etiology and the other with septic shock. A total of 45 patients diagnosed with "pure" DN and a sufficient number of glomeruli for the diagnosis were included.

In all the cases, the nephrologist was the specialist who recommended the biopsy, considering all the available data. We classified the indications for renal biopsy into three groups: nephrotic proteinuria with or without nephrotic syndrome; rapidly progressive kidney injury (RPKI); and CKD. All the renal biopsies were revised by a nephropathologist to confirm the glomerular classification type, the grade of interstitial fibrosis and tubular atrophy (IFTA), interstitial inflammation, arteriolar hyalinosis and the presence of large vessel arteriosclerosis on the basis of the criteria previously described[17]. Four types are described: Glomerular Class Ⅰ, glomerular basement membrane thickening; Class Ⅱ, mesangial expansion, mild (Ⅱa) or severe (Ⅱb); Class Ⅲ, nodular sclerosis (Kimmelstiel-Wilson lesions); and class Ⅳ, advanced diabetic glomerulosclerosis.

The demographic, clinical, and laboratory data and comorbid conditions of every patient at the time of biopsy were extracted from clinical records. We recorded the date when the nephrologist began follow-up and whether the patients were receiving treatment with renin-angiotensin aldosterone system inhibitors (RAASIs), statins or antiplatelet drugs.

In 39 patients, we had available information on baseline renal function from 1.1 to 24.1 mo before renal biopsy. We recorded the last follow-up serum creatinine or the starting date of RRT for all the patients.

The glomerular filtration rate was estimated according to the CKD-EPI formula[1] at baseline, at renal biopsy and at the last follow-up visit. The eGFR and albuminuria categories of CKD were classified according to the KDIGO 2012 classification[18]. In this manner, six eGFR categories were recognized (mL/min per 1.73 m^2): G1 = eGFR $\geqslant$ 90; G2 = 60-89; G3a = 45-59; G3b = 30-44; G4 = 15-29; and G5 < 15. Proteinuria categories were described based on protein-to-creatinine ratio (mg/g) or protein excretion rate (mg/24 h) as follows: A1 $\leqslant$ 150; A2 = 150-500; and A3 $\geqslant$ 500.

Table 1 Clinical characteristics at renal biopsy *n* (%)

Total	*n* = 45
Age (yr) (range)	58.3 ± 13.3 (28-84)
Sex (men)	32 (71.1)
Diabetes type 2	38 (84.4)
Diabetes duration (yr) (range)	9.6 ± 7.8 (0-35)
BMI (kg/m^2) (range)	29.3 ± 5.3 (27.8-47.8)
Obesity BMI > 30 kg/m^2	18 (40.9)
Hypertension (yes)	42 (93.3)
Smoker, active or past (yes)	34 (75.6)
Dyslipidemic (yes)	33 (73.3)
Ischemic heart disease (yes)	7 (15.6)
CVA (yes)	6 (13.3)
Peripheral arterial disease (yes)	8 (17.8)
Any CVD	16 (35.6)
Hematuria (yes)	18 (41.9)
Serum albumin (g/dL) (range)	3.4 ± 0.7 (2-5)
HbA1c% (range)	6.5 ± 1.4 (4.1-9.3)
Total cholesterol (mg/dL)	177.9 ± 58.7
Previous nephrology care (yr) (range)	1.21 ± 2.4 (0-12)
RAASI treatment	40 (88.9)
Statin treatment	33 (73.3)
Antiplatelet drug treatment	21 (46.7)

BMI: Body mass index; CVD: Cardiovascular disease; RAASI: Renin-angiotensin aldosterone system inhibitor; CVA: Cerebrovascular accident.

The presentation of RPKI was considered in those cases in which a decrease in eGFR greater than 25% was seen between baseline and biopsy, independent of biopsy indication. The final end-point was defined as RRT initiation or progression of CKD according to the KDIGO 2012 definition as a in CKD category and a decrease in eGFR of 25% or more. The follow-up period was considered from biopsy until endpoint, death or last follow-up.

The silent diabetic nephropathy variable was defined for cases that showed an atypical disease pattern or in which DN was not suspected. This variable grouped patients with RPKI without significant proteinuria (< 0.5 g/d) and/or a duration of DM of less than 5 years and/or the need to start RRT less than 1.5 years from renal biopsy.

Statistical analysis

The statistical analysis was performed using SPSS, version 17.0 for Windows, and the STATA software, version 12. Quantitative data are described by means ± SDs or medians (interquartile ranges). Qualitative data are described by counts and percentages [*n* (%)].

Survival median was estimated by the Kaplan-Meier function. The log-rank test was used to compare survival functions. To study factors associated with renal events, univariate analysis was performed, adjusting Cox regression models. The proportionality of hazards assumption was checked graphically. Finally, a multivariate predictive model was adjusted, including statistically significant variables and clinically relevant factors. The model was adjusted by the enter method and including the least number of covariates necessary. Harrell's c-index[19] was calculated to evaluate the model's predictive ability. This index measures the ability of a predictor to separate groups with different answers and is still acceptable greater than approximately 0.85. An exploratory descriptive analysis was performed to compare the two samples, defined by the silent DN variable. Association was studied by the χ^2 test or Fisher's exact test and the Mann-Whitney *U* test. To estimate silent DN's effects on the risk of renal events, we adjusted the multivariate Cox regression model, including possible confounding factors (complete model). We defined a confusion factor as a difference of more than 10% between the adjusted hazard ratio (HR) and the complete model. HRs are presented with 95%CIs. All the tests were two-tailed, and a significance level ⩽ 0.05 was considered statistically significant.

RESULTS

Data from 45 patients were included in this study. The patients' characteristics at the time of biopsy are detailed in Table 1. Most patients with biopsy-proven DN in our series had type 2 diabetes and were hypertensive, dyslipidemic and smokers. Seventy-one percent were men with a mean age of 58.3 ± 13.3 years old and a DM evolution time of 9.6 ± 7.8 years. Thirty-five percent had cardiovascular disease, 40% had retinopathy, and 40% had microhematuria. Their values of HbA1c were normal, according to international recommendations for these patients, but their cholesterol levels were not normal, although 73% of the patients were on statins. Furthermore, 89% of the subjects were on treatment with RAASIs, as well as 47% on antiplatelet drugs at the time of the biopsy.

In Table 2, we show the evolution of the renal parameters during follow-up. In 62% of the cases, the biopsy indication was a nephrotic range of proteinuria, with or without nephrotic syndrome. Nine percent of the patients presented proteinuria ⩽ 0.5 g/24 h at the time of the biopsy. Although 48.8% of the patients showed baseline creatinine ⩽ 1.4 mg/dL, 68% of them showed eGFRs at time of biopsy < 45 mL/min per 1.73 m^2, and 15.6% were in the grade 5 eGFR category.

Thirty-three percent of the subjects were classified with RPKI, three of them without significant proteinuria (< 0.5 g). Seven of these patients needed dialysis, two of them only for a mean time of 8 d and the others permanently.

Twenty-eight patients (62%) reached the final event, and 21 of them required RRT. The median renal survival 3.4 years (95%CI: 2.1-4.7).

In Table 3, we describe the clinical and histopathological findings, classified according to the type of glomerular lesions. Most cases (23 patients) presented glomerular class Ⅲ or nodular sclerosis, and 4 subjects (9%) had advanced diabetic glomerulosclerosis (class Ⅳ) that was not suspected when the biopsy has been recommended. The four patients whose diagnoses of DN coincided with the DM diagnosis had advanced forms of DN: 2 cases with class Ⅱ b, 1 case with class Ⅲ and

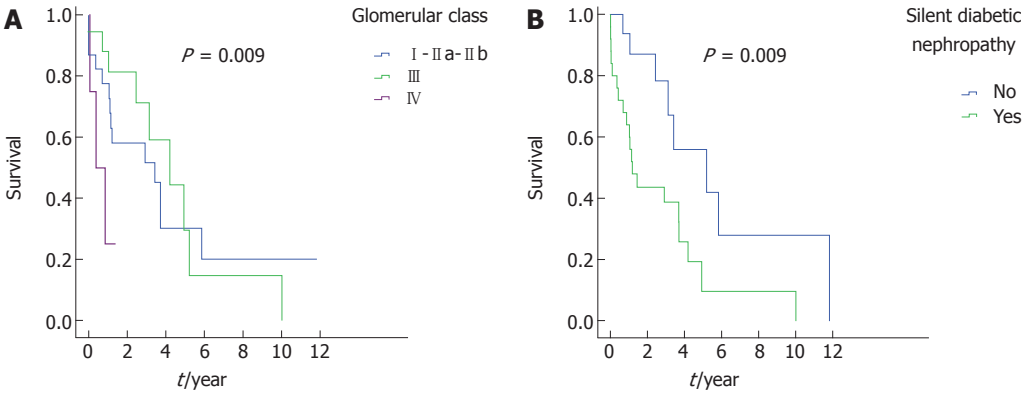

Figure 1 Renal survival. A: Depending on glomerular classification. For renal survival analysis using the Kaplan-Meier method, histopathological classes Ⅰ, Ⅱa and Ⅱb were grouped together as there were insufficient cases for separate analysis. The median renal survival in class Ⅰ/Ⅱa/Ⅱb was 4.2 years (95%CI: 1.8-6.6), in class Ⅲ, it was 3.4 (95%CI: 0.6-6.2), and in class Ⅳ, it was 0.4 (95%CI: 0-1.2). We found statistically significant differences (P = 0.009) when comparing class Ⅳ with the other classes and also when comparing class Ⅲ to classes Ⅰ and Ⅱa-Ⅱb; B: In silent and non-silent diabetic nephropathy. Renal survival of patients with silent and non-silent diabetic nephropathy (DN) was compared. The median of renal survival in patients with silent DN was 5.2 years (95%CI: 1.1-9.4) and 1.2 years (95%CI: 0.5-1.8) in cases of patients with non-silent DN (P = 0.009).

1 case with class Ⅳ.

The patients who had advanced diabetic glomerulosclerosis were younger, had more cardiovascular diseases and retinopathy, and had worse renal function and lower figures of serum albumin than other histopathological types. Additionally, this group of patients showed a higher proportion and greater severity of interstitial fibrosis and tubular atrophy but no differences in vascular lesions or inflammation scores. Renal survival was variable in the different glomerular classes, not only comparing class Ⅳ with the other classes but also comparing class Ⅲ to classes Ⅰ and Ⅱa-Ⅱb (Figure 1A); the median in class Ⅰ/Ⅱa/Ⅱb was 4.2 years (95%CI: 1.8-6.6), in class Ⅲ, the median was 3.4 (95%CI: 0.6-6.2), and in class Ⅳ, it was 0.4 (95%CI: 0-1.2; P = 0.009).

Twenty-five patients were considered to have silent DN: thirteen patients with less than 5 years of duration of DM at biopsy, 4 of them diagnosed with diabetes at the same time as renal biopsy; 1 patient with RPKI and proteinuria < 0.5 g/d; and 14 patients who began RRT before 1.5 years after biopsy (3 with less than 5 years of duration of DM). As shown in Table 4, compared to the remainder of the patients, the silent DN subjects presented with a shorter evolution time of diabetes, had worse renal function at the time of biopsy, had a higher frequency of RPKI and less HbA1c, and had more advanced histopathological forms, and they presented more renal events. They frequently had more cardiovascular disease, although this difference was not statistically significant. To estimate the risk of silent DN, we adjusted a multivariate regression model including possible bias factors: age, eGFR, proteinuria, glomerular class, CVD and HbA1c. The final model (Table 5) estimated the risk for silent DN of 2.1 (95%CI: 0.8-5.1), adjusted for cardiovascular disease and HbA1c. The remainder of the factors were discarded as they were considered confounders. Figure 1B illustrates the renal survival curves in silent DN, compared to the other subjects.

The results of univariate Cox proportional hazard analysis, according to clinical variables and histopathological variables, are shown in Tables 6 and 7, respectively. Clinical variables statistically significantly associated with renal end point were: baseline and renal biopsy eGFR and serum creatinine; BMI < 30 kg/m^2; Hb A1 < 7%; RPKI; silent DN; and coexistence of cardiovascular disease. Of the histopathological variables, only glomerular class Ⅳ and percentage of global glomerulosclerosis were statistically significantly associated with the renal end point.

The results of multivariate Cox proportional hazard models are shown in Table 8. We found that eGFR, cardiovascular disease and HbA1c at the time of biopsy were risk factors for progression of DN (initiation of renal replacement therapy or decline ⩾ 25% and change in CKD category), adjusted for age and sex. For every 10 mL/min per 1.73 m^2 decrease in eGFR, we obtained a DN progression risk of HR = 2 (1.3-3.0) (P = 0.001). Patients with cardiovascular disease were at greater risk for DN progression (HR = 2.8, 1.1-7.1; P = 0.032). Although diabetic patients with CKD and HbA1c < 7% showed greater renal progression risk than patients with HbA1c ⩾ 7%, with an HR of 2.9 (1.0-8.4), this effect was not statistically significant (P = 0.054). Harrel's c index was 0.823, indicating acceptable predictive ability.

DISCUSSION

The present study analyzed clinical and histopathological factors associated with worse renal prognosis in a cohort of patients with biopsy-proven diabetic nephropathy, mostly type 2 diabetics. Two thirds of the patients had an eGFR at the time of the biopsy < 45 mL/min per 1.73 m^2, that is, irreversible damage to renal function, and half of the patients reached ESRD in a median period of 3.4 years.

In our series, eGFR at time of biopsy was a determinative factor for CKD progression, as is already well known. In contrast, proteinuria was not associated with

Table 2 Renal parameters and evolution *n* (%)

		Previous renal data (*n* = 39)	Renal biopsy (*n* = 45)	End of follow-up (*n* = 24)
Time prior to biopsy (mo) (range)		7.3 ± 5.2 (1.1-24.1)		
Follow-up period (yr) (range)				3.4 ± 2.9 (0.2-11.8)
Renal biopsy indication	RPKI		8 (17.8)	
	Nephrotic proteinuria		28 (62.2)	
	CKD		9 (20)	
Serum creatinine (mg/dL) (range)		1.6 ± 0.8 (0.8-4.5)	2.3 ± 1.5 (0.8-6)	2.3 ± 1.8 (0.7-9.1)
eGFR (mL/min per 1.73 m^2) (range)		51.4 ± 20.9 (14.7-97.6)	39.1 ± 22.5 (8.1-101.2)	40.8 ± 25 (5.1-107.1)
[1]eGFR category	G5 < 15		7 (15.6)	24 (53.3)
	G4 15-30		8 (17.8)	6 (13.3)
	G3b 30-45		15 (33.3)	5 (11.1)
	G3a 45-60		7 (15.6)	7 (15.6)
	G2 60-90		7 (15.6)	1 (2.2)
	G1 > 90		1 (2.2)	2 (4.4)
> 25% drop in eGFR prior to biopsy		13 (33.3)		
[2]Proteinuria (range)		3.7 ± 3.4 (0-12.9)	4.5 ± 2.7 (1-8.9)	
[3]Proteinuria category	A1 < 150	3 (8.8)	0	
	A2 150-500	2 (5.9)	4 (8.9)	
	A3 > 500	29 (85.3)	41 (91.1)	
RRT		2	5	21 (46.7)
[4]CKD progression				7 (15.6)
eGFR improvement > 25%				4 (8.9)
Exitus				5 (11.1)

[1]GFR categories (mL/min per 1.73 m^2): G1 = GFR ⩾ 90; G2 = 60-89; G3a = 45-59; G3b = 30-44; G4 = 15-29; G5 < 15. [2]Baseline proteinuria was measured using protein/creatinine ratio in spot urine (mg/g). Renal biopsy proteinuria was measured using excretion rate over 24 h (g/d). [3]Proteinuria categories are described based on protein-to-creatinine ratio (mg/g) or protein excretion rate (mg/24 h) in: A1 ⩽ 150, A2 = 150-500, A3 ⩾ 500. [4]CKD progression: 25% or greater eGFR decline, accompanied by a decrease in GFR category. RPKI: Rapidly progressive kidney injury; eGFR: Estimated glomerular filtration rate; CKD: Chronic kidney disease; RRT: Renal replacement therapy.

worse renal prognosis, although the majority of patients showed proteinuria > 500 g/d, but most of patients without proteinuria also experienced renal events.

Although our study included selection bias, which was the clinical indication for renal biopsy, our series included only cases of DN in which other causes of renal damage had been excluded. Therefore, our findings, even if they cannot be extrapolated to all patients with DN, could increase understanding of why some patients with diabetes have atypical clinical courses and are diagnosed in advanced stages of renal disease, with minimal therapeutic possibilities.

Although the majority of patients had been medically followed up before biopsy, this fact did not prevent negative evolution or late diagnosis of the illness. The RPKI presentation form, predominant in 33% of the patients, was associated with a poor renal prognosis, although it behaved as a confounding factor and not as an independent risk factor.

It was shown[16] that, in DN, relatively small elevations in serum creatinine could significantly underestimate the degree of renal damage, and these elevations were unpredictable most of the time.

Without a doubt, this fact contributed to the large proportion of patients in our series that we classified with silent diabetic nephropathy, that is, cases that went unnoticed until advanced stages. These patients had shorter diabetes evolution times; they presented a higher frequency of RPKI, a major loss of renal function at the moment of the biopsy, and they had a higher proportion of renal events. Although they had more cardiovascular diseases compared to the remainder of the group, this difference was not statistically significant.

All these data support that serum creatinine is not a good parameter for monitoring renal function in diabetic patients and that even with normal serum creatinine levels, eGFR should be a routine test. It is probable that an eGFR at less than 90 mL/min per 1.73 m^2, we should recommend several tests per year in these patients to detect CKD progression and optimize their treatment.

In a study of 22 biopsy-proven diabetic nephropathy cases[16], these authors found in the majority of cases evidence of acute kidney injury in their biopsies, including tubular necrosis and interstitial inflammation, although seven subjects had similar rates of progression and yet undetectable acute events. In our series, we excluded those patients in whom we suspected renal failure secondary to another etiology. However, it is possible that some cases of functional loss might have existed, especially in nephrotic patients, because in four patients, we observed an improvement in renal function during follow-up. Other authors found that interstitial lesions, but not glomerular class, was a significant predictor of renal prognosis in diabetic nephropathy in type 2 diabetes[20],

Table 3 Clinical and histopathological findings according to glomerular classification of diabetic nephropathy *n* (%)

		Ⅰ-Ⅱa-Ⅱb (*n* = 18)	Ⅲ (*n* = 23)	Ⅳ (*n* = 4)
Age (yr)		59.9 ± 12.2	58.1 ± 14.1	52.3 ± 15.4
Years of diabetes		9.1 ± 7.4	9.3 ± 6.3	15.1 ± 18
BMI (kg/m^2)		29 ± 4.9	30.1 ± 6	26.8 ± 2.4
Serum creatinine (mg/dL)		2 ± 1.2	2.2 ± 1.4	4.4 ± 1.9
eGFR (mL/min per 1.73 m^2)		42.3 ± 21.9	40.4 ± 22.9	17.6 ± 12.3
HbA1c (%)		6.7 ± 1.4	6.3 ± 1.3	6.7 ± 2
Proteinuria (g/d)		3.1 ± 2.6	5.2 ± 4	4.7 ± 5.6
Serum albumin (g/dL)		3.6 ± 0.7	3.3 ± 0.7	2.9 ± 0.7
Serum cholesterol (mg/dL)		165.4 ± 71.6	191.5 ± 34.6	213 ± 0
Hypertension		16 (88.9)	22 (95.7)	4 (100)
Diabetic retinopathy		7 (38.9)	9 (39.1)	2 (50)
CVD		7 (38.9)	6 (26.1)	3 (75)
RPKI		0	6 (33.3)	7 (30.4)
Patients with renal events		9 (50)	15 (65.2)	4 (100)
RRT		6 (33.3)	11 (47.8)	4 (100)
[1]Years from biopsy to renal event		4.2 ± 1.2	3.4 ± 1.4	0.4 ± 0.4
% of global glomerulosclerosis		18.1 ± 12.8	18.4 ± 15.1	77.3 ± 16.9
Interstitial fibrosis	0	1 (5.6)	0	0
and tubular atrophy	1	10 (55.6)	13 (56.5)	0
	2	5 (27.8)	9 (39.1)	1 (25)
	3	2 (11.1)	1 (4.3)	3 (75)
Interstitial inflammation	0	3 (16.7)	0	0
	1	14 (77.8)	22 (95.7)	4 (100)
	2	1 (5.6)	1 (4.3)	0
Arteriolar hyalinosis	0	1 (5.6)	1 (4.3)	1 (25)
	1	3 (16.7)	2 (8.7)	0
	2	14 (77.8)	20 (87)	3 (75)
Large vessel arteriosclerosis (yes)		17 (94.4)	19 (86.4)	4 (100)

[1]Years from biopsy to renal event are expressed as medians ± SEs, estimated by Kaplan-Meier method. CVD: Cardiovascular disease; BMI: Body mass index; RPKI: Rapidly progressive kidney injury; RRT: Renal replacement therapy; eGFR: Estimated glomerular filtration rate.

Table 4 Clinical differences at the time of biopsy between silent and non-silent diabetic nephropathy *n* (%)

	Non-silent DN (*n* = 20)	Silent DN (*n* = 25)	*P* value
Age (yr)	55.8 ± 12.2	60.3 ± 14.1	
Sex (women)	5 (25)	8 (32)	
BMI (kg/m^2)	30.9 ± 6.3	28 ± 4.1	
T2 DM	17 (85)	21 (84)	
Duration of diabetes (year)	12.5 ± 5.3	7 ± 8.9	0.005
Follow-up period (year)	3.4 ± 3.5	3.5 ± 2.7	
Smoking, active or past	16 (80)	18 (72)	
Retinopathy	8 (40)	10 (40)	
CVD	6 (30)	10 (40)	
HbA1c (%)	7 ± 1.2	6.2 ± 1.5	0.03
Serum creatine at biopsy (mg/dL)	1.8 ± 1	2.7 ± 1.6	0.03
eGFR at biopsy (mL/min per 1.73 m^2)	47 ± 22.5	32.9 ± 20.8	0.04
Proteinuria (g/d)	3.5 ± 2.6	5 ± 4.3	
Hematuria	8 (44.4)	10 (40)	
RPKI	3 (15)	12 (48)	0.03
CKD progression	3 (15)	10 (50)	0.04
Renal events	8 (40)	20 (80)	0.01
Histopathological class AP Ⅲ-Ⅳ	9 (45)	18 (72)	
Glomerular sclerosis percentage	18.8 ± 14.3	27.2 ± 26.4	
IFTA 0-1	12 (60)	12 (48)	
IFTA 2	7 (35)	8 (32)	
IFTA 3	1 (5)	5 (20)	
Severe arteriolar hyalinosis	15 (75)	22 (88)	
Large vessel arteriosclerosis	18 (94.7)	22 (88)	
RAASI treatment	18 (90)	22 (88)	
Statins treatment	15 (75)	18 (72)	

IFTA: Interstitial fibrosis and tubular atrophy; CVD: Cardiovascular disease; RPKI: Rapidly progressive kidney injury; RRT: Renal replacement therapy; CKD: Chronic kidney disease; eGFR: Estimated glomerular filtration rate; BMI: Body mass index; RAASI: Renin-angiotensin aldosterone system inhibitor; DN: Diabetic nephropathy; DM: Diabetes mellitus.

Table 5 Multivariate Cox proportional model of renal end point by the variable silent diabetic nephropathy

Variables in the equation	HR	95%CI	
		Lower	Upper
CVD	3.943	1.649	9.429
HbA1c%	0.724	0.516	1.016
Silent DN	2.137	0.819	5.573

Silent DN: Histologic diagnosis of DN and RPKI without significant proteinuria (< 0.5 g/d) and/or a DM with less than 5 years of evolution and/or the need to begin RRT before 1.5 years after renal biopsy. CVD: Cardiovascular disease; DN: Diabetic nephropathy; HR: Hazard ratio; DM: Diabetes mellitus.

but it was a small series of 69 type 2 diabetic patients, all with overt proteinuria.

The only histopathological finding of our series that proved to be a risk factor for renal progression was advanced diabetic glomerulosclerosis. It is possible that if our sample had been larger, we would have been able to demonstrate prognostic values of more benign histological types, as observed by the different lengths of renal survival seen in our series, which was worse for type Ⅲ nodular sclerosis, compared to patients with types Ⅰ and Ⅱa-Ⅱb.

Another risk factor associated with poor renal prognosis was a BMI < 30 kg/m^2. Although obesity is a risk factor for CKD and ESRD[21], its effects have not been clear in patients with T2DM[22]. Although in our study, BMI seemed to have a paradoxical effect, similar to that described in the survival of patients with T2DM in TSR[23], it was not an independent risk factor for renal progression.

In the present study, HbA1c < 7% was correlated with worse renal prognosis in patients with established DN. Important large, randomized, controlled, multicenter trials have shown that intensive glycemic control in T2DM reduces the risk of albuminuria and proteinuria[24], but evidence has been lacking that intensive glycemic control reduces the risk of significant clinical renal outcomes, such as doubling of serum creatinine level, ESRD or death from renal disease[25]. In these trials, severe hypoglycemia was clearly increased among intensively treated patients[26]. In contrast, we know that the individuals with progressive renal dysfunction are at increased risk for hypoglycemia, which is multifactorial.

These trials have either failed to demonstrate a benefit of glucose lowering for CVD risk or have even suggested an increased CVD risk with very tight glycemic control, most likely explained by the adverse effects of hypoglycemia on the heart and blood vessels[27]. Acute hypoglycemia triggers a cascade of physiologic responses, including the activation of inflammatory pathways, release of counter-regulatory hormones, including epinephrine, and reduced blood flow to the myocardium.

In a recent prospective study in older adults with diabetes[28], an association between dementia and having presented hypoglycemic episodes during 12 years of follow-up was found. The authors indicated as possible etiopathogenic mechanisms hypoxia by vasoconstriction, neuronal loss, hyperinsulinemia, exacerbation of the oxidative stress and inflammatory mediators. Although this series was adjusted for cardiovascular events, small vessel vascular disease was not discharged, so it is possible that cerebral microinfarcts played some part in cerebral atrophy and cognitive deterioration.

In the present study, a past history of CVD was identified as an independent risk factor for CKD progression in DN, almost tripling the risk of progressive CKD. Although the effects that diabetic CKD has on CV risk are well known[29], the renal risk of CVD in DN has not been defined. Some authors have advocated that vascular disease of the kidney can explain nonalbuminuric progressive DN[30]. In our series, the prevalence of CVD was similar or even slightly lower than that reported by these authors (37% in patients with reduced eGFR), but we have already mentioned that this form of onset was very rare in our series. Similar degrees of intrarenal vascular disease, measured by the Doppler resistance index of the interlobar renal arteries, were found in diabetic patients with reduced GFR, regardless of their albuminuria status.

Our data sustain that regardless of albuminuric phenotype, past history of CVD is a risk factor for progressive renal function decline in DN, as other authors have found[31]. In support of this theory, a recent study[32] linked cerebral microinfarcts, diagnosed by magnetic resonance imaging, with low eGFR and worse renal prognosis in type 2 DM, regardless albuminuria. The risk of doubling of the serum creatinine concentration or the need for dialysis was significantly greater for patients with silent cerebral infarction (HR = 4.79, 95%CI: 2.72-8.46) than for patients without silent cerebral infarction. The authors believe that this association might have been due to the similarity between renal and cerebral vascular hemodynamic behaviors.

Therefore, it is interesting that in our series, we found that cardiovascular disease and tighter glycemic control were DN progression risk factors. Although our findings cannot be extrapolated to the totality of patients with diabetic nephropathy, we can speculate that at least in diabetic patients with vascular disease, the benefits of strict glycemic control do not improve renal prognosis when kidney failure has already been established. It is possible that on an already damaged renal parenchyma, hypoglycemia could induce the release of proinflammatory mediators by means of hypoxia, which could explain the accelerated evolution of renal failure in patients with an inflamed substrate prone to cardiovascular disease.

Some studies have revealed that serum levels of various proinflammatory cytokines, chemokines and adhesion molecules, particularly TNF-α and IP-10, were associated with the severity of DN and of atherosclerosis[33]. These molecules could be useful markers for the progression of DN and atherosclerosis.

In conclusion, in our study of a cohort of patients with biopsy-proven diabetic nephropathy and kidney failure, we found that a history of CVD was an inde-

Table 6 Univariate Cox proportional hazard analysis of renal end point, according to clinical variables

	HR	95%CI		*P* value
		Lower	Upper	
Age (yr)	1.00	0.97	1.03	
Sex (men)	1.19	0.50	2.85	
Diabetes type (2/1)	0.76	0.31	1.91	
Diabetes duration (years)	1.01	0.95	1.07	
BMI < 30 (yes/no)	2.94	1.09	7.69	0.03
Smoker (yes/no)	0.92	0.34	2.50	
Hypertension (yes/no)	1.01	0.13	7.69	
CVD (yes/no)	4.56	1.94	10.69	0.000
Retinopathy (yes/no)	1.24	0.56	2.75	
Baseline Serum creatinine (mg/dL)	2.18	1.14	4.16	0.02
Baseline eGFR (mL/min per 1.73 m^2)	0.98	0.95	1.00	
[1]Baseline proteinuria (g/g)	1.12	0.98	1.27	
eGFR drop > 25% before biopsy	3.96	1.54	10.18	0.004
Serum creatinine (mg/dL) at biopsy	2.97	1.91	4.61	0.000
eGFR (mL/min per 1.73 m^2) at biopsy	0.94	0.92	0.97	0.000
RPKI	2.74	1.21	6.24	0.02
[2]Proteinuria at biopsy (g/d)	1.09	0.97	1.23	
Hematuria	1.65	0.72	3.82	
Serum albumin (g/dL)	0.75	0.41	1.35	
HbA1c % (**< 7/≥ 7**)	3.37	1.23	9.25	0.02
Total cholesterol (mg/dL)	0.98	0.96	1.01	
RAASI treatment (yes/no)	0.59	0.22	1.59	
Statin treatment	0.66	0.27	1.62	
Silent DN	3.04	1.26	7.3	0.02

[1]Baseline proteinuria was measured using protein/creatinine ratio in spot urine (g/g). [2]Renal biopsy proteinuria was measured using excretion rate in 24 h (g/d). BMI: Body mass index; CVD: Cardiovascular disease; eGFR: Estimated glomerular filtration rate; RPKI: Rapidly progressive kidney injury; RAASI: Renin-angiotensin aldosterone system inhibitor; DN: Diabetic nephropathy.

Table 7 Univariate Cox proportional hazard analysis of renal end point, according to histological variables

		HR	95%CI		*P* value
Glomerular class	Ⅲ/Ⅰ-Ⅱa-Ⅱb	1.2	0.5	2.9	
	Ⅳ/Ⅰ-Ⅱa-Ⅱb	5.6	1.6	19.7	0.007
	Ⅳ/Ⅲ	4.6	1.4	15.1	0.01
% of global glomerulosclerosis		1.0	1.0	1.0	0.01
IFTA	(> 25 ≤ 25%)	1.2	0.5	2.5	
Arteriolar hyalinosis	Severe/mild	0.7	0.2	2.2	
Large vessel arteriosclerosis (yes)	1-2/0	1.2	0.4	4.1	

HR: Hazard ratio; IFTA: Interstitial fibrosis and tubular atrophy.

Table 8 Multivariate Cox proportional hazard model of renal end point, adjusted for age and sex

	HR	95%CI for HR		*P* value
		Lower	Upper	
CVD	2.75	1.07	7.11	0.036
RPKI	1.29	0.46	3.64	0.626
eGFR (10 mL/min per 1.73 m^2)	1.96	1.28	3.00	0.001
HbA1c% (**< 7/≥ 7**)	2.88	0.98	8.44	0.054

CVD: Cardiovascular disease; HR: Hazard ratio; RPKI: Rapidly progressive kidney injury; eGFR: Estimated glomerular filtration rate.

pendent progression factor for diabetic nephropathy and that levels of HbA1c less than 7% could favor renal progression, especially in cases with associated vascular disease. Whether this accelerated progression is due to renal vascular disease or to an underlying inflammatory state could not be clarified in this study.

It is necessary to diagnose diabetic patients at risk for cardiovascular disease and kidney disease progression before these lesions become irreversible. The biochemical parameters normally used in clinical settings are not good markers of renal progression. Prospective studies should be undertaken to evaluate the usefulness more refined parameters, such as cystatine C clearance and inflammatory and early vascular damage markers, in diabetic patients to detect and treat these patients earlier.

ACKNOWLEDGMENTS

We thank Juan Orive and Kim Ball for their inestimable assistance in translating this article.

COMMENTS

Background

Diabetes mellitus (DM) is one of the leading causes of end-stage kidney disease. Different forms of presentation and progression of diabetic nephropathy have been described, both in DM1 and DM2.

Research frontiers

The prognostic factors of diabetic nephropathy (DN) have not been well established, nor have been the indicators for identifying patients at greater risk for progression. Further translational studies should be performed to increase knowledge of the etiopathogenic mechanisms and treatment of this type of nephropathy.

Innovations and breakthroughs

This study supports that glomerular lesions were the basic substrates responsible for renal insufficiency in a subgroup of diabetic patients. DN sometimes presents with rapid progression despite proteinuria. It is probable that glomerular lesions and cardiovascular disease in diabetic patients share a common substrate that implies a worse prognosis for these patients. Further studies are needed to support the theory of a possible negative renal effect of strict metabolic control in patients with established diabetic nephropathy.

Applications

Serum creatinine and proteinuria are not early markers to detect the risk of progression in DN. The threshold of eGFR, less than which renal function must be monitored, should be much higher in diabetic patients than in other chronic kidney disease patients, especially if there are associated cardiovascular risk factors. Authors should be cautious in metabolic control of patients with cardiovascular disease and DN.

Terminology

Diabetic nephropathy: Renal complications of diabetes; Histopathological diagnosis: The histopathological diagnosis of DN is based on light and electron microscopic glomerular lesions. Tubulointerstitial and vascular lesions often accompany glomerular changes, but they are not specific to diabetes; Silent disease: This term describes ischemic heart disease in diabetic patients who presents as myocardial ischemia without angina. In this study, the authors have extrapolated this term to nephropathy to refer to the way it presents, with hardly any clinical renal expression until advanced stages of illness.

Peer review

This is an interesting observational study on the clinical course of DN, focusing in particular on a novel phenotype called silent DN.

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P- Reviewers: Duan SB, Olowu WA, Kirmizis DA
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World Journal of Nephrology

Online Submissions: http://www.wjgnet.com/esps/
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World J Nephrol 2014 February 6; 3(1): I-V
ISSN 2220-6124 (online)

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Name of journal

World Journal of Nephrology

ISSN

ISSN 2220-6124 (online)

Launch date

February 6, 2012

Frequency
Quarterly

Editorial office
Jin-Lei Wang, Director
Xiu-Xia Song, Vice Director
World Journal of Nephrology
Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
Beijing 100025, China
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Fax: +86-10-85381893
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Publisher
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16 **Pagedas AC**, inventor; Ancel Surgical R&D Inc., assignee. Flexible endoscopic grasping and cutting device and position-

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World Journal of *Nephrology*

World J Nephrol 2014 May 6; 3(2): 16-23

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NAME OF JOURNAL
World Journal of Nephrology

ISSN
ISSN 2220-6124 (online)

LAUNCH DATE
February 6, 2012

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Quarterly

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MINIREVIEWS

Pediatric lupus nephritis: Management update

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Received: May 30, 2013 Revised: August 28, 2013
Accepted: March 3, 2014
Published online: May 6, 2014

Abstract

Childhood-onset systemic lupus erythematosus (cSLE) is a severe multisystem autoimmune disease. Renal involvement occurs in the majority of cSLE patients and is often fatal. Renal biopsy is an important investigation in the management of lupus nephritis. Treatment of renal lupus consists of an induction phase and maintenance phase. Treatment of childhood lupus nephritis using steroids is associated with poor outcome and excess side-effects. The addition of cyclophosphamide to the treatment schedule has improved disease control. In view of treatment failure using these drugs and a tendency for non-adherence, many newer agents such as immune-modulators and monoclonal antibodies are being tried in patients with cSLE. Trials of these novel agents in the pediatric population are still lacking making a consensus in the management protocol of pediatric lupus nephritis difficult.

Key words: Pediatric; Lupus nephritis; Management; Monoclonal antibody; Cyclophosphamide; Mycophenolate mofetil

Core tip: Childhood-onset systemic lupus erythematosus (cSLE) is a rare but severe autoimmune disease with multisystem involvement. Renal disease occurs in 50% to 75% of all cSLE patients and is a major cause of increased morbidity and mortality. Originally SLE nephritis was treated with steroids with a poor outcome which improved markedly with the introduction of cyclophosphamide, but at the cost of increased side effects which resulted in a further search for a less toxic, but equally effective regime. Here we discuss some newer drugs including immune-modulators and monoclonal antibodies in addition to azathioprine and mycophenolate mofetil, however, most of the evidence on these medications is restricted to adult literature and pediatric data are extrapolated from these trials.

Sinha R, Raut S. Pediatric lupus nephritis: Management update. *World J Nephrol* 2014; 3(2): 16-23 Available from: URL: http://www.wjgnet.com/2220-6124/full/v3/i2/16.htm DOI: http://dx.doi.org/10.5527/wjn.v3.i2.16

INTRODUCTION

Epidemiology

Childhood-onset systemic lupus erythematosus (cSLE) is a rare but severe autoimmune disease with multisystem involvement, the incidence is 0.3/100000-0.9/100000 children-per year with a prevalence of 3.3/100000-8.8/100000 children[1]. A higher frequency of cSLE is reported in Asians, African Americans, Hispanics, and native Americans[2]. Median age of onset of cSLE is between 11 and 12 years (rare below 5 years), and 80% of patients are female[3]. cSLE follows a more severe disease course than adult-onset SLE, with higher morbidity and lower survival rates[4].

Diagnosis of cSLE

Four out of 11 American College of Rheumatology (ACR) criteria have a sensitivity and specificity greater than 95% for the diagnosis of cSLE. These criteria are as follows: malar rash, discoid rash, photosensitivity rash, oral or nasopharyngeal ulceration, nonerosive arthritis,

Table 1 International society of nephrology/renal pathology society 2003 classification of lupus nephritis[11,12]

Class	Name			Light microscopy	Immunofluorescence
I	Minimal mesangial LN			Normal	Mesangial immune deposits
II	Mesangial proliferative LN			Mesangial hypercellularity or mesangial matrix expansion	Mesangial immune deposits
III	Focal LN	A	Active lesions	Segmental, or global glomerulonephritis (< 50% of glomeruli)	Diffuse subendothelial immune deposits
		A/C	Active and chronic lesions		
		C	Chronic lesions		
IV	Diffuse LN	A	Active lesions	Segmental, or global glomerulonephritis LN (> 50% glomeruli)	Diffuse subendothelial immune deposits
		A/C	Active and chronic lesions		
		C	Chronic lesions		
V	Membranous LN				Global or segmental subepithelial immune deposits
VI	Advanced sclerosing LN			LN (> 90% globally Sclerosed glomeruli without residual activity)	

Class V may occur in combination with class III or IV, in which case both will be diagnosed. LN: Lupus nephritis.

serositis, renal disorder, neurologic disorder, hematologic disorder, immunologic disorder, and antinuclear antibody[5]. Although still not widely used, the newer systemic lupus international collaborating clinics (SLICC) criteria have been introduced for the classification of SLE[6]. According to the SLICC rule for the classification of SLE, the patient must satisfy at least 4 criteria, including at least one clinical criterion and one immunologic criterion OR the patient must have biopsy-proven lupus nephritis in the presence of antinuclear antibodies or anti-double-stranded DNA antibodies.

Renal involvement

Renal disease occurs in 50% to 75% of all cSLE patients, mostly within the first 2 years after diagnosis[7]. Lupus nephritis (LN) is more common in males and in non-White populations[8,9]. Initial manifestations of renal disease range from minimal proteinuria and microscopic hematuria to nephrotic-range proteinuria, urinary casts, severe hypertension, peripheral edema, and renal insufficiency or acute renal failure. SLE most commonly affects the glomerulus (*i.e.*, lupus nephritis), but can also involve the renal interstitium. It can also present with features of thrombotic microangiopathy including both atypical hemolytic uremic syndrome as well as thrombotic thrombocytopenic purpura.

CASE DEFINITION FOR LN

As per the ACR criteria, LN is defined as: persistent proteinuria *i.e.*, 0.5 g per day [a spot urine protein/creatinine ratio of 0.5 can be substituted) or greater than 3+ by dipstick; and/or cellular casts including red blood cells (RBCs), granular, tubular, or mixed][10]. An additional, perhaps optimal, criterion is a renal biopsy sample demonstrating immune complex-mediated glomerulonephritis compatible with LN[11]. As the severity of the nephritis may not correlate with the severity of the clinical signs and symptoms, a renal biopsy should be performed for any suspicion of glomerulonephritis, including persistent mild proteinuria.

INDICATIONS FOR RENAL BIOPSY IN PATIENTS WITH CSLE[12]

Increased serum creatinine without compelling alternative causes, such as sepsis, hypovolemia, or medication.

Confirmed proteinuria of 1.0 g per 24 h (either 24-h urine specimens or spot protein/creatinine ratios are acceptable).

Combinations of the following, assuming the findings are confirmed in at least 2 tests performed within a short period of time and in the absence of alternative causes: (1) Proteinuria: 0.5 g per 24 h plus hematuria, defined as 5 RBCs per hpf; and (2) Proteinuria: 0.5 g per 24 h plus cellular casts.

Renal biopsy should be reported as per the International Society of Nephrology/Renal Pathology classification (Table 1). Recent modifications of the activity and chronicity index not only help in acute management, but also help in prognostication.

TREATMENT

Originally SLE nephritis was treated by steroids with a poor outcome which improved markedly with the introduction of cyclophosphamide. The first controlled trial reporting the short-term efficacy of cyclophosphamide for lupus nephritis in adults was published in 1971[13]. Initially this combination was advocated for a prolonged period, but unfortunately the improved outcome was found to be associated with long-term side effects, which resulted in a further search for a less toxic, but equally effective regime. Most of the studies have been performed in adults and to a large extent the current recommendations are borrowed heavily from adult studies. The current therapeutic strategy for SLE nephritis distinguishes two distinct phases of treatment. The first phase is INDUCTION therapy which aims to control disease activity by inducing remission of disease flare (which may be the initial presentation or represent a new flare). It is at this point that potential organ-threatening and/or life-

threatening disease needs to be aggressively treated. The second phase is MAINTENANCE, wherein the target is to avoid relapses and control the disease by limiting inflammation and damage.

Class Ⅰ/Ⅱ LN are milder and generally do not require immunosuppressive treatment, whereas class Ⅲ /Ⅳ needs to be treated aggressively[14]. Research studies over the last decade have shown increasing evidence of the efficacy of mycophenolate mofetil (MMF)/azathioprine (AZA) with a better side effect profile as compared to cyclophosphamide (CyC). Despite this, as shown by the recently conducted consensus meeting of pediatric rheumatologists and nephrologists in North America, the majority still prefer CyC as the induction agent[15]. Most of the studies on the use of MMF in children have only been case series. The largest series included 31 children or young people who were treated with MMF (either initially or switched from AZA) and showed that 73% had a good response without any recorded major side-effects[16]. Among the multiple adult studies, the first comparative study on MMF compared with CyC was published in Hong Kong in 2000[17]. MMF and CyC showed similar rates of improvement and of complete remission, 81% and 76%, respectively. Patients experienced fewer side-effects with MMF treatment. Subsequently Contreras *et al*[18], studied 59 adults with lupus nephritis who were initially treated with 4-7 mo infusions of CyC and then randomized to quarterly infusions of CyC or oral MMF or AZA. Patients treated with AZA or MMF showed fewer flares than those treated with CyC, six, three, and eight flares, respectively. Patients treated with MMF experienced fewer side-effects than those treated with CyC except for an increased risk of gastrointestinal symptoms and diarrhea with MMF. To date, the biggest study, the aspreva lupus management study (ALMS) attempted to determine the efficacy of MMF as an induction agent for LN. The study included 370 patients with class Ⅲ through Ⅴ lupus nephritis[19] and consisted of one 24-wk induction phase and thereafter a 3-year maintenance phase. The results did not show any difference between the percentages of patients responding to treatment (56.2% in the MMF group, and 53.0% in the CyC group). There was also no significant difference in the rate of side-effects and a tendency for more severe adverse events in the MMF group (P = 0.07). In a sub-analysis of the ALMS, Isenberg *et al*[20] showed that response varied with race, in that Black and Hispanic patients responded better to MMF (60.4%) compared to CyC (38.5%), P = 0.03. In a recently published meta-analysis, Touma *et al*[21] looked at the cumulative evidence for MMF/CyC as induction treatment. Four trials with 668 patients were included and no difference in clinical efficacy was found between the two drugs. MMF did, however, show significantly less alopecia (RR = 5.77; 95%CI: 1.56-21.38), but other side-effects were not significantly different. Researchers have also studied patients with class Ⅴ nephritis (*i.e.*, a membranous pattern on kidney biopsy) and found no differences between the MMF and CyC-treated groups[22]. Based on these studies, the ACR has published their recommendation on SLE nephritis, albeit targeted primarily towards the adult population.

ACR recommendation

As per the ACR recent recommendation for class Ⅲ/Ⅳ LN, MMF and glucocorticoids (GC) can be used as induction agents for African-American and Hispanic patients, whereas Cyc and GC remain the first choice for White populations. MMF and GC are agents of choice in isolated class Ⅴ LN[10]. In mixed cases such as class Ⅴ with Ⅲ or Ⅳ LN, the treatment should be similar to that for class Ⅲ/Ⅳ LN. Other induction modalities that can be tried in refractory cases include intravenous immunoglobulin, plasma exchange and B-lymphocyte depletion agents such as Rituximab[23,24] (Figures 1 and 2).

The ACR recommends either MMF or AZA and low dose steroid for the maintenance phase. MMF has been shown to be statistically better than AZA in terms of time to treatment failure (a composite including death, end stage renal disease, doubling of serum creatinine, and renal flare)[25].

In patients who fail to respond after 6 mo of treatment with GC plus MMF or CyC, the ACR recommend a switch of immunosuppressive agent from either CyC to MMF or from MMF to CyC, and these changes are accompanied by Ⅳ pulses of GCs.

Adherence

Non-adherence to immunosuppressive agents can be common in the adolescent age group[14], resulting in relapse of symptoms and sometimes an acute presentation with renal failure after initial successful treatment. Intravenous agents, such as CyC or rituximab can be considered in this situation, to ensure adherence and disease control.

RECENT ADVANCES IN SLE MEDICATIONS

Despite advances in treatment, mortality related to SLE nephritis has been static over the last decade and morbidity continues to be a major factor. Hence, there is a strong need for more effective drugs with, if possible, fewer side-effects. Studies are particularly required in children, as due to a lack of pediatric studies drugs trialed on adults are still been tried in children with severe lupus nephritis. We will discuss some of the newer medicines, however, most of the evidence on these medications is restricted to adult literature.

Rituximab

Rituximab, a chimeric antibody targeting CD20-positive cells, was first used by Tullus[26] in 2000 in a girl with class Ⅴ lupus nephritis and therapy-resistant nephrotic syndrome. The therapeutic response was remarkable and her proteinuria improved so much that her serum albumin normalized. Many other clinicians have had similar clinical experiences and several case series have published positive results[14,27]. Unfortunately the first randomized

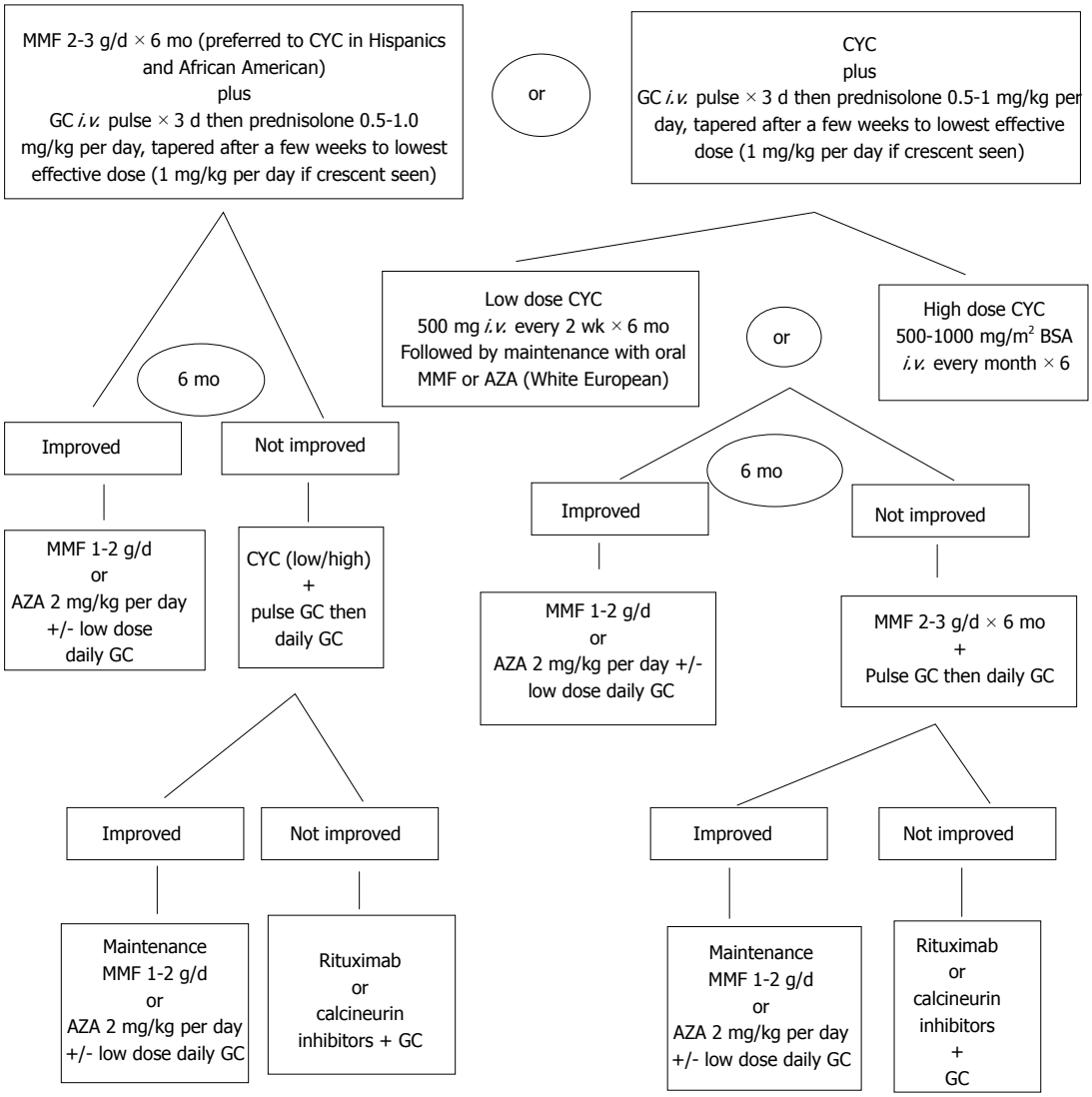

Figure 1 Class Ⅲ/Ⅳ lupus nephritis induction therapy. MMF: Mycophenolate mofetil; CYC: Cyclophosphamide; GC: Glucocorticoids; iv: Intravenous; AZA: Azathioprine; BSA: Body surface area.

controlled trial, EXPLORER, which included 237 patients with moderate to severe extra-renal lupus did not find any difference between rituximab and placebo[28]. Another large randomized placebo-controlled study on rituximab, the LUNAR trial[29], which included 144 patients with class Ⅲ or Ⅳ lupus nephritis showed that only 30.6% of the patients in the placebo group and 25.4% of the rituximab-treated patient fulfilled the criteria for a complete response. Greater improvements in complement levels (P = 0.025) and antibodies to dsDNA (P = 0.007) were recorded in the rituximab group compared to the placebo group[30].

Belimumab

Belimumab is a fully humanized monoclonal antibody that binds to soluble B-lymphocyte stimulator (BLyS) and acts as a specific inhibitor of its biological activity. BLyS, also known as B-cell activating factor (BAFF) is an immunomodulatory cytokine that promotes B-cell survival, B-cell differentiation, and immunoglobulin class switching. A phase Ⅲ randomized double-blind placebo controlled study showed significant benefit, but to date, there is not enough data to recommend its use in children with lupus nephritis[31].

Ocrelizumab

Ocrelizumab is a fully humanized antibody that targets CD20-positive B cells. It is a "next-generation rituximab" and the potential problem of human anti-chimeric antibody development is hopefully ameliorated. The BELONG study, 2010 was set up to study the efficacy of ocrelizumab in 381 patients with lupus nephritis[32] and the results are awaited.

Epratuzumab

Epratuzumab is a monoclonal antibody against CD22, another B-cell-specific surface antigen. Early open data in a few patients have shown positive results[33]. A study involving 227 patients found that a dose of 600 mg weekly was associated with greater british isles lupus activity

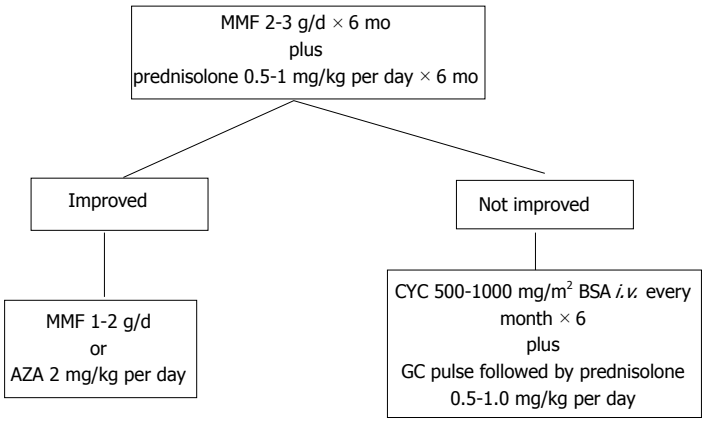

Figure 2 Treatment of class Ⅴ without proliferative changes and with nephrotic range proteinuria (> 3 g/24 h). MMF: Mycophenolate mofetil; AZA: Azathioprine; CYC: Cyclophosphamide; GC: Glucocorticoids.

Table 2 Monitoring schedule for systemic lupus erythematosus nephritis[10]

Stage of disease	Blood pressure	Urinalysis	Protein/creatinine ratio	Serum creatinine	C3/C4 level	Anti-DNA
Active nephritis at onset of treatment	1	1	1	1	2	3
Previous active nephritis, none currently	3	3	3	3	3	6
No prior or current nephritis	3	6	6	6	6	6

grading improvement[34].

Tocilizumab

Tocilizumab is a humanized monoclonal antibody against the interleukin-6 receptor. In a phase I study of 16 patients with mild to moderate lupus, tocilizumab was found to be well tolerated, significantly reducing plasma cells and dsDNA suggesting a good clinical response[35]. Further studies seem warranted.

Abetimus

Abetimus is an immunomodulating agent designed to induce tolerance in B cells directed against dsDNA and to reduce anti-dsDNA levels. It is a synthetic molecule consisting of four double-stranded oligodeoxyribonucleotides attached to non-immunogenic polyethylene glycol. Abetimus works through the formation and clearance of drug antibody complexes and by tolerating anti-dsDNA specific B cells. Cardiel *et al*[36], 2008 in a 22-mo study of 317 patients showed that abetimus failed to prolong time to flare compared to the placebo.

Atacicept

Atacicept is a receptor analogue that binds both BAFF and a proliferation-inducing ligand to related members of the tumor necrosis factor superfamily. A phase Ⅱ study of atacicept showed a marked reduction in B cells and immunoglobulin levels and a short term side-effect profile similar to placebo[37].

Rigeromid

Rigerimod is a spliceosomal peptide that is recognized by $CD4^+$ T cells from patients with lupus, but not from those with other autoimmune diseases. In a 12-wk study, 150 patients with lupus and high SLE Disease Activity Index scores (Safety of estrogens in lupus erythematosus national assessment) were given three infusions of two different doses of rigerimod or placebo followed by 12 wk of observation. The treatment seemed to be well tolerated and a statistically significant reduction in disease activity was recorded[38]. Longer-term studies are needed.

Abatacept

Abatacept is a fusion protein composed of an immunoglobulin fused to the extracellular domain of CTLA-4, a molecule capable of binding B7 which selectively modulates the CD80/CD86:CD28 co-stimulatory signal. A recent 12-mo double-blind placebo-controlled study in 118 lupus patients failed to meet the primary end point of a reduction in new flares[39]. Serious adverse events were higher in the abatacept group compared with the placebo group (20% *vs* 7%).

Infliximab

The use of infliximab in lupus has been surrounded with major worries. Long-term use of infliximab in lupus patients can cause severe side-effects, including severe infections and even cerebral lymphoma[40].

ADJUNCTIVE TREATMENTS

The ACR recommended that all SLE patients with nephritis be treated with a background of hydroxychloroquine (HCQ), unless there is a contraindication[11]. HCQ treatment may reduce the risk of renal damage and clotting events in SLE[41-43]. Medications to control high blood pressure (anti-hypertensive), fluid overload (diuretics), proteinuria (angiotensin converting enzyme inhibitors), hypercoagulability (aspirin or other anticoagulants) and lipid control (statins)[22,44,45] are mainstays of adjunc-

tive treatment. Dietary restriction may be necessary in terms of sodium, protein and calories. Unlike adults, these recommendations must be adjusted to take into account the growth and developmental status of the child. Infection is the most common cause of death in cSLE due to immune suppression[46]. Infections can be difficult to differentiate from a flare of SLE disease activity. C-reactive protein monitoring can be useful as most SLE patients have normal levels, except during inter-current infections.

RECOMMENDED MONITORING OF LUPUS NEPHRITIS

SLE is a chronic disease with the possibility of frequent flare-ups. Hence, these children need to be followed up regularly (Table 2). Compliance can be a major issue and needs to be addressed during each clinic visit[47].

CONCLUSION

In conclusion, the outcome of lupus nephritis is primarily dependent on histological classification at presentation. Early renal biopsy in all children with features of lupus nephritis to decide on induction therapy, aggressive treatment of hypertension and other adjunct therapy is recommended to improve mortality and morbidity of such patients.

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P- Reviewers: Martins LSS, Pedersen EB, Sheashaa HA, Tanaka H **S- Editor**: Gou SX **L- Editor**: Webster JR **E- Editor**: Wu HL

ISSN 2220-6124 (online)

World Journal of *Nephrology*

World J Nephrol 2014 August 6; 3(3): 24-121

Published by Baishideng Publishing Group Inc

WJN

World Journal of Nephrology

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AIM AND SCOPE

World Journal of Nephrology (*World J Nephrol*, *WJN*, online ISSN 2220-6124, DOI: 10.5527) is a peer-reviewed open access academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

WJN covers topics concerning kidney development, renal regeneration, kidney tumors, therapy of renal disease, hemodialysis, peritoneal dialysis, kidney transplantation, diagnostic imaging, evidence-based medicine, epidemiology and nursing. Priority publication will be given to articles concerning diagnosis and treatment of nephrology diseases. The following aspects are covered: Clinical diagnosis, laboratory diagnosis, differential diagnosis, imaging tests, pathological diagnosis, molecular biological diagnosis, immunological diagnosis, genetic diagnosis, functional diagnostics, and physical diagnosis; and comprehensive therapy, drug therapy, surgical therapy, interventional treatment, minimally invasive therapy, and robot-assisted therapy.

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INDEXING/ABSTRACTING

World Journal of Nephrology is now indexed in PubMed Central, PubMed, and Digital Object Identifier.

FLYLEAF I-III Editorial Board

NAME OF JOURNAL
World Journal of Nephrology

ISSN
ISSN 2220-6124 (online)

LAUNCH DATE
February 6, 2012

FREQUENCY
Quarterly

EDITORIAL OFFICE
Jin-Lei Wang, Director
Xiu-Xia Song, Vice Director
World Journal of Nephrology
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No. 62 Dongsihuan Zhonglu, Chaoyang District,
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E-mail: editorialoffice@wjgnet.com
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
http://www.wjgnet.com

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Baishideng Publishing Group Inc
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Pleasanton, CA 94588, USA
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Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
http://www.wjgnet.com

PUBLICATION DATE
August 6, 2014

INSTRUCTIONS TO AUTHORS
Full instructions are available online at http://www.wjgnet.com/2218-6220/g_info_20100722175812.htm.

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DOI: 10.5527/wjn.v3.i3.24

World J Nephrol 2014 August 6; 3(3): 24-30
ISSN 2220-6124 (online)

REVIEW

Kidney regeneration: Where we are and future perspectives

Joao Paulo Zambon, Renata S Magalhaes, Inkap Ko, Christina L Ross, Giuseppe Orlando, Andrea Peloso, Anthony Atala, James J Yoo

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Received: April 18, 2014 Revised: June 27, 2014
Accepted: July 25, 2014
Published online: August 6, 2014

Abstract

In 2012, about 16487 people received kidney transplants in the United States, whereas 95022 candidates were on the waiting list by the end of the year. Despite advances in renal transplant immunology, approximately 40% of recipients will die or lose graft within 10 years. The limitations of current therapies for renal failure have led researchers to explore the development of modalities that could improve, restore, or replace the renal function. The aim of this paper is to describe a reasonable approach for kidney regeneration and review the current literature regarding cell sources and mechanisms to develop a bioengineering kidney. Due to kidneys peculiar anatomy, extracellular matrix based scaffolds are rational starting point for their regeneration. The perfusion of detergents through the kidney vasculature is an efficient method for delivering decellularizing agents to cells and for removing of cellular material from the tissue. Many efforts have focused on the search of a reliable cell source to provide enrichment for achieving stable renal cell systems. For an efficient bioengineered kidney, these cells must be attached to the organ and then maturated into the bioractors, which simulates the human body environment. A functional bioengineered kidney is still a big challenge for scientists. In the last ten years we have got many improvements on the field of solid organ regeneration; however, we are still far away from the main target. Currently, regenerative centers worldwide have been striving to find feasible strategies to develop bioengineered kidneys. Cell-scaffold technology gives hope to end-stage renal disease patients who struggle with morbidity and mortality due to extended periods on dialysis or immunosupression. The potential of bioengineered organ is to provide a reliable source of organs, which can be refunctionalized and transplanted.

Key words: Kidney regeneration; Stem cells; Decellularization; Extra cellular matrix; Regenerative medicine

Core tip: In 2012, about 16487 people received kidney transplants in the United States, whereas 95022 candidates were on the waiting list by the end of the year. Despite advances in renal transplant immunology, 20% of recipients will experience an episode of acute rejection within 5 years of transplantation, and approximately 40% of recipients will die or lose graft function within 10 years. The aim of this paper is to describe a reasonable approach for kidney regeneration and review the current literature regarding possible cell sources and mechanisms to develop a bioengineering kidney.

Zambon JP, Magalhaes RS, Ko I, Ross CL, Orlando G, Peloso A, Atala A, Yoo JJ. Kidney regeneration: Where we are and future perspectives. *World J Nephrol* 2014; 3(3): 24-30 Available from: URL: http://www.wjgnet.com/2220-6124/full/v3/i3/24.htm DOI: http://dx.doi.org/10.5527/wjn.v3.i3.24

INTRODUCTION

In the United States, approximately 1 million patients live with end-stage renal disease (ESRD), with over 100000 new diagnoses every year. Although hemodialysis has in-

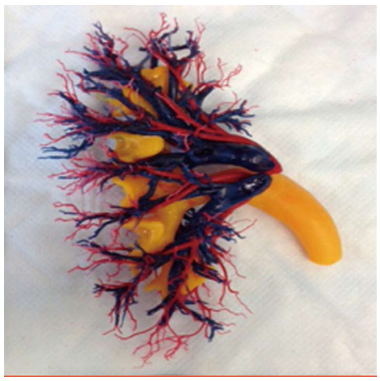

Figure 1 Vascular and collecting system casting of a kidney. Red: Arteries; Blue: Veins; Yellow: Ureter and collecting system. Permission of Wake Forest Institute for Regenerative Medicine.

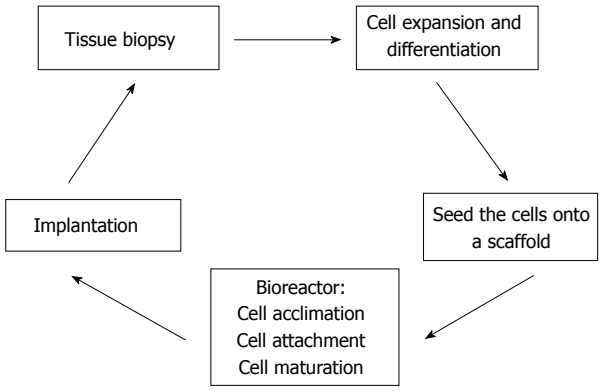

Figure 2 Regeneration of a tissue/organ.

creased the survival of patients with ESRD, kidney transplantation remains the only potential curative treatment. In 2012, about 16487 people received kidney transplants in the United States, whereas 95022 candidates were on the waiting list by the end of the year. Despite advances in renal transplant immunology, 20% of recipients will experience an episode of acute rejection within 5 years of transplantation, and approximately 40% of recipients will die or lose graft function within 10 years[1-4].

The limitations of current therapies for renal failure have led researchers to explore the development of alternative modalities that could improve, restore, or replace either partial or total renal function. Tissue engineering and regenerative medicine represents one of the newest innovations in modern-day science. It represents a broad spectrum of methodologies and techniques aiming to repair, augment, and regenerate damaged organs and tissues. The basis of tissue engineering is that cells can be expanded *in vitro,* placed on a tissue scaffold made of suitable biomaterial, and then implanted into the host[5-7].

Within the field of organ bioengineering, the methodology of seeding cells on supporting scaffolding material has shown great promise for generating viable organs. A simple structure such as vessels, bladders, upper airways, and urethras have been implanted into patients with acceptable results in the short and midterms. The structural simplicity of these organs enables them to meet the oxygen and nutrient requirements *via* diffusion from adjacent host tissues while angiogenesis has time to occur. Unfortunately, complex organs such as the kidney cannot be viably incorporated without the reconnection of new structure to the host vasculature, a task that has presented insurmountable challenges experimentally let alone clinically[8-12].

The aim of this paper is to describe a reasonable approach for kidney regeneration and review the current literature regarding possible cell sources and mechanisms to develop a bioengineering kidney.

STRUCTURAL AND FUNCTIONAL CONSIDERATIONS OF HUMAN KIDNEYS

The kidneys are the primary organs for maintaining fluid, electrolyte, and acid-base balance. They produce hormones such as rennin, erythropoietin, and convert a precursor of vitamin D, 1,25-dihydroxyvitamin D, to active metabolite. Each kidney has more than thirty different cell types, approximately 2 million glomeruli, numerous arterioles, capillaries, and tubules that interconnect in a three dimensional pattern to filter the blood and excrete waste through the collecting system[13]. Figure 1 represents an example of a kidney's vascular cast.

WHOLE KIDNEY REGENERATION

Ideally, a bioengineered kidney must be biocompatible, non-immunogenic and support cell growth. The basic idea for solid organ regeneration is to harvest a tissue biopsy from a donor and expand these cells in culture. Subsequently, they are seeded into a scaffold, and placed into a bioreactor in order to promote cell acclimation, attachment, and maturation. Once this is achieved, the cell-seeded scaffold can be implanted on the host[14,15]. Figure 2 represents a potential methodology for solid organ regeneration.

KIDNEY DECELLULARIZATION AND EXTRACELLULAR MATRIX PRESERVATION-BIOLOGICAL SCAFFOLD PRODUCTION

Extracellular matrix (ECM) is the naturally occurring scaffold material secreted and manufactured by the resident cells of each tissue and organ. The complex 3D organization of the ECM and its components are dictated by the tissue from which ECM is derived. The structural and functional molecules of the ECM are in a state of dynamic equilibrium within the surrounding tissues, and also provide the means by which cells communicate with each other and the external environment. The ECM contains growth factors and others bioinductive factors, which facilitates the remodeling process, cell attachment and tissue integration[16,17].

Due to their peculiar anatomy and physiology, ECM based scaffolds are a rational starting point for kidney re-

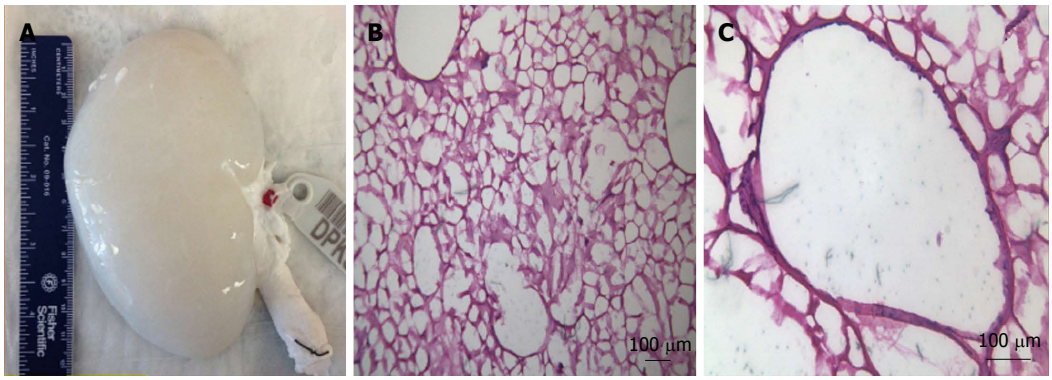

Figure 3 A decellularized pig kidney scaffold and its extra cellular matrix after decellularization. A: Decellularized pig kidney scaffold; B: Hematoxylin and eosin staining of the decellularized pig kidney scaffold shows a decellularized extracellular matrix (× 200); C: Hematoxylin and eosin staining of the decellularized pig kidney scaffold shows a decellularized extracellular matrix (× 400). Permission of Wake Forest Institute for Regenerative Medicine.

generation. For this purpose, several protocols have been described for whole kidney decellularization. The perfusion of detergents and enzymes (*e.g.*, DNase) through the kidney vasculature is an efficient method for delivering decellularizing agents to cells and for removing of cellular material from the tissue. However, in spite of being effective decellularization agents, the effects of these agents on kidney microvasculature have not been established, and further studies are necessary to elucidate them[18-20]. Figure 3 represents a decellularized pig kidney scaffold and its extra cellular matrix after decellularuzation.

CELL EXPANSION AND DIFFERENTIATION

The kidney has approximately 30 different specialized cell types. For an efficient scaffold, all cells must be characterized for repopulation, which represents a challenging task. Many efforts have focused on the search of a reliable cell source and optimal growth conditions to provide adequate enrichment for achieving stable renal cell expansion systems[4].

Regarding vascular anatomy, the main renal artery splits into segmental, interlobar, interlobular, and arcuate arteries. The venous system is formed by a complex net of veins, which drains to the main renal vein. Each kidney has approximately 2 million glomeruli, which are responsible for renal filtration process and an extensive net of capillaries[13]. Figure 4 represents a pig kidney's glomerulus.

ADULT KIDNEY STEM CELLS

The regenerative capacity of a tissue is determined in part by whether it contains endogenous stem cells. These stem cell populations are housed in a niche, which regulates stem cell survival, self-renewal, and differentiation. In a normal environment, stem cells remain quiescent in the niche for long periods until they are activated by the requirement of new cells to maintain the tissue or because of the tissue damage. With regards to renal adult stem cells, a subset has been found in the Bowman's capsule, glomeruli, pericytes, proximal tubules, and renal papilla. These cells expressed stem cell markers such as CD24, CD133, CD146 and Pax-2[21-25].

Regenerative mechanisms after acute renal failure have not been well established, but appear that tubular cells, growth factors and cytokines are involved in this process as well demonstrated by Humphreys *et al*[26]. They reported *via* a genetic maping technique, that tubular epithelial cells were the predominant source of regeneration after kidney ischemic injury, and distal tubular cells were more involved with growth factor production such as epidermal, IGF-1, and hepatocyte growth factor[26,27].

Harari-Steinberg *et al*[28] identified in human kidneys nephron progenitor cells (hNPCs), which were capable of generation of kidney structures and functional repair of chronic renal disease. These cells expressed NCAM1$^+$ and had a high clonogenic potential. Moreover, when grafted in aggregates into a chorioallantoic membrane of the chick embryo they generate renal structures. Ultimately, hNPCs were injected directly into the kidney's parenchyma of mice with chronic kidney disease. They reported that treatment with hNPCs halted disease progression and increased creatinine clearance throughout the 12-wk study period[28].

Buzhor *et al*[29] also demonstrated that human adult kidney epithelial cells (hKEpCs) positive for NCAM1$^+$ overexpressed nephron progenitor markers, acquired a mesenchymal fate and produce epithelial renal tissue on single-cell grafting in chick chorioallantoic membrane and mouse.

Rinkevich *et al*[30] demonstrated an *in vivo* clonal analysis of progenitor cells found in mammalian kidneys. They used a long-term *in vivo* genetic lineage tracing and clonal analysis of individual cells isolated from kidneys and demonstrated that tissue and lineage-restricted precursors cells from tubules and non tubules structures such as glomerulus are directly involved in the kidney recovery after injury. As a future direction, the isolation and characterization of kidney precursor cells offer a

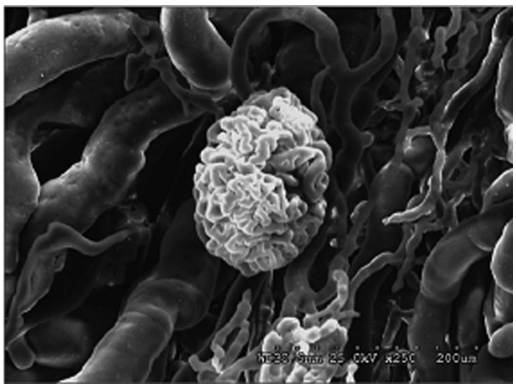

Figure 4 Normal kidney glomerulous visualized through scanning electron microscopy (magnification × 250). Permission of Wake Forest Institute for Regenerative Medicine.

therapeutic target to increase or restore the regenerative capacity of the mamallian kidney[30].

BONE MARROW DERIVED STEM CELLS

The bone marrow contains two major populations of stem cells, hematopoietic stem cells (HSCs), and mesenchymal stromal cells (MSC), which provide stromal support for HSCs. These stem cell therapies derived from bone marrow have been used to repair a variety of organs in experimental models. A possible explanation for apparent plasticity of these cells is a mechanism of transdifferentiation, dedifferentiation or cell fusion[31].

Despite studies have shown promising results with bone marrow derived stem cells, the biological relevance and clinical importance have not been well demonstrated for kidney regeneration. Initially, bone-marrow stem cells have been considered as a source of "replacement" cells that could be used for the treatment of different diseases. However, studies in experimental transplantation models or direct injection of these cells into tissue have shown that the contribution of bone-marrow cells to nonhematopoietic cell fates is uncommon. Therefore, the potential of these cells for whole organ regeneration is far from being considered as a treatment option[32,33].

MESENCHYMAL STEM CELLS

Mesenchymal stem cells (MSC) are stromal cells that can be found and isolated from different tissues. Because of their multilineage differentiation potential and their ability to migrate to the site of injury, they have been studied in the last 10 years as a therapeutic agent in kidney injury. It has been demonstrated that MSC are involved in immune response through the activation of T and B-lymphocytes. In addition, they stimulate interleukins, β-TGF, hepatocyte, fibroblast, and vascular endothelial growth factors[34,35].

Adipocyte derived stem cells (ADSC) are a type of mesenchymal stem cell that in the last decade have been explored as an attractive source of cells with regenerative properties. These cells are abundant, easily harvested with low morbidity, and seem to stimulate angiogenesis. Regarding kidney regeneration, de Almeida *et al*[36] demonstrated in an experimental model of acute renal failure that the injection of ADSC in mice reduced renal fibrosis at six weeks; however, they did not find the injected cells in the kidney. Chen *et al*[37] also demonstrated in rats that an intra-renal injection of ADSC attenuated the deterioration of renal function at 14 d, improved angiogenesis, and preserved the architecture integrity[36,37].

Despite being an attractive source of stem cells for kidney regeneration, the exact mechanism of action of MSCs has not been established. Most of the experimental studies have been performed with rodents and have short follow up. Further studies with a longer follow up and using large animal models should be performed before translating to clinical trials.

HUMAN AMNIOTIC FLUID AND PLACENTA DERIVED STEM CELLS

Amniotic fluid, due to its contact with the fetus, has been considered an interesting source for undifferentiated or partially differentiated cells. The molecular composition of amniotic fluid and the presence of nutritive substances play a key role in the proliferation and differentiation of different cell types.

Human amniotic stem cells (HASC) express surface markers and transcription factors distinctive of embryonic stem cells (ESCs). These include octamer-binding transcription factor 4 (OCT-4) and stage specific embryonic antigen (SSEA-4)[38]. HASCs have high replicative self-renewal potential and multilineage differentiation capacity. Perin *et al*[39,40] showed that HASC integrated into metanephric structures after being injected into embryonic kidneys, which improved repair/recovery of kidneys with acute tubular necrosis[39-41].

In the field of cell therapy and regenerative medicine, many studies have been done to establish reliable animal models for different types of disease targeting the feasibility and benefits of human amniotic stem cell therapy.

EMBRYONIC STEM CELLS

ESC are pluripotent cells derived from blastocysts. These cells propagate readily and remain undifferentiated when cultured with leukemia inhibitory factor (LIF). When LIF is withdrawn, ESCs form aggregates called embryoid bodies that generate a variety of specialized cell types. However, the extraction of these cells involves the destruction of embryos, therefore their use is associated with controversial ethical dilemmas[42].

In spite of the self-renewing potential and the capability of differentiation into tissues derived from the three germ layers, ESCs are associated with uncontrolled growth and teratoma formation. Kidney regeneration studies have demonstrated that renal progenitor cells derived from ESCs differentiated into glomerular-like

structures and integrated into renal proximal tubules when implanted *in vivo*[42,43].

GENERATION OF KIDNEY TISSUE FROM EMBRYONIC RUDIMENTS

This approach for generating kidney tissue can be achieved by the use of undifferentiated or partially developed kidney precursor cells derived from early embryos and fetal tissue. Dekel *et al*[44] transplanted in immunodeficient mice human or pig kidney precursors, which were obtained from 7 to 8 wk human fetus or 3.5 to 4 wk pig gestation. The rudimentar kidneys survive, grow and complete nephrogenesis, forming a functional organ able to produce urine. The successful organogenesis was achieved only when early progenitors with mesenchymal cells and ureteric bud branches were transplanted. Nevertheless, as well as embryonic cells, this approach also involves the destruction of embryos and is associated with controversial ethical dilemmas[44].

SOMATIC NUCLEAR CELL TRANSFER

The first renal tissue created *via* therapeutic cloning techniques has been described by Lanza *et al*[45] in a bovine model. In this study, a skin fibroblast nucleus was microinjected into an enucleated oocyte that was transplanted *in vitero* for 12 wk. Cloned renal cells were then seeded into a biodegradable scaffold and transplanted *in vivo* to follow the growth of the construct. The authors reported that the kidney-like organ was capable of secreting urinary fluid, confirming that the implant contained cells capable of filtration, reabsorption and secretion. However, like ESCs, this technique also is associated with controversial ethical dilemmas[45,46].

INDUCED PLURIPOTENT STEM CELLS

Induced pluripotent stem cells (iPSC) were first described by Takahashi and Yamanaka in 2006 when they reprogrammed human fibroblasts to become pluripotent stem cells by the addition of four different genes: *Oct3/4*, *Sox2*, *c-Myc*, and *Klf4*. Despite being a good source of cells, not all adult stem cells can be reprogrammed using the same method, which means that each cell type may have critical factors. Unlike ESCs, iPS cells have no ethical issues and no immune rejection. On the other hand, these cells are reprogrammed through the addition of oncogenes, which increase the risk of uncontrolled growth[47-49].

The surrogate application of iPSC as representative of kidney disease is increasingly becoming reality given recent advances involving the production of iPSC from both mesangial and epithelial cells derived from urine[45]. In addition to that, iPSCs have been generated from proximal tubular cells and podocytes[46]. Despite promising results, some issues should be highlighted before clinical application. First of all, there are no established differentiation protocols for moving from pluripotent state to functional kidney cell. Second, there are no optimal culture conditions for targeting cells. Third, a stepwise differentiation depends on specific factors, which must be identified[50,51].

BIOREACTOR CULTURE-CELL ACCLIMATION, ATTACHMENT AND MATURATION

For whole organ regeneration, cells need to be attached to the organ and then maturated. The bioreactor simulates the human body environment; however, each organ depends on different conditions such as perfusion rate, temperature, CO_2 concentration, growth factors, and nutrients, *etc*. Due to kidney complexity, the recellularization of a functional kidney is still a big challenge for scientists. In 2013, Song *et al*[52] published the first experimental orthotopic transplantation of a bioengineered kidney in rats. They repopulated acellular rat kidneys with endothelial and epithelial cells through the renal artery and ureter respectively. Engrafted epithelial cells were found to reestablish polarity and organize in tubular structures expressing Na/K-ATPase and aquaporin similar to native proximal tubular epithelium. Also, epithelial cells formed structures resembling native distal tubular epithelium and lined the renal pelvis similar to native transitional epithelium. Transmission and scanning electron microscopy of regenerated kidneys showed perfused glomerular capillaries with engrafted podocytes and formation of foot processes. However, as they suggested, before translating this technology, it will require optimization of cell seeding protocols, upscaling of biomimetic organ culture, as well as isolation, differentiation, and expansion of the required cell types[52].

FUTURE DIRECTIONS

In the field of regenerative medicine and whole organ regeneration many efforts have been carried out to translate this technology for clinical practice. The ultimate goal is to provide a feasible and reliable therapy for different types of diseases. In this context, although there are many improvements on kidney regeneration, there are still many hurdles to be overcome, and as our knowledge improves, more complex questions remain unclear.

A functional bioengineered kidney is still a big challenge for scientists. In the last ten years we have got many improvements on the field of solid organ regeneration; however, we are still faraway from the main target. Currently, regenerative centers worldwide have been striving to find feasible strategies to develop bioengineered kidneys. Cell-scaffold technology gives hope to end-stage renal disease patients who struggle with morbidity and mortality due to extended periods on dialysis or immunosupression. The potential of bioengineered organ is to provide a reliable source of organs, which

can be refunctionalized and transplanted.

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P- Reviewer: Olowu WA **S- Editor**: Ji FF
L- Editor: A **E- Editor**: Wu HL

World Journal of Nephrology

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DOI: 10.5527/wjn.v3.i3.31

World J Nephrol 2014 August 6; 3(3): 31-49
ISSN 2220-6124 (online)

REVIEW

Chronic kidney disease prediction is an inexact science: The concept of "progressors" and "nonprogressors"

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Received: April 13, 2014 Revised: June 13, 2014
Accepted: July 25, 2014
Published online: August 6, 2014

Abstract

In 2002, the National Kidney Foundation Kidney Disease Outcomes Quality Initiative (NKF KDOQI) instituted new guidelines that established a novel chronic kidney disease (CKD) staging paradigm. This set of guidelines, since updated, is now very widely accepted around the world. Nevertheless, the authoritative United States Preventative Task Force had in August 2012 acknowledged that we know surprisingly little about whether screening adults with no signs or symptoms of CKD improve health outcomes and that we deserve better information on CKD. More recently, the American Society of Nephrology and the American College of Physicians, two very well respected United States professional physician organizations were strongly at odds coming out with exactly opposite recommendations regarding the need or otherwise for "CKD screening" among the asymptomatic population. In this review, we revisit the various angles and perspectives of these conflicting arguments, raise unanswered questions regarding the validity and veracity of the NKF KDOQI CKD staging model, and raise even more questions about the soundness of its evidence-base. We show clinical evidence, from a Mayo Clinic Health System Renal Unit in Northwestern Wisconsin, United States, of the pitfalls of the current CKD staging model, show the inexactitude and unpredictable vagaries of current CKD prediction models and call for a more cautious and guarded application of CKD staging paradigms in clinical practice. The impacts of acute kidney injury on CKD initiation and CKD propagation and progression, the effects of such phenomenon as the syndrome of late onset renal failure from angiotensin blockade and the syndrome of rapid onset end stage renal disease on CKD initiation, CKD propagation and CKD progression to end stage renal disease all demand further study and analysis. Yet more research on CKD staging, CKD prognostication and CKD predictions is warranted. Finally and most importantly, cognizant of the very serious limitations and drawbacks of the NKF K/DOQI CKD staging model, the need to individualize CKD care, both in terms of patient care and prognostication, cannot be overemphasized.

Key words: Acute kidney injury; Chronic kidney disease; Chronic kidney disease staging; Estimated glomerular filtration rate; End stage renal disease; National Kidney Foundation Kidney Disease Outcomes Quality Initiative; Renal replacement therapy; Serum creatinine; Syndrome of late onset renal failure from angiotensin blockade; Syndrome of rapid onset end stage renal disease

Core tip: In 2002, the National Kidney Foundation established a novel chronic kidney disease (CKD) staging paradigm. In 2012, the authoritative United States Preventative Task Force questioned the validity of asymptomatic CKD screening. The American Society of Nephrology and the American College of Physicians have opposite recommendations regarding this controversy.

We examined the evidence-base and limitations of CKD staging. Furthermore, we show clinical evidence of pitfalls of the current CKD staging model and the failings of current CKD prediction models. We called for more research into CKD to end stage renal disease translations including the impact of acute kidney injury on this continuum. CKD care and prognostication must be individualized.

Onuigbo MAC, Agbasi N. Chronic kidney disease prediction is an inexact science: The concept of "progressors" and "nonprogressors". *World J Nephrol* 2014; 3(3): 31-49 Available from: URL: http://www.wjgnet.com/2220-6124/full/v3/i3/31.htm DOI: http://dx.doi.org/10.5527/wjn.v3.i3.31

INTRODUCTION

In 2002, the National Kidney Foundation Kidney Disease Outcomes Quality Initiative (NKF KDOQI) instituted new guidelines that established a novel chronic kidney disease (CKD) staging paradigm[1,2]. In this model, by use of pre-specified estimated glomerular filtration rates (eGFR) ranges, CKD was characterized into five stages: Ⅰ, Ⅱ Ⅲ Ⅳ and Ⅴ[1,2]. More recent updates of the NKF KDOQI guidelines have incorporated the degree of associated proteinuria to plausibly enhance the utility, applicability, validity and clinical relevance of this CKD staging paradigm[3]. Furthermore, it must be acknowledged that the entire paradigm of the NKF K/DOQI 2002 CKD staging and the design of various CKD risk stratification scoring models and published CKD progression prediction formulas are all firmly predicated on the validity of this albeit unproven and only assumed archetype of a predictable linear and time-dependent progression of CKD through stages Ⅰ, Ⅱ, Ⅲ, Ⅳ and Ⅴ, and finally to inexorably end in end stage renal disease (ESRD) and the need for renal replacement therapy[1-3]. Thus, note worthily, this NKF KDOQI CKD staging archetype assumes that CKD patients follow this linear, predictable, smoothly progressive and time-dependent curve to advance through the increasing CKD stages before inexorably reaching ESRD and the need for renal replacement therapy (RRT). Indeed, current nephrology literature on ESRD outcomes in CKD patients, is unquestionably primarily and almost entirely predicated on this presumed theory of a smooth, continuous, predictable and time-dependent linear progressive loss of eGFR[4-8]. This presumption is so strongly held that several reports over the decades have described CKD progression to ESRD in terms of annual rates of GFR decline or as mean annualized eGFR slopes in mL/min per 1.73 m^2 every year[4,6,7,9-18]. A detailed investigative analysis of these reports has been recently published in a recent review[19]. Undeniably, this generally accepted consensus classic view of CKD-ESRD progression presumes that functional nephron loss in chronic progressive kidney disease, and therefore as measured by serum creatinine, is orderly and mathematically definable[9].

However, it must be acknowledged that such a premise of predictable linear time-dependent progressive step-wise decline in kidney function, with mathematically linear falling eGFR over time, and with eGFR methodically marching through these incremental projected CKD stages Ⅰ through Ⅴ and then inexorably ending in symptomatic ESRD and the need for renal replacement therapy is unproven, untested and potentially flawed[20-32]. Contrary to the classic premise of a smooth predictable linear decline of renal function from CKD to ESRD described in the preceding section of this chapter, more and more data have over the years suggested that the natural pattern of progression from CKD to ESRD followed a more staccato and unpredictable course[20-32]. Moreover, and again contrary to the NKF schema of a predictable linear CKD staging referred to above, the results of new analysis of CKD databases suggest a high degree of variability of CKD staging among CKD patients, and this CKD staging variability is often unpredictable, and with significant intra-patient variability, such that our current knowledge of this very important patient group can, at best, be described as limited and incomplete[19,33-36].

A recent publication in the *Kidney International* journal put CKD smack back into the realms of public discourse when it was reported that 59% of Americans had a lifetime risk of developing CKD and that Americans had a 1 in 3 chance of CKD risk[37]. Whereas, it has been known for decades that eGFR declines in parallel with age[37,38], to what extent this is true and linearly predictable at the individual (older) CKD level remains unclear[19,33-36]. To further buttress the latter sentiment and concerns regarding the validity of current CKD staging paradigms, in August 2012, the authoritative United States Preventative Task Force had in a very comprehensive analysis acknowledged that we know surprisingly little about whether screening adults with no signs or symptoms of CKD improve health outcomes and that we deserve better information on CKD[39].

HOW MUCH DO PHYSICIANS AND OTHER HEALTHCARE PROVIDERS EMBRACE THE NKF K/DOQI 2002 CKD GUIDELINES?

Without a doubt, the introduction of the K/DOQI 2002 CKD guidelines has heralded a growing appreciation of the impact of CKD in health outcomes[3,40]. Undoubtedly, there has followed an exponential increase in nephrology specialty referrals and nephrology specialty office visits for purported "CKD follow up" since the introduction of the 2002 NKF KDOQI CKD clinical practice guidelines[41-49]. However, given the above limitations and constraints of the K/DOQI CKD clinical practice guidelines model, one interesting question is to what extent physicians and providers have embraced its recommenda-

tions and structure[50]. An online survey, developed by the NKF KDOQI Education Committee, was sent to 16323 healthcare providers of which 951 completed the survey[50]. Whereas 78% of all providers reported using the NKF KDOQI guidelines in their practice, the most common barrier perceived by physicians was lack of evidence supporting the KDOQI guidelines[50]. Notably, compared to physician extenders and allied health professionals, not surprising, we must admit, a larger proportion of nephrologists cited lack of evidence and too much influence from industry as barriers to the implementation of the KDOQI guidelines[50]. Surely and undoubtedly, and this fact was recognized very much earlier on, the validity of the clinical practice guidelines of the NKF K/DOQI 2002 report is limited in some areas because of the paucity of evidence-based data[40].

UTILITY OF CKD PREDICTION MODELS IN THE NEPHROLOGY LITERATURE

Tangri *et al*[51] in a recent 2011 publication devised and validated different new CKD prediction models using demographic, clinical, and laboratory data from two independent Canadian cohorts of patients with CKD stages Ⅲ to Ⅴ (estimated GFR, 10-59 mL/min per 1.73 m^2) who were referred to nephrologists between April 1, 2001, and December 31, 2008[51]. The most accurate model included age, sex, estimated GFR, albuminuria, serum calcium, serum phosphate, serum bicarbonate, and serum albumin (C statistic, 0.917; 95%CI: 0.901-0.933 in the development cohort and 0.841; 95%CI: 0.825-0.857 in the validation cohort)[51]. In the validation cohort, this model was more accurate than a simpler model that included age, sex, estimated GFR, and albuminuria (integrated discrimination improvement, 3.2%; 95%CI: 2.4%-4.2%; calibration (Nam and D'Agostino 2 statistic, 19 *vs* 32); and reclassification for CKD stage 3 (NRI, 8.0%; 95%CI: 2.1%-13.9%) and for CKD stage 4 (NRI, 4.1%; 95%CI: -0.5%-8.8%)[51]. The conclusion was that a model using routinely obtained laboratory tests can accurately predict progression to kidney failure in patients with CKD stages Ⅲ to Ⅴ[51].

In a subsequent 2013 review of CKD prediction models, 13 studies describing 23 models were analyzed[52]. Eight studies (11 models) involved kidney failure, five studies (6 models) involved all-cause mortality, and three studies (6 models) involved cardiovascular events[51-64]. Measures of eGFR or serum creatinine level were included in 10 studies (17 models), and measures of proteinuria were included in 9 studies (15 models)[51-64]. Only 2 studies (4 models) met the criteria for clinical usefulness, of which 1 study (3 models) presented reclassification indices with clinically useful risk categories[52]. A 2013 VA study of 1866 participants aged 65 and older with CKD with an eGFR less than 30 mL/min per 1.73 m^2 body surface area (BSA), concluded that a new CKD prediction model using commonly available clinical measures (age, congestive heart failure, systolic blood pressure, eGFR, potassium, and albumin) showed excellent ability to predict the onset of ESRD within the next year in elderly adults[65]. Peeters et al in an analysis of 595 Canadian CKD patients in stages Ⅲ-Ⅴ CKD, developed an eight-variable model [including age, sex, eGFR, albuminuria, calcium, phosphate, bicarbonate, albumin] and the conclusion was that this more complex model also accurately predicted the progression to kidney failure[66]. Despite these foregoing claims and assertions regarding the utility of CKD prediction models in patient care and in individual patient CKD prognostication, from direct clinical experience, and after a critical unbiased review of currently available CKD progression prediction models, and related CKD literature, we have concluded that all currently published CKD progression prediction models remain untested, not validated, and of limited use for individual CKD patient prognostication, at any given pre-specified point in time[19-36].

LIFETIME CKD RISK MODELING AMONG THE US POPULATION–HOW ACCURATE AND RELIABLE CAN SUCH POPULATION WIDE ESTIMATES BE?

Grams *et al*[37], in a Markov Monte Carlo model simulation study of current United States black and white populations had concluded that from birth, the overall lifetime risks of CKD stages 3a+, stage 3b+, stage 4+, and ESRD were 59.1%, 33.6%, 11.5%, and 3.6%, respectively[37]. According to this very publicized report, the risk of CKD increased with age, with approximately one-half the CKD stage 3a cases developing after 70 years of age[37]. However, to what extent this is true and linearly predictable at the individual (older) CKD level remains unclear[67]. The clinical relevance, the validity and real life implications of these estimates remain to be more appropriately elucidated[29-32]. Moreover, as is evident from the analysis in the next paragraph, such population-based disease risk estimates can often be shown to be very significantly off-mark, more so when validated against actual reported ESRD incidence values as available in the United States Renal Data System (USRDS).

OVERESTIMATION OF ESRD INCIDENCE RATES AMONG US CKD COHORTS-REPORT OF A COMPARATIVE ANALYSIS

A 2007 United States of America Centers for Disease Control report revealed that 16.5% of the United States. population 20 years of age and older had CKD as defined by eGFR < 60 mL/min per 1.73 m^2 BSA[68]. This CKD prevalence estimate, at the time of this report, translated that > 20 million adult Americans had CKD[68]. Besides, three United States studies published between 2004 and 2009, among different CKD patient population cohorts,

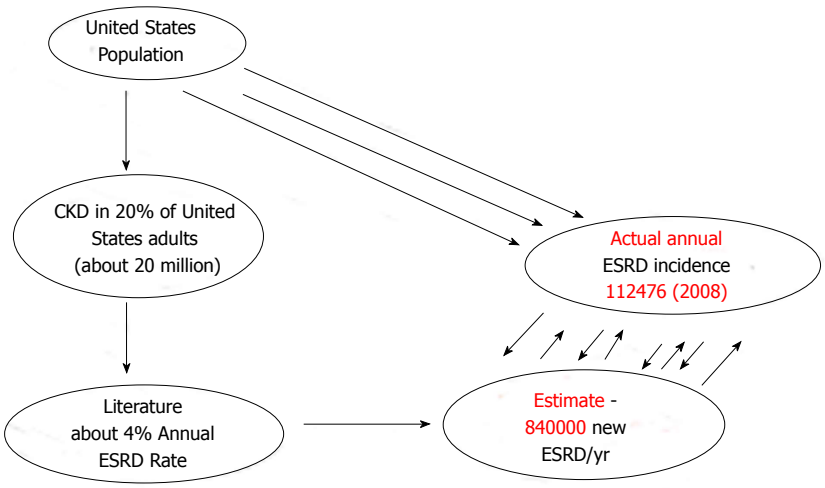

Figure 1 Gross overestimation of annual incident end stage renal disease patients for 2008 from projected estimates based on annualized end stage renal disease rates from three cited concurrent United States chronic kidney disease cohort studies. CKD: Chronic kidney disease; ESRD: End stage renal disease.

had demonstrated annualized ESRD rates among CKD patients of 4%, 8.2% and 4.5%, respectively[29,69,70]. A meta-analysis of these three studies produced a weighted average annualized ESRD incidence rate of 4.2%[30-32]. This weighted average annualized ESRD incidence rate of 4.2% among the entire United States CKD population from 2007 would produce an estimated 840000 new ESRD cases/year for 2008[30-32]. This represented a gross over-estimation when compared to the actual reported USRDS incident ESRD for 2008 of 112476[30-32,71] (Figure 1). Thus, the estimated ESRD incidence for 2008 of 840000 was a whopping 650% gross overestimate over the actual recorded number of new ESRD patients in the United States for 2008 of 112476[30-32,71].

EVIDENCE OF CKD STAGE VARIABILITY AND UNPREDICTABILITY AMONG STUDIED CKD COHORTS: THE PROBABLE IMPACT OF THE EFFECTS OF THE SYNDROME OF LATE ONSET RENAL FAILURE FROM ANGIOTENSIN BLOCKADE

In a 2011 retrospective analysis, Sikaneta *et al*[35], analyzed the longitudinal changes during a 1.1-year observation period of eGFR and CKD stages among 1262 patients, mean age 71.25 years, drawn from two large Canadian renal clinics. This study demonstrated CKD stage variability (defined by changes in CKD stages) and reported that CKD stage changed in 40% of the cohort (including 7.4% in whom CKD stage improved) whereas CKD stage remained static in 762 (60.4%) patients, the majority of this CKD cohort[35]. Furthermore, although CKD stage had remained static in 762 (60.4%) patients, that is to say, they did not experience a change in CKD stage during the initial observation period, 204 (40%) of 512 patients from this subgroup who were available for follow-up 2.3 years later still ended up on dialysis, suggesting subsequent acute unanticipated yet irreversible ESRD, a picture that is consistent with the newly described syndrome of rapid onset end stage renal disease which syndrome is expatiated in greater detail in a following section of this review[72]. This Canadian study demonstrated the clear unpredictability of CKD staging and prognostication at the individual patient level[35,36]. Moreover, the observation that 7.4% of this Canadian CKD cohort actually demonstrated improved CKD stage is still more dramatic[35,36]. In an editorial correspondence, we had speculated that some of these CKD patients who revealed improved CKD stages during follow up may indeed represent some CKD patients who had experienced acute worsening kidney function while on angiotensin blockade and who following withdrawal of angiotensin inhibition had exhibited improved kidney function[36]. This would only further strengthen the existence of the previously unrecognized syndrome of late onset renal failure from angiotensin blockade, a syndrome that we first described in 2005[73-77]. In another Canadian study, Levin et al studied 4231 CKD Ⅳ patients characterized by an index eGFR of less than 30 mL/min per 1.73 m^2, at least 3 subsequent eGFR values available for analysis, and no less than 4 months of follow-up between January 2000 and January 2004[33]. In this CKD cohort, mean age of 67 years, median follow-up of 31 mo, during the first 2 years of follow-up, 24% started dialysis therapy, 1% received a transplant, 7% died, and 1% was lost to follow-up[33]. The conclusion from this study was that the clinical course of patients with CKD stage 4 was unpredictably variable[33].

A JUNE 2011 MAYO CLINIC LABORATORY DATABASE TWO-YEAR SNAP SHOT ANALYSIS OF CKD STAGE CHANGES IN CKD IV PATIENTS

In June 2011, we pulled by IT reporting, all stage Ⅳ CKD patients with eGFR in the 15.0-29.9 mL/min per 1.73 m^2 BSA range in a Mayo Clinic Laboratory Database reported between April 19, 2009 and April 19, 2011[19,30,32,75]. All patients who had received renal replace-

ment therapy for acute kidney injury (AKI) or ESRD were excluded from the analysis. We included for analysis, all patients with at least three recorded eGFR values, and with a minimum of 6 mo between the first and the last reported eGFR estimations[19,30,32,78]. After excluding 62 ESRD patients, and all who received renal replacement therapy for AKI, 241 patients qualified for this analysis - 102 males and 139 females. In over 95% of the patients, eGFR remained stable and did not vary by as much as 5 eGFR points (< 25% from baseline) over the two-year study period[19,30,32,78]. The conclusion following this snapshot cross sectional analysis was that eGFR in the majority of CKD stage Ⅳ patients remained stable after two years of follow up[19,30,32,78].

EVIDENCE IN THE CKD LITERATURE OF THE EXISTENCE OF CKD" PROGRESSORS" AND CKD "NONPROGEESSORS"

Our experience studying the natural history of CKD at the Mayo Clinic Health System Renal Unit in Northwestern Wisconsin, United States, suggests that some CKD patients are able to maintain stable, albeit diminished eGFR levels over several years, the so-called "nonprogressors" or "asymptomatic" CKD patients, whereas, other CKD patients, for often unclear reasons, have an apparent enhanced propensity to progressively lose eGFR over time, the so-called "progressors" or "symptomatic" CKD[31,79].

In a 2012 retrospective report from South Korea, of 347 CKD Ⅲ patients, enrolled between January 1997 and December 1999, who were followed up through June 2010, a period of 10 years, 167 patients (48.1%) did not progress, 60 (17.3%) progressed to stage 4 and 120 (34.6%) progressed to stage 5, with 91 (26.2%) starting dialysis[80].

Other investigators, as reviewed below, have also documented such disparate behavior of individual patient CKD stages over time, with the additional introduction of the terms, "improvers" and "nonimprovers", to describe this dichotomy of observed CKD nonprogression *vs* progression, respectively, with follow up[81-85]. In a 2-year follow-up of the MDRD study, GFR remained stable in 19% of patients and improved in 11%[81]. In the African American Study of Kidney Disease and Hypertension (AASK) trial, however, over a longer follow up period of 8.8 years and with Bayesian models, eGFR improved among only 3.3%, with a mean slope of +1.06 mL/min per 1.73 m^2 per year[82]. In another study of individual GFR progression trajectories over 12 years of follow-up in participants in AASK trial, Li *et al*[83] demonstrated that many patients with CKD have a nonlinear GFR trajectory or a prolonged period of nonprogression. In this study, 352 (41.6%) patients showed a > 0.9 probability of having either a nonlinear trajectory or a prolonged nonprogression period; in 559 (66.1%), the probability was > 0.5. Baseline eGFR > 40 mL/min per 1.73 m^2 and urine protein-creatinine ratio < 0.22 g/g were associated with a higher likelihood of a non progression period. 74 patients (8.7%) had both a substantial period of stable or increasing eGFR and a substantial period of rapid eGFR decrease[83]. In another population with mild CKD receiving primary care through a large integrated health care system between 2004 and 2009, eGFR rose over time among 41.3%[84]. In yet another retrospective study of patients before nephrology referral, eGFR did not progress among 16% of those with stages 3-5 of CKD[85].

CKD "IMPROVERS" AND "NONIMPROVERS" IN A 2013 FRENCH REPORT

Recently, French investigators examined 406 patients in the NephroTest cohort with measured glomerular filtration rates (mGFR) measured by 51Cr-EDTA clearance at least 3 times during at least 2 years of follow-up[86]. Individual examination of mGFR trajectories by 4 independent nephrologists classified patients as "improvers", defined as those showing a sustained mGFR increase, or "nonimprovers"[86]. Twelve patients with erratic trajectories were excluded. Measured GFR improved over time in 62 patients (15.3%). Their median mGFR slope was + 1.88 (IQR, 1.38, 3.55) mL/min per year; it was 22.23 (23.9, 20.91) for the 332 "nonimprovers". "Improvers" had various nephropathies, but not diabetic glomerulopathy or polycystic kidney disease[86]. They did not differ from "nonimprovers" for age, sex, cardiovascular history, or CKD stage, but their urinary albumin excretion rate was lower. GFR improvement is possible in CKD patients at any CKD stage through stage 4-5[86]. It is noteworthy that this GFR improvement is associated with a decrease in the number of metabolic complications over time. In conclusion, this French report showed that renal function can improve over time in a significant proportion of CKD patients, even at a severe stage of CKD[86]. Indeed, the 15.3% prevalence of GFR improvement observed in this cohort is consistent with the few reports previously published.

These observations warrant further study of CKD progression or nonprogression among stable population-based asymptomatic CKD patients to improve our knowledge of the natural history of CKD and to help optimize CKD care around the world - our soon to be introduced new IT Software Program, The CKD Express© IT Software Program, which would help accelerate the harnessing of such new information about the natural history of CKD will be discussed in a later section of this review[49,87,88].

GRAPHICAL CASE SUMMARIES OF SELECTED INDIVIDUAL PATIENT-LEVEL

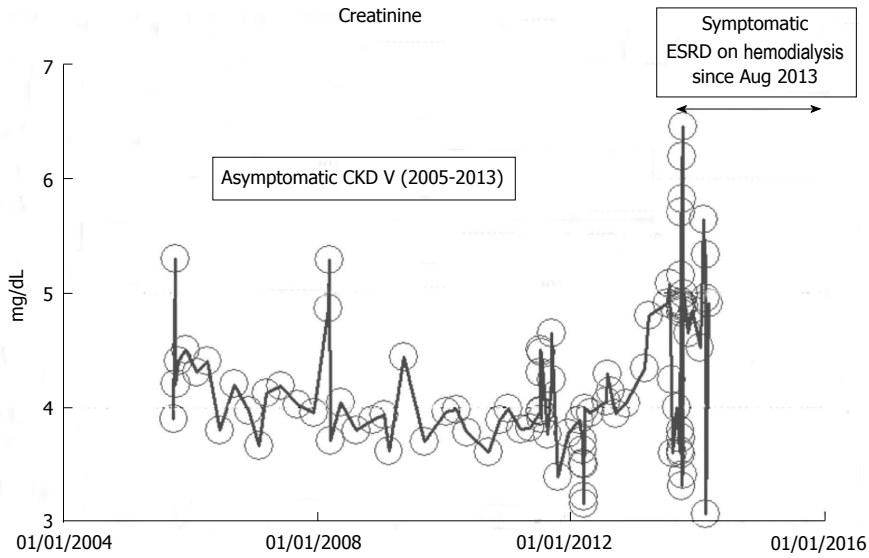

Figure 2 A now 82-year-old white male on maintenance hemodialysis August 2013-March 2014, after 8 years of asymptomatic chronic kidney disease V, as monitored and tracked through a simulation of The chronic kidney disease Express© IT Software Program. CKD: Chronic kidney disease; ESRD: End stage renal disease.

SERUM CREATININE TRAJECTORIES OVER SEVERAL YEARS TO DEMONSTRATE CKD STAGING UNPREDICTABILITY AS SEEN AT THE MAYO CLINIC HEALTH SYSTEM RENAL UNIT, NORTHWESTERN WISCONSIN, UNITED STATES, 2002-2014: THE CONCEPT OF "SYMPTOMATIC" *VS* "ASYMPTOMATIC" CKD

From our experience of studying the natural history of CKD at the Mayo Clinic Health System Renal Unit, Northwestern Wisconsin, United States, during the period, 2002-2014, and from a dispassionate review of the nephrology literature, it would appear that some CKD patients are able to maintain stable, albeit diminished eGFR levels over years, the so-called "non-progressors" or "asymptomatic" CKD patients, whereas, other CKD patients, for often unclear reasons, have the enhanced propensity to progressively lose eGFR over time, the so-called "progressors" or "symptomatic" CKD[32,78-86]. We will show in this section of this review, graphical representations, some selected CKD patients that we have encountered in the last 12 years in our Mayo Clinic Health System Renal Unit, Northwestern Wisconsin, United States, that demonstrate these patterns of behavior: (1) as at March 2014, a now 82-year-old white male, ex-smoker, with a history of hypertension and CKD has been on maintenance hemodialysis for symptomatic ESRD since August 2013. However, for 8 years, between 2005 and 2013, despite a stable CKD stage V with serum creatinine of 4.0-5.0 mg/dL (eGFR 8-14 mL/min per 1.73 m^2 BSA), he had remained asymptomatic. He had an AVF created in June 2005 which was subsequently revised for non-maturation in January 2006. For unclear reasons, he then became symptomatic in July 2013 with nausea, vomiting and anorexia, and has been on maintenance hemodialysis since August 2013 (Figure 2); (2) as at February 2014, a now 78 year-old white male, with a history of hypertension, type Ⅱ diabetes mellitus, obesity, recurrent UTI and CKD V, has over the last 7 years, between 2006 and 2013, despite a stable CKD stage V with serum creatinine of 4.5-5.5 mg/dL (eGFR 8-11 mL/min per 1.73 m^2 BSA), had remained asymptomatic. He continues on alternate monthly course of prophylactic short course of oral Levofloxacin for recurrent UTI prophylaxis. (Figures 3 and 4); and (3) as at early 2014, a now 78-year-old white male, diagnosed with Wegener's Granulomatosis with AKI on CKD in 2005. Serum creatinine had increased from 2.0 mg/dL to 3.5-4.0 mg/dL. The Wegener's Granulomatosis was treated with standard chemotherapy (Prednisone), and he has been in remission since 2006, albeit with a new baseline serum creatinine of 3.5-4.0 mg/dL (eGFR 16-22 mL/min per 1.73 m^2 BSA). He has remained otherwise an asymptomatic CKD IV patient between 2006 and early 2014 (Figures 5 and 6).

UNPREDICTABILITY OF THE IMPACT OF AKI EVENTS ON CKD PROGRESSION-A BRIEF REVIEW OF SELECTED CASES FROM THE MAYO CLINIC HEALTH SYSTEM RENAL UNIT, EAU CLAIRE, NORTHWESTERN WISCONSIN, UNITED STATES

In a just published book chapter, we had extensively reviewed the multifaceted nature of renal outcomes following AKI in CKD patients[89-91]. As a result of the var-

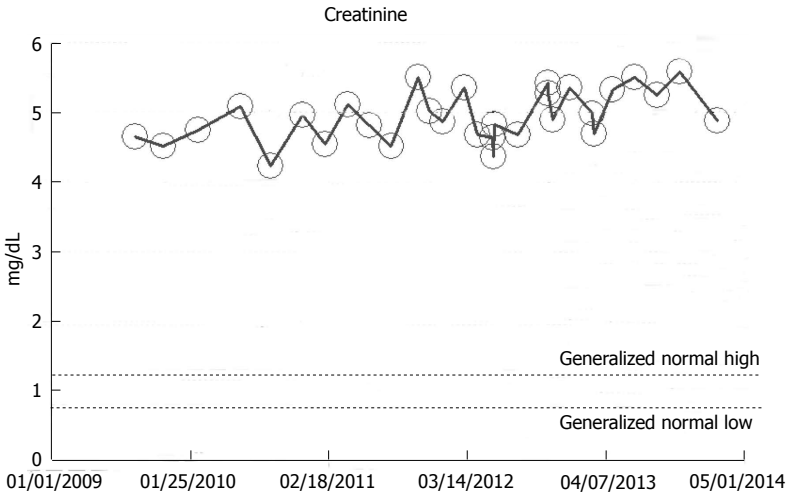

Figure 3 A now 78-year-old white male on with asymptomatic stable chronic kidney disease V for 8 years, 2006-2014, serum creatinine of 4.5-5.5 mg/dL (eGFR 8-11 mL/min per 1.73 m^2 Body Surface Area), as monitored and tracked through a simulation of The chronic kidney disease Express© IT Software Program. eGFR: Glomerular filtration rates.

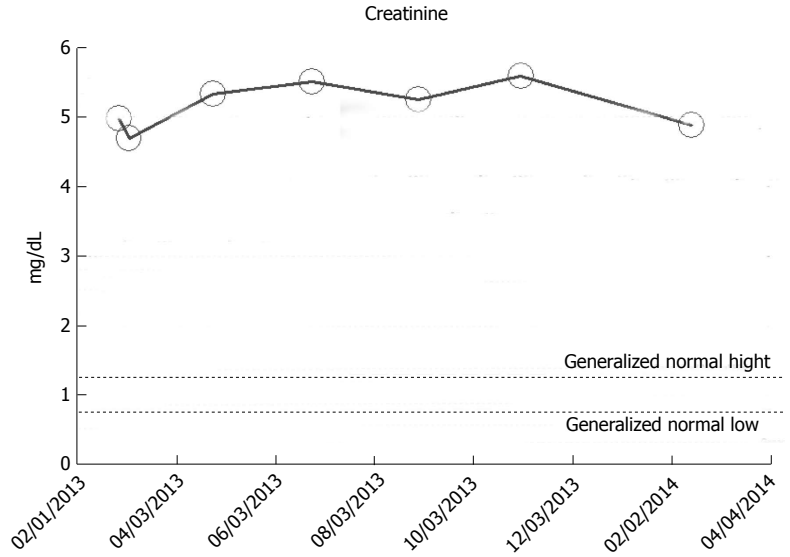

Figure 4 A now 78-year-old white male on with asymptomatic stable chronic kidney disease V for 8 year, 2006-2014, serum creatinine of 5.0-5.5 mg/dL (eGFR 8-11 mL/min per 1.73 m^2 Body Surface Area), in the last one year, February 2013-March 2013, as monitored and tracked through a simulation of The chronic kidney disease Express© IT Software Program. eGFR: Glomerular filtration rates.

ied nature of these renal outcomes, we dubbed this "the rainbow syndrome of too many colors"[91]. We would briefly describe three patients with AKI on CKD and three observed different outcomes to demonstrate the array of possible renal outcomes following AKI on CKD - complete recovery of renal function back to baseline, partial recovery following AKI, and a third outcome where despite multiple repeated AKI events over several years, a 92-year-old Caucasian female was still able to maintain her baseline serum creatinine (Figure 7). In the following section, we shall describe the previously unrecognized syndrome of acute yet irreversible ESRD following unpredictably on an AKI event in a CKD patient, the syndrome of rapid onset end stage renal disease or (SORO-ESRD)[19,30,72,89].

A-48 year-old obese hypertensive Caucasian male patient, with otherwise stable stage Ⅱ CKD, baseline serum creatinine of 1.2 mg/dL (eGFR > 60 mL/min per 1.73 m^2 BSA), in January 2013, developed AKI on CKD following fever of unknown origin (FUO) complicating methicillin-resistant Staphylococcus Aureus (MRSA) bacteremia. He at the time of admission in January 2013 was on hydrochlorothiazide 25 mg daily, Lotrel (5 mg of amlodipine + 10 mg of benazepril) daily. Lotrel was promptly discontinued on admission and Amlodipine only was continued. MRSA septicemia was treated with parenteral antibiotics, guided by antibiogram[91]. A kidney biopsy revealed acute interstitial nephritis. Peak serum serum creatinine was 3.3 mg/dL. He exhibited full recovery of kidney function as at March 2013 (Figure 8).

An 83-year-old white woman with hypertension and otherwise stable CKD Ⅲ had undergone a right hemicolectomy procedure together with 150 cm small intestinal resection with end-to-end ileo-colic anastomosis for colon cancer in October 2011. This was further complicated by dehydration and diarrhea and the need for a second laparoscopic procedure in November 2011. She experienced AKI in October 2011 following the initial surgical procedure and another AKI episode in November 2011 from hypovolemic dehydration with peak creatinine values as shown in Figure 9. She subsequently partially recovered kidney function and since December 2011, she has maintained a serum creatinine of 1.8-2.2 mg/dL through March 2014. She otherwise remains asymptomatic, and continues to feel great at her current age of 85 years. Her baseline serum creatinine was 1.2 mg/dL in

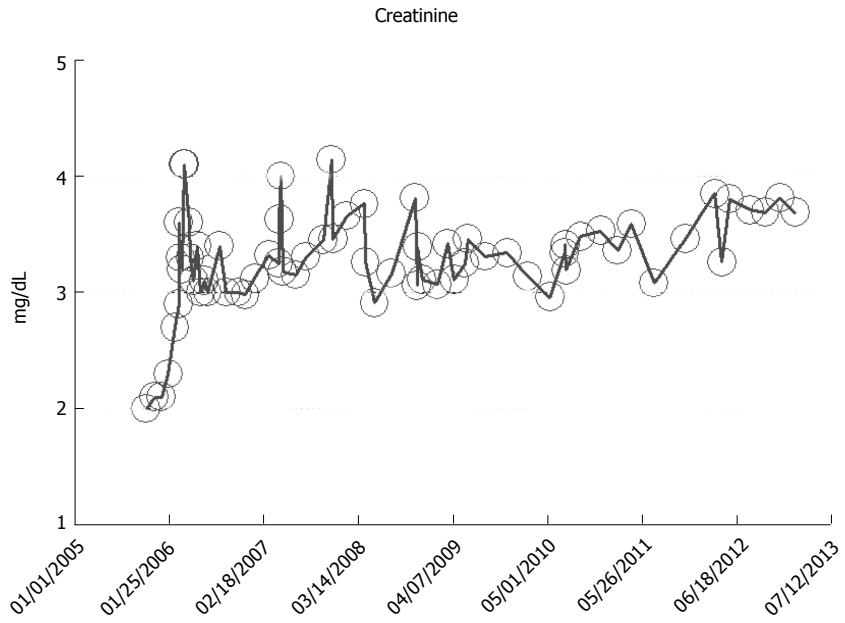

Figure 5 A now 78-year-old white male with treated Wegener's Granulomatoisis in remission since 2006, with new stable chronic kidney disease IV 2008-2014, following initial acute kidney injury on chronic kidney disease in 2005, as monitored and tracked through a simulation of The chronic kidney disease Express© IT Software Program.

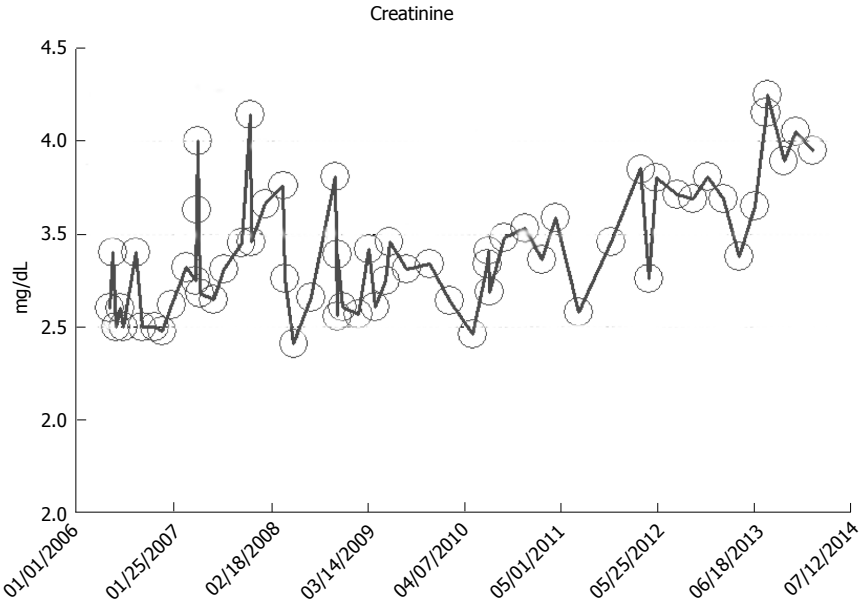

Figure 6 The same patient as in Figure 5 has remained otherwise an asymptomatic chronic kidney disease IV patient between 2006 and early 2014, serum creatinine of 3.5-4.0 mg/dL (eGFR 16-22 mL/min per 1.73 m^2 Body Surface Area), as monitored and tracked through a simulation of The chronic kidney disease Express© IT Software Program. eGFR: Glomerular filtration rates.

January 2006, 1.3-1.4 mg/dL in 2010-2011 (CKD stage Ⅲ, eGFR approximately 36-38 mL/min per 1.73 m^2 BSA), AKI intervened in October-November 2011, and she has had a new higher baseline serum creatinine of 2.0-2.6 mg/dl (eGFR 20-25 mL/min per 1.73 m^2 BSA), CKD stage IV, in the last three years, between December 2011 and March 2014 (Figure 9).

A 92-year-old white woman in March 2014, with a history of multiple AKI events between 2005 and 2014, over nearly 10 years, but is still able to generally maintain an otherwise asymptomatic CKD Ⅳ status with serum creatinine of 1.7-2.0 mg/dL (CKD stage Ⅳ, eGFR 20-29 mL/min per 1.73 m^2 BSA), through March 2014, the same baseline serum creatinine she had 10 years ago in 2005 (Figure 7). The repeat episodes of AKI events are shown in Figure 7 and were caused by the following causes: (1) Pyelonephritis, treated with Bactrim, June 2006, peak creatinine was 3.0 mg/dL; (2) Left lower lobe pneumonia with diarrhea, dehydration and hypotension, July 2007, peak creatinine was 3.43 mg/dL; (3) Pyelonephritis, September 2009, peak creatinine was 2.37 mg/dL; and (4) Acute gastroenteritis and dehydration, March 2012, peak creatinine was 2.75 mg/dL.

THE SYNDROME OF RAPID ONSET END STAGE RENAL DISEASE

The commonly held consensus of the propagation of CKD to ESRD is that of a predictable, linear, progressive, relentless, time-dependent and knowable decline in renal function, with predictably increasing serum creatinine or falling eGFR, leading inexorably to ESRD and the need for renal replacement therapy[1-4,6,7,9-18]. This universally accepted paradigm of CKD-ESRD progression will be referred to in this review as the "classic" pattern

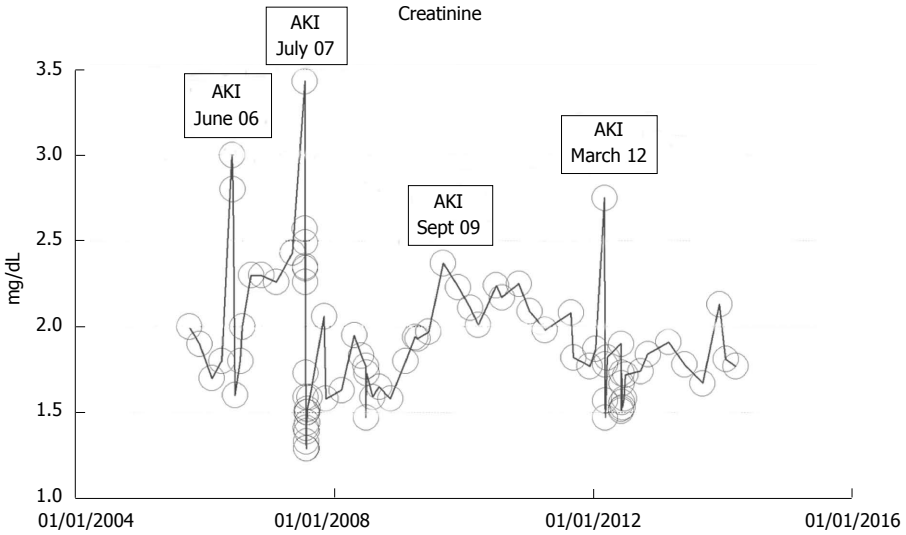

Figure 7 Serum creatinine trajectory in a now 92-year-old Caucasian female patient who despite multiple repeated AKI episodes between 2006 and 2012, has otherwise maintained a baseline serum creatinine of 1.7-2.0 mg/dL through March 2014, as monitored and tracked through a simulation of The chronic kidney disease Express IT Software Program. AKI: Acute kidney injury.

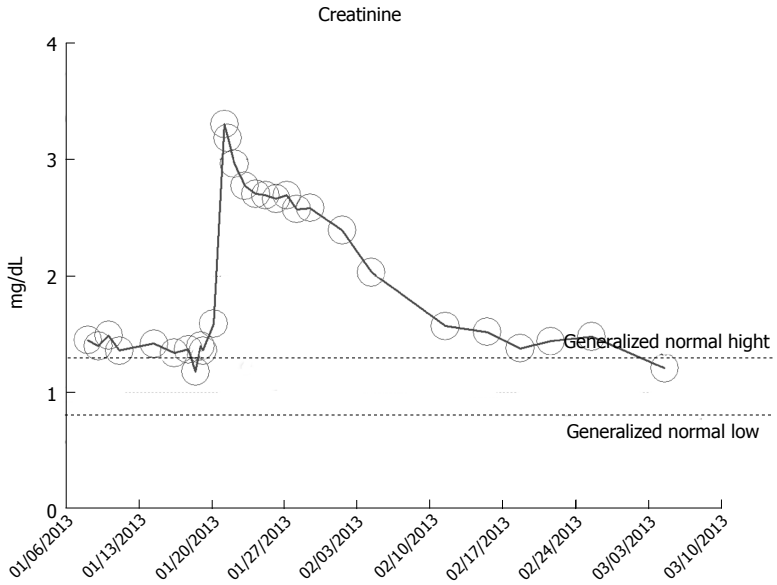

Figure 8 Serum creatinine trajectory in an 48-year-old obese hypertensive male patient who developed acute kidney injury from biopsy-proved acute interstitial nephritis complication methicillin-resistant Staphylococcus Aureus septicemia, with full renal recovery after one month, as monitored and tracked through a simulation of The chronic kidney disease Express© IT Software Program. AKI: Acute kidney injury.

of CKD to ESRD progression[19,92].

What's more, whereas 25.2% of ESRD Medicare patients all experienced antecedent AKI, for many decades now, this relationship between antecedent AKI and irreversible ESRD had been fortuitously blamed on the so-called "residual confounding"[93]. However, in 2010, we put to rest any doubts as to the direct causality between antecedent AKI and the precipitation of acute yet irreversible ESRD, when we first described the previously unrecognized syndrome of rapid onset end stage renal disease or SORO-ESRD in the journal, *Renal Failure*[72]. We have defined the syndrome of rapid-onset ESRD as the unpredictable, unanticipated and accelerated progression from a-priori stable CKD to irreversible ESRD, requiring permanent RRT, following a new episode of AKI precipitated by antecedent new medical/surgical events, with the interval between AKI and the need for RRT represented by a period of often less than less than two weeks, measured only in days following surgically induced AKI[19,30,72,92,94,95]. We have also described this syndrome of acute yet irreversible ESRD in renal transplant recipients[19,92,94,95]. We would briefly describe in this section, CKD patients who demonstrate features of the "classic" CKD to ESRD translation (Figures 10 and 11), and patients who on the other hand exhibit the features of the syndrome of rapid onset end stage renal disease or SORO-ESRD (Figure 12).

We were the first to aptly circumscribe this syndrome of rapid onset ESRD as a distinct clinical entity[19,30,72,92,94,95]. Our findings have received significant collaborating support following the publication of several more recent reports demonstrating the not uncommon occurrence of SORO-ESRD, in incident adult ESRD populations in both the US and Canada[96-99]. In fact, a recent 2012 review in the journal *Kidney International* concluded that AKI can cause ESRD directly, and increase

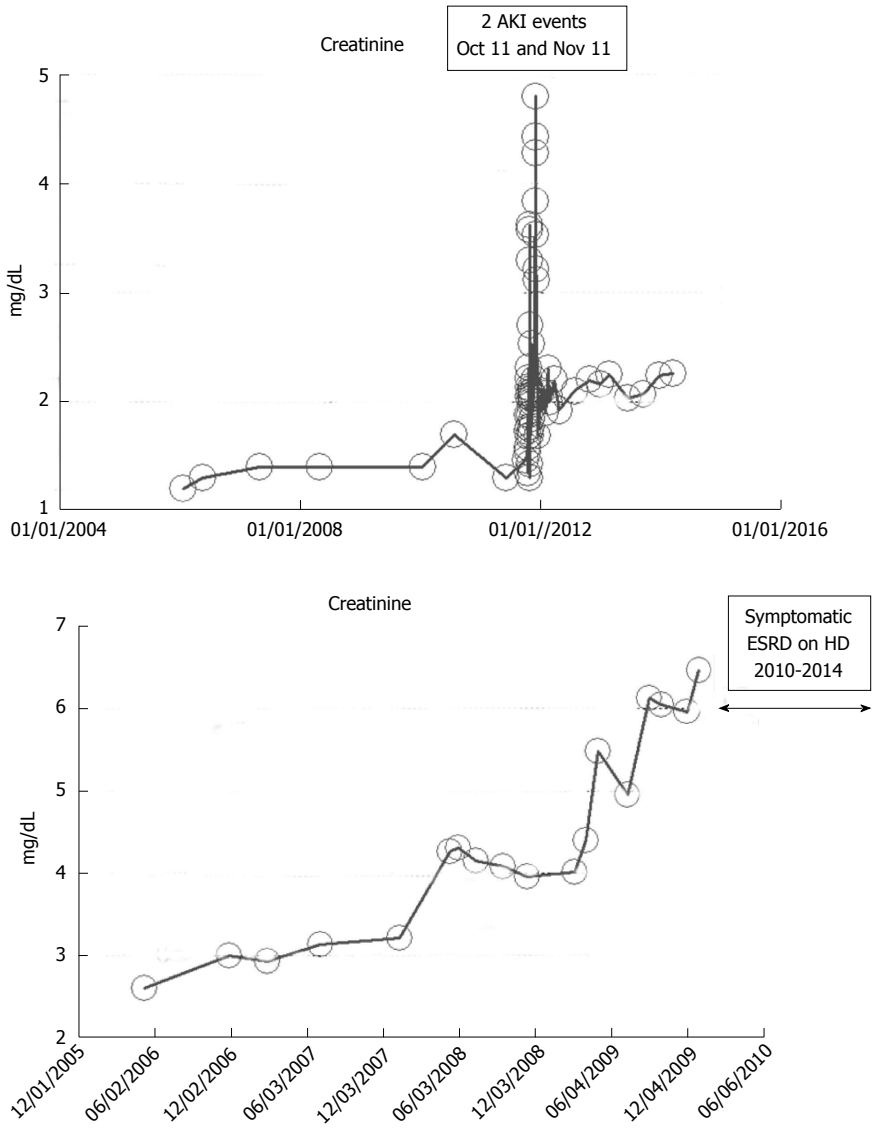

Figure 9 Serum creatinine trajectory in an 85-year-old obese hypertensive female patient who developed acute kidney injury following a complicated right hemicolectomy with small intestinal resection and ileo-colic anastomosis in October 2011, with partial but stable renal recovery (chronic kidney disease IV) after one month, as monitored and tracked through a simulation of The chronic kidney disease Express© IT Software Program.

Figure 10 Serum creatinine trajectory in a 82-year-old white male patient with features of the predictable linear chronic kidney disease to end stage renal disease progression from 2006 through 2010 when he started hemodialysis for symptomatic uremia, as monitored and tracked through a simulation of The chronic kidney disease Express© IT Software Program. ESRD: End stage renal disease; HD: Hemodialysis.

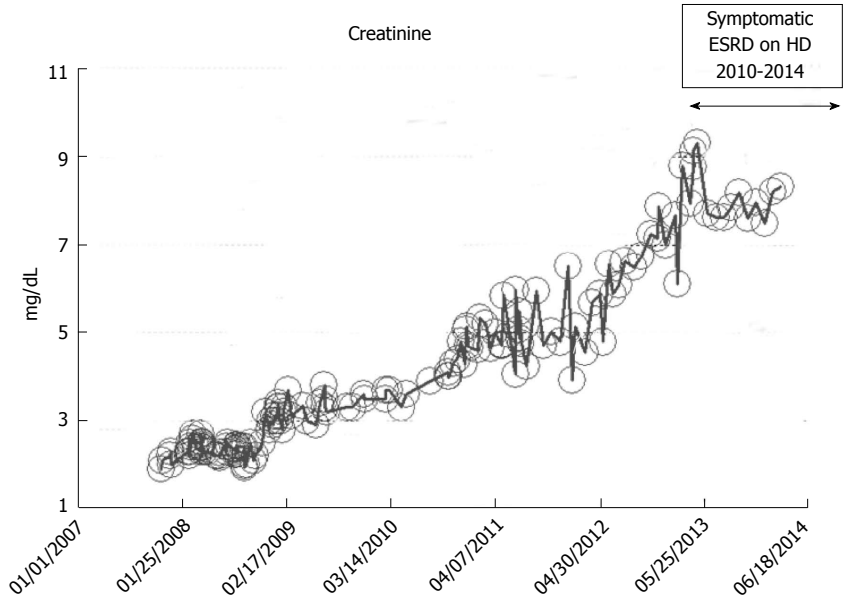

Figure 11 Serum creatinine trajectory in a 52-year-old white male patient with features of the predictable linear chronic kidney disease to end stage renal disease progression from 2007 through 2010 when he started hemodialysis for symptomatic uremia, as monitored and tracked through a simulation of The chronic kidney disease Express© IT Software Program. ESRD: End stage renal disease; HD: Hemodialysis.

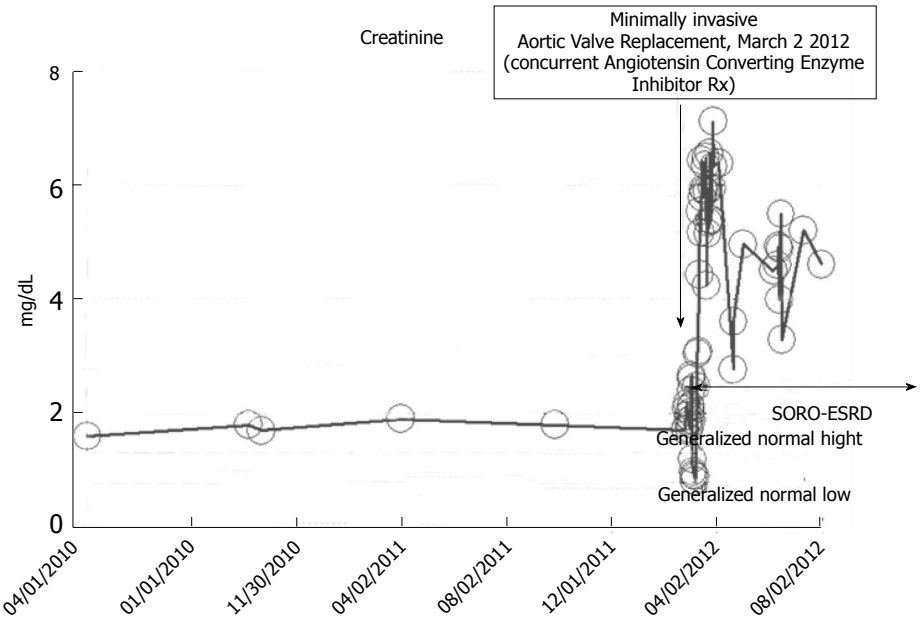

Figure 12 Serum creatinine trajectory, 2010-2012, with post-operative acute kidney injury precipitating acute yet irreversible end stage renal disease in March 2012 while on concurrent ACE inhibition, following minimally invasive aortic valve replacement in a then 73-year-old thin white woman. SORO-ESRD: Syndrome of rapid onset end-stage renal disease.

the risk of developing incident CKD and worsening of underlying CKD and even went further to posit that the distinction between AKI and CKD may be artificial[100].

"Classic" CKD to ESRD pattern

An 82-year-old while male with a history of hypertension, low ejection fraction ischemic cardiomyopathy, type II diabetes mellitus, coronary artery disease, obesity and hypothyroidism developed progressively worsening renal failure with predictable linear increase in serum creatinine from May 2006 when serum ceatinine was 2.6 mg/dL through to January 2010 when serum creatinine exceeded 6.0 mg/dL and he developed features of uremia and started renal replacement therapy in the form of in-center outpatient hemodialysis in January 2010 (Figure 10). He died in early 2014, while still on maintenance hemodialysis, from failure to thrive. No laboratory data were available for this patient before May 2006.

"Classic" CKD to ESRD pattern

A 52-year-old while male with a history of hypertension, low ejection fraction ischemic cardiomyopathy, and hypothyroidism developed progressively worsening renal failure with predictable linear increase in serum creatinine from November 2007 when serum ceatinine was 1.9 mg/dL through to December 2010 when serum creatinine exceeded 7.0 mg/dL and he developed features of uremia and started renal replacement therapy in the form of in-center outpatient hemodialysis (Figure 11). He continues on maintenance hemodialysis as at March 2014, the date of this present review.

"SORO-ESRD" pattern in native kidneys

A 73-year-old obese male patient with a baseline serum creatinine of approximately 1.7 mg/dL, 2010-2012, with a past medical history for hypertension, type Ⅱ diabetes mellitus, on concurrent ACE inhibition with Lisinopril 40 mg daily, was admitted to the coronary care unit (CCU) in February 2012 with acutely decompensating heart failure. Acute coronary syndrome was ruled out and the patient subsequently underwent minimally invasive aortic valve replacement for symptomatic aortic stenosis with a 25 mm St. Judes Epic stented tissue valve March 2 2012. He rapidly developed post-operative AKI on CKD and required hemodialysis on the 1st post-operative day (Figure 12). He has since remained on outpatient in-center maintenance hemodialysis for ESRD, from March 2012 through to March 2014, the date of present reporting.

"SORO-ESRD" pattern in a renal transplant recipient

A 53-year-old Caucasian type 1 diabetic woman with simultaneous pancreas-kidney transplantation in 2000 for ESRD and type I diabetes mellitus, maintained on chronic transplant immunosuppression with tacrolimus, cellcept, and prednisone had maintained her allograft CKD stage Ⅲ status throughout most of 2010 with a baseline serum creatinine of 1.6 mg/dL. In January 2011, she suffered from acute transplant pyelonephritis precipitating AKI, further complicated by dehydration from acute gastroenteritis[94]. The patient's serum creatinine rose quickly within days to 5.16 mg/dL (Figure 13). She required hemodialysis for symptomatic renal failure. A transplant kidney biopsy revealed acute tubular necrosis with chronic transplant glomerulopathy but no rejection. She was maintained on in-center outpatient hemodialysis for oliguric irreversible ESRD until January 2012, twelve months later, when she received a new renal allograft re-transplant, a living-related kidney allograft from her then 32-year-old son at Mayo Clinic, Rochester. Additional clinical details of this renal transplant recipient are de-

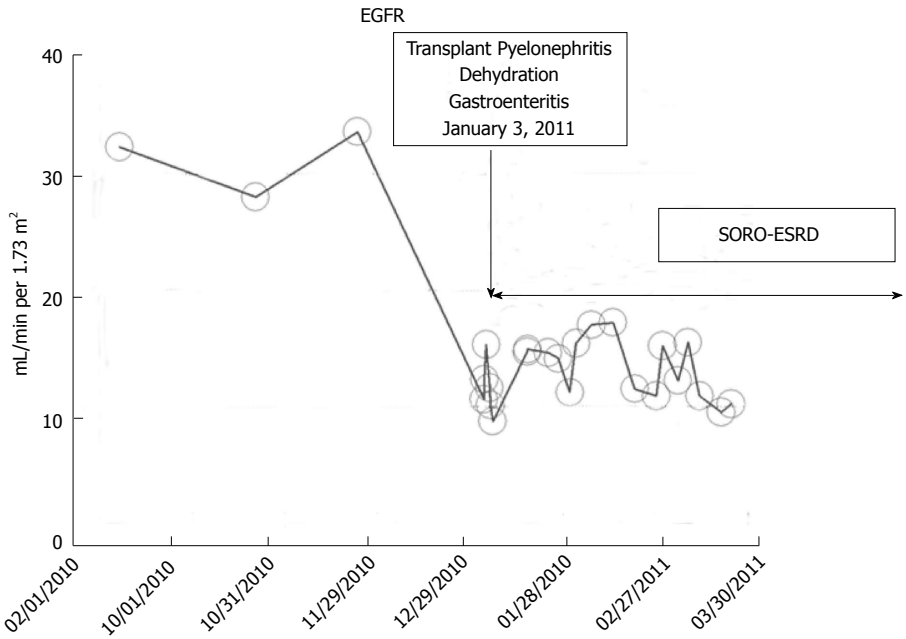

Figure 13 Estimated glomerular filtration rates trajectory in a renal transplant recipient following acute kidney injury and Syndrome of Rapid Onset End-Stage Renal Disease in January 2011, was on maintenance in-center hemodialysis for 12 mo and received a second living related kidney transplant from 32-year-old son in January 2012 at Mayo Clinic, Rochester. SORO-ESRD: Syndrome of rapid onset end-stage renal disease. EGFR: Estimated glomerular filtration rates.

scribed in our recent publication[94].

CKD SCREENING REVISITED-CKD SCREENING CONTROVERSY BETWEEN ACP AND ASN: TO SCREEN OR NOT TO SCREEN?

To further emphasize on the inexactness of CKD prediction and prognostication, the August 2012 United States Preventive Services Task Force (USPSTF) report on CKD screening concluded that we know surprisingly little about whether screening adults with no signs or symptoms of CKD will improve health outcomes and that clinicians and patients deserve better information on CKD[39]. This unsettled state of affairs regarding the appropriateness or otherwise of CKD screening within the asymptomatic population came to the fore and became part of the public discourse when two very respected professional United States medical associations, the American Society of Nephrology (ASN) and the American College of Physicians (ACP) both simultaneously came down on opposite sides of the argument for and against CKD screening among asymptomatic populations, respectively[101,102]. The ASN position was that "while acknowledging the need for further and larger scale clinical research into CKD and how the disease progresses in its early stages, ASN believes current evidence strongly supports the value of early detection of, and screening for, CKD"[102]. Given the present level of evidence-base, and more so from our experience at the Mayo Clinic Health System Renal Unit, in Northwestern Wisconsin, United States, we would side with the USPSTF in cautioning against CKD screening among the otherwise asymptomatic population. Such a wide-out CKD screening model will most likely result in more unnecessary care, escalating costs of CKD care to Medicare, increased referrals to nephrologists, and all of this without any evidence of improved CKD outcomes[32,46,49,103,104]. Additionally, we could not agree more with the USPSTF on the urgent need for more research into CKD progression and prognostication.

MULTIPLE PUTATIVE MECHANISMS OF CKD INITIATION AND PROPAGATION

Clearly, from accruing evidence in the literature, there is a continuing elongating list of putative mechanisms that have been variously incriminated in the initiation and propagation of CKD progression[105-128]. We have analyzed and summarized these mechanisms in an extensively referenced recent review[105]. A critical review of current literature clearly demonstrates that culprit pathogenetic molecule(s) or mechanistic factor(s) responsible for the initiation and propagation of diabetic and/or non-diabetic nephropathy and subsequent progression of CKD to ESRD and the need for permanent renal replacement therapy, remain unverified, unconfirmed, uncertain, and possibly unknown[105-128]. Undeniably, several independent and often conflicting lines of evidence in the literature, from both human and experimental studies, propose a variety of presumed pathogenetic culprit mechanisms and factors[105-128]. Putative roles are ascribed in the literature for oxidative stress, inflammation, underlying genetic predispositions including variations of the non-muscle myosin heavy chain 9 gene (*MYH9*) on chromosome 22 and variants at chromosome 6q24-27 among African-Americans, advanced glycosylation end products and the interaction of these end products on the multili-

gand receptor of the immunoglobulin superfamily receptor for advanced glycation end products, intrarenal angiotensin II and/or renin production, lipid toxicity, podocyte injury and apoptosis, cytokine/chemokine/growth factor release causing renal injury, asymmetric dimethylarginine, and uric acid[105-128]. We posit that our current understanding of these different plausible pathways remain infantile at best, and that more studies are warranted[105]. We surmise that our better understanding of these mechanisms and processes that influence CKD initiation and CKD propagation, and our ability to decipher the relative contributions of these pathways and/or factors in CKD-ESRD translation, together with the impact of AKI on this continuum of CKD pathogenesis would implicitly help improve our overall ability to prognosticate and care for our CKD patients, in general[31,32,79,105].

THE CKD EXPRESS©-A NEWLY INTRODUCED IT SOFTWARE TO ENHANCE AND OPTIMIZE CKD CARE

From the foregoing review, it is very clear that there remains a significant knowledge gap of our understanding of the natural history of CKD, in general. As a result of these inherent deficiencies in the CKD knowledge base, in an MBA "New Product" class at the University of Wisconsin Consortium in 2011-2012, the first author together with a group of fellow MBA students devised a new IT Software Program called The CKD Express©, still currently in United States patent application[32,88,89,129]. This innovative IT Software Program with its unique artificial intelligence (AI), decision support tools (DSS) and other enhancements that include algorithmic components would enable a trained Nurse Practitioner, under the supervision of a nephrologist, to remotely track and remotely manage CKD patients by tracking serum creatinine and simultaneous eGFR trajectories of individual CKD patients indefinitely over time, through established IT system networks linked with the various associated EMR systems[32,87,88,129].This way, CKD patients will carry out pre-specified blood tests including the basic metabolic profile at pre-determined time intervals as determined by the IT Software. Only targeted and identified CKD patients would then need to either be referred to see a nephrologist, or repeat a blood test, urgently be seen in an Urgent Care Center or even the Emergency department as determined by the inbuilt algorithms in The CKD Express©[32,87,88,129]. This would ensure the delivery of convenient, affordable, effective and efficient CKD care around the world[32,49,87,88,129].

CONCLUSION

CKD staging is a useful guide, but its evidence-base is shaky, at best. However, CKD care must be individualized. Clearly, despite decades of painstaking research into the dynamics, processes and mechanisms of translation from CKD to ESRD and the need for renal replacement therapy, the medical community in general, and nephrologists in particular, arguably still remain at a considerable loss in understanding the nuances of such translations. We suggest a complete reappraisal of current nephrology practices and a new push to begin to develop new models of CKD care that correctly recognize the diversity of CKD as representative of a wide spectrum of disease states[31,32]. Most appropriately, Bansal and Hsu, in a 2008 analysis of the long-term outcomes of patients with CKD had strongly echoed the observation that the apparently conflicting and disparate ESRD and/or mortality rates reported in various CKD population cohorts in the literature only emphasized the heterogeneity of different CKD populations[130]. In a recent analysis, in comparison with patients with other underlying causes of CKD, patients with APKD and IgA nephropathy had a statistically significant slower progression rate of CKD to ESRD[131]. Nephrologists must therefore not rely on CKD staging alone to direct management of or risk stratification of patients with CKD[130]. Nephrologists must always consider the etiology and rate of progression of kidney disease, patient age and a wide array of renal cardiovascular disease risk factors[130,131]. Nephrologists must recognize that CKD prediction is at best an inexact science. CKD prediction models are very limited in scope and applicability. Inter-patient and intra-patient CKD stages variability over time is immense. There are "progressors" and "nonprogressors", "improvers" and "nonimprovers", and sometimes the same CKD patient often then exhibits contrasting CKD progression or non-progression patterns at different time periods. The impact of AKI on CKD is multi-faceted, the so-called "rainbow syndrome"[19,30,72,92,94,95]. Moreover, the role of AKI in CKD initiation is even far less well understood[132].

The overarching need to always individualize CKD care cannot be overemphasized as CKD represents a whole wide spectrum of distinctly different clinical disease entities, with each individual patient often subject to a multitude of aggravating factors, some of which often remain unrecognized[19,30,31,72,92,94,95,132,133]. This is in fact how we practice medicine - one patient, and only one patient at a time[19,31]. A soon to be published longitudinal retrospective United Kingdom analysis of approximately 600000 patients strongly emphasized this paramount need for individualized CKD care[134]. This study identified a 5.9% incidence of CKD in the United Kingdom in 2010, and with follow up, CKD stages were static in 50% of patients, progressed in 10%-15% of patients and actually improved in 25%-30% of patients[134].

Finally, we hope that the acceptance and subsequent introduction by Medicare of our newly devised IT Software, The CKD Express© into general CKD care in the United States, will generate enough prospective patient-level serum creatinine trajectories' data over the next few years to begin to help bridge the yawning gaps in our current knowledge of the natural history of CKD[32,87,88,129,135-137]. Every effort to reduce, if not eliminate AKI (Renoprevention) must be emphasized in both general medicine care and in nephrology specialty care,

respectively[32,87,88,129,135,136]. Such reengineering protocols will reduce AKI incidence, potentially save hundreds of millions of healthcare dollars and invariably lead to less ESRD incidence[32,87,88,129,138,139]. A more forceful and pragmatic application of renoprevention strategies in the CCU that aggressively enunciate and articulate the preemptive withholding (from all CKD patients) of nephrotoxics including renin angiotensin aldosterone system blockers, the adoption of measures to aggressively prevent or treat perioperative hypotension, the avoidance of nephrotoxic exposure such as iodinated contrast and nephrotoxic antibiotics, whenever possible, would collectively lead to less AKI, and therefore potentially less SORO-ESRD[32,87,88,129,138-140]. Such paradigms of care would translate into better patient outcomes, hugely significant renal salvage, and indeed massive dollar savings[32,138,139]. Such paradigm shifts would constitute major rethinking in current nephrology practice, a form of nephrology practice reengineering[32,138-140].

ACKNOWLEDGMENTS

This work is dedicated to the memory of our very dear friends, Mr. Ikechukwu Ojoko (Idejuogwugwu), and Chigbo Eduzor MD (CC), who passed away back home in Nigeria, several years ago, after reported brief illnesses. Idejuogwugwu and CC, you are truly missed. This work is also dedicated to the memories of the 153 Nigerians who died in a fiery plane crash in Lagos, Nigeria, on June 3, 2012. May their souls rest in perfect peace. Finally, we dedicate this work to the majority millions of Nigerians who continue to suffer in poverty, destitution, deprivation amidst plenty, to Nigerians who remain continuously exposed daily to indefensible insecurity in the face of paralyzed, corrupt and weak governments and derelict governance, and we pray for better days for that country. It remains our hope and prayers that someday, we would be celebrating an effective, efficient, affordable, accessible and potent health care delivery system in Nigeria that would veritably and equitably serve all Nigerians, both rich and poor, both privileged and underprivileged. Lastly, we look forward to enhanced CKD care, together with accessible affordable ESRD care to all needy Nigerians.

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P- Reviewer: Bhimma R, Duan SB **S- Editor**: Ji FF
L- Editor: A **E- Editor**: Wu HL

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World J Nephrol 2014 August 6; 3(3): 50-63
ISSN 2220-6124 (online)

REVIEW

Obesity in kidney disease: A heavyweight opponent

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Supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (São Paulo Research´s Foundation, FAPESP) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, INCT Complex Fluids and Renal Immunopathology Laboratory INSERM/CNPq), No. 12/15205-4, 12/02270-2, 12/16794-3, 12/23347-3
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Received: March 18, 2014 Revised: April 18, 2014
Accepted: June 10, 2014
Published online: August 6, 2014

Abstract

Obesity is an important worldwide challenge that must be faced in most developed and developing countries because of unhealthy nutritional habits. The consequences of obesity and being overweight are observed in different organs, but the kidney is one of the most affected. Excess adipose tissue causes hemodynamic alterations in the kidney that can result in renal disease. However, obesity is also commonly associated with other comorbidities such as chronic inflammation, hypertension and diabetes. This association of several aggravating factors is still a matter of concern in clinical and basic research because the pathophysiologic mechanisms surrounding chronic kidney disease development in obese patients remain unclear. This review will discuss the consequences of obesity in the context of renal injury.

Key words: Overweight; Obesity; Kidney disease; Renin-angiotensin system; Diabetes

Core tip: Obesity is unquestionably one of the biggest health challenges the modern world will face this century. It has vast effects on systemic function including cardiovascular disease, metabolic dysfunction and chronic inflammation. All of these factors have a great impact on kidney function, and current data indicate a significant correlation between obesity and kidney disease because of irregular immune activation, altered renal hemodynamics and metabolic mediator signaling. This review focuses on the most recent findings that have begun to elucidate the relationship between obesity and its effect on the kidneys.

Felizardo RJF, Burgos-Silva M, Aguiar CF, Câmara NOS. Obesity in kidney disease: A heavyweight opponent. *World J Nephrol* 2014; 3(3): 50-63 Available from: URL: http://www.wjgnet.com/2220-6124/full/v3/i3/50.htm DOI: http://dx.doi.org/10.5527/wjn.v3.i3.50

INTRODUCTION

Obesity is unquestionably one of the biggest health challenges the world population faces this century. Statistics indicate that more than 1.4 billion adults over 20 (35%) are overweight, with 11% falling into the obesity category according to World Health Organization statistics. Although the state of being obese and overweight has usually been associated with developed countries and high income, globalization and widespread unhealthy nutritional habits have caused these phenomena to reach epidemic proportions. As a consequence to the rise in obesity rates, comorbidities linked to this disease such as diabetes, car-

Table 1 Recent major multicenter studies regarding the impact of obesity and overweight on the incidence of kidney disease, renal function prognosis and patient survival

Cohort	Number of patients	Country	Result	Ref.
Dialysis patients	1957	Netherlands	Higher mortality with very high or low BMI (< 65 yr)	[157]
Kidney transplant	1810	Netherlands	Higher mortality and kidney graft failure	[158]
Native population	1924	Sweden	Higher Chronic Renal Failure	[13]
National Health and Nutrition Examination Survey Ⅲ	5659	United States	Higher micro-albuminuria with metabolic syndrome	[159]
Hipertension and obesity	4585	Spain	Higher risk of renal insufficiency	[160]
Native population	2585	United States	Higher risk of kidney disease	[12]
Native population	5403	Japan	Higher risk of proteinuria	[161]
Kidney transplant	51927	United States	Lower patient and graft survival. Higher chronic graft failure and delayed graft function	[162]

diovascular disease, and cancer have also increased[1].

Adipose tissue has a great impact on metabolic homeostasis and immunological function. The conjunction of the main obesity-related risk factors defines a clinical condition termed Metabolic Syndrome. This syndrome aggregates a variety of pathologies, including dyslipidemia, thrombosis, low-grade systemic inflammation, elevated blood pressure, hyperglycemia and insulin resistance. Adipose tissue possesses an important influence over the immune response profile *via* direct and indirect mechanisms through the secretion of nonesterified fatty acids, cytokines and endocrine mediators defined as adipokines. Together, these factors contribute to a systemic change in the way the body works, adapts and responds to challenges.

Although many studies have associated obesity with higher morbidity rates and obesity-related diseases[2], some groups argue the contrary. Overweight and obese patients reportedly display higher survival, while patients with low body mass are at a higher risk of general mortality and cardiovascular and many non-cardiovascular disease incidence, a phenomenon referred to as the "obesity paradox"[3,4]. These findings also highlight the complex relationship that obesity has with different pathologies and demonstrates that a closer look is needed to understand the particular effects of being obese and overweight on the organism.

OBESITY AND THE KIDNEY

Obesity affects the function of many organs. The heart is one of the main organs affected by metabolic syndrome, and obesity significantly increases the chances of cardiac dysfunction because of chronic hemodynamic burden, which causes dyspnea, edema, ongoing systemic inflammation, metabolic alterations and other related comorbidities[5]. Other organs such as the liver are also affected by this pathology, with lipid accumulation causing non-alcoholic fatty liver disease[6]. Lung function is also compromised by adipose tissue around the abdomen, rib cage and visceral cavity[7].

The kidney is also responsive to obesity. Several multicenter studies have identified a direct correlation between obesity and renal complications (Table 1). Obesity has a multifactorial mechanism and is considered an independent factor in chronic kidney disease (CKD) development and progression to end-stage renal disease (ESRD)[8]. Studies demonstrate that obesity-induced hypertension and diabetes are strong determinants of CKD. Analyses relating obesity and kidney transplantation revealed that in 1987, 11.6% of adults awaiting a kidney transplant were obese, and in 2001, obesity among adults rose to 25.1%[9]. Concomitantly, body mass index (BMI) among patients initiating dialysis increased from 25.7 kg/m^2 to 27.5 kg/m^2 from 1995 to 2002[10]; and when compared with normal weight persons (BMI, 18.5-24.9 kg/m^2), there is a directly proportional relationship between increased BMI and increased CKD and ESRD risk[11,12]. A study conducted by Ejerblad *et al*[13] examined the association between degrees of obesity and CKD. After making adjustments for many covariates, the investigators found a 2.8-fold increased risk of nephrosclerosis and a 7-fold increased risk of diabetic nephropathy among adults who had a BMI of 35 kg/m^2 or higher compared with a lifetime highest BMI lower than 25 kg/m^2. In adults with no diabetes or hypertension, a lifetime highest BMI of 35 kg/m^2 or higher was associated with a 2-fold increased risk of CKD. Conversely, obese patients had better recovery and benefitted from reduced body weight by diminishing proteinuria[14]. Obesity was recently demonstrated to accelerate IgA nephropathy progression[15]. In this scenario, obesity could be one of the few preventable risk factors for CKD development because it also mediates diabetes and hypertension, which are related to kidney disease progression[14,16,17].

The occurrence of obesity during early life is linked to low glomerular filtration rates (GFRs), while being overweight during adulthood doubles the chances of chronic kidney disease[18]. Many researchers have described the direct impacts obesity has on the kidneys, which include hyperfiltration, elevated glomerular tension, and podocyte stress[19]. Some researchers have also correlated obesity-related inflammation and adipokine deregulation to kidney function. The present review will focus on the impact of obesity on kidney function and discuss its influence on the progression of kidney disease.

Obesity-induced inflammation

Adipose tissue is known for its roles in lipid storage, ther-

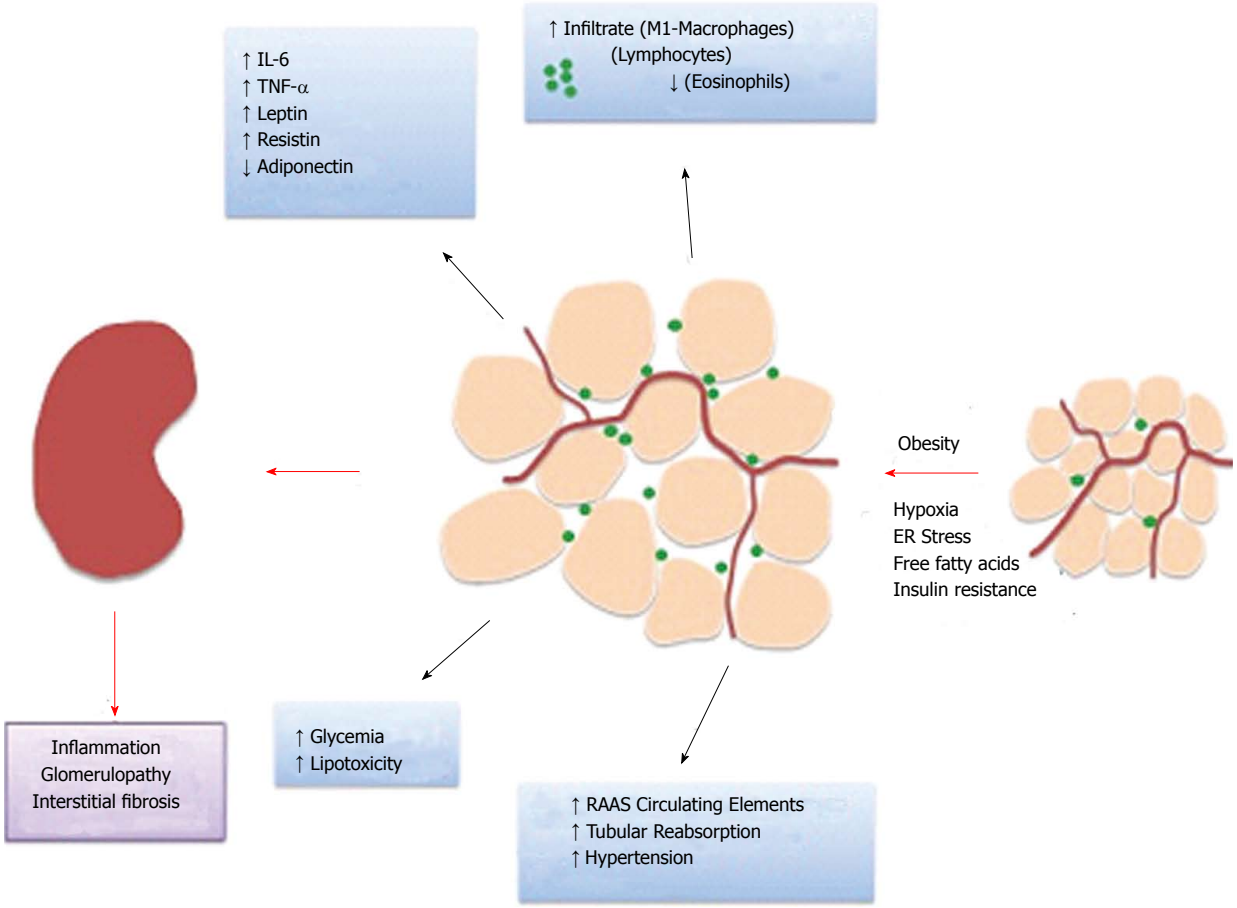

Figure 1 Main factors involved in obesity-induced inflammation, metabolic stress and hemodynamic disorder that participate in kidney function impairment. RAAS: Renin-angiotensin-aldosterone system; ER: Endoplasmic reticulum; TNF-α: Tumor necrosis factor alpha.

mogenesis and metabolic regulation. However, in recent years, focus has been given to its endocrine properties such as cytokine and adipokine secretion (Figure 1).

As previously described, obesity and diabetes are conditions that present a state of low-grade inflammation. Significant evidence supports the concept of adipose tissue as an immunomodulatory organ. Adipose tissue harbors a considerable amount of immune cells such as macrophages, lymphocytes and eosinophils. In obesity, the frequency of infiltrated cells rises, and they acquire a pro-inflammatory profile[20]. Excess free fatty acids that are present in obesity activate diverse inflammatory pathways involving endoplasmic reticulum stress[21], toll-like receptor[22,23], inflammasome and nuclear factor-κB (NF-κB) signaling activation[24,25]. In parallel, adipose tissue becomes hypoxic with adipocyte hypertrophy, which induces a change from aerobic to anaerobic glycolysis and lactate production.

With obesity, adipocyte hypertrophy and hypoxia induce cell death and resident immune cell activation, which in turn promotes inflammatory cell recruitment[26]. Macrophages constitute the principal population of resident and recruited cells in adipose tissue, which have a role in maintaining tissue homeostasis by assisting with the clearance of dead cells and debris. Because of lipid accumulation and adipocyte cell death, non-inflammatory tissue-resident M2 type macrophages and recruited monocytes undergo proliferation and macrophage M1 polarization[27-29]. These cells in turn secrete higher levels of inflammatory cytokines such as TNF-α, IL-6 and MCP-1 and lower levels of anti-inflammatory mediators such as arginase 1[28-30]. IL-4-expressing eosinophil counts also decrease with obesity, which contributes to inflammation[31]. Furthermore, $CD8^+$ and $CD4^+$ Th1 lymphocyte counts also increase while Treg numbers reduce with obesity. In accordance, B cell pro-inflammatory immunoglobulin G2c (IgG2c) production also participates in cell activation[32-35].

Proinflammatory cytokines are also produced by the renal parenchyma in response to hyperglycemia as well as vasoactive peptides such as angiotensin II and endothelin[36]. These molecules activate signaling second messengers such as protein kinase C, MAP kinase and NF-κB, which alter the gene expression of several cytokines and growth factors.

Increased TNF-α levels reduce the expression of nephrin and podocin, causing podocytopathy[37]. Similarly, IL-6 promotes adhesion molecule expression, which increases oxidative stress[38], and IL-6 receptor blockade can inhibit the progression of proteinuria, renal lipid deposition and mesangial cell proliferation[39]. An additional important growth factor for renal injury is transforming growth factor (TGF)-β, which induces podocyte apoptosis, extracellular matrix synthesis and mesangial cell pro-

liferation, thus exacerbating the development of the glomerular lesions associated with diabetes and obesity[40].

While many studies demonstrate the effect of metabolism on the immune system, studies have demonstrated that the reverse also happens; immune cell activation in adipose tissue is a determinant of obesity-linked metabolic changes such as insulin resistance[41]. For example, in response to inflammatory mediators, adipose tissue also down regulates glucose transporter GLUT4 expression, which increases insulin resistance.

Obesity and the adipokine imbalance

In addition to cytokines, adipose tissue is also responsible for the production of many endocrine mediators termed adipokines, which regulate inflammation, food consumption and link immune and metabolic functions. Amongst these are leptin, adiponectin, visfatin, resistin, intelectin and others. These factors are mostly secreted by adipocytes and have imbalanced expression in obesity. Many studies have documented the importance of these cytokines in the regulation of metabolism and inflammation and suggest a role for these cytokines in obesity-related metabolic and inflammatory distortion. Although there is still much to elucidate regarding the role of adipokines in kidney disease, recent studies now have begun to clarify the influence of these mediators in kidney pathology.

Leptin and kidney disease

Leptin was the first adipokine to be characterized and is the best described in the literature. It is secreted by different adipose compartments and induces signaling through Ob-a to Ob-f subtype receptor activation, and the Ob-Rb receptor is the most important. Its main actions are on the nervous system and kidneys. Leptin acts on the nervous system by stimulating neuropeptides that promote satiety and energy consumption. It has been suggested that one develops leptin resistance in obesity because of the absence of many of its effects despite elevated adipokine levels. Hyperleptinemia has also been associated with many cardiovascular and immunologic dysfunctions[42].

Many reports have linked obesity and leptin to hypertension. Studies indicate that this adipokine activates the sympathetic nervous system and may suppress parasympathetic nerve activity, which alters baroreflex control[43,44]. Leptin also increases renal sympathetic nerve activity, as demonstrated by studies on ObR deletion in the central nervous system[45]. Because the sympathetic nervous system contributes to CKD, leptin hypertensive actions may promote kidney disease.

Leptin also holds important pro-inflammatory activity. Its structure resembles other cytokines such as IL-2 and can stimulate many immune cells. Studies demonstrate that leptin induces the production of inflammatory cytokines as IL-6 and TNF-α by monocytes and additionally induces chemokine ligands, reactive oxygen species production and macrophage and monocyte proliferation[42]. Leptin also polarizes $CD4^+$ lymphocytes toward a Th2 profile, which increases the production of inflammatory cytokines such as IL-2 and IFN-γ[42]. Therefore, excess leptin, which is characteristic during obesity, is an important mediator of obesity-related immune and metabolic dysfunction.

Recent studies have also suggested that leptin imposes an important action in the kidneys, as this mediator localizes mainly to the organ after injection[46]. CKD patients demonstrate high leptin levels, as do ESRD patients, and hemodialysis fails to lower these values[47,48]. The kidneys also express the Ob-Ra and Ob-Rb leptin receptor isoforms[49]. In vitro, leptin induces glomerular endothelial cell proliferation, which is augmented in the presence of angiotensin Ⅱ and increases TGF-β1 production. Furthermore, leptin infusion into rats *in vivo* also induced proteinuria, glomerular endothelial cell proliferation and TGF-β1 production and increased collagen type Ⅳ expression[50]. This adipokine also induced type I collagen in mesangial cells, confirming data that link obesity, glomerulosclerosis and glomerulomegaly, which is defined as obesity-related glomerulopathy[51,52].

Adiponectin and kidney disease

Adiponectin is another adipokine with immunomodulatory and metabolic actions. It is present in plasma at a considerable concentration[53], and its receptors R1, R2 and T cadherin are expressed by a wide range of tissues. Adiponectin is negatively correlated with hypertension[54]. It exerts its metabolic actions by increasing glucose uptake and fatty acid oxidation and inhibiting gluconeogenesis. In addition to improving insulin sensitivity, it also possesses potent anti-inflammatory properties[42].

Unlike leptin, low serum adiponectin levels are found in obese patients, and its production is reduced by hypoxia, inflammatory mediators such as IL-6 and oxidative stress[55-57]. Hypoadiponectinemia has been linked to diverse complications in obesity. Mice lacking adiponectin display increased susceptibility to high-fat diet-induced insulin resistance[58]. Moreover, adiponectin overexpression in high-fat diet-fed animals caused less fat accumulation and reduced adipose tissue macrophage infiltration, and it prevented premature death[59].

Recent studies have begun to elucidate the role of adiponectin in kidney injury. Current data demonstrate that adiponectin is secreted not only by adipocytes but also renal tubular cells[60]. Research indicates that plasma adiponectin is inversely correlated with albuminuria in obese patients[61]. Adiponectin-null mice also develop albuminuria and podocyte damage as well as glomerular oxidative stress[62]. These mice also display more expressive albuminuria, fibrosis and macrophage infiltration after 5/6 nephrectomy[63]. Moreover, mice overexpressing adiponectin recover more rapidly and exhibit less interstitial fibrosis after podocyte-specific damage[64]. Metabolic syndrome has also been associated with low adiponectin levels and worse prognosis after kidney transplantation[65]. These data are controversial, however, as some studies describe a direct link between adiponectin levels

and mortality in advanced CKI and kidney transplant patients[66,67]. While recent work suggests that adiponectin causes less intense ischemia-reperfusion kidney injury[68], the contrary was observed when exogenous adiponectin was administered[69]. Furthermore, kidney function also influences adiponectin levels because the kidneys are responsible for its elimination, and kidney transplantation significantly reduces the adiponectin concentration[70].

Resistin and visfatin

Resistin is a recently discovered adipokine with inflammatory properties. Some works suggest that this mediator increases insulin resistance, while others fail to find this correlation[71,72]. Although in mice, it is expressed mainly by adipocytes, in humans it is produced principally by macrophages and monocytes. Although there are still few data on its impact on renal function, some research indicates that serum resistin levels are strongly associated with decreased GFRs and inflammatory biomarkers in CKD[71].

Adipose tissue and the kidneys also synthesize visfatin, and this is upregulated in type-2 diabetic rats, inducing fibrosis and inflammatory pathway activation[73]. In CKD patients, higher visfatin levels also are correlated with decreased GFR and endothelial dysfunction[74,75]. Furthermore, another study with human plasma determined that this mediator was also linked to creatinine levels, inflammation and endothelial damage in kidney recipients, which is negatively related to plasma albumin levels[19].

OBESITY AND RAAS IN KIDNEY DISEASE

The pathophysiologic mechanism surrounding CKD development in obese patients remains unclear, but many events must be linked to ESRD such as altered renal hemodynamics, insulin resistance, hyperlipidemia, inflammation and oxidative stress (Figure 1). Hemodynamic alterations such as higher renal plasma flow, GFR and filtration fraction were linked to obesity when compared with the levels in non-obese patients[76,77]. The effect of BMI on renal hemodynamics was also proven by another work in which GFR and effective renal plasma flow (ERPF) were evaluated with a high-sodium diet. According to this study, ERPF and the GFR were statistically increased when individuals were exposed to a high-sodium diet and compared to another group that was exposed to a low-sodium diet without a change in filtration fraction (FF). However, increased sodium intake-induced changes in the GFR and FF were significantly greater in people with a BMI $\geqslant$ 25 kg/m^2[78]. The hemodynamic effects of overweight on kidney function and albuminuria are enhanced with hypertension, which itself is a clinical complication of obesity. Chagnac *et al*[79] demonstrated that glomerular hyperfiltration could have a relevant role in development of hypertension in obese patients by increasing postglomerular oncotic pressure and proximal tubular sodium reabsorption.

As an individual gains weight, renal mass as well as the glomerular diameter increases[80]. Podocytes are highly specialized cells that support the glomerular basement membrane (GBM) and play an important role in the glomerular filtration barrier *via* their foot processes. With glomerular hypertrophy, podocytes must cover a larger area by expanding these processes. If this podocyte enlargement is not proportional to glomerular hypertrophy, this adaptation could cause podocyte detachment and consequently a loss selectivity of serum protein selectivity[81,82]. Considering that podocytes are cells with limited capacity for cell division and replacement, proteinuria may be detected as is commonly observed in obese patients. Supporting this hypothesis, individuals who reduced their body mass also had significant reductions in proteinuria[14,83].

Extensive studies demonstrate that a lack of podocytes covering the GBM results in the formation of denuded areas, which trigger matrix deposition resulting in glomerulosclerosis in experimental models as well as in human biopsies[84-87]. As kidney injury persists, kidney fibrosis becomes an inevitable outcome in which epithelial-mesenchymal and endothelial-mesenchymal transition events generate matrix-producing fibroblasts in the interstitial space that contribute to renal fibrosis. Accumulation of matrix elements caused by the fibrotic process progressively alters normal kidney architecture by contraction and increased stiffness, resulting in disrupted blood flow supply and nephron function[88,89].

Once a number of podocytes are injured, a vicious cycle starts in which other podocytes also become damaged, accelerating podocyte deterioration and glomerulosclerosis[90]. The extensive loss of glomeruli imposes excessive stress on the remaining glomeruli because of hemodynamic alterations and glomerular hypertrophy, which can subsequently cause further sclerosis of the remaining glomeruli[91]. This could explain the progressive spreading of glomerular damage in later disease stages in which patients develop chronic renal failure[90]. The approach of using new agents to avoid podocyte lesions in different models of acute and chronic kidney disease resulted in less matrix deposition and consequent glomerulosclerosis[92,93].

In obesity, the renin-angiotensin-aldosterone system (RAAS) is commonly activated and is one of the strongest links to renal injury. All of the major components necessary to generate angiotensin Ⅱ (Ang Ⅱ) are found in the kidney[94]. The RAAS is a well-known mechanism to regulate blood pressure, fluids and electrolyte balance[95], and its activation impairs normal pressure natriuresis, increases renal tubular sodium reabsorption, and causes volume expansion. Physical compression of kidneys by visceral adipose tissue in obesity exacerbates these responses and increases blood pressure, leading to hypertension in obese subjects.

RAAS effects are obtained when angiotensinogen (AGT), the precursor of bioactive angiotensin peptides, is cleaved by both renin and angiotensin converting enzyme (ACE) to generate Ang Ⅱ. Ang Ⅱ, which is the active peptide and is the main effector of RAAS, pos-

sesses a dual role in physiology. Ang Ⅱ helps maintain long-term blood pressure and blood volume in the body; conversely, it has also been considered a multifunctional cytokine that plays a role in cell proliferation, hypertrophy, superoxide production, inflammation and extracellular matrix deposition[96]. Ang Ⅱ plays an endocrine role, and its participation in the development of obesity was evidenced by several works in which AGT, ang Ⅱ and ang Ⅱ receptor-deficient mice were protected against high-fat diet-induced obesity[97-99].

There are several pathophysiological conditions, including hypertensive models, in which Ang Ⅱ, in response to increased arterial blood pressure, increases efferent glomerular arteriole resistance and induces TGF-β production[100]. It also impairs the auto-regulation of afferent arterioles by avoiding vasoconstriction[101]. Taken together, Ang Ⅱ directly and indirectly enhances capillary filtration pressure and promotes proteinuria, which is one of the most important factors involved in renal disease progression. Moreover, Ang Ⅱ is also involved in nephrin dephosphorylation during podocyte apoptosis[102], which is a protein that is part of the slit diaphragm and binds to the adjacent nephrins of other podocytes. Ang II decreases the synthesis of negatively charged proteoglycans that are present on the glomerular basement membrane, which impairs the filtration of high molecular weight proteins by electrostatic repulsion[103].

Human adipose tissue expresses all of the RAAS components, including angiotensin, ACE, renin and the AT1 and AT2 receptors. Consequently, the AGT produced by adipose tissue contributes significantly to circulating AGT levels. In humans and mice, a strong relationship has been observed between increased AGT gene expression and obesity[104], supporting a role for adipose AGT in hypertensive obese patients. Weight reduction reduced blood pressure through systemic RAAS suppression and decreased AGT, renin and aldosterone levels in adipose tissue and plasma[105]. Mice with adipose tissue-restricted AGT expression were normotensive, whereas when adipose AGT was overexpressed, the mice became hypertensive[106]. Ang Ⅱ is also involved in adipocyte metabolism by influencing leptin and adiponectin release. Once leptin levels are increased, Ang Ⅱ promotes a number of cellular processes that attenuate leptin signaling and contribute to leptin resistance, which is common in obesity[107]. Conversely, adiponectin was upregulated when RAS was blocked by an ACE inhibitor or Ang II receptor blocker, suggesting Ang Ⅱ participation in the inhibition of adiponectin release[108].

Not only AGT but also aldosterone levels are increased in obese patients. Aldosterone is a mineralocorticoid hormone that is produced in the adrenal glands in response to Ang Ⅱ and a high extracellular potassium concentration, which increases blood pressure *via* sodium retention in the collecting duct. Aldosterone is correlated with increased blood pressure[109] and can also be produced by adipocytes through pathways that are dependent on the Ang Ⅱ-ATI receptor axis and calcineurin signaling[110] as well as pathways that are independent of Ang Ⅱ, in which adipocytes secrete factors that may stimulate the adrenal gland and increase circulating aldosterone levels, resulting in mineralocorticoid receptor activation and increasing blood pressure and hypertension[111]. Aldosterone binds to cytosolic mineralocorticoid receptors and promotes cell signaling pathways, endothelial dysfunction, inflammation and fibrosis independently and in concert with Ang Ⅱ[112]. Moreover, Ang Ⅱ activates the mineralocorticoid receptor in the absence of aldosterone and promotes kidney injury[113,114]. Blocking the mineralocorticoid receptors with antagonists attenuates obesity-induced hypertension and glomerular hyperfiltration[115].

Many clinical trials have been performed to mitigate the effects caused by RAAS. Multiple pharmacological strategies are used to treat CKD patients to diminish proteinuria and blood pressure. These strategies comprehend the use of RAAS-blocking agents alone or combined with ACE inhibitors, angiotensin-receptor blockers, direct renin inhibitors and mineralocorticoid-receptor antagonists[116]. The combination of a pharmacological therapy with reduced sodium intake was a better choice to diminish blood pressure and proteinuria than combined therapies[117]. Attempts to antagonize aldosterone receptors demonstrated promising results to diminish glomerulosclerosis[118].

In summary, the obesity-RAAS-hypertension axis is closely related to renal disease, as the increased release of adipose tissue derived-RAAS elements into the circulation can alter hemodynamic homeostasis. Increased Ang Ⅱ, AGT and aldosterone levels promote increased tubular reabsorption, leading to arterial hypertension and renal vasodilation. These events contribute to glomerular hypertension, which is an important factor in glomerulosclerosis and CKD progression.

OBESITY AND DIABETES IN RENAL DISEASE

Obesity is an important risk factor for hypertension and type 2 diabetes development, which are the leading causes of end-stage renal disease. The relationship between obesity, diabetes and kidney disease is very close because obesity and diabetes alter renal function, leading to renal disease. These renal alterations in both cases include anatomical, physiological and pathological changes (Figure 1).

Physiological and hemodynamic alterations are largely responsible for the subsequent anatomical and histopathological modifications. Among the major hemodynamic changes in obese and/or diabetic patients are increased GFR and intraglomerular capillary pressure[119,120]. Such alterations lead to diabetic nephropathy, increases in kidney weight and size, increased glomerular size, podocyte hypertrophy and mesangial matrix expansion[121].

Diabetes-related renal injuries can be grouped into

Table 2 Summary of the most important changes in the kidney during diabetes

Stages	Features
1 and 2	Hyperfiltration and renal hypertrophy
3	Microalbuminuria and hypertension as clinical features. As histological features: arteriolar hyalinosis, glomerular basement membrane thickening and mesangial matrix expansion
4 (Diabetic Nephropathy)	Proteinuria, nephrotic syndrome and decreased GFR
5	End-stage renal disease

Adapted from Amann *et al*[150]. GFR: Glomerular filtration rate.

five stages that comprise the remodeling that occurs throughout diabetic nephropathy. These stages are summarized in Table 2.

Although obesity and diabetes *per se* are responsible for renal injury, some other factors usually present in these conditions significantly aggravate renal damage such as blood pressure, hyperlipidemia, hyperglycemia, genetic factors[122] and inflammation. Some of these conditions are described in the following sections.

Hypertension in diabetes-related kidney disease

Hypertension and diabetes are two important risk factors in the development of kidney diseases, and when they are present simultaneously, they aggravate renal injury.

Hypertension-induced kidney damage in obesity and diabetes follows a very similar sequence of events including increased renal tubular sodium reabsorption as well as RAAS and sympathetic nervous system activation[123-125]. Such an increase in blood pressure along with increased glomerular capillary pressure and GFR are main contributors to the initial renal damage in obesity and diabetic nephropathy[126].

Given the importance of hypertension in worsening renal injury, especially in diabetic nephropathy, many studies have been performed to demonstrate the importance of controlling blood pressure when treating diabetic nephropathy, and the recommended blood pressure is less than 130/80 mmHg[127]. Several clinical trials have also been developed and have demonstrated renal protection when low blood pressure is achieved[128].

Renin-angiotensin system blockade is an important treatment for controlling blood pressure and decreasing proteinuric kidney disease progression[129-131]. Angiotensin Ⅱ-receptor blocker (ARB) therapy helps prevent the progression from normoalbuminuric (Albumin-Creatinine Ratio < 30 mg/g creatinine) to albuminuric stages (ACR 30-100 mg/g creatinine)[132]. Another important strategy is combined therapy with ARB and ACE inhibitors, which demonstrates a greater decrease in proteinuria than monotherapy[133].

Hyperlipidemia in obesity and diabetes

Dyslipidemia is an important component of metabolic syndrome and is often directly related to obesity and diabetes. Patients with diabetic nephropathy usually have several changes in their lipid profile[134], and the presence of increased blood lipid levels is a risk factor for albuminuria[135]. Several studies have demonstrated a correlation between triglyceride and cholesterol levels with renal function markers. Ravid and colleagues[136] observed a significant and positive correlation between total cholesterol and albuminuria in type 2 diabetic patients in a five-year cohort. Similarly, Klein *et al*[137] noted that type 1 diabetic patients with elevated total cholesterol and low HDL levels also had higher incidence of renal failure.

Although these studies demonstrate significant correlations between dyslipidemia and impaired renal function in diabetic subjects, little is known about the mechanisms by which the increased lipid profile causes kidney damage. Studies have demonstrated lipid deposits in the glomeruli and in the mesangium of obese individuals, suggesting that these lipids may cause kidney damage and lipotoxicity[138]. This glomerular lipotoxicity would be because of renal sterol-regulatory element-binding protein (SREBP-1 and 2) expression, whereas lipotoxicity causes tubulointerstitial fibrosis and inflammation in the proximal tubule epithelial cells[139]. Furthermore, alterations in the coagulation-fibrinolytic system, increased atherosclerosis and endothelial cell damage can also cause or aggravate diabetic nephropathy[140].

Hence, the importance of lipid control in the maintenance of kidney function in diabetic patients has been postulated[141].

Hyperglycemia in diabetes-related renal injury

Vascular alterations in diabetes are largely due to increased blood glucose levels. Hyperglycemia promotes microvascular injury by several mechanisms. The most important mechanisms are as follows: increased intracellular advanced glycated end product (AGE) formation; interaction between AGEs and their receptors, with consequent disruption of cell signaling and function; constant protein kinase C activation[142]; and increased hexosamine pathway activity[143]. Renal endothelial and mesangial cells are susceptible to such hyperglycemia-induced changes[144]. Thus, the hyperglycemia-induced alterations that occur in the kidney are similar to those described above but generate characteristic damage to renal cells. Because of AGE-driven structural changes in extracellular matrix proteins, metalloproteinases lose their ability to degrade the matrix efficiently, which causes basement membrane thickening[145]. In the mesangium, AGE-induced changes include increased pericyte apoptosis and increased vascular endothelial growth factor expression, and these changes in turn cause glomerular hyperfiltration[146].

Because hyperglycemia causes severe damage to the kidneys and other organs, several studies were developed to demonstrate the importance of glycemic control to prevent diabetic nephropathy. One of these clinical trials, the Diabetes Control and Complications Trial, compared conventional and intensive insulin therapy in type 1 diabetic patients. Over approximately 6.5 years, decreased risks for microalbuminuria and overt nephropathy were

observed with intensive glycemic control[147,148]. The Action in Diabetes and Vascular disease: Preterax and Diamicron-MR Controlled Evaluation clinical trial, which was based on type 2 diabetic patients, also observed a reduction in albuminuria and nephropathy progression with insulin therapy intensification in late disease[149].

TREATMENTS AND PHARMACOLOGICAL INTERVENTION

Most treatments and approaches to reduce kidney injury in obese patients focus on managing associated risk factors such as hypertension, diabetes and hyperlipidemia using strategies as nutritional counseling, pharmacological interference and in some cases, surgery.

Dietary treatment consists on the change in nutritional habits and lifestyle. Eating smaller portions, increasing water consumption, minimizing salt ingestion and practicing physical activities are essential for weight reduction. Such practices can prevent and treat obesity which in turn reduces the risk of CKD. However this is a measure that brings long-term results. Treatment of patients with severe obesity focuses on reduction of proteinuria levels. Currently, several studies point out to the combined therapy of RAS inhibitors (ACE inhibitors and Ang Ⅱ receptor antagonists); low calories and low salt diets as presumably the best therapeutic options for obese patients with high levels of proteinuria[117].

Weight loss is also an important factor in this treatment regimen. Surgical intervention to treat obesity is a strategic option that can diminish levels of proteinuria in obese patients by mainly reducing hyperfiltration, attenuating obesity-mediated dyslipidemia and insulin resistance, reducing blood pressure and altering adipokine levels such as leptin and adiponectin which have direct a effect on podocytes, therefore improving kidney function[14,151,152]. Even modest weight loss has been associated with a substantial reduction in blood pressure and risk of diabetes[153]. The benefits of bariatric surgery are attributed to sympathetic nervous system suppression, decreasing therefore overall renal sympathetic activity and reduction on sodium reabsorption[154].

Once patients begin to lose weight, longer-term maintenance is difficult and even with continued treatment, patients may regain their normal condition. To prevent this, there is a need for adjunctive therapies for patients who are not able to lose weight or sustain weight loss solely with lifestyle changes[155]. In this scenario, the introduction of pharmacological treatment by the use of, for instance, noradrenergic agents, gastrointestinal lipase inhibitors and serotonin receptor agonists become an alternative and efficient strategy towards weight loss[156].

CONCLUSION

Obesity has great influence on end-stage renal disease, and it can be either the cause of renal alterations and kidney injury or an aggravating factor when other conditions such as hypertension and diabetes are established. All of these factors represent severe insults to the kidney, resulting in high costs to health systems to manage dialysis patients as well as those with post- cardiovascular events. Therefore, studies that relate these factors are important for developing new strategies to treat obese patients with renal disease to reduce patient mortality and improve quality of life.

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P- Reviewer: Aramwit P, Landry DL **S- Editor**: Song XX
L- Editor: A **E- Editor**: Wu HL

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DOI: 10.5527/wjn.v3.i3.64

World J Nephrol 2014 August 6; 3(3): 64-76
ISSN 2220-6124 (online)

REVIEW

Renin-angiotensin system in the kidney: What is new?

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Author contributions: Ferrão FM, Lara LS and Lowe J all contributed to this paper, filling all the three conditions established by Baishideng Publishing Group.
Supported by Carlos Chagas Filho Rio de Janeiro State Research Foundation (FAPERJ), National Institute of Science and Technology for Structural Biology and Bioimaging, Brazilian National Research Council (CNPq)
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Received: May 26, 2014 Revised: July 7, 2014
Accepted: July 25, 2014
Published online: August 6, 2014

Abstract

The renin-angiotensin system (RAS) has been known for more than a century as a cascade that regulates body fluid balance and blood pressure. Angiotensin Ⅱ (Ang Ⅱ) has many functions in different tissues; however it is on the kidney that this peptide exerts its main functions. New enzymes, alternative routes for Ang Ⅱ formation or even active Ang Ⅱ-derived peptides have now been described acting on Ang Ⅱ AT_1 or AT_2 receptors, or in receptors which have recently been cloned, such as Mas and AT_4. Another interesting observation was that old members of the RAS, such as angiotensin converting enzyme (ACE), renin and prorenin, well known by its enzymatic activity, can also activate intracellular signaling pathways, acting as an outside-in signal transduction molecule or on the renin/(Pro)renin receptor. Moreover, the endocrine RAS, now is also known to have paracrine, autocrine and intracrine action on different tissues, expressing necessary components for local Ang Ⅱ formation. This *in situ* formation, especially in the kidney, increases Ang Ⅱ levels to regulate blood pressure and renal functions. These discoveries, such as the ACE2/Ang-(1-7)/Mas axis and its antangonistic effect rather than classical deleterious Ang Ⅱ effects, improves the development of new drugs for treating hypertension and cardiovascular diseases.

Key words: Renin-angiotensin system; Angiotensin Ⅱ; Kidney; Hypertension treatment; Receptor

Core tip: Activation of the angiotensin converting enzyme (ACE)/ Angiotensin Ⅱ (Ang Ⅱ)/AT_1 axis leads to vasoconstriction, anti-diuresis, anti-natriuresis, release of aldosterone and anti-diuretic hormone, which can result in hypertension, renal and cardiovascular diseases. Inhibition of renin and ACE or blocking AT_1 receptor is the most used therapies for heart failure and hypertension. Nevertheless, the discovery of local Ang Ⅱ synthesis, new Ang Ⅱ metabolites, receptors and axis of this system, makes possible the development of new drugs and strategies for renal and cardiovascular diseases treatment, such as activation of ACE2/Ang-(1-7)/Mas axis, which presents opposite effects of AT_1 activation by Ang Ⅱ.

Ferrão FM, Lara LS, Lowe J. Renin-angiotensin system in the kidney: What is new? *World J Nephrol* 2014; 3(3): 64-76 Available from: URL: http://www.wjgnet.com/2220-6124/full/v3/i3/64.htm DOI: http://dx.doi.org/10.5527/wjn.v3.i3.64

RAS IS NOT ONLY AN ENDOCRINE SYSTEM

The first observation that the arterial blood pressure could be regulated was in 1898 after the discovery of a soluble protein extracted from the kidney that increased

Table 1 Comparison of the components from classic and recent renin-angiotensin system

	Classic RAS	Recent RAS
Hormone process	Endocrine	Paracrine Autocrine Intracrine
Bioactive peptide	Ang Ⅱ	Ang Ⅱ Ang III Ang IV Ang-(1-7) Ang-(3-4) Ang A Alamandine
Receptor	AT_1 AT_2	AT_{1a} AT_{1b} AT_2 Mas Mrg AT4 PRR ACE

Ang: Angiotensin; ACE: Angiotensin converting enzyme; AT_1: Angiotensin type 1 receptor; AT_2: Angiotensin type 2 receptor; AT_4: Angiotensin type 4 receptor; Mas: Ang-(1-7) Mas receptor; Mrg: Mas-related G-protein-coupled receptor; PRR: Renin/(Pro)renin receptor; RAS: Renin-angiotensin system.

blood pressure in rabbits, called "renin"[1]. Over 30 years later, Goldblatt *et al*[2] associated the decrease of blood pressure in kidneys with hypertension by using a silver clamp to partially constrict dogs renal arteries, resulting in reno-vascular hypertension. Using the same methodology as Goldblatt, Braun-Menendez *et al*[3] in 1940 isolated a vasoconstrictor substance responsible for the reno-vascular hypertension from renal venous blood of the hypertensive kidney dog, calling it "hypertensin". Page *et al*[4] independently described a vasoconstrictor substance by injecting renin into cats, called "angiotonin". The same group also described angiotensinogen, first referred to as a "renin activator"[4]. The name "angiotensin" for the vasoconstrictor substance "hypertensin" by Braun-Menendez and "angiotonin" by Page emerged in 1958 after they both agreed on this hybrid name, since these 2 substances proved to be the same potent vasoactive octapeptide. The World Health Organization and the American Heart Association in 1987 suggested the abbreviation Ang for angiotensin, numbering the amino acids residues of angiotensin I (Ang I) as a reference for all angiotensin-derived peptides[5]. The decapeptide Ang I has no physiological effect, but is hydrolyzed by angiotensin converting enzyme (ACE) generating angiotensin Ⅱ (Ang Ⅱ), which was considered the only peptide in renin-angiotensin system (RAS) with biological actions[6].

More than a century since the discovery of renin by Robert Tigerstedt and Bergman[1], the RAS, remains a fascinating subject for research. Although it is well known the distinct roles of RAS in different tissues, such as brain, adipose tissue, gastrointestinal tract and cardiovascular system[7-10], it is on the kidney that Ang Ⅱ has its main function on regulating body fluid content and blood pressure by alterating Na^+ and water homeostasis, intrarenal hemodynamics and glomerular filtration[11,12]. Ang Ⅱ stimulates anti-diuretic hormone secretion in the pituitary gland with increased water reabsorption in the collecting duct, and also increases aldosterone secretion, a steroid hormone synthesized mainly by the adrenal cortex, and a downstream effector of Ang Ⅱ that induces sodium reabsorption and concomitant potassium and hydrogen ion excretion by the kidney[13].

Many new findings suggest new properties of this system, with new enzymes, different routes for Ang Ⅱ formation, new receptors and active Ang Ⅱ-derived peptides (Table 1). The classical axis ACE/Ang Ⅱ/AT_1 is not the only signaling pathway within RAS, since others such as angiotensin converting enzyme 2 ACE2/Ang-(1-7)/Mas receptor and Angiotensin IV/AT_4 indicate new activities for this cascade[14,15]. Besides the inhibition of renin and ACE, and also angiotensin type 1 receptor (AT_1) blockade, activation of the ACE2/Ang-(1-7)/Mas axis is a possible alternative target for new drugs, since some protective effects on renal and cardiovascular function have been reported[14,16-18]. Ang Ⅱ is not the only active peptide of the RAS, there now being physiological properties associated with many Ang Ⅱ-derived peptides[14,15,19]. Ang Ⅱ can be hydrolyzed by > 13 "angiotensinases", proteolytic enzymes such as aminopeptidases, carboxipeptidases, endopeptidases, ACE2 and neprilysin, generating angiotensin Ⅲ (Ang Ⅲ), angiotensin Ⅳ (Ang Ⅳ), angiotensin-(1-7) [Ang-(1-7)], angiotensin-(3-4) [Ang-(3-4)], angiotensin A (Ang A), and alamandine, which can bind to specific receptors or act on the same receptors as Ang Ⅱ[14,15,19-22]. Although AT_1 and AT_2 receptors are the most studied receptors for Ang Ⅱ, two other receptors - Mas receptor for Ang-(1-7), and AT_4 receptor for Ang Ⅳ - have been cloned[14,15]. Ang Ⅱ-derived peptides could have similar effects to Ang Ⅱ, or counteract its effects on renal function. For instance, like Ang Ⅱ, Ang-(1-7) can increase intracellular Ca^{2+} *via* AT_1 receptor, but has the opposite effect to Ang Ⅱ, since it can induce antiproliferative and protective effect through the Mas receptor[23,24]. New functions for well known members of the RAS have been found. For example, ACE, known for its catalytic action on Ang I, also functions as a signal transduction molecule, initiating a series of intracellular events when stimulated[25,26]. Besides increasing catalytic activity of renin and prorenin, the renin/(Pro)renin receptor (PRR), cloned in 2002[27], can also induce an intracellular signaling pathway generating effects in an angiotensin-independent manner[6,27].

It is now considered that RAS assumes paracrine, autocrine and intracrine mechanisms of action in hormone signaling[6,28]. Many tissues and cells, including kidneys, have all the necessary RAS components to form Ang Ⅱ *in situ*[29-31]. Renal levels of Ang Ⅱ are much higher than in the plasma[32], indicating that the source of Ang Ⅱ within the kidney is not only provided by filtered plasma Ang Ⅱ. The kidney expresses all the major components of the RAS, such as angiotensinogen, renin and

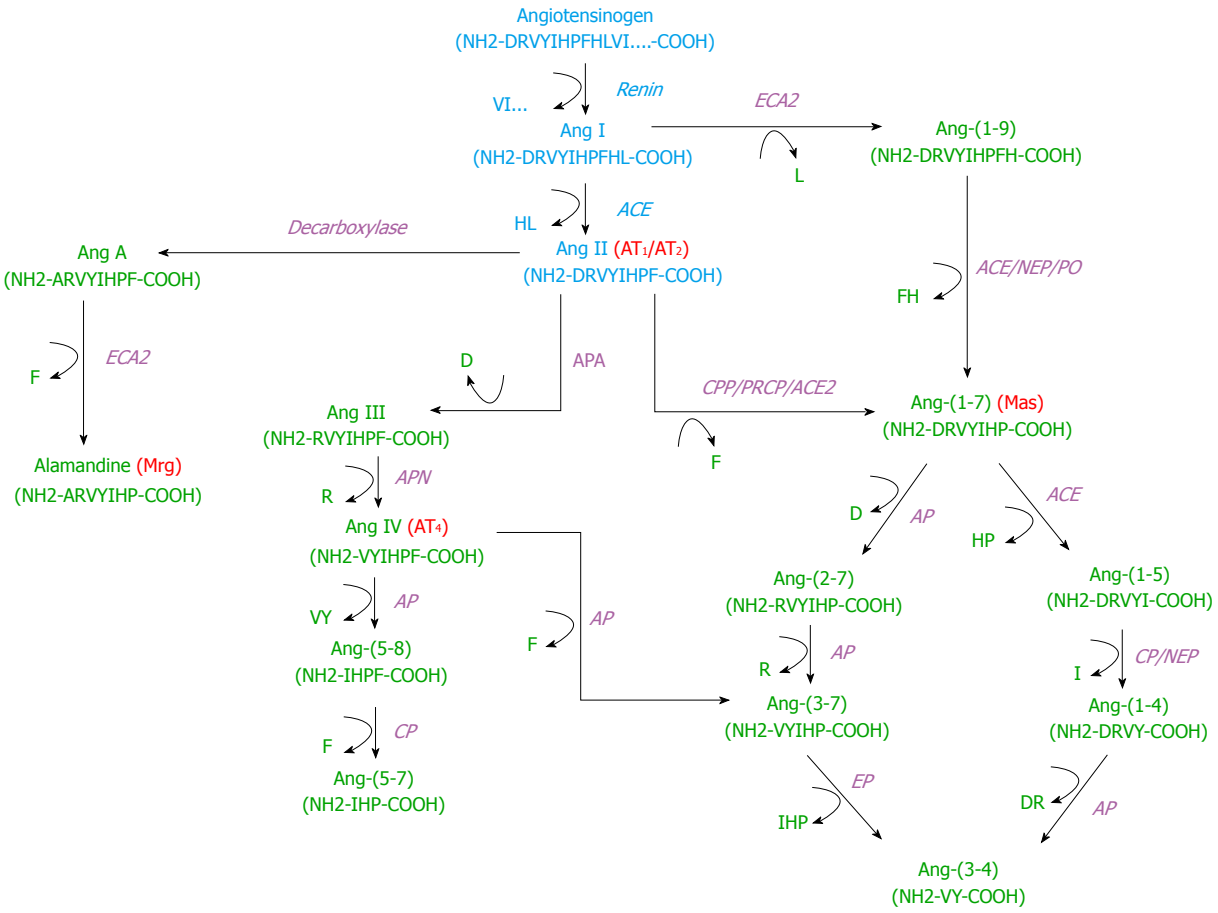

Figure 1 Classic view of renin-angiotensin system cascade (blue) and recent view of renin-angiotensin system cascade (green). AP: Aminopeptidase; APA: Aminopeptidase A; APN: Aminopeptidase N; CP: Carboxypeptidase; EP: Endopeptidase; ACE: Angiotensin converting enzyme; ACE2: Angiotensin converting enzyme 2; CPP: Carboxypeptidase P; PRCP: Prolyl carboxypeptidase; NEP: Neprilysin; PO: Prolyl oligopeptidase; Mas: Ang-(1-7) Mas receptor. Adaptated from Axelband *et al*[20] with permission.

ACE[29-31]. Locally synthesized Ang Ⅱ can act on cell surface, nuclear and cytoplasmic AT_1 and AT_2 receptors[33-35].

We will describe a novel view of the classic RAS that includes new members, routes, receptors, and new drugs and targets for the treatment of heart failure and hypertension. Due to the high Ang Ⅱ concentration in different compartments of the kidney, and the importance of Ang Ⅱ effects on renal function in physiological and physiopathological conditions, the focus will be on the intrarenal RAS, especially its paracrine and intracrine functions. This new aspect of RAS will improve our present understanding of RAS and the role of its new members, which should benefit the development of new treatments for hypertension and kidney diseases.

NEW MEMBERS OF RAS: ANG Ⅱ-DERIVED PEPTIDES

Classically, renin is secreted by juxtaglomerular cells in response to 3 stimuli: (1) decreased arterial blood pressure, detected by baroreceptors; (2) decreased sodium levels in the macula densa ultrafiltrate; and (3) increased sympathetic nervous system activity. Renin is an enzyme with only one known substrate, angiotensinogen. The reaction catalyzed by renin, generating the decapeptide Ang Ⅰ, is the rate-limiting step in Ang Ⅱ formation. Ang Ⅰ is then converted to Ang Ⅱ by ACE, a monomeric glycoprotein that acts as an exopeptidase to cleave dipeptides from the C-terminus of Ang Ⅰ -(1–10) (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu) into the octapeptide Ang Ⅱ-(1-8)[36] (Figure 1). The main Ang Ⅱ effects are mediated by the AT_1 receptor, such as vasoconstriction, anti-diuresis, anti-natriuresis, release of aldosterone and anti-diuretic hormone, whereas AT_2 activation counterbalances these effects[19,36,37].

It is widely accepted that small peptides derived from Ang Ⅱ have local physiological effects, especially in the kidney (Figure 1). ACE2 is a transmembrane glycoprotein that shares a 42% of homology with ACE and contains a single active site domain more closely to the N domain of ACE[16,38]. Unlike ACE, ACE2 is a monocarboxypeptidase, generating Ang-(1-7) by the cleavage of a single Phe residue from Ang Ⅱ, and Ang-(1-9), removing the C-terminal Leu residue from Ang Ⅰ [16,38]. Within the renal brush-border vesicles of the rat, Ang-(1-7) is preferentially hydrolyzed by aminopeptidases and neprilysin (NEP) after aminopeptidase blockade, generating Ang-(1-4)[39]. In the basolateral membrane, brush-border vesicles of the pig and purified preparations of

renal NEP Ang I is hydrolyzed primarily to Ang-(1-7) and Ang-(1-4)[40,41]. In sheep proximal tubules, urine and serum, Ang Ⅱ is converted to Ang-(1-7) by both membrane-bound and soluble forms of ACE2[38].

The physiological importance of Ang-(1-7) has become increasingly evident, especially after Santos *et al*[14] found a G protein-coupled receptor for Ang-(1-7), the Mas receptor, using a selective Ang-(1-7) antagonist. The Mas protooncogene was cloned and sequenced in 1986, after being detected by its tumorigenicity in mice[42]. This gene encodes a protein with 7 hydrophobic transmembrane domains, first considered as an "orphan" G protein-coupled receptor[43]. Ang-(1-7) exerts many effects on renal function, such as diuresis and natriureses, and it can be detected in human urine[44]. This peptide is of importance during late gestation in rats, where RAS overactivity is associated with increased kidney and urine levels of Ang-(1-7) and enhanced kidney immunostaining of Ang-(1-7) and ACE2[45].

Diuretic/natriuretic effects of Ang-(1-7) may also be due to the regulation of Na^+ reabsorption within the proximal tubule. *In vivo* and *in vitro* studies showed that Ang-(1-7) is a potent inhibitor of Na^+ reabsorption in this nephron segment, acting on different receptors[46-49]. Ang-(1-7) can bind to distinct receptors and induces different cellular responses depending on the cell type. For instance, in distal tubule cell (MDCK), Ang-(1-7) inhibits ($Na^+ + K^+$)-ATPase activity through the AT_1 receptor to stimulate the PI-PLC/PKC signaling pathway[47], whereas in the proximal tubule, it inhibits Na^+-ATPase *via* the AT_2/G(i/o) protein/cGMP/PKG pathway[48]. Moreover, at different concentrations of Ang-(1-7) (10^{-12}, 10^{-9}, or 10^{-6} mol/L) used in intratubular perfusion in the absence or presence of the Mas receptor antagonist (A779) of rat isolated proximal tubules, it was shown that Ang-(1-7) has a biphasic dose-dependent effect on the Na^+/H^+ exchanger mediated by Mas receptor and gave a moderate increase in intracellular Ca^{2+} levels ($[Ca^{2+}]_i$)[49]. Increased $[Ca^{2+}]_i$ stimulated by Ang-(1-7) also occurred in MDCK cells, but through the AT_1 receptor, which in turn stimulated Ca^{2+} release from endoplasmic reticulum *via* the PLC pathway and Ca^{2+} influx through PLA2-dependent store-operated Ca^{2+} entry[24]. In this way, ACE2/Ang-(1-7)/Mas axis can counteract most of the deleterious effects of ACE/Ang Ⅱ/AT_1. It has been corroborated that acute intravenous infusion of Ang-(1-7) induces diuresis, natriuresis and renal vasodilatation[50].

Like to Ang-(1-7), there is another heptapeptide derived from Ang Ⅱ having the opposite effect to Ang Ⅱ, namely Ang-(2-8), also known as Ang Ⅲ. Ang Ⅱ can be hydrolyzed by aminopeptidase A, generating Ang Ⅲ[51] (Figure 1). Heretofore there has been no evidence of a specific receptor for Ang Ⅲ, and Ang Ⅲ normally binds to AT_1 with greater affinity than to the AT_2 receptor inducing natriuresis on rats[52,53]. Intrarenal Ang Ⅲ induces natriuresis *via* the AT_2 receptor in the proximal tubule by a cGMP-dependent mechanism[51].

Ang Ⅲ can be hydrolyzed by aminopeptidase N generating Ang-(3-8), also called Ang IV, which can be also generated directly from Ang Ⅱ by D-aminopeptidase[20,54]. The receptor for Ang IV, AT_4, was initially detected in the guinea pig hippocampus[15]. Protein purification and peptide sequencing showed that the AT_4 receptor is an insulin-regulated aminopeptidase[54]. AT_4 receptor is also found in the kidney, where this angiotensin-derived fragment can elicit many responses[55]. Aminopeptidases A and N are abundant in the kidney, especially in proximal nephron, and Ang Ⅳ is formed in the glomerulus[56,57]. Ang Ⅳ increases blood flow in the kidney and decreases in Na^+ transport in proximal tubules[55]. Ang Ⅳ induces Ca^{2+} mobilization in human proximal tubule cells[58] through the AT_1 receptor. In AT_4 knockout (-/-) mice, Ang Ⅳ mediated its renal vasoconstrictor effects through AT_{1a} receptors[59].

Ang Ⅱ can also be hydrolyzed to dipeptides that are biological active, and we have found an alternative pathway for Ang-(1-7) formation from Ang Ⅱ by carboxypeptidase N, and posterior generation of Ang-(3-4) with Ang-(1-5) and Ang-(1-4) as intermediate peptides[20] (Figure 1), using isolated basolateral membranes from sheep proximal tubules and different peptidase inhibitors. Ang-(3-4) could counteract inhibition of plasma membrane Ca^{2+}-ATPase promoted by nanomolar concentrations of Ang Ⅱ through conformational changes in the AT_2 receptor and the cAMP/PKA pathway[19,20,37].

Ang (3-4) is remarkably stable in human blood serum and has antihypertensive effects in spontaneously hypertensive rats (SHR)[60,61]. Dias *et al*[62] showed that oral administration of Ang-(3-4) inhibited Na^+-ATPase activity in membranes of SHR and blocked the stimulation of Na^+-ATPase induced by Ang Ⅱ in normotensive rats *via* the AT_2 receptor and the PKA signaling pathway. This effect leads to increased urinary Na^+ concentration, and simultaneous decrease in systolic arterial blood pressure in SHR, but not in normotensive rats[62].

The presence of another angiotensin derived fragment, known as Ang A (Ala-Arg-Val-Tyr-Ile-His-Pro-Phe), occurs in the plasma of healthy humans and in high levels in end-stage patients with renal failure[21,63]. Decarboxylation of Asp^1 of Ang Ⅱ, in the presence of mononuclear leukocytes leads to Ang A generation, which has higher affinity for AT_2 than Ang Ⅱ and the same affinity for the AT_1 receptor[21,63]. As the other Ang Ⅱ-derived peptides, Ang A exerts its effects on the kidney, inducing renal vasoconstrictor responses in normotensive and hypertensive rats, and also in genetically modified mice[64]. Ang A can also be hydrolyzed by ECA2 in rats, mice and humans generating the heptapeptide alamandine (Ala-Arg-Val-Tyr-Ile-His-Pro), a novel peptide of the RAS[22]. Alamandine has long-term antihypertensive effect in SHRs and antifibrotic effects in isoproterenol-treated rats *via* the Mas-related G-protein-coupled receptor, member D (MrgD), and independent of Mas and AT_2 receptor, the known vasodilator receptors of the RAS, since it is blocked by $D\text{-}Pro^7$-angiotensin-(1-7) and PD123319, but not by the Mas antagonist A-779[22]. Most members of Mas-related

G-protein-coupled receptor (Mrg), a novel class of RAS-related receptor, are orphan, with no identified endogenous ligand, but MrgD has been identified as a binding site for alamandine[22].

NEW MEMBERS OF RAS: RECEPTORS

Classically, there are 2 well described Ang Ⅱ receptors, AT_1 and AT_2 receptors. However, newer work on RAS and its effects shows that there are novel members of this system.

Besides the newly described Ang Ⅱ-derived peptides and their corresponding receptors, there are enzyme members of RAS whose actions depend on interaction with receptors. Nguyen *et al*[27] in 2002 cloned the PRR, which contains a specific binding site for renin and its inactive precursor, prorenin; this interaction stimulates their catalytic activity, increasing RAS activation. Prorenin has a "handle" region that binds to the receptor with a 3-4 fold higher affinity than renin and is important in enzymatic activation of prorenin[65].

After binding, renin and prorenin can also act as agonists to its receptor, generating effects in an Ang Ⅱ-independent manner. In the human kidney, PRR is expressed in glomerular mesangial cells, the subendothelium of renal arteries[27], in the distal nephron[66], collecting ducts, and mostly at the apical surface of intercalated cells, where, due to its high expression it stimulates cyclooxygenase-2 (COX-2)-derived prostaglandins to attenuate the anti-natriuretic and vasopressor effects of Ang Ⅱ[67].

However, activation of PRR in kidney is also associated with many pathological conditions. Activation of human PRR and MAPK through an Ang Ⅱ-independent mechanism contributes to the development of nephropathy in prorenin/renin transgenic rats overexpressing the human receptor[68]. PRR is important through the same signaling pathway in diabetic nephropathy by its activation of glomerular ERK. These studies used an AT_{1a} receptor-deficient mice[69] and db/db mice to show that the receptor-bound prorenin leads to the development of nephropathy in type 2 diabetes[70]. In HEK cells, renin and prorenin activate its receptor to promote fibrosis in an Ang II-independent manner[71].

Kohlstedt *et al*[25] in 2004 revealed another unexpected function of the RAS enzymes. Human ACE, usually known by its catalytic action on Ang I in generating Ang Ⅱ, could also function as an outside-in signal transduction molecule. Binding of ACE substrates or inhibitors to this enzyme can stimulate intracellular signaling pathways: ACE inhibitors (perindoprilat and ramiprilat), like the ACE substrate (bradykinin), could also increase COX-2 expression, ACE phosphorylation at Ser1270 and activation of JNK in endothelial cells[25]. The modulation of gene expression in endothelial cell by ACE inhibitors and JNK/c-Jun pathway requires ACE dimerization through the C domain of the enzyme[26]. This indicates that, although ACE is not a cell surface receptor, it is involved in cell functions. Nevertheless, whether ACE works only as a catalytic enzyme or as a signaling molecule in the kidney remains to be elucidated.

BREAKING PARADIGMS

A newly recognized view of RAS assumes that Ang Ⅱ acts beyond cell surface receptors, with endocrine and paracrine action of RAS. Ang Ⅱ also acts through intracellular receptors. Local RAS was first described within the kidney over 20 years ago[29-32], where the levels of Ang Ⅱ are much higher than in plasma[32,72]. Intrarenal Ang Ⅱ levels and local formation in the kidney have been reported by Navar and colleagues[11,32,73-76].

In addition to Ang Ⅱ synthesis in the kidney, there are other well-described mechanisms that play a critical role in high renal Ang Ⅱ levels, and these occur after Ang II endocytosis with the AT_1 receptor[77,78]. Since AT_1 receptors are expressed in different parts of the kidney, such as in the mesangial cells, afferent and efferent arterioles, glomerular podocytes, macula densa and both basolateral and luminal membranes of different nephron segments[79,80], intracellular Ang Ⅱ accumulation by coupled-receptor internalization is one of main sources of renal Ang Ⅱ accumulation.

In Ang Ⅱ-dependent hypertension several groups have shown that Ang Ⅱ can positively amplify it, leading to its high intrarenal levels. Zhuo *et al*[77] showed increased intracellular Ang Ⅱ levels in cortical endosomes, and Ang Ⅱ-infused hypertensive rats mediated by AT_1 receptors. Ang Ⅱ-infused rats through an osmotic minipump also had increased Ang Ⅱ levels in renal interstitial fluid, which is mediated by the AT_1 receptor[81]. Ang Ⅱ endocytosis with AT_1 receptor has been confirmed by the absence of renal Ang Ⅱ accumulation in AT_{1a} receptor-deficient mice ($Agtr1a^{-/-}$)[82,83]. Another possible pathway for increasing the intrarenal Ang Ⅱ level is due to endogenous Ang Ⅱ production, *via* markedly augmentation on angiotensinogen[11,84] and renin expression in collecting ducts[85,86], the secretion of renin and prorenin by these cells into the luminal fluid, leading to its increased urinary levels in Ang Ⅱ-infused hypertensive rats[87]. These results indicating a positive feedback by Ang Ⅱ in the kidney contradict the well-established view that Ang Ⅱ has a negative feedback mechanism in the expression and activity in the RAS, where high Ang Ⅱ levels suppress the release of renin in juxtaglomerular cells and Ang Ⅱ production in the kidney[88], demonstrating the complexity of the system.

Both Ang Ⅱ receptors (AT_1 and AT_2) are expressed in adult kidneys, although AT_2 receptor is less expressed than AT_1 receptor[79]. This intensely local synthesis of high renal levels of Ang Ⅱ, and the wide expression of Ang Ⅱ receptors within the kidney, provides evidence of the pivotal role of Ang Ⅱ in renal physiology, regulating water and solute reabsorption and renal hemodynamic processes that contribute to Na^+ balance and blood pressure regulation. AT_1 receptors in the kidney are responsible for the development of hypertension[89-91]. And AT_1 receptors within the kidney are necessary for cardiac

hypertrophy and hypertension[90,92].

Ang Ⅱ has many effects on different parts of the kidney. As in the systemic circulation, intrarenal Ang Ⅱ also is important in renal hemodynamics. Thereby, long-term treatment with Ang Ⅱ receptor blockers induced unusual proliferative changes in afferent arteriolar smooth muscle cells, narrowing arteriolar lumens and reducing glomerular pressure[93]. Administration of Ang Ⅱ through an osmotic minipump in hypertensive rats leads to marked suppression of Na^+ excretion as well as renal and medullary blood flow[94]. Peritubular capillary Ang Ⅱ infusion enhanced proximal tubular reabsorption and reduced single nephron glomerular filtration rate in rats[95].

Different targets and signaling pathways regulate Na^+ balance within the kidney; rats infused with Ang Ⅱ showed enhanced ENaC expression[96] and activation of the renal Na^+:Cl^- cotransporter[97,98]. *In vitro* studies using isolated basolateral membrane fractions from pig kidney have demonstrated that Ang II stimulates the renal proximal tubule Na^+-ATPase activity *via* PI-PLCβ/PKC pathway[99,100].

It is widely known that intracellular Ca^{2+} mobilization in proximal tubule cells leads to the activation of many Ca^{2+}-dependent intracellular signaling pathways, including those associated with Na^+ reabsorption[101]. Ang Ⅱ microperfusion techniques in rabbit superficial segment of proximal tubules *in vitro* regulated Na^+ reabsorption *via* PKC and intracellular Ca^{2+}[102]; low concentrations of Ang Ⅱ inhibited membrane Ca^{2+}-ATPase *via* AT_1/AT_2 receptors heterodimers and PKC in isolated fractions of basolateral membranes of proximal tubule, increasing cytosolic Ca^{2+} concentration in proximal tubule cells[37,103]. Luminal Ang Ⅱ stimulates AT_1/AT_2 receptors heterodimerization that increases sarco/endoplasmic reticulum Ca^{2+}-ATPase activity and promotes Ca^{2+} mobilization in proximal tubule cells[101].

The intracrine/intracellular system is new paradigm. Cells that express all the necessary components for synthesis can generate Ang Ⅱ internally[28,29]. Ang Ⅱ can be secreted and exert autocrine effects, or remain inside the cell and have its effects[6,35]. An alternative way for the intracellular source of Ang Ⅱ is the internalization of extracellular Ang Ⅱ after binding to the AT_1 surface receptor[82,83]. Not all internalized Ang Ⅱ-AT_1 complex is degraded in lysosomes, thereby increasing its concentration within the cell, and the AT_1 receptor may be relocated to other organelles, including the nucleus[101,104-108]. Indeed, subcellular localization of ^{125}I-labeled Ang Ⅱ in the pig kidney indicates that Ang Ⅱ generation is predominantly extracellular, followed by AT_1 receptor-mediated endocytosis leading to higher intracellular Ang Ⅱ levels[109]. In accord with this, internalization is seen to be important for AT_{1a} receptor function in polarized proximal tubule epithelial cells, where apical AT_{1a} receptor internalize before interaction with G proteins, which stimulates phospholipase C and cAMP to increase proximal tubule Na^+ reabsorption[110,111].

Within the kidney, cells from different segments can generate Ang Ⅱ or internalize Ang Ⅱ through the AT_1 receptor[109-111]. *In vitro* and *in vivo* studies showed that extracellular Ang Ⅱ accumulates within the kidney *via* AT_{1a} receptor-mediated endocytosis[82,83,107]. Although many have demonstrated different Ang Ⅱ intracellular effects, the precise role of intracellular Ang Ⅱ in nephron segments remains poorly understood. Renal intracellular Ang Ⅱ increases blood pressure and decreases 24 h urinary Na^+ excretion in rats and mices[89,105], suggesting that, like intrarenal Ang Ⅱ, intracellular Ang Ⅱ within the kidney also increases Na^+ reabsorption and blood pressure.

Endocytosis of Ang Ⅱ through the AT_1 receptor within proximal tubule cells occurs through 2 main pathways: the clathrin-dependent and the microtubule-associated pathway[106]. The canonical clathrin-dependent endocytosis pathway for Ang Ⅱ occurs in different cell types, such as vascular smooth muscle and human embryonic kidney (HEK-293) cells through the AT_1 receptor, c-Src and clathrin Adapter Protein 2[112]. In rabbit proximal tubule cells, the alternative microtubule-associated endocytic pathway rather than the clathrin-dependent pathway participates in the AT_1 receptor-mediated uptake of Ang Ⅱ[113].

Another alternative endocytic pathway for Ang Ⅱ internalization in proximal tubule cells has been described by Gonzalez-Villalobos *et al*[114], where anti-megalin antisera interferes with Ang Ⅱ binding in cell brush-border membrane vesicles extracted from mice, indicating that Ang Ⅱ internalization is a megalin-dependent process.

Angiotensin receptors are present in the intracellular organelles, including the sarco/endoplasmic reticulum, Golgi apparatus and the nucleus, indicating that Ang Ⅱ can have many intracellular effects, including modulation of gene expression[33-35]. Proximal tubule cells express angiotensinogen, renin, and ACE mRNAs, suggesting high levels of intracellular Ang Ⅱ[28,32,73]. Thus, microinjection of Ang Ⅱ directly in single rabbit proximal tubule cells increased intracellular Ca^{2+} mobilization through its intracellular AT_1 receptors and Ca^{2+} release from intracellular stores[115]. Ang Ⅱ induced transcriptional responses of mRNAs for MCP-1, NHE-3 and TGF-β1 stimulating the AT_{1a} receptor in freshly isolated intact rat renal cortical nuclei, indicating that internalized and/or intracellular Ang Ⅱ acts on nuclear receptors to mediate growth, proinflammatory responses and Na^+-retaining effects[108]. Furthermore, in isolated nuclei from kidney cortex from sheep in the absence of cytoplasm, all RAS components (angiotensinogen, ACE and renin) have been identified[116], showing that Ang Ⅱ can indeed be synthesized within the nucleus.

Another interesting role for intracellular Ang Ⅱ is encountered in pathological situations. It is thought that intracellular Ang Ⅱ levels could be altered in different diseases, such as diabetic nephropathy and cardiomyopathy, where hyperglycemia might induce intracellular Ang Ⅱ production. Indeed, a high glucose concentration induced an increase of ACE mRNA, synthesis and secretion of renin and Ang Ⅱ in an immortalized murine mesangial cell line[117-119]. Interestingly, an alternative pathway

Table 2 Most common drugs already established for clinical use and emerging drugs and new targets for the treatment of hypertension, cardiovascular and renal diseases

Target	Drug	Therapy	Clinical use
Renin	Aliskiren	HTN, RF	+
	Remikiren, enalkiren	HTN	+
ACE	Captopril, lisinopril, trandolapril	HTN, HF, LVD, DN	+
	Enalapril, enalaprilat, fosinopril, ramipril	HTN, HF	+
	Moexipril, quinapril, perindopril, benazepril	HTN	+
AT_1	Losartan, azilsartan, valsartan, ibesartan, candesartan, telmisartan, eprosartan, omesartan	HTN, HF	+
Mas	AVE 0991	HTN	-
	Ang-(1-7)-CyD	HF	-
ACE2	Xanthenone	HTN, RF, HF	-

+: Already used in clinic; –: Not used in clinic yet. ACE: Angiotensin converting enzyme; ACE2:Angiotensin converting enzyme 2; AT1: Angiotensintype 1 receptor; Mas: Ang-(1-7) Mas receptor; RF: Renal failure; HF: Heart failure; HTN: Hypertension; LVD: Left ventricular dysfunction; DN: Diabetic nephropathy.

was found for the synthesis of intracellular Ang Ⅱ in the presence of high glucose in vascular smooth muscle cells. Under normal glucose levels, Ang Ⅱ is generated by cathepsin D and ACE; however, Ang Ⅱ is obtained by cathepsin D and chymase action in the presence of high glucose[120,121].

NEW TARGETS FOR HYPERTENSION TREATMENT

RAS is important in the development of hypertension and cardiovascular diseases[1-4,90]; one of the most common treatments for these diseases is pharmacological inhibition of enzymes and blockade receptors of RAS[122]. Inhibition of renin, the enzyme that initiates RAS, presents a strategy for hypertension therapy (Table 2). Aliskiren is a more selective and potent inhibitor of human renin than other orally active renin inhibitors, remikiren and enalkiren[123]; it can block the generation of active renin in both normotensive and hypertensive human subjects[124]. Aliskiren is as effective as losartan, valsartan and ibesartan (AT_1 receptor blockers), atenolol (β blocker) and amlodipine (Ca^{2+} channel blocker), and has an antihypertensive effect comparable to other major classes of antihypertensive drugs[124,125]. Besides decreasing blood pressure, aliskiren is also renoprotective in diabetic and nondiabetic models of chronic kidney disease, preventing albuminuria in rats[126]. In humans, aliskiren significantly decreases blood pressure, and also the urinary albumin and creatinine ratio in 15 patients with type 2 diabetes mellitus[127].

ACE is another enzyme of the RAS that can be pharmacology inhibited so as to decrease hypertension (Table 2). A total of 17 small orally active ACE inhibitors have recently been synthesized for clinical use, all binding to the active site of the enzyme and interfering with ACE's ability to bind and cleave its substrates (Ang I and bradykinin, among others)[128,129]. Many ACE inhibitors were approved for hypertension treatment, heart failure and left ventricular dysfunction (*e.g.*, captopril, lisinopril, trandolapril), as also captopril for diabetic nephropathy[129].

Ang Ⅱ promotes cardiovascular disorders and hypertension *via* the AT_1 receptor, which can be blocked to treat these pathological conditions (Table 2). A total of 8 non-peptide angiotensin-receptor blockers (ARBs) orally active are used clinically for hypertension and cardiovascular diseases (namely losartan, azilsartan, valsartan, ibesartan, candesartan, telmisartan, eprosartan, omesartan), which are all well-tolerated[129,130]. Telmisartan seems more efficacious in decreasing blood pressure than the other ARBs[131,132].

Many patients with hypertension require combination regimens to achieve a significant decrease in blood pressure. In this case, the most commonly used drugs are ARBs and ACE inhibitors, Ca^{2+} channel blockers (CCB) and diuretics[130]. Long-term treatment triple therapy with olmesartan medoxomil (ARB), amlodipine besylate (CCB) and hydrochlorothiazide (diuretic) in 2112 hypertensive patients with moderate to severe hypertension resulted in 44.5%-79.8% of participants having a decreased the mean blood pressure from 168.6/100.7 mm Hg to 125.0-136.8/77.8-82.5 mmHg, reaching the blood pressure goal[133]. The same triple therapy also proved to be efficient in hypertensive Hispanic/Latin patients[134].

However, even with the successful results obtained by inhibiting the enzymes and receptors of the RAS, many patients do not respond as expected, and cardiovascular disease risks have not decreased to those in normotensive people. Due to the high death rates by heart diseases in the world, which are higher than from many cancers[135], it is important to devise new strategies for the treatment of cardiovascular diseases and hypertension.

Because of the discovery of new components in the RAS that have herein been described, novel Ang Ⅱ-derived peptides have emerged as excellent target for heart diseases. Since the ACE2/Ang-(1-7)/Mas axis has an opposite and protective effect from the deleterious ACE/Ang Ⅱ/AT_1 axis, it is now the main target for these drugs[14]. Besides inhibiting ACE activity and blocking AT_1 receptors responsible for the inhibition of ACE/Ang Ⅱ/AT_1 axis, activation of the ACE2/Ang-(1-7)/Mas axis is a promising alternative means for the treatment of the heart diseases. Nevertheless, this new strategy presents certain problems. First, as a peptide, Ang-(1-7) is proteolytically degraded in the gastrointestinal tract[18]; and second, Ang-(1-7) has a short half-life, complicating its use as an oral pharmacotherapy for hypertension and cardiovascular disease.

The difficulty was overcome after the synthesis of the first nonpeptide compound able to mimic Ang-(1-7) and bind selectively to the Mas receptor[136], the AVE 0991 5-formyl-4-methoxy-2-phenyl-1-{[4-(2-ethyl-ami-

nocarbonylsulfonamido-5-isobutyl-3-thienyl)-phenyl]-methyl}-imidazole (Table 2)[137]. Although this molecule is an antihypertensive candidate because it stimulates NO release in endothelial cells[137], promotes vasorelaxation in mouse and rat aortic rings[138], and attenuates hypertension in SHR[139], clinical trials are needed to see its effects in humans.

Another important achievement has been the inclusion of the heptapeptide in hydroxypropyl-β-cyclodextrin [Ang-(1-7)-CyD], avoiding its proteolytic degradation in the gastrointestinal tract and permitting its oral administration (Table 2)[18]. Cyclodextrins are amphiphilic oligosaccharides that increase drug stability and absorption[140]; after oral administration, they are split up into small saccharides in the colon, leaving Ang-(1-7) to be absorbed[18]. Chronic oral administration of Ang-(1-7)-CyD in isoproterenol-treated rats increases plasma Ang-(1-7) levels, with attenuation of myocardial infarction associated with cardioprotective effects[141].

Another option for the treatment of the deleterious effects of Ang Ⅱ is activation of ACE2, which, besides increasing Ang Ⅱ degradation, enhances Ang-(1-7) production; ACE2 activators are an alternative source for controlling hypertension (Table 2). Acute intravenous administration of xanthenone (XNT), which interact with ACE2 in specific sites, promotes conformational changes and increases ACE2 activity. Consequently, it decreases blood pressure, improves cardiac function and decreases renal fibrosis in SHR[142]. It also has antihypertensive effects in rats with pulmonary hypertension[143].

These results together suggest that, besides inhibition of renin and ACE, associated or not with the blocking of AT_1 receptor, activation of the ACE2/Ang-(1-7)/Mas axis and its protective effects is emerging as an excellent alternative therapy for the treatment of hypertension and cardiovascular diseases.

CONCLUSION

The data presented herein show that RAS has passed from being simply an endocrine system to one with paracrine, autocrine and intracrine functions, increasing Ang Ⅱ concentration in different tissues including the kidney. After years of research, the RAS - previously seen as a simple system with only 2 receptors (AT_1 and AT_2), and one active peptide (Ang Ⅱ), turns out to be a complex system, with many new members continuing to be described. In addition to (ACE)/Ang Ⅱ/AT_1 and AT_2 axis, other signaling pathways in the RAS, such as ACE2/angiotensin-(1-7)/Mas and Ang IV/AT_4, and other active peptide of the RAS, with physiological relevance as Ang Ⅲ, Ang-(3-4), Ang A and alamandine, are now widely recognized. These newly discovered fragments derived from Ang Ⅱ can act on the same classic Ang Ⅱ receptors, AT_1 and AT_2, or on specific receptors (Mas and AT_4) having the same or the opposite effects of Ang Ⅱ depending on the triggered signaling pathway, in the kidney and other tissues, with many roles seen in physiological and physiopathological conditions. The discovery of renin and prorenin as agonists of PRR receptor, stimulating intracellular pathways and having effects on different cells types in an Ang Ⅱ-independent manner, raised another axis for this system, namely the prorenin/PRR/MAPK ERK1/2 axis.

Finally, activation of the new ACE2/Ang-(1-7)/Mas axis with opposite and protective effects, compared with ACE/Ang Ⅱ/AT_1 axis, with different drugs such as AVE 0991, the nonpeptide compound mimicking Ang-(1-7) effects, the Ang-(1-7)-CyD, and the XNT, the activator of ACE2 activity, now leading to improved and greater fall in blood pressure creates new possibilities for patients who do not respond as expected to conventional antihypertensive drugs.

A thorough understanding of RAS and all the new possibilities described on this review will certainly contribute to the development of pharmacological approaches, discovery of new drugs and alternative treatments for hypertension, cardiovascular and kidney diseases.

ACKNOWLEDGEMENTS

We express our appreciation for the support given by our laboratory colleagues and we especially acknowledge BioMedES for the English revision of the manuscript.

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P- Reviewer: Wong KL **S- Editor**: Ji FF **L- Editor**: A
E- Editor: Wu HL

World Journal of Nephrology

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DOI: 10.5527/wjn.v3.i3.77

World J Nephrol 2014 August 6; 3(3): 77-84
ISSN 2220-6124 (online)

MINIREVIEWS

Therapeutic target for nephrotic syndrome: Identification of novel slit diaphragm associated molecules

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Received: March 26, 2014 Revised: May 15, 2014
Accepted: June 27, 2014
Published online: August 6, 2014

Abstract

The slit diaphragm bridging the neighboring foot processes functions as a final barrier of glomerular capillary wall for preventing the leak of plasma proteins into primary urine. It is now accepted that the dysfunction of the sit diaphragm contributes to the development of proteinuria in several glomerular diseases. Nephrin, a gene product of *NPHS1*, a gene for a congenital nephrotic syndrome of Finnish type, constitutes an extracellular domain of the slit diaphragm. Podocin was identified as a gene product of *NPHS2*, a gene for a familial steroid-resistant nephrotic syndrome of French. Podocin binds the cytoplasmic domain of nephrin. After then, CD2 associated protein, NEPH1 and transient receptor potential-6 were also found as crucial molecules of the slit diaphragm. In order to explore other novel molecules contributing to the development of proteinuria, we performed a subtraction hybridization assay with a normal rat glomerular RNA and a glomerular RNA of rats with a puromycin aminonucleoside nephropathy, a mimic of a human minimal change type nephrotic syndrome. Then we have found that synaptic vesicle protein 2B, ephrin-B1 and neurexin were already downregulated at the early stage of puromycin aminonucleoside nephropathy, and that these molecules were localized close to nephrin. It is conceivable that these molecules are the slit diaphragm associated molecules, which participate in the regulation of the barrier function. These molecules could be targets to establish a novel therapy for nephrotic syndrome.

Key words: Podocyte; Slit diaphragm; Synaptic vesicle protein 2B; Ephrin-B1; Neurexin

Core tip: The slit diaphragm located between neighboring foot processes of a glomerular podocyte functions as a final barrier to retain plasma proteins. Recently several molecules such as nephrin and podocin were identified as functional molecules of the slit diaphragm. However, the precise molecular compositions of the slit diaphragm are still unclear and the mechanism regulating its barrier function is not fully understood yet. Recently we have reported that synaptic vesicle protein 2B, ephrin-B1 and neurexin are expressed in podocyte and the decreased function of these molecules participates in the initiation of proteinuria. These molecules could be targets for a novel therapy for proteinuria.

Fukusumi Y, Miyauchi N, Hashimoto T, Saito A, Kawachi H. Therapeutic target for nephrotic syndrome: Identification of novel slit diaphragm associated molecules. *World J Nephrol* 2014; 3(3): 77-84 Available from: URL: http://www.wjgnet.com/2220-6124/full/v3/i3/77.htm DOI: http://dx.doi.org/10.5527/wjn.v3.i3.77

INTRODUCTION

A glomerular capillary wall preventing the leak of plasma proteins is consisted of three layers: an endothelial cell, a glomerular basement membrane, and a glomerular epithelial cell (podocyte). Podocyte is characterized as its

highly sophisticated shape with the primary processes and the interdigitating secondary processes. The secondary process is called as a foot process[1]. The interdigitating foot processes are bridged by a structure called slit diaphragm. In 1988, Orikasa *et al*[2] of our group reported that the murine monoclonal antibody recognizing the extra-cellular site of the slit diaphragm caused massive proteinuria if injected into rats. The finding clearly indicated that the slit diaphragm is one of the essential structures of the barrier of the glomerular capillary wall[2]. In 1998, Kestilä *et al*[3] found the responsible gene for the Finnish type congenital nephrotic syndrome, and reported that its gene product, they called nephrin, was an extracellular component of the slit diaphragm[3,4]. Podocin was identified by Bout *et al*[5] as a protein coded by the responsible gene for familial steroid-resistant nephrotic syndrome in 2000. Following nephrin and podocin, CD2 associated protein (CD2AP), NEPH1 and canonical transient receptor potential-6 (TRPC6) were identified as crucial molecules of the slit diaphragm[6-10]. Several studies showed that the functional loss of these molecules participated in the initiation of proteinuria in acquired glomerular diseases[11-18]. It is now accepted that the slit diaphragm is a final barrier of glomerular capillary wall preventing proteinuria[19-23]. To explore the targets for the novel therapy of proteinuria, the subtraction hybridization assay was done with a normal rat glomerular cDNA and cDNA of rats showing proteinuria. We identified some molecules downregulated at proteinuric states. In this article, first we review the characteristics of the critical slit diaphragm molecules previously reported, and then we introduce the novel slit diaphragm-associated molecules, synaptic vesicle protein 2 (SV2) B, ephrin-B1 and neurexin.

CRITICAL MOLECULES CONSTITUTING THE SLIT DIAPHRAGM

Zonula occludens-1

Zonula occludens-1 (ZO-1) was originally identified as a molecule of the tight junction[24]. ZO-1 belongs to the membrane-associated guanylate kinase homologue (MAGUKs)[25]. It is reported that ZO-1 was expressed at the slit diaphragm in podocyte[26]. ZO-1 is the first protein reported to constitute the slit diaphragm. Splicing variants, ZO-1 α^+ and ZO-1 α^- which lacks motif α have been reported[27]. Both are expressed in tight junctions of the tubular epithelial cells, but only ZO-1α^- is expressed at the slit diaphragm[28].

Nephrin

Nephrin is a product of gene mutated in Finish type congenital nephrotic syndrome[3]. Nephrin is now accepted as the most important component of the slit diaphragm. Nephrin is a transmembrane protein of 1241 amino acid residues of the immunoglobulin super family. Nephrin contains eight Ig-like modules and a single fibronectin type Ⅲ module. The nephrin homologues of mouse[29] and rat[12,30] were cloned. Rat nephrin has 82.2% homology to human nephrin. We have shown the anti-slit diaphragm antibody previously reported, which cause proteinuria if injected into rat, binds the extracellular site of rat nephrin[12,31,32], indicating that nephrin is an essential slit diaphragm molecule.

Podocin

Podocin was found as a protein coded by *NPHS2*, the responsible gene of autosomal recessive steroid-resistant nephrotic syndrome. Podocin is reported to bind nephrin and is accepted to be a slit diaphragm molecule[5]. Podocin is a 42 kDa protein with a single transmembrane domain. Because immunoelectron microscopic study demonstrated that both N- and C- termini were in cytoplasm, podocin is considered to have a hairpin-like structure[33]. Podocin homologues of rat and mouse were cloned. Identity between rat and mouse, mouse and human, rat and human, are 92.7%, 86%, 84.3%, respectively[14]. It is demonstrated that podocin interacts with nephrin and CD2AP[34]. It is reported that podocin is a raft-associated component of the slit diaphragm and to serve a scaffolding function.

CD2AP

CD2AP is understood to be one of critical molecules of the slit diaphragm[6]. CD2AP was originally reported to be an adaptor protein binding the cytoplasmic domain of CD2, a membrane protein on natural killer cell and T cell[35]. CD2AP is an 80 kDa protein containing an actin-binding site at the N terminus. CD2AP bound nephrin and anchored nephrin to the cytoskeleton[6]. It is reported that mice lacking CD2AP exhibit loss of foot process, a nephrotic range proteinuria and advance renal failure. It is reported that a mutation of the gene for CD2AP were detected in two human patients with focal segmental glomerulosclerosis (FSGS)[36].

NEPH1

NEPH1 is a nephrin associated protein identified by a gene trap method[7]. NEPH1 has five extracellular immunoglobulin-like domains[7]. NEPH1 interacts with C-terminal domain of podocin[8], ZO-1 and nephrin[37]. The foot processes effacement and proteinuria were detected in NEPH1 knockout mice. All NEPH1 knockout mice died before 8 week of age[7], indicating that NEPH1 is an essential molecule in podocyte.

TRPC6

In 2005, it was reported that the mutation in TRPC6 channel can cause familial FSGS[9,10]. TRPC6 belongs to the transient receptor potential superfamily of non-selective cation channels. TRPC6 is understood to be a receptor-operated channel leading to the influx of calcium in response to phospholipase C-mediated signals[38]. Immunoelectron microscopy study showed TRPC6 localized at major processes, foot processes and at the slit diaphragm[9,10,18,39]. TRPC6 is reported to be colocalized with nephrin, podocin, and CD2AP. In addition, nephrin

and podocin are co-immunoprecipitated with TRPC6 in cultured podocyte[10]. Winn *et al*[9] reported that the mutation of TRPC6, proline-to-glutamine substitution at position 112 (P112Q) which is detected in patients of familiar FSGS leads to both increased amplitude and duration of calcium influx in the over expression system. Reiser *et al*[10] showed that R895C and E897K, other TRPC mutants detected in the patients, displayed increased current amplitude in the system with HEK293 cells. It is understood that the FSGS-associated mutations could lead to be a gain-of-function alteration in activity and thus increased calcium influx. Recently, Eckel *et al*[40] showed that albuminuria caused by continuous injection of angiotensin Ⅱ was significantly less in TRPC-deficient mice than in wild type mice and discussed that TRPC6 promotes albuminuria by promoting angiotensin II-dependent increase in calcium. It is now accepted that TRPC6 channel activity at the slit diaphragm is essential for proper regulation of podocyte structure and function.

THE MAJOR SLIT DIAPHRAGM MOLECULES ARE DOWNREGULATED IN NEPHROTIC STATES

It was reported that the expression of nephrin decreased in patients of minimal change nephrotic syndrome (MCNS)[13,41]. Our group has investigated the expression of nephrin in rat puromycin aminonucleoside (PAN)-induced nephropathy[12]. PAN nephropathy is widely used as the model of MCNS. Our group showed mRNA expression for nephrin declined already at 1h after PAN injection into rats. We also observed that the immunofluorescence staining of nephrin changed to a discontinuous pattern from a continuous pattern along glomerular capillary wall on day 10 of PAN nephropathy when proteinuria peaked. The downregulation of nephrin in PAN nephropathy was reported by another group[11]. We reported that podocin is colocalized with nephrin in normal rats, whereas the podocin staining is apart from nephrin in rats with PAN nephropathy[14]. These observation showed that the molecular structure of the slit diaphragm was rearranged in PAN nephropathy, and that these molecular rearrangement led proteinuria in this model. It is conceivable that these alterations of the slit diaphragm participate in the development of MCNS. It is reported that nephrin staining changed to a discontinuous pattern in patients with membranous nephropathy[15]. It is reported that such an alteration in the staining of nephrin was detected in rats of passive Heymann nephritis model, mimic of human membranous nephropathy[42]. We observed that CD2AP and podocin were already apart from nephrin at the early phase of passive Heymann nephritis before the onset of proteinuria[43]. These reports showed that the altered localization of the slit diaphragm molecules is involved in the development of proteinuria also in membranous nephropathy. We reported that the expressions of nephrin, podocin and NEPH1 altered in rats of adriamycin (ADR)-induced nephropathy, which is accepted as a mimic of FSGS[44]. The rats with ADR nephropathy showed severe and continuous proteinuria. The dissociation of NEPH1 from nephrin was observed at the early phase of ADR nephropathy, when the dissociation of podocin from nephrin is not observed. It is postulated that the NEPH1- nephrin dissociation initiates proteinuria in this disease. In contrast to other slit diaphragm molecules, it was discussed that TRPC6 expression in podocytes is up-regulated in several diseases[18].

EXPLORING THE NOVEL FUNCTIONAL MOLECULES REGULATING THE BARRIER FUNCTION OF THE SLIT DIAPHRAGM

Although some critical molecules maintaining the barrier function of the slit diaphragm were identified, the precise molecular compositions of the slit diaphragm and the mechanism regulating its barrier function were not fully understood yet. To further analyze the molecular compositions of the slit diaphragm and to identify the target molecule for the therapy for nephrotic syndrome, we tried to purify the novel molecules and a subtraction assay with cDNA from normal rat glomeruli and nephrotic syndrome rat glomeruli was done[45]. It is plausible that, nephrin, podocin, CD2AP and NEPH1 were downregulated at the proteinuric states. Therefore, it is conceivable that the molecule whose expression decreased at the proteinuric state might be a molecule relating the development of proteinuria. We investigated the localization and the role of the molecules identified by the subtraction assay in podocyte. In addition, those of their associated molecules were also investigated. We focused on synaptic vesicle protein 2B[45], ephrin-B1[46] and neurexin[47] in these molecules. It is considered that these molecules are essential molecules of the slit diaphragm (Figure 1), and that they are the candidates for the target of novel therapy for nephrotic syndrome. The nature of these molecules is described below.

SV2B

SV2 has three isoforms, SV2A, SV2B and SV2C. Our group found SV2B was downregulated in PAN nephropathy. The expression of mRNA of SV2B was already reduced when abnormal proteinuria was not detected yet. It is considered that the observation showed the decrease of the SV2B expression is not a mere consequence of proteinuria but has an etiological significance causing proteinuria. The decrease of the expression of SV2B was also observed from the early phase in anti-nephrin antibody-induced nephropathy. The observation indicated that SV2B is the slit diaphragm-associated molecule.

SV2 is known to regulate calcium-mediated synaptic transmission. It is understood that SV2 plays an essential role in vesicle trafficking[48-50]. SV2 was understood to be exclusively expressed in the neuronal tissue. However,

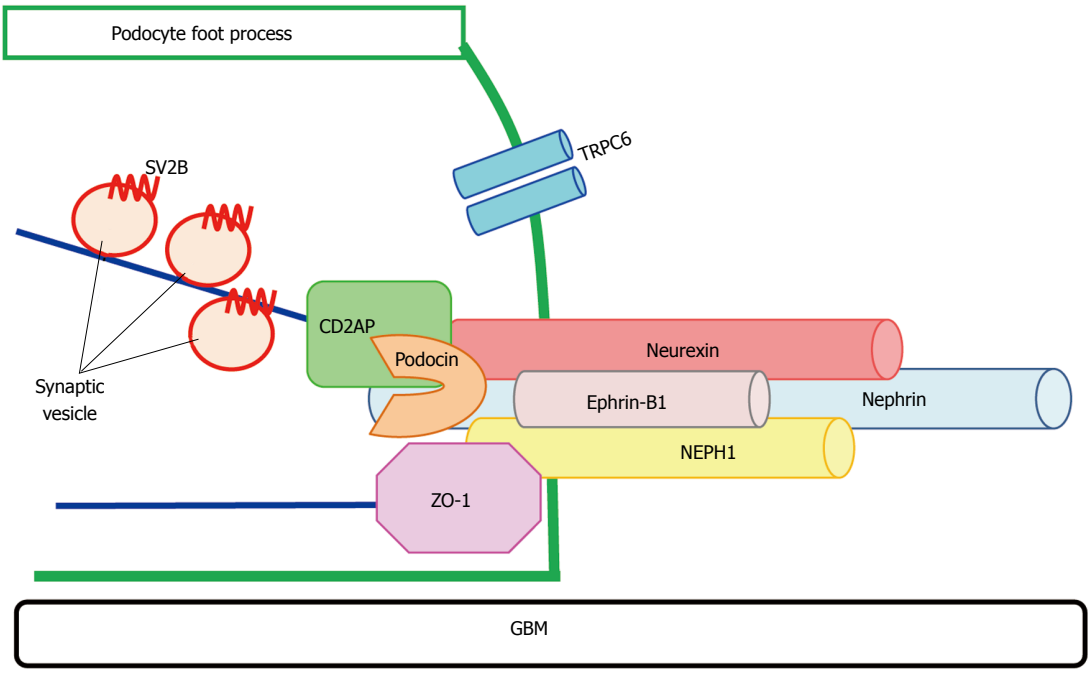

Figure 1 Molecules constituting the slit diaphragm. Nephrin and NEPH1 are the transmembrane proteins constituting the extracellular site of the slit diaphragm. Nephrin interacts with CD2 associated protein (CD2AP), podocin and NEPH1. NEPH1 interacts with ZO-1. Canonical transient receptor potential-6 (TRPC6) is located at the slit diaphragm area. SV2B is expressed at the slit diaphragm area and contributes to the proper localization of CD2AP. Ephrin-B1 and neurexin are transmembrane proteins of the slit diaphragm. GBM: glomerular basement membrane; ZO-1: Zonula occludens-1.

some reports have shown that SV2s are expressed in several other tissues[51,52]. SV2B is detected in the microvesicles of pinealocytes. Both pinealocyte and podocyte are characterized by their specialized processes[52]. These properties also suggested that SV2B plays a role in the trafficking to the terminal of processes.

To analyze the role of SV2B in podocyte, RNA silencing analysis was performed using cultured podocytes, and the expression of CD2AP, one of critical molecules of the slit diaphragm[45] was analyzed. CD2AP was detected at the tip of the process in control cells, whereas the CD2AP staining was detected mainly at the cytoplasm in the cells treated with siRNA for SV2B. From these observations, we concluded that SV2B contributed to the maintaining of the normal molecular structure of the slit diaphragm. It is postulated that the dysfunction of SV2B is involved in the development of proteinuria via the redistribution of proteins of the slit diaphragm such as CD2AP in PAN nephropathy and in other proteinuric states. Not only SV2B but also Rab3A, another synaptic vesicle molecule and rabphilin-3a, an effecter of Rab3A were expressed in the podocyte, and played an important role in maintaining the podocyte function[53]. More elucidation of the role of the synaptic vesicle like vesicle expressing SV2B and Rab3A in podocyte is awaited.

Ephrin-B1

The molecules belonging to the Eph-ephrin family were identified by the subtraction assay. Both Ephs and ephrins are transmembrane proteins and they function as ligand-receptor pairs[54-56]. Eph-ephrin family have many biological functions such as the cell migration and axon guidance[57-59]. It is also reported that the Eph-ephrin-B family play a role in the regulation of the permeability between epithelial cells[57]. These characteristics of Eph-ephrin-B prompted us to analyze a role of Eph-ephrin-B in podocyte. Ephrin-B1, ephrin-B2, EphB1 and EphB2 mRNA expressions were detected in normal rat glomeruli and in murine cultured podocytes. Our group observed that the mRNA expression and immunofluorescence findings of ephrin-B1 were found to be decreased at 24 h of the nephropathy caused by the anti-nephrin antibody injection, whereas EphB1 or ephrin-B2 was not altered. Ephrin-B1, an original name Lerk-2, is a membrane-anchored protein[54,55]. Ephrin-B1 contains a cytoplasmic tail, a single transmembrane domain and an extracellular domain[55,56]. An immunoelectron microscopic study showed that ephrin-B1 was detected at the slit diaphragm[46]. Interaction of ephrin-B1 with nephrin was observed by the immuno-precipitation assay with glomerular lysate. It is conceivable that these observations showed ephrin-B1 is a slit-diaphragm-associated protein. The expression of ephrin-B1 was decreased already at the early phase of the anti-nephrin antibody-induced nephropathy when the alteration of nephrin staining is not remarkable yet. The podocyte injury in this model is caused by the binding of antibody to nephrin. The observation that the expression of ephrin-B1 altered more rapidly than nephrin in this model is very interesting, and we believe that the findings suggested that ephrin-B1 is highly associated with nephrin.

To investigate the function of ephrin-B1 in podocyte, a knockdown system with siRNA was done in cultured podocyte. CD2AP was detected at the tip end of the pro-

Table 1 Summary of the critical slit diaphragm and the novel slit diaphragm associated molecules

Ref.	Molecules	Predicted molecule weight	Functions in the slit diaphragm
Schnabe *et al*[26]	ZO-1	225 kDa	Interact with NEPH1
Kestilä *et al*[3]	Nephrin	180 kDa	Maintaining the barrier function
Schwarz *et al*[34]	Podocin	42 kDa	A raft-associated component and interact with nephrin and CD2AP
Shih *et al*[6]	CD2AP	80 kDa	Interact with nephrin and anchor nephrin to the cytoskeleton
Liu *et al*[37]	NEPH1	110 kDa	Interact with ZO-1 and nephrin
Winn *et al*[9], Reiser *et al*[10]	TRPC6	About 110 kDa	Interact with nephrin and podocin
Miyauchi *et al*[45]	SV2B	80 kDa	The proper arrangement of CD2AP
Hashimoto *et al*[46]	Ephrin-B1	50 kDa	Maintaining the slit diaphragm structure
Saito *et al*[47]	Neurexin	150 kDa	Regulating the slit diaphragm function

ZO-1: Zonula occludens-1; CD2AP: CD2 associated protein; TRPC6: Transient receptor potential-6.

cesses in control cells, whereas the staining of CD2AP was detected in cytoplasmic area around the nuclei. The finding suggested that ephrin-B1 plays a role for the trafficking of CD2AP to the tip of process. All of these results suggest that ephrin-B1 is also a component of the slit diaphragm complex. Recently, Wnuk *et al*[60] reported that Eph-B4 and ephrin-Bs were expressed in podocyte and that the expressions were altered in glomerulonephritis model. These findings suggested that members of the Eph-ephrin-B family could be targets for a novel therapy for proteinuria.

Neurexin

After the discovery of SV2B as a critical molecule of the slit diaphragm, we analyzed the expression of several synaptic vesicle associated molecules in podocyte, and found that neurexin is expressed in rat and human podocyte[47]. Neurexin was originally identified as a cell surface receptor for α-latrotoxin, a component of black widow spider venom, and was considered to play a critical role in cell-cell interaction in across the synapse[61-65]. Neurexin has an interaction with synaptotagmin, a synaptic vesicle associated molecule, and is postulated to play a role in synaptic vesicle docking[66,67]. It is also reported that neurexin binds calcium/calmodulin-dependent serine protein kinase (CASK), a member of the MAGUK family[68]. It is reported that CASK interacts with nephrin[69]. CASK is also accepted to be an essential molecule of the slit diaphragm. Our group demonstrated that neurexin was restrictedly expressed in the glomeruli of the kidney[47]. Dual-labeling immunofluorescence studies showed that neurexin located close to CD2AP. It was also detected that some portions of the neurexin staining are coincident with the staining of Rab3A, a synaptic vesicle molecule. We observed that the staining intensity of neurexin in the glomeruli was clearly reduced, and their staining pattern shifted to a discontinuous patchy pattern in PAN nephropathy and in anti-nephrin antibody induced nephropathy. The alteration in the staining of neurexin in these models was detected more clearly and rapidly than that in the nephrin staining. These observations suggest that neurexin is one of the essential molecules regulating the slit diaphragm function. It is postulated that neurexin is a candidate of the targets for a novel therapy for nephrotic syndrome.

CONCLUSION

It is now widely understood that the dysfunction of the slit diaphragm participates in the initiation of proteinuria in several kinds of glomerular diseases. We summarized the nature of the major slit diaphragm molecules in Table 1. We reviewed the novel slit diaphragm associated molecules SV2B, ephrin-B1 and neurexin. Because proteinuria is an independent risk factor for the vascular episode in brain, heart and other organs[70], the novel more effective therapy for proteinuria should be established. It is reported that dysregulation of the synaptic vesicle function is involved in several neuronal diseases, and the drug targeting synaptic vesicle is used for the treatment for epilepsy. SV2B and other synaptic vesicle associated proteins could be novel therapeutic targets for nephrotic syndrome.

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P- Reviewer: Kong WY, Nihalani D, Quiroga B, Tanaka H, Yorioka N **S- Editor**: Ji FF **L- Editor**: A **E- Editor**: Wu HL

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DOI: 10.5527/wjn.v3.i3.85

World J Nephrol 2014 August 6; 3(3): 85-91
ISSN 2220-6124 (online)

ORIGINAL ARTICLE

Kinin B_2 receptor does not exert renoprotective effects on mice with glycerol-induced rhabdomyolysis

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Supported by The National Council of Scientific and Technological Development - CNPq, No. 135020/2011-5
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Received: December 4, 2013 Revised: April 24, 2014
Accepted: May 14, 2014
Published online: August 6, 2014

Abstract

AIM: To investigate a potential protective role of the kinin B_2 receptor in a glycerol-induced rhabdomyolysis mouse model.

METHODS: We separated 28 C57Bl/6 male mice into 4 groups: untreated WT animals, untreated B_2 knockout mice, glycerol-treated WT and glycerol-treated B_2 knockout mice. Glycerol-treated animals received one intramuscular injections of glycerol solution (50% v/v, 7 mL/kg). After 48 h, urine and blood samples were collected to measure creatinine and urea levels. Additionally, kidney samples were extracted for histological evaluation, and the mRNA expression levels of kinin B1 and B2 receptors and inflammatory mediators were measured by real-time polymerase chain reaction.

RESULTS: Serum creatinine and urea levels showed differences between untreated wild-type and glycerol-treated wild-type mice (0.66 ± 0.04 *vs* 2.61 ± 0.53 mg/dL, $P < 0.01$; and 33.51 ± 2.08 *vs* 330.2 ± 77.7 mg/dL, $P < 0.005$), and between untreated B_2 knockout mice and glycerol-treated knockout mice (0.56 ± 0.03 *vs* 2.23 ± 0.87 mg/dL, $P < 0.05$; and 42.49 ± 3.2 *vs* 327.2 ± 58.4 mg/dL, $P < 0.01$), but there was no difference between the glycerol-treated wild-type and glycerol-treated knockout mice. Glycerol was able to induce a striking increase in kinin B_2 receptor expression (> 30 times, 31.34 ± 8.9) in kidney. Animals injected with glycerol had a higher degree of tubular injury than untreated animals. Wild-type and knockout mice treated with glycerol intramuscularly present kidney injury, with impairment in renal function. However, B_2 knockout mice treated with glycerol did not show a different phenotype regarding kidney injury markers, when compared to the wild-type glycerol-treated group.

CONCLUSION: We conclude that the kinin B_2 receptor

does not have a protective role in renal injury.

Key words: Kinins; acute kidney injury; Animal models; Rhabdomyolysis; Skeletal muscle

Core tip: In this work we are showing that glycerol-treated animals experienced impairment in renal function. Furthermore, we worked with kinin B_2 receptor knockout mice and our results suggest that kinin B_2 receptor does not exert renoprotective effects in this rhabdomyolysis model. In addition, we are presenting results of kidney expressions and we investigated several candidates that can participate in the kidney injury induced by glycerol.

Gattai PP, Mafra FFP, Wasinski F, Almeida SS, Cenedeze MA, Malheiros DMAC, Bacurau RFP, Barros CC, Câmara NOS, Araujo RC. Kinin B_2 receptor does not exert renoprotective effects on mice with glycerol-induced rhabdomyolysis. *World J Nephrol* 2014; 3(3): 85-91 Available from: URL: http://www.wjgnet.com/2220-6124/full/v3/i3/85.htm DOI: http://dx.doi.org/10.5527/wjn.v3.i3.85

INTRODUCTION

Acute kidney injury (AKI) is a common life-threatening disease that places a heavy burden on the health system[1]. It was reported that the mortality rate for this disease ranges from 10% to 80%. Other studies suggest that AKI may be a step in the progression toward chronic kidney disease[2], in humans and animals[3].

The main factors that predispose patients to AKI include hemodynamic instability, hypovolemia, hypoxia, ischemia and reperfusion (I/R), and burns, among others[4]. There are several models used to study this disease; one of them is the glycerol-induced rhabdomyolysis model in which AKI occurs after muscle injury. In this nephrotoxic experimental model, the waste products from metabolism, such as enzymes and other molecules (*e.g.*, uric acid and myoglobin), cause kidney injury to the proximal and distal tubules[4]. Such injuries cause an abrupt (in a matter of hours or days) decline in renal function.

In renal inflammatory conditions such as AKI, the kallikrein-kinin system (KKS) plays an important role in glucose homeostasis[5,6]. This system is also involved in kidney inflammatory and vasodilation processes[7], which are directly involved in the inflammatory response mechanism of AKI. The AKI inflammatory response involves induction of local vascular ischemia, hypoxia and tubular injury[4]. KKS exerts its actions by activating two receptors: B_1 (B_1R) and B_2 (B_2R)[8]. The activation of B_1R is inducible and occurs under pathological conditions such as in ischemia[9], while B_2R is constitutively active under normal physiological circumstances[8]. Furthermore, some authors[10] have shown that there are fewer B_2R in rat nephrons affected by renal disease. It is well established that kinins are rapidly generated after tissue injury and that they have a central role in the development and maintenance of inflammatory processes, whether they are acute or chronic[1].

There is a renal KKS[11] that can respond in a more specific way to AKI. Another system that could be involved in the mechanism of AKI, as observed in the glycerol-induced rhabdomyolysis model, is the renin-angiotensin system (RAS). A drop in blood pressure, typically observed during a hypovolemic state, will activate this system and induce renal perfusion[12]. It is important to note that, the renal KKS can activate RAS[13]. In a recent study from our laboratory[14], we showed that there is a relationship between the genetic polymorphisms of ACE and B_2R, where the former can modulate the kinins in transplanted kidney patients. In other animal models of renal injury, it has been shown that B_2 has a possible protective role[15], while others have shown that it has a deleterious role[16].

It is described in the literature that the B_2R are in all portions of the nephron, except in podocytes of human kidney[17]. Moreover, it is described that the NO levels fall in rhabdomyolysis[4], and that the B_2R activation is related with NO production[18]. Thus, the purpose of this study is to investigate the role of B_2R in the kidney in an animal model of glycerol-induced rhabdomyolysis.

MATERIALS AND METHODS

Experimental design

This study began only with approval of the ethics committee of the Federal University of Sao Paulo (UNIFESP, nº 0300/11), and experiments were performed in accordance with the guidelines established by the Brazilian College for Animal Experimentation. The animals were provided by the Center for Development of Experimental Medicine and Biology (CEDEME) at UNIFESP. Twenty-eight male 3-month-old C57Bl/6 mice were used. Animals were randomly divided into 4 groups: WT (n = 5), glycerol WT (GWT, n = 9), B_2KO (n = 6), and glycerol B_2KO (GB$_2$KO, n = 8). All animals were placed in individual cages (Alesco, Brazil) in an environment with controlled temperature (21 ℃), a light/dark cycle of 12 h for one week and water and standard chow *ad libitum*.

The WT and B_2KO groups were control groups and received no treatment. The animals in the treated groups were deprived of water for 18 h to better evidence of AKI. Animals of both treatment groups (GWT and GB$_2$KO) were then slightly sedated with an intraperitoneal injection of ketamine-xylazine solution (150 μL, Vetnil, Brazil; at 10 μL/g, Vetbrands Brazil, Brazil), and received an i.m. injection of glycerol solution (50% v/v, Merk, Brazil, 7 mL/kg), with half a dose in each gastrocnemius muscle. Then, animals were placed back in their cages in a heated environment until recovery.

The urine samples were placed in 2 mL tubes and stored at -20 ℃ until analysis. Then, 48 h after the injec-

tions, the animals were anesthetized with *i.p.* ketamine-xylazine solution (300 μL at 10 μL/g), and blood samples were collected by intracardiac puncture into non-heparinized 1.5 mL tubes. These samples were incubated for approximately 20 min at room temperature and were then centrifuged at 4 ℃ at 4000 rpm for 10 min. The serum was collected and stored in a 1.5 mL tube at -20 ℃ until analysis. After blood sample collection, the animals were sacrificed by cervical dislocation, and each kidney was removed, weighed and transversally cut. One piece was placed in one 2 mL tube and immediately immersed in liquid nitrogen and then stored at -80 ℃ until analysis, while the other half was placed in a 2 mL tube with 10% buffered formaldehyde solution for 24 h, then placed into a new tube with a 70% ethanol solution and stored until sectioning.

Renal function analysis

For renal function analysis, we measured serum creatinine (S_{Cr}) levels and urine creatinine levels (U_{Cr}) according to the method described by Jaffé with slight modifications[19]. Briefly, we deproteinized the samples by adding 100 μL of each serum sample to a 1.5 mL tube containing 200 μL of 1.84 % H_2SO_4 and 300 μL of sodium tungstate and thoroughly mixed the resulting solution. The tubes were maintained at room temperature for 15 min and then centrifuged for 10 min at 4000 rpm at room temperature. The supernatant was then collected and placed in another 1.5 mL tube. Then, 5.28 mL of picric acid was mixed with 1.32 mL of a 10% NaOH solution in a 14 mL falcon tube. Next, 100 μL of picric acid (Labtest Diagnostica SA, Brazil) was added to 63 wells of a 96-well flat-bottomed ELISA microplate (Cral plast, Brazil), followed by pipetting and mixing of 200 μL of Milliq H_2O (Millipore, United States) in triplicate for blank samples, 200 μL of standard protein at 5 mg/mL in triplicate, and 200 μL of each deproteinized sample in duplicate. The plate was incubated for 20 min and then read in an EPOCH spectrophotometer (Biotek Instruments Inc., United States) at 450 nm. To measure U_{Cr} levels, we used the same method, but we did not deproteinize the samples. Instead, we diluted the samples (1:125) and multiplied the results by 25. The blood urea nitrogen (BUN) level were measured by colorimetric reaction kit (Labtest diagnostica, Brazil) in spectrophotometer at 600 nm, following the manufacturer's instructions.

Urine collection

Urine was collected by direct punction in bladder, for measure of creatinine and urea levels. The urine was collected in 1.5 mL tubes.

We also quantified the total urine protein (mg/dL) by colorimetric assay at 660 nm using the Pierce Protein Assay Kit (Thermo Scientific, United States).

Gene expression quantification

Total RNA was extracted using the TRIzol Reagent method (Invitrogen, United States) from tissues and stored at -80 ℃. The samples were run on 1% agarose gels to evaluate the integrity of the samples, and the samples were then quantified using a nanoDrop (NanoDrop Technologies, Inc., United States). Single-stranded cDNA was synthesized for each sample, using MML-V reverse transcriptase (Promega, United States). The qRT-PCR was carried out using Taqman (Applied Byosystem, United States) probes for GAPDH, B_1R and B_2R gene expression, and SYBR Green Real-time polymerase chain reaction (PCR) (Applied Byosystem, United States) probes for β-actin (sense - 5' CTG GCC TCA CTG TCC ACC TT 3' , antisense - 5' CGG ACT CAT CGT ACT CCT GCT T 3'), TGF-β1 (sense - 5' TTA GGA AGG ACC TGG GTT GG 3' , antisense - 5' AAG TTG GCA TGG TAG CCC TT 3') and IL-1β (sense - 5' AGG AGA ACC AAG CAA CGA CA 3' , antisense - 5' CGT TTT TCC ATC TTC TTC TTT 3') gene expression. The plates were placed in a 7500 real-time PCR system (Applied Biosystems, United States) for reading. The fold change was calculated by taking the $2^{-\Delta CT}$ of each sample and dividing it by the wild type (WT) group average.

Histological analysis

Slices 5 μm thick were cut from the samples stored in 70% ethanol solution and stained with HE. The samples were analyzed using an optical microscope at 200 × magnification. The criteria used to analyze the tubular injury in the samples included epithelium desquamation, cellular debris in the lumen, flattening of epithelium, the presence of cylinders and dilation of the lumen. Based on these criteria, an expert classified and scored the injuries: grade Ⅰ (< 10%), grade Ⅱ (10%-25%), grade Ⅲ (25-50%), and grade Ⅳ (> 50%).

Statistical analysis

The values are expressed as the mean ± SE. Statistical analyses were carried out by one-way ANOVA (analysis of variance) followed by Tukey's *post-hoc* test, and associations were made by the Spearman correlation test. P values < 0.05 were considered statistically significant. The statistical software used for graphs and analysis was GraphPad Prism 5.

RESULTS

Renal function evaluation

The S_{Cr} levels were different between the control wild-type and glycerol, and knockout control group and glycerol, with glycerol groups greater about 4 times than control groups (Figure 1A). There were no significant differences in the U_{cr} levels, despite the wild-type showed about 11 times higher levels than glycerol wild-type group (Figure 1B). The BUN levels were different about 10 times between the control wild-type and glycerol, and about 8 times between control knockout mice and glycerol (Figure 1C). Urinary urea content differed between the control and glycerol groups, about 24 times for wild-type groups and about 33 times for knockout groups

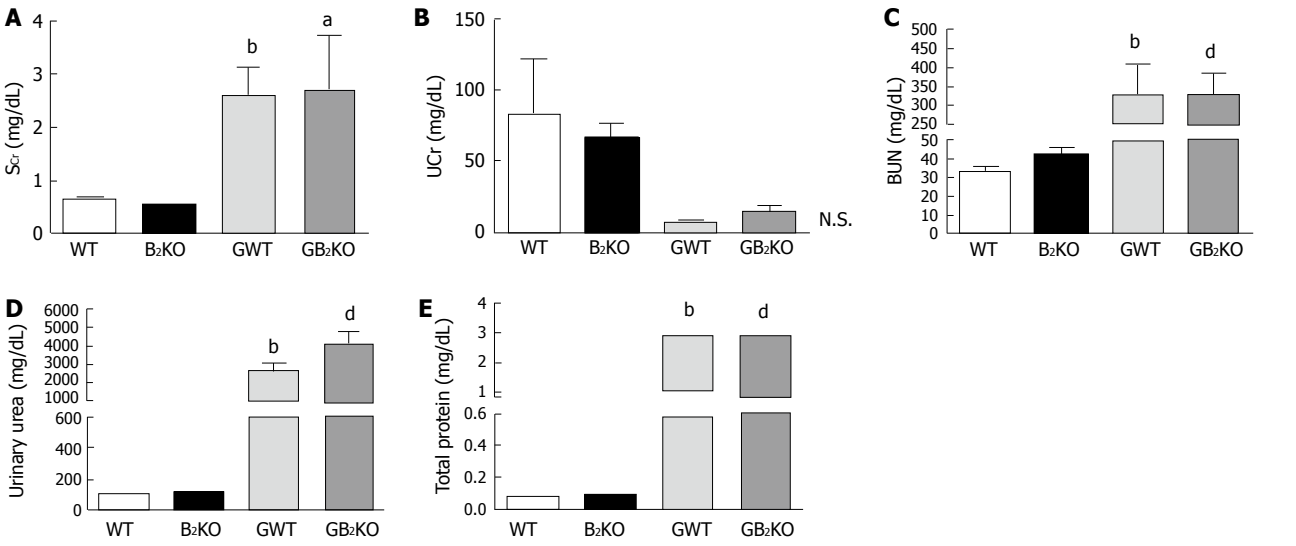

Figure 1 Serum and urinary parameters. A: S_{Cr} levels ($^aP < 0.05$ *vs* B_2KO, $^bP < 0.01$ *vs* WT); B: U_{Cr} levels; C: BUN levels ($^bP < 0.005$ *vs* WT, $^dP < 0.01$ *vs* B_2KO); D: Urinary urea ($^bP < 0.01$ *vs* WT, $^dP < 0.005$ *vs* B_2KO); E: Total urine proteins ($^bP < 0.005$ *vs* WT, $^dP < 0.005$ *vs* B_2KO). WT: Wild type; B_2KO: Kinin B2 receptor knockout mice; GWT: Glycerol wild type; B_2KO: Glycerol kinin B2 receptor knockout mice; BUN: Blood urea nitrogen.

Table 1 Serum and urinary creatinine

Variable	Groups			
	WT	B_2KO	GWT	GB_2KO
S_{Cr}	0.6621 ± 0.041	0.5584 ± 0.027	2.613 ± 0.536[b]	2.233 ± 0.867[a]
U_{Cr}	50.31 ± 29.24	32.80 ± 15.44	4.540 ± 2.137	9.084 ± 3.931

$^aP < 0.05$ *vs* B_2KO; $^bP < 0.01$ *vs* WT. B2KO: Kinin B2 receptor knockout mice; WT: Wild type; GB_2KO: Glycerol kinin B2 receptor knockout mice.

(Figure 1D). The levels of total urine proteins were different between the wild-type and glycerol group (around 42 times), while the difference between knockout and glycerol group was approximately 36 times. There were no difference between wild-type glycerol and knockout glycerol group (Figure 1E).

Gene expression evaluation

The fold changes in B_1R, B_2R, β-actin, IL-1β and TGF-β1 renal expression are shown in Figure 2. Also shown are the associations between these expression levels. The fold change of the B_2R was different between the control and glycerol groups about 30 times (Figure 2A). The B_1R fold change was different between the control and glycerol groups about 14 times, and between glycerol groups almost 2 times (Figure 2B). The associations were not different between the B_1R and B_2R (Figure 2C), but showed difference between B_2R and IL-1β, where greater expression of B_2R results in lower expression of IL-1β (Figure 2D), and between B_1R and TGF-β1 groups, with greater expression of B_1R when the expression of TGF-β1 is lower (Figure 2E).

Histological evaluation and histomorphometry

The histological evaluation and histomorphometry shown in Figure 3 demonstrate that both sets of animals (WT and B_2KO) had a high degree of tubular injury when injected with glycerol (Figure 3F and H), but WT had a higher degree IV compared with B_2KO.

DISCUSSION

It is described that the NO levels fall in rhabdomyolysis[4], and that the B_2R activation is related with NO production[18] we considered investigate the role of kinin B2 receptor in glycerol-induced rhabdomyolysis model.

Our findings demonstrate that the renal expression of B_2R in animals that received glycerol is 30 times greater than that of the controls. Another study showed a 5-fold increase of B_2R expression[15]. These results suggest that B_2R may be involved in the kidney inflammatory process. Interestingly, the peak renal expression of B_2R coincided with a significant reduction in renal function, which could suggest that the renal upregulation of these receptors occurs in response to renal injury.

Renal function was evaluated by measuring serum creatinine and BUN levels. In mice, particularly male C57Bl/6 mice, the normal serum creatinine level (S_{Cr}) is approximately 0.8 ± 0.1 mg/dL[20], which is similar to the values found in the control groups (Table 1). In a recent study[21], the authors showed using the glycerol model in rats, that the peak of renal function impairment occurs between 48 and 72 h, as evidenced by S_{Cr} levels. In our study, we investigated the peak of injury.

Kidney injury in the glycerol model is due to the release of nephrotoxic molecules and proteins (*e.g.*, myoglobin) into the bloodstream, causing damage upon reaching the kidney[4]. The literature describes, using rat and cell culture models[22-27], the deleterious role of this protein, specifically the heme prosthetic group, in the tubular endothelium. This role is associated with the production of reactive oxygen species (ROS) and free radicals in the mitochondria, which initiate lipid peroxidation reactions.

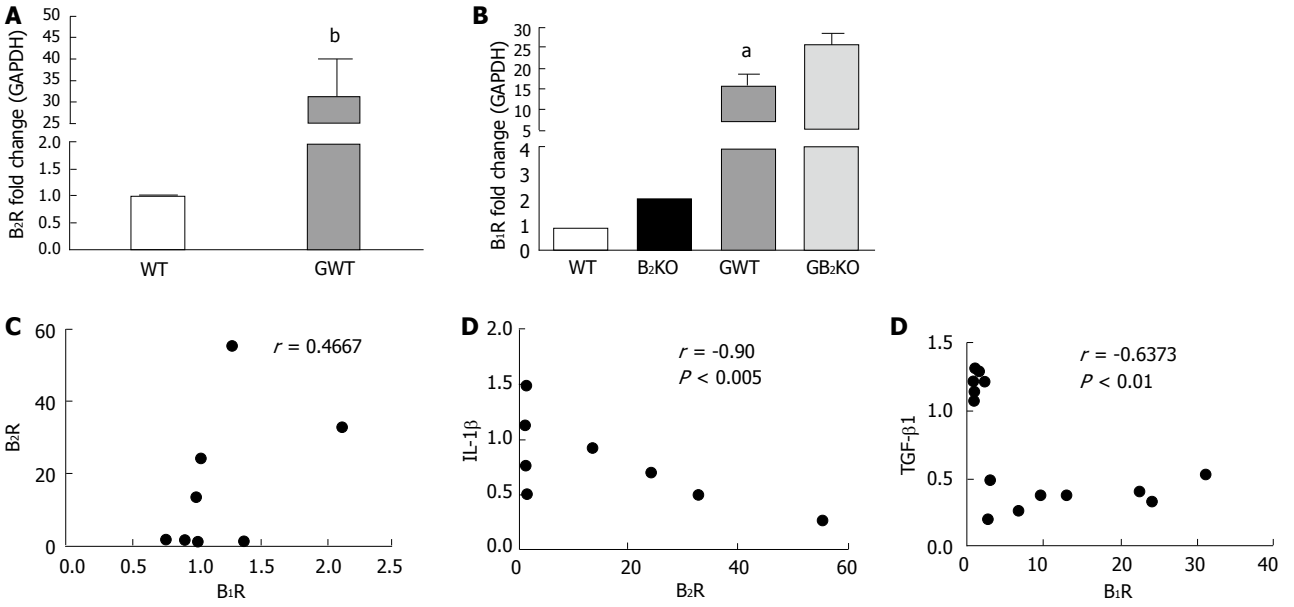

Figure 2 Renal gene expression and association between genes. A: B_2R fold change ($^bP < 0.01$ *vs* WT); B: B_1R fold change ($^aP < 0.05$ *vs* B_2KO); C: Association between B_2R and B_1R; D: Association between IL-1β and B_1R; E: Association between TGF-β1 and B_1R. WT: Wild type; B_2KO: Kinin B2 receptor knockout mice; GWT: Glycerol wild type; GB_2KO: Glycerol kinin B2 receptor knockout mice; BUN: Blood urea nitrogen.

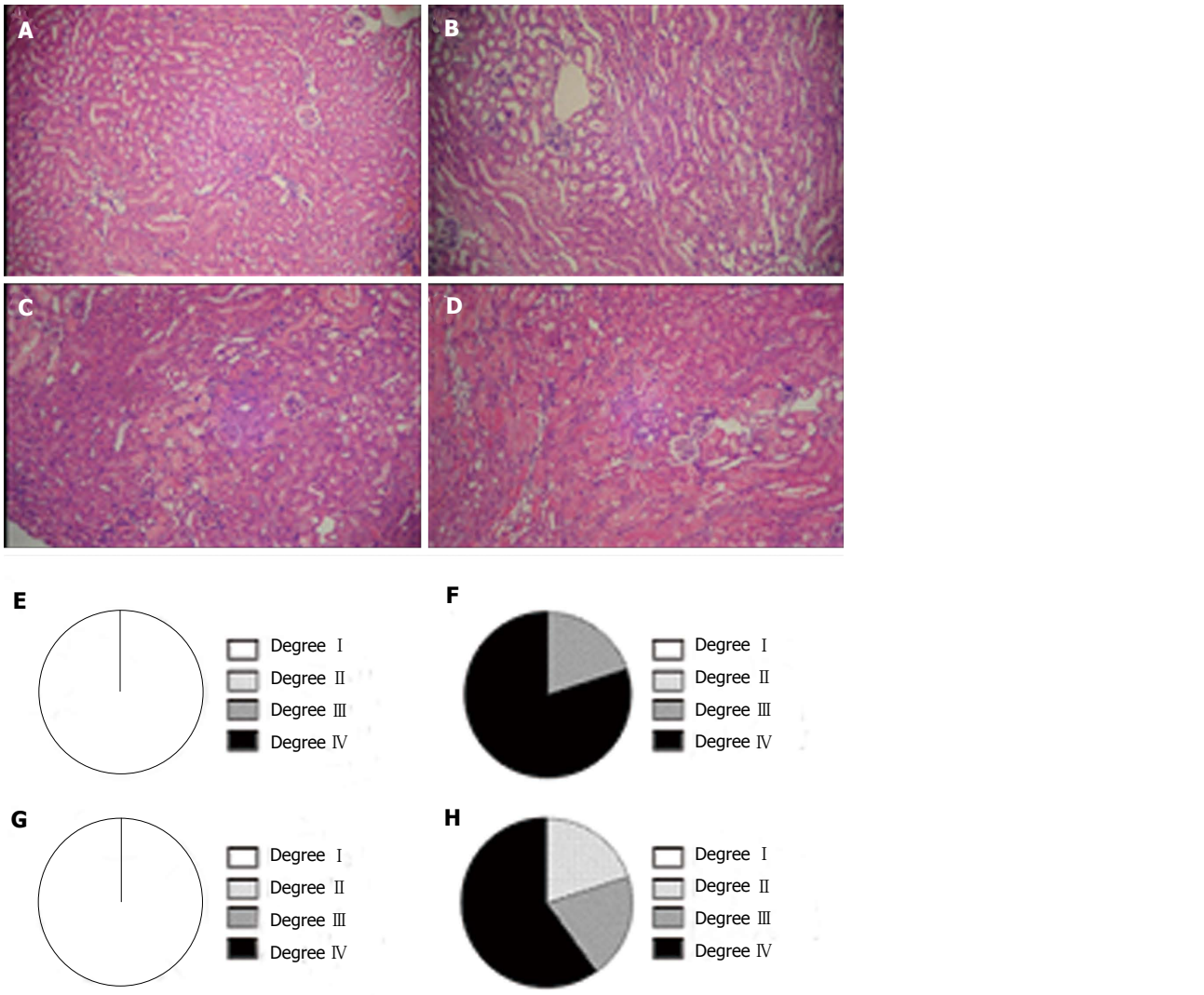

Figure 3 Histological evaluation and Graphs show the degrees of renal injury. A: Degree Ⅰ; B: Degree Ⅱ; C: Degree Ⅲ; D: Degree Ⅳ; E: WT; F: GWT; G: B_2KO; H: GB_2KO. WT: Wild type; B_2KO: Kinin B2 receptor knockout mice; GWT: Glycerol wild type; GB_2KO: Glycerol kinin B2 receptor knockout mice;

It has been shown that human skeletal muscle can survive for up to 3 or 4 h during circulatory ischemia[28]. In other studies[24], the authors showed that in the rat glycerol-induced rhabdomyolysis model, the renal mitochondria were already markedly degenerated 3 h after the glycerol application. Twenty-four hours after treatment, there were clear signs of tubular necrosis (proximal and distal). The authors attributed this to the formation of intra-tubular aggregates. This conclusion suggests that disruption of skeletal muscle can occur approximately 3 h after the glycerol administration.

Recent studies[28-31] show that deletion of B_1R and B_2R exacerbates the renal phenotype in diabetic mouse models, suggesting that both receptors have a protective effect on diabetic nephropathy by suppressing oxidative stress *via* NO and prostaglandins. However, because the absence of one of the receptors causes increased expression of the other, it is difficult to determine the precise function of each receptor[28]. In other models, such as the I/R model[15] the receptors' role was also studied. Blocking B_1R showed an antifibrotic effect, which therefore has a protective effect. Some authors[16] have demonstrated that renal injury by ischemia and reperfusion is significantly increased by B_2R activation and that this activation is related to increased production of ROS, suggesting that B_2R activation is deleterious.

Kinin receptors in renal tissue were studied in the I/R model, where it was shown that both receptors have a protective role in this type of injury[30]. However, a different study[15] found that during I/R in mice, the double knockout showed an extremely high S_{Cr} along with a proinflammatory profile. The renal B_2R expression alternated, beginning with low expression after 4 h, high expression after 24 h, and 48 h after the reperfusion onset, its expression level was similar to that observed at 4 h and then increased to values similar to those observed at 24 h post-reperfusion.

An important difference between the previous two studies[15,24] and ours is that in the other studies, ischemia and reperfusion were induced exclusively in the kidneys, whereas in our work, nephrotoxic kidney injury occurs after induction of primary skeletal muscle injury. Furthermore, in the model we use, there is no time for reperfusion to occur because the peak of injury would be 48 h later, allowing the kidneys to therefore remain in an ischemic state.

Regarding the release of proinflammatory cytokines (*e.g.*, IL-1β) and growth factors with a known profibrotic effect (TGF-β1), some authors[21] have shown that in the rat glycerol model, the mRNA expression levels of IL-1β peak 48 h after treatment, while in our work, the same expression levels were observed in GB_2KO animals, whereas GWT animals showed expression levels similar to those found 24 h post-treatment by these authors. In our study, we found a strong negative association between the expression of B_2R and IL-1β, which may indicate that B_2R does not induce inflammation in the kidneys.

Previous studies show[21] that renal TGF-β1 expression 48 h after *im* glycerol injection in mice is 1.5 times greater than our results indicate. It is important to note that the lineage of the animals used was different and that the endogenous gene studied was also different. Furthermore, in our work, we did not find any association between renal expression of B_2R and TGF-β1 (data not shown). Meanwhile, B_1R and TGF- β1 had a strong negative association.

Another important issue regarding analysis of TGF-β1 expression is the methodological difference between the studies. Although both studies use rodents, rats and mice have differences in several genes, including TGF-β1; the rat version is on chromosome 1, while the murine version is located on chromosome 7.

In conclusion, our results suggest that B_2R does not have a renoprotective role in mice with glycerol-induced rhabdomyolysis.

COMMENTS

Background

Acute kidney injury (AKI) is a common life-threatening disease that places a heavy burden on the health system and may be a step in the progression toward chronic kidney disease. It was reported that the mortality rate for this disease ranges from 10% to 80%, depending on the population studied. According to some authors, prevention is the key to avoiding the morbidity and mortality associated with AKI.

Research frontiers

Kinins is related with several models of inflammatory process in different organs as well in kidney. However, there is a lack of acknowledgments about kinins and rhabdomyolysis. The kinins are related with pathologies as well in physiology process. However, the absence of B2 receptor can induces the expression of the kinin B1 receptor and it difficult the determination of the precise function of each receptor. In this study, the authors demonstrated that the overexpression of kinin B_2 receptor could be involved with kidney injury once that the peak renal expression of kinin B_2 receptor coincided with a significant reduction in renal function.

Innovations and breakthroughs

This is the first study reporting that kinin B_2 receptor is over-expressed in kidney in rhabdomyolysis model. Furthermore, these results suggest that the overexpression may be one of the causes of kidney injury in this animal model.

Applications

Kinin B2 receptor antagonist could be tested in future to avoid kidney injury in rhabdomyolysis process.

Terminology

Rhabdomyolysis is a skeletal muscle disease where the muscle tends to break, that commonly result in acute kidney injury. Bradykinin is a nonapeptide messenger that is enzymatically produced from kallidin and that act via activation of two membrane receptors: kinin B_1 receptor and kinin B_2 receptor. Kinin B_1 receptor is a bradykinin receptor that is induced in response to inflammation, it may play a role in chronic inflammation. Kinin B_2 receptor is a bradykinin receptor that is constitutively expressed and may play a role in the acute phase of inflammation.

Peer review

Gattai *et al* reported that the kinin B_2 receptor knockout. The authors provided detailed data on this and the manuscript is well written.

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P- Reviewer: Fujigaki Y, Watanabe T **S- Editor**: Song XX
L- Editor: A **E- Editor**: Wu HL

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Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.5527/wjn.v3.i3.92

World J Nephrol 2014 August 6; 3(3): 92-100
ISSN 2220-6124 (online)

RETROSPECTIVE STUDY

Role of endoscopic ultrasound fine-needle aspiration evaluating adrenal gland enlargement or mass

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Author contributions: Martinez M and DeWitt J contributed equally to this work, abstracted data from medical records and analyzed data; Martinez M, DeWitt J, Al-Haddad M, Sherman S and LeBlanc J wrote the paper.

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Received: December 4, 2013 Revised: February 18, 2014
Accepted: May 8, 2014
Published online: August 6, 2014

Abstract

AIM: To report the clinical impact of adrenal endoscopic ultrasound fine-needle aspiration (EUS-FNA) in the evaluation of patients with adrenal gland enlargement or mass.

METHODS: In a retrospective single-center case-series, patients undergoing EUS-FNA of either adrenal gland from 1997-2011 in our tertiary care center were included. Medical records were reviewed and results of EUS, cytology, adrenal size change on follow-up imaging ≥ 6 mo after EUS and any repeat EUS or surgery were abstracted. A lesion was considered benign if: (1) EUS-FNA cytology was benign and the lesion remained < 1 cm from its original size on follow-up computed tomography (CT), magnetic resonance imaging or repeat EUS ≥ 6 mo after EUS-FNA; or (2) subsequent adrenalectomy and surgical pathology was benign.

RESULTS: Ninety-four patients had left (n = 90) and/or right (n = 5) adrenal EUS-FNA without adverse events. EUS indications included: cancer staging or suspected recurrence (n = 31), pancreatic (n = 20), mediastinal (n = 10), adrenal (n = 7), lung (n = 7) mass or other indication (n = 19). Diagnoses after adrenal EUS-FNA included metastatic lung (n = 10), esophageal (n= 5), colon (n = 2), or other cancer (n = 8); benign primary adrenal mass or benign tissue (n = 60); or was non-diagnostic (n = 9). Available follow-up confirmed a benign lesion in 5/9 non-diagnostic aspirates and 32/60 benign aspirates. Four of the 60 benign aspirates were later confirmed as malignant by repeat biopsy, follow-up CT, or adrenalectomy. Adrenal EUS-FNA diagnosed metastatic cancer in 24, and ruled out metastasis in 10 patients. For the diagnosis of malignancy, EUS-FNA of either adrenal had sensitivity, specificity, positive predictive value and negative predictive value of 86%, 97%, 96% and 89%, respectively.

CONCLUSION: Adrenal gland EUS-FNA is safe, minimally invasive and a sensitive technique with significant impact in the management of adrenal gland mass or enlargement.

Key words: Adrenal gland neoplasms/diagnosis; Adrenal glands/pathology; Adrenal gland/ultrasonography; Adrenal gland neoplasms/secondary; Endosonography; Biopsy; Fine-needle

Core tip: Studies evaluating endoscopic ultrasound fine-needle aspiration (EUS-FNA) of the adrenal gland generally include patients with underlying malignancy only and most lack follow-up for benign lesions. We report the clinical utility of adrenal gland EUS-FNA in a retrospective study that included 94 patients who underwent EUS-FNA of either adrenal for various indications and provide follow-up information for those with benign EUS-FNA cytology results. For the diagnosis of malignancy, EUS-FNA of either adrenal had sensitivity, specificity, positive predictive value and negative predictive value of 86%, 97%, 96% and 89%, without

serious adverse events.

Martinez M, LeBlanc J, Al-Haddad M, Sherman S, DeWitt J. Role of Endoscopic Ultrasound Fine-needle aspiration evaluating adrenal gland enlargement or mass. *World J Nephrol* 2014; 3(3): 92-100 Available from: URL: http://www.wjgnet.com/2220-6124/full/v3/i3/92.htm DOI: http://dx.doi.org/10.5527/wjn.v3.i3.92

INTRODUCTION

The development of modern imaging techniques such as ultrasonography, computed tomography (CT), and magnetic resonance imaging (MRI), has led to increased detection of adrenal masses, which are found in up to 5% of patients undergoing CT of the abdomen[1]. The incidence of an adrenal incidentaloma (detection of an otherwise unsuspected adrenal mass on imaging), ranges from 0.2%-7% as reported in autopsy series[2]. Most of these incidentally found lesions are non-functioning adenomas, but 2% are metastatic lesions[3].

About 75% of adrenal masses identified during staging of patients with cancer are metastatic lesions which are most commonly metastases from lung, breast, stomach and kidney, as well as, melanomas and lymphomas[2]. The sensitivity and specificity of imaging techniques are currently insufficient to differentiate benign from malignant masses, therefore, patients with a high index of suspicion for malignancy are often referred for percutaneous biopsy[4].

Image-guided fine needle aspiration (FNA), using either ultrasound (US) or CT and percutaneous approach, have traditionally been used for sampling of the adrenal glands[5,6]. However, this technique yields non-diagnostic samples in up to 14% of patients and is associated with adverse events in 0.4%-12%[7,8].

Endoscopic ultrasound guided-fine needle aspiration (EUS-FNA) of either adrenal offers a minimally invasive and accurate method for sampling the adrenals with a low risk profile[3,9-12]. However, studies to date have mostly included patients with underlying malignancy and the great majority lack follow-up imaging for benign lesions or include follow-up for few patients[4,12,13]. This study reports the utility of EUS-FNA in patients with known adrenal gland enlargement or a mass, and the impact of the EUS-FNA cytology result on patient care, final diagnosis and adverse events from the procedure.

MATERIALS AND METHODS

This retrospective single-center case series was approved by the institutional Review Boards at the Indiana University Health School of Medicine in Indianapolis Indiana. Cytology and EUS databases between October 1997 and December 2011 were reviewed to identify all patients who underwent EUS-FNA of either adrenal gland. The original 38 patients were previously described in a 2007 publication from our hospital[3]. Medical records were reviewed and results of imaging (CT and MRI) prior to the procedure, EUS indications and findings, cytological investigations and complications were recorded. In addition, follow-up clinical information and any repeat adrenal imaging or surgery of the adrenal gland was abstracted. For patients without available follow-up on our medical records, referring physicians were contacted by phone to obtain this information. Through institutional protocol, all patients were called within 48 h after EUS to assess for any short-term adverse events not already identified. Adverse events were defined as: systolic blood pressure less than 80 mmHg at any time during the procedure, hypoxemia (oxygen saturation less than 85% on room air or on baseline oxygen supplementation), bradycardia (heart rate less than 50 beats per minute), bleeding recognized during EUS or subsequent imaging studies with hemoglobin drop of $\geqslant$ 2 g/dL from baseline, need for blood transfusion within 48 h of the procedure, pneumothorax, abdominal pain, hypertensive urgency and, requirement for hospitalization.

EUS

After obtaining written informed consent, patients received conscious sedation using various combinations of intravenous midazolam, meperidine, fentanyl or propofol under appropriate cardiorespiratory monitoring. All procedures were done by or under the supervision of one of seven attending endoscopists. Radial endosonography (Olympus GFUM-20, GFUM-130, GFUM-160 or GFUE160-AL5; Olympus America, Center Valley, PA; United States), was performed initially in some patients. Linear EUS (Olympus GF-UC30P, Olympus GF-UC140P, or Pentax 32-UA or 36-UX; Pentax Medical, Montvale, NJ; United States), was performed in all patients.

The left adrenal gland was visualized by one of 2 methods. First, the descending aorta was followed to the celiac axis; once this was seen, the left adrenal gland was visualized after a slight clockwise rotation and withdrawal movement. Alternatively, the splenic vein posterior to the body of the pancreas was identified by transgastric imaging; clockwise rotation and withdrawal of the echo endoscope following the splenic vein laterally then permitted the identification of the left adrenal gland superior to the upper pole of the left kidney. Transduodenal imaging of the right adrenal gland with EUS was performed with the echoendoscope in the long position along the greater curvature of the stomach. The inferior vena cava or the right kidney was then visualized, and then right adrenal gland was uniformly present between the superior pole of the right kidney, the liver and the inferior vena cava. EUS exams for patients in this study attempted to image a known or suspected adrenal mass or enlargement and did not routinely attempt to visualize both adrenal glands

The size of the adrenal gland for study purposes was the maximal cross-sectional diameter of the gland. An adrenal gland mass was considered to be a focal enlarge-

ment of the gland with a notable discrete mass, whereas, adrenal gland enlargement was considered when the gland was diffusively increased size without a visible discrete mass.

EUS-FNA was performed using a 19, 22 or 25 gauge, 8 cm needle (Cook-Medical, Winston-Salem, NC; United States or Boston Scientific, Natick, MA; United States). Minimal clotting parameters required to perform EUS-FNA were a platelet count of $\geqslant$ 50000 and INR $\leqslant$ 1.5. Color Doppler imaging was used to ensure the absence of intervening vascular structures along the anticipated needle path. After needle puncture of the adrenal gland, the stylet was removed. At the discretion of the endosonographer, suction was applied to the proximal end of the needle with a vacuum containing syringe. If excess blood was present in the initial specimen, subsequent passes with the same needle were attempted without suction. There was no maximum number of biopsy attempts allowed. Biopsy attempts were performed at the discretion of the endosonography until considered that useful clinical information was provided, or that further attempts would be futile. According to our routine endoscopy unit protocol, patients were monitored in the recovery area after EUS imaging for at least 60 min before discharge. No additional monitoring was performed after adrenal biopsy.

Cytological examination

Aspirates were expressed and smeared onto 2 glass slides. One slide was air-dried and stained with a modified Giemsa stain for on-site interpretation, while the other slide was alcohol-fixed and stained using the Papanicolaou method. A cytotechnologist and/or cytopathologist, not blinded to the patient's clinical history, were available on-site for real-time preliminary interpretation for all procedures; this added an additional 2-3 min to the procedure for each FNA pass. Additional aspirates were submitted for immunocytochemical analysis at the discretion of the cytopathologist to confirm metastatic malignancy when required.

Cytology reports were characterized as "diagnostic for malignancy", "benign adrenal tissue", or "non-diagnostic". The following were considered to be cytologic features of benign adrenocortical tissue: clusters of cells with a foamy cytoplasm and smoothly contoured, round to oval nuclei, all within a vacuolated or foamy background with occasional single cells[13]. Diagnostic cytology specimens were considered to include any of the following: benign-appearing cytologic features of the adrenal gland, primary adrenal neoplastic tissue, or metastatic malignant cells. Non-diagnostic cytology specimens had none of these three features but did show any of the following: amorphous debris, blood, or gastric contaminant.

Study definitions

The final diagnosis was made on the basis of the surgical pathology if resection was performed, unequivocal cytology from EUS-FNA, clinical follow-up, or the stability of lesion size as assessed by subsequent imaging studies. An adrenal lesion was considered stable (and therefore benign) if size was within 1 cm by follow-up imaging (CT or MRI) obtained at least 6 mo after EUS-FNA[14]. EUS-FNA of either adrenal gland was considered to have had an impact on patient care if the cytology resulted in either: (1) benign cytology which excluded adrenal metastasis and permitted resection of the primary tumor; or (2) initial diagnosis of malignancy, distant metastasis, tumor recurrence or primary adrenal neoplasm.

Statistical analysis

For analysis, continuous variables were described as means and standard deviations, and dichotomous variables were expressed as simple proportions, with or without 95%CI. Student's *t* test and Fisher's exact tests were used to test for differences in comparisons between continuous and dichotomous variables, respectively. For calculating test characteristics of EUS-FNA for the diagnosis of malignancy, only aspirates interpreted as diagnostic for malignancy on cytological examination were considered as true positives. Patients with subsequent adrenalectomy, percutaneous adrenal biopsies or follow-up abdominal imaging of the adrenal at least 6 mo after EUS were utilized to calculate the test characteristics of EUS-FNA for the diagnosis of non-malignant (benign or non-diagnostic) specimens. 95% confidence intervals were calculated when appropriate. A *P* value less than 0.05 was considered statistically significant.

RESULTS

94 consecutive patients (52% men; median age: 66 years, range 32-86) underwent 95 attempted EUS-FNA of the left (n = 90) and/or right (n = 5) adrenal gland during the study period. There were no adverse events related to these procedures. Patient characteristics and EUS findings by results of diagnostic and non-diagnostic biopsies are summarized in Table 1. Patients with diagnostic malignant biopsies had smaller lesions than those with diagnostic benign lesions (P = 0.027) otherwise the clinical and EUS features of the two groups were similar. Indications for EUS in all 94 patients are summarized in Table 2. Known adrenal gland enlargement, fullness or mass according to previous imaging was present in 55 (59%). A previous diagnosis of cancer was present in 40 patients (42%) (Table 3).

Prior attempt with percutaneous CT-guided approach for adrenal biopsy was performed and unsuccessful in 3 patients, two of them subsequently had a diagnostic adrenal EUS-FNA (1 malignant, 1 benign); the third patient had a non-diagnostic EUS-FNA of the adrenal gland.

EUS findings and cytology

The mean maximal diameters for the right and left adrenal masses were 3.5 $\pm$ 0.88 cm and 2.72 $\pm$ 1.36 cm, respectively. EUS identified an adrenal mass in the 5 (100%) patients who underwent right adrenal EUS-FNA and in 75/90 (83%) who underwent left EUS-FNA. The left 15 adrenals

Table 1 Patients characteristics and endoscopic ultrasound findings *n* (%)

Characteristics	Diagnostic (*n* = 85)		Non-diagnostic (*n* = 9)	*P* value
	Benign (*n* = 60)	Malignant (*n* = 25)		
Age (mean ± SD)	67 ± 11	63 ± 14		0.16[1]
	66 ± 12		66 ± 11	0.99[2]
Race				
White	57 (95)	25 (100)	7 (78)	
African American	3 (5)	0 (0)	2 (22)	
Hispanic	0 (0)	0 (0)	0 (0)	
Gender				⩾ 0.99[3]
Male	26 (27)	19 (20)	5 (5)	
Female	34 (36)	6 (7)	4 (5)	
Adrenal biopsied				⩾ 0.99[4]
Left	58 (61)	23 (24)	9 (10)	
Right	2 (2)	3 (3)	0 (0)	
EUS image of adrenal				
Mass	49 (52)	25 (26)	6 (6)	0.14[5]
Diffuse enlargement	11 (12)	1 (1)	3 (3)	0.09[6]
Size by EUS, cm				
Mean ± SD	3.4 ± 1.6	2.6 ± 1.2		0.027[7]
Mean ± SD	2.8 ± 1.4		2.4 ± 1.2	0.41[8]
Range	0.7-5.2	1.3-7.0	1.0-4.0	
Echogenicity				
Hypoechoic	40 (42)	22 (24)	4 (4)	
Hyperechoic	1 (1)	0 (0)	1 (1)	0.14[9]
Not reported or unavailable	19 (20)	4 (4)	4 (4)	
Number of FNA passes				
Mean ± SD	3.0 ± 1.7	3.0 ± 1.3	3.1 ± 1.5	0.461

[1]Mean age diagnostic vs non-diagnostic; [2]Diagnostic *vs* non-diagnostic cytology result based on gender; [3]Adrenal Gland FNA side and Diagnostic vs non-diagnostic cytology result; [4]Presence or absence of an adrenal mass and diagnostic *vs* non-diagnostic cytology result; [5]Presence of absence of an adrenal mass and benign *vs* malignant FNA cytology; [6]Median size by EUS (cm) and malignant *vs* benign FNA cytology; [7]Median size by EUS (cm) and Diagnostic *vs* non-diagnostic cytology; [8]Adrenal Echogenicity on EUS and Diagnostic *vs* non-diagnostic cytology; [9]Number of FNA passes; and Diagnostic *vs* non-diagnostic FNA; [10]Mean age benign vs malignant. EUS: Endoscopic ultrasound; FNA: Fine-needle aspiration.

without mass demonstrated only diffuse enlargement (one patient had bilateral adrenal EUS-FNA) (Table 1).

Nine aspirations were non-diagnostic (9.5%). Four of these, had a previous diagnosis of cancer and 6 had an identified adrenal mass during EUS with a mean mass diameter of 2.4 ± 1.2 cm. Non-diagnostic aspirations occurred mostly before 2004, however the frequency before and after 2004 was not different ($P = 0.14$), and this was considered to be related to operator's learning curve (Table 4).

Diagnostic cytology was obtained in 86 biopsies after a mean of 3.2 ± 1.4 needle passes. There was no statistical significance between the number of needle passes for diagnostic biopsies and non-diagnostic biopsies ($P = 0.98$). All nondiagnostic biopsies were from the left adrenal gland; all right adrenal biopsies were diagnostic. Ninety-one fine-needle aspirations were performed with a 22G needle and included all the specimens that yielded a non-diagnostic sample. Only 3 and 1 biopsies on these series were obtained with a 25 G and a 19 G needle, respectively.

Table 2 Indications for endoscopic ultrasound

Indication for EUS	*n* (%)
Cancer staging[1]	26 (27)
Suspected cancer recurrence[2]	5 (6)
Abnormal CT/PET-CT or MRI	
Pancreatic mass	20 (21)
Mediastinal mass	10 (11)
Lung mass	7 (7)
Adrenal mass	7(7)
Gastric mass	2 (2)
Liver mass	3 (3)
Kidney mass	1 (1)
Retroperitoneal mass	1 (1)
Other[3]	12 (13)
Total of patients	94

[1]Esophageal cancer (*n* = 3), gastric cancer (*n* = 2), breast (*n* = 1), jejunal adenocarcinoma (*n* = 1), renal cell cancer (*n* = 2), cholangiocarcinoma (*n* = 1), lung cancer (*n* = 16); [2]Suspected recurrence of oral cancer (*n* = 1), breast cancer (*n* = 1), hepatoma (*n* = 1), lung adenocarcinoma (*n* = 1), esophageal adenocarcinoma (*n* = 1); [3]Chronic pancreatitis (*n* = 3), abnormal upper endoscopy (*n* = 3), common bile duct stricture (*n* = 2), celiac nerve block (*n* = 1), suspected metastatic disease on imaging (*n* = 1), Barrett's esophagus with high grade dysplasia (*n* = 1), ectatic pancreatic duct (*n* = 1). EUS: Endoscopic ultrasound; PET: Positron emission tomography; CT: Computed tomography.

Table 3 Previous diagnosis of cancer in patients undergoing endoscopic ultrasound guided fine-needle aspiration

Previous diagnosis of cancer (*n* = 40)	Benign cytology on EUS-FNA (*n* = 21)	Malignant cytology on EUS-FNA (*n* = 15)	Non-diagnostic cytology on EUS-FNA (*n* = 4)
Penile cancer	0	1	0
Oral SCC	0	1	0
Lung cancer	15	3	1
Renal cell carcinoma	0	2	1
Esophageal ADC	1	3	0
Breast cancer	1	1	0
Gastric ADC	1	1	0
Hepatocellular carcinoma	0	1	0
Pulmonary carcinoid	0	0	1
Colon ADC	0	1	1
SCC of the duodenum	1	0	0
Basal cell cancer of the skin	1	0	0
Bladder cancer	1	0	0
Melanoma	0	1	0

EUS-FNA: Endoscopic ultrasound guided fine-needle aspiration; SCC: Squamous cell carcinoma; ADC: Adenocarcinoma.

Adrenal gland FNA was malignant in 26% (*n* = 25) and benign in 64% (*n* = 60). Details about adrenal gland EUS-FNA cytology results are summarized in Table 5.

Clinical follow-up

Follow-up was available for 36/60 (60%) patients with benign adrenal cytology. The remaining 24 patients either were: lost to follow-up (*n* = 4), did not get repeat adrenal gland imaging (*n* = 5) or died (*n* = 15). The 15 patients died a mean of 28 ± 36 mo after EUS without follow imaging.

Table 4 Timing of diagnostic and non-diagnostic biopsies *n* (%)

Timing of EUS-FNA	Diagnostic EUS-FNA	Non diagnostic EUS-FNA	Total EUS-FNA
Before 01/2004	31 (33)	6 (7)	37
After 2004	54 (57)	3 (3)	57
Total	85 (90)	9 (10)	94

Non diagnostic EUS-FNA before 2004 vs after 2004 (P = NS). EUS-FNA: Endoscopic ultrasound guided fine-needle aspiration.

Available follow-up for 5/9 (55%) patients with non-diagnostic biopsies, demonstrated a stable adrenal lesion on repeat CT or MRI; the remaining four died before follow-up imaging (Figure 1). Median follow-up for benign and non-diagnostic biopsies was 24 mo (range 4-96) and 12 mo (range 7-36), respectively.

In 36 patients with benign adrenal cytology, available follow-up from imaging in 28 showed a stable adrenal lesion on CT (n = 27) or repeat EUS (n = 1). Five additional patients underwent adrenalectomy and without repeat imaging in 4. In these five, surgical pathology was benign in 4 and demonstrated an adrenocortical carcinoma in 1 (Table 6). For the remaining three, 2 had subsequent CT-guided adrenal biopsy showing metastatic non-small cell lung cancer in one (4 mo after EUS) and large cell neuroendocrine tumor in another (EUS-FNA biopsy of the pancreas had previously showed neuroendocrine tumor). Finally, one patient had follow-up CT 6 mo after EUS that demonstrated a new contralateral adrenal mass with findings of metastatic disease to the adrenals (Table 6).

In one additional patient with history of melanoma, CT scan for surveillance revealed a left adrenal mass. EUS-FNA of the mass was malignant, however, adrenalectomy 1 mo later showed benign pathology.

Clinical impact of EUS-FNA

For the diagnosis of malignancy EUS-FNA of the adrenal gland had a sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of 86% (95%CI: 68%-95%), 97% (95%CI: 83%-100%), 96% (95%CI: 79%-100%) and 89% (95%CI: 74%-96%), respectively. The sensitivity, specificity, positive predictive value and negative predictive value of adrenal gland EUS-FNA for benign lesions was 97% (95%CI: 83%-100%), 86% (95%CI: 68%-95%), 89% (95%CI: 74%-96%) and 96% (95%CI: 79%-100%), respectively.

The diagnostic accuracy of adrenal gland EUS-FNA was 92% for both benign and malignant lesions.

Only 2 patients died within 6 mo of the procedure. If these two were hypothetically included as false-negative biopsies, test characteristics for the diagnosis of malignancy would change to: sensitivity 80%, specificity 97%, positive predictive value 96% and negative predictive value to 84%.

In patients with benign adrenal gland cytology, EUS-FNA ruled out adrenal metastasis in 10 patients with underlying malignancy available follow-up (adrenalectomy or follow-up imaging). EUS-FNA of the adrenal gland made the initial diagnosis of stage Ⅳ cancer in 18 patients (lung cancer in 10, undifferentiated carcinoma in 1 and, esophageal in 4, colon in 2 and pancreatic adenocarcinoma in 1), and initial diagnosis of cancer recurrence in 6 patients (RCC in 2, oral SCC in 1, HCC in 1, esophageal cancer in 1 and breast cancer in 1).

Table 5 Cytology results from adrenal gland endoscopic ultrasound guided fine-needle aspiration

EUS-FNA cytologic diagnosis	*n*
Malignant EUS-FNA cytology (26%, n = 25)	
Metastatic lung cancer	10
Metastatic esophageal adenocarcinoma	5
Metastatic colon adenocarcinoma	2
Metastatic renal cell carcinoma	2
Metastatic breast adenocarcinoma	1
Metastatic pancreatic adenocarcinoma	1
Metastatic melanoma	1
Metastatic oral squamous cell carcinoma	1
Metastatic hepatocellular carcinoma	1
Undifferentiated carcinoma	1
Benign EUS-FNA cytology (64%, n = 60)	
Benign adrenal tissue	57
Aldosteronoma	1
Paraganglioma	1
Pheochromocytoma[1]	1

[1]Previously negative normal plasma catecholamines and, 24-h urine normetanephrines, vanillylmandelic acid and metanephrines. EUS-FNA: Endoscopic ultrasound guided fine-needle aspiration.

Benign cytology and exclusion of metastases in 10/36 patients with malignancy or a precancerous lesion (non-small cell cancer in 7, gastrointestinal stromal tumor in 1, esophageal adenocarcinoma in 1, and gastric adenocarcinoma in 1) permitted subsequent surgery. EUS-FNA of the adrenal gland confirmed an initial diagnosis of unsuspected pheochromocytoma in one patient. Finally, unnecessary surgery was avoided in 18 patients with metastatic disease and 6 patients with cancer recurrence.

DISCUSSION

Adrenal gland adenomas are discovered in 5% of abdominal CT exams, in 2%-9% of autopsy studies and up to 4%-7% of patients with potentially resectable lung cancer, therefore accurate characterization of these lesions in cancer patients is essential[12,15]. Unfortunately, sensitivity and specificity of imaging techniques are currently insufficient to differentiate benign from malignant masses and, false-negative and false-positive rates by CT scan both average 10%[4].

Distinguishing a metastatic lesion from a primary adrenal tumor is aided by the knowledge of past cancer type and contrast-enhanced CT of the chest, abdomen and pelvis. Fluorodeoxyglucose (FDG)-positron emission tomography (PET)/CT is increasingly used in re-staging protocols for FDG-avid malignant tumors and can aid to document other extra-adrenal metastatic lesions[16]. According to the AACE/AAES (American As-

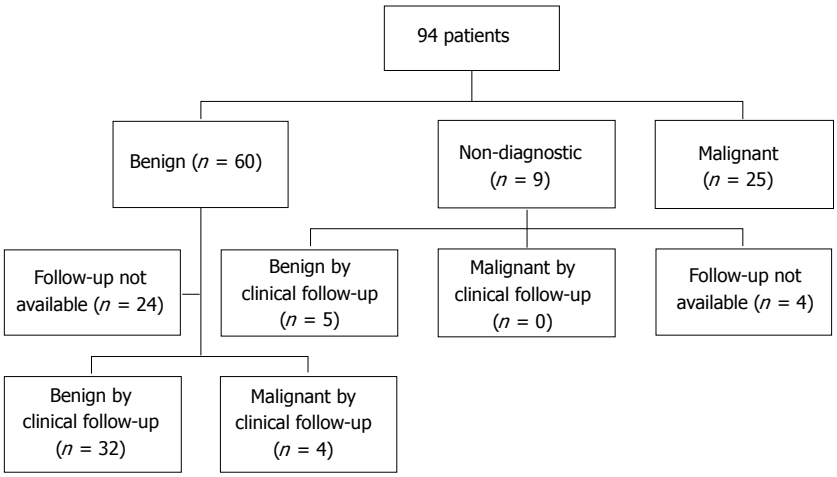

Figure 1 Patient flowchart .

Table 6 Final diagnosis for patients with non-malignant biopsies for who follow up was available

Final diagnosis	Benign FNA	Non-diagnostic FNA
Confirmed benign on follow up	32[1]	5[1]
Confirmed malignant on follow up	4[2]	0
Total of patients with follow up	36	5

[1]Follow up CT; [2]Subsequent CT-guided adrenal biopsy (*n* = 2), enlargement on repeat CT (*n* = 1) or adrenalectomy (*n* = 1). FNA: Fine-needle aspiration; CT: Computed tomography.

sociation of Clinical Endocrinologists and American Association of Endocrine Surgeons) guidelines, CT-guided FNA of an adrenal lesion can be performed to confirm metastatic disease if a definitive diagnosis is needed for oncologic treatment planning[16].

In our series, the sensitivity, specificity, PPV and NPV of adrenal gland EUS-FNA for the diagnosis of malignancy was of 86%, 97%, 96% and 89%, respectively. These results are similar to other series which report adrenal gland EUS-FNA sensitivity and negative predictive value rates ranging from 86%-100% and 70%-100%, respectively yet most of these studies have only included patients with underlying lung cancer[6,13].

With the widespread availability of CT and therefore percutaneous CT-guided fine-needle aspiration of the adrenals, the use of EUS-FNA to obtain adrenal gland biopsy could be questioned. While percutaneous CT-guided adrenal gland EUS-FNA of lesions of 2.8-5 cm in size, has been reported to be reliable and predict a benign course on long term follow up in patients with a benign cytology result[17], the reported rate of complications from percutaneous CT-guided adrenal gland EUS-FNA ranges from 0%-12% with an overall rate of 5.3%[7,15]. The most frequent adverse events related to percutaneous adrenal biopsies include hemorrhage and pneumothorax. Less common adverse events are pain, pancreatitis, and rarely needle-tract seeding. In our study we identified no short term (< 48 h) adverse events in any patient and no adverse events in those with available long term follow-up. In the current series, we performed diagnostic left adrenal biopsies in 2 of 3 patients in whom percutaneous approach of the left adrenal gland had been previously attempted unsuccessfully. These findings have been reported by others and emphasize that EUS-FNA may be utilized as a rescue procedure for those in whom percutaneous biopsies are contraindicated or unsuccessful[9]. Taken together, EUS-FNA appears to be a safe procedure and an acceptable alternative to percutaneous sampling of the adrenal glands.

About 5% of all incidentally discovered adrenal lesions are pheochromocytomas, and 25% of all pheochromocytomas are discovered incidentally. Typical features of pheochromocytomas include paroxysmal hypertension, headaches, sweating and palpitations; but, patients may not present with classical symptoms and up to 8% may be asymptomatic[18]. Sood *et al*[18] reported 3 cases of patients with catecholamine secreting tumors who underwent CT-guided percutaneous mass biopsy, including one with a pheochromocytoma and did not experience any adverse events related to the biopsy. In our series, one patient was unexpectedly diagnosed with pheochromocytoma by EUS-FNA and did not experience any adverse events from the procedure.

EUS shows a normal or minimally enlarged left adrenal gland in 98% of patients compared with only a 69% by transabdominal ultrasound[19]. A normal or minimally enlarged right adrenal gland, however, is seen in only 30% of patients on EUS, whereas transabdominal ultrasound permits detection in nearly all patients. Therefore, left adrenal EUS-FNA is attempted more often than right adrenal biopsies[19]. Recently, Uemura reported a rate of visualization of the right adrenal gland of 87.3% (*n* = 150) on EUS[13]. To date, there have been only a few reports of successful right adrenal gland EUS-FNA, but no large case-series[9-12,20]. The utility of EUS-FNA of right adrenal masses requires further clarification.

In our case series, the median adrenal gland diameter was higher in patients with diagnostic benign biopsies compared to malignant FNA specimens. This is in contrast with the results reported by Eloubeidi *et al*[12] who found larger masses in patients with malignancy (3.1 cm) compared to those with benign lesions (2.3 cm). A potential reason for this difference is that our group has

Table 7 Comparison of different Studies evaluating adrenal gland endoscopic ultrasound guided fine-needle aspiration

Ref.	Year	Number of patients	Patient population	EUS-FNA Left adrenal, *n*	Patient population	EUS-FNA Left adrenal, *n*	EUS-FNA Right adrenal, n	Benign EUS-FNA cytology, *n*	Malignant EUS-FNA cytology (*n*)	Non-Diagnostic rate	Sensitivity	Specificity	PPV	NPV	F/U for benign lesions	Method for F/U
Current research	2014	94	Patients undergoing EUS-FNA of either adrenal	94	Patients undergoing EUS-FNA of either adrenal	90	5	60	25	10%	86%	97%	96%	89%	Available on 36/60	CT/MRI, repeat EUS at ⩾ 6 mo or surgical pathology from adrenalectomy
[1]Uemura *et al*[13]	2013	150	Potentially resectable lung cancer	150	Potentially resectable lung cancer	91	51	7	4	0%	100%	100%	100%	100%	Available in 4/7	F/U CT at 6 months
Schuurbiers *et al*[17]	2011	85	Lung cancer	150	Lung cancer	85	0	25	55	6%	86%	96%	91%	70%	Available in 23/30	Clinical (n = 11) or F/U CT (n = 10)[2]
Eloubeidi *et al*[12]	2010	59	Known or suspected malignancy	59	Known or suspected malignancy	54	5	37	22	0%	NR	NR	NR	NR	Clinical F/U for 37	Not part of study protocol
Bodtger *et al*[4]	2009	40	Known or suspected lung cancer	40	Known or suspected lung cancer	40	0	29	11	0%	94%	43%	91%	55%	Available	Survival at 2 yr
Ang *et al*[21]	2007	119	Lung cancer	119	Lung cancer	4	0	2	2	0%	NR	NR	NR	NR	N/A	N/A

[1]EUS-FNA was done in 11 patients, 3 had bilateral EUS-FNA; [2]Two patients had CT at 3 mo. EUS-FNA: Endoscopic ultrasound guided fine-needle aspiration; PPV: Positive predictive value; NPV: Negative predictive value; FNA: Fine-needle aspiration; F/U: Follow up; N/A: Not available; NR: Not reported; CT: Computerized Tomography.

aggressively biopsied adrenal masses over 3 cm in size in the following patients: (1) a history of malignancy; (2) a new diagnosis of cancer; or (3) a suspected recurrence due to the significant impact a diagnosis of metastatic malignancy has in this population.

Various techniques have been used to estimate the probability of malignancy of an adrenal mass, including its size, imaging characteristics and growth rate on serial imaging[16]. Asymptomatic patients with an indeterminate initial imaging study are advised to have follow-up imaging in 3-12 mo to assess for growth[16]. Surgical resection is recommended for lesions that grow; however, the threshold increase in size and growth rate that triggers resection have not been determined[16]. Guidelines from the AACE/AAES in 2009 on the management of adrenal incidentalomas recommend that benign appearing lesions smaller than 4 cm should have repeat adrenal imaging at 3-6 mo and then annually for 1-2 years. These same guidelines recommend surgery for growth rate more than 1cm or development of a hormonally active lesion (grade 3, Level C evidence)[16]. Based on these recommendations above, we utilized adrenal growth rate of ⩽ 1 cm at follow imaging 6 mo or longer after EUS to correct confirm benign cytology as a benign lesion.

Other studies evaluating adrenal gland EUS-FNA and its clinical impact in patients with established or suspected malignancy, have either used survival at ⩾ 2 years as confirmation for benignancy or not reported follow-up for benign lesions[4,12,21]. Schuurbiers *et al*[17] reported follow-up imaging for 10/30 patients with either benign or non-diagnostic EUS-FNA of the left adrenal gland. Similarly, Uemura *et al*[13] reported follow-up imaging at 6 mo for 4/7 patients with benign EUS-FNA and underlying lung cancer. To our knowledge, our series represents the first large study to utilize growth rates to confirm benign adrenal lesions and utilize these data to calculate test characteristics of EUS-FNA in non-cancer patients undergoing right and/or left adrenal gland EUS-FNA (Table 7).

False positive results for malignancy have been reported with EUS-FNA and its incidence varies anywhere from 1% to 15%[22]. Our rate was 1% and it was considered to be secondary to cytological misinterpretation.

Potential limitations of this study include limited assessment of long-term adverse events after EUS-FNA due to inability to contact patients within weeks of the pro-

cedure. Nevertheless, a careful review of the available records was performed and all patients were contacted for short term events within 48 h of the procedure. Secondly, many patients with benign adrenal gland FNA cytology had underlying cancer and died before follow-up CT or never followed up, which could have affected the final diagnosis of the nature of the adrenal gland abnormality. However, because follow-up imaging was not available for these patients, they were excluded from the sensitivity analysis.

Another potential limitation is that during several years of the study time period, PET scan was not available and therefore is not applicable to this case series. With the advent of PET, any decision to pursue a biopsy for a positive or indeterminate PET scan is generally at the discretion of the referring physician. With widespread metastatic disease, a positive scan within either adrenal is likely considered as diagnostic for metastatic disease and therefore a biopsy would not be necessary. However, in a patient with known or suspected malignancy and a positive adrenal gland on PET in isolation, we advocate EUS-FNA of the adrenal as this may signify novel metastatic disease which may merit additional or novel chemotherapy or possibly adrenalectomy.

In conclusion, EUS-FNA of the adrenal is a safe, minimally invasive and sensitive technique with significant impact in the management of patients with malignancy diagnosed either prior or during the procedure. It permits surgical treatment for cancer in patients with localized malignancy and a benign adrenal lesion. This technique also diagnoses metastatic disease and cancer recurrence, avoiding unnecessary invasive surgical procedures in patients with established metastatic disease by adrenal biopsy.

COMMENTS

Background

Different modalities can be used to sample the adrenal glands. Image guided fine-needle aspiration using either CT and ultrasound guidance have traditionally been used. With the advent of new endoscopic techniques, endoscopic ultrasound guidance for fine-needle aspiration (EUS-FNA) of either adrenal gland has become a very plausible technique for this matter. There have been reports of adrenal gland EUS-FNA and this has shown to be a very safe and minimally invasive procedure.

Research frontiers

When sampling adrenal gland lesions, especially in patients with known or suspected underlying malignancy, it is of supreme importance not only the technique used possesses a great deal of diagnostic accuracy, but also to understand how did previous studies obtain that diagnostic accuracy; this relates to the method for follow up of lesions with benign cytology results. This is a very important area of research in this subject.

Innovations and breakthroughs

Most publications regarding EUS-FNA have universally included patients with underlying malignancy and, have had small patient numbers and/or have not included repeat imaging to document follow up on lesions with benign cytology results. According to the recommendations of the American Association of Clinical Endocrinologists and American Association of Endocrine Surgeons, adrenal lesions with benign appearance and smaller than 4cm, should have repeat adrenal imaging at 3-6 mo. These same guidelines also recommend surgery if the growth rate exceeds 1cm or if the lesion becomes hormonally active. In our study, we included 94 patients that had EUS-FNA of either adrenal gland, reviewed records and, abstracted information about EUS indication, EUS findings, EUS-FNA results, clinical and follow up imaging if this was available. A true diagnosis of a benign lesion was considered when there was a benign EUS-FNA result and the lesion had not grown more than 1 cm from its original size on follow up CT/MRI or repeat EUS or if the patient underwent adrenalectomy when the surgical pathology was benign. The clinical impact of adrenal EUS-FNA was analyzed on a case by cases basis. In the present study, the authors showed that adrenal gland EUS-FNA is a sensitive, specific, and safe minimally invasive diagnostic technique that has a great impact in patient care. Adrenal gland EUS-FNA ruled out metastatic disease in patients with underlying malignancy, therefore permitting surgery for primary tumor; it also made the initial diagnosis of stage Ⅳ cancer or recurrent malignancy in others.

Applications

This study suggests that adrenal gland EUS-FNA is a clinically useful, accurate and a safe technique in patients with adrenal gland mass or enlargement regardless or the presence of underlying malignancy.

Terminology

Endoscopic ultrasound (EUS) or echo-endoscopy is a procedure in which endoscopy is combined with ultrasound to obtain images of the internal anatomy. Combined with Doppler imaging, nearby blood vessels can be evaluated. During the performance of this procedure, abnormal structures can be biopsied using a fine-needle aspiration technique.

Peer review

This is a retrospective single-center case-series evaluating the impact of EUS-FNA (Endoscopic ultrasound guided fine-needle aspiration) in the evaluation of patients with left and/or right adrenal gland lesions discovered at EUS as part of a staging procedure or incidentally for other indications. The authors should be congratulated in their effort to present real clinical impact of EUS-FNA in patients with both malignant and benign adrenal lesions/findings that has never been done before, where patient population were mainly patients with cancer who were undergoing EUS-FNA for staging purposes.

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DOI: 10.5527/wjn.v3.i3.101

World J Nephrol 2014 August 6; 3(3): 101-106
ISSN 2220-6124 (online)

OBSERVATIONAL STUDY

Gait speed and hospitalization among ambulatory hemodialysis patients: USRDS special study data

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Supported by National Institutes of Health contract HHSN267200715004C, ADB No. N01-DK-7-5004 (Dr. Kutner)
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Received: April 13, 2014 Revised: June 17, 2014
Accepted: July 15, 2014
Published online: August 6, 2014

Abstract

AIM: To assess the association of measured gait speed with hemodialysis (HD) patients' hospitalization, in conjunction with, and apart from, recent fall history.

METHODS: Gait speed was measured by a standard protocol and falls during the past 12 mo were ascertained for a prevalent multi-center HD cohort (n = 668) aged 20-92. Hospitalization during the past 12 mo was identified in the patient's clinic records, and the first hospitalization after gait speed assessment (or the competing event of death) was identified in the 2013 United States Renal Data System Standard Analysis Files.

RESULTS: Slow gait speed, defined as < 0.8 m/s, characterized 34.7% of the patients, and 27.1% had experienced a recent fall. Patients with slow gait speed but without a history of recent falls were 1.79 times more likely to have been hospitalized during the past 12 mo (OR = 1.79, 95%CI: 1.11-2.88, P = 0.02), and patients with slow gait speed and a history of recent falls were over two times more likely to have been hospitalized (OR = 2.10, 95%CI: 1.19-3.73, P = 0.01), compared with patients having faster gait speed and no recent fall history. Prospective examination of gait speed/fall history status in relation to first hospitalization (or death) incurred by the end of follow-up December 31, 2011 also showed that slow gait speed was associated with these events in conjunction with a history of falls (HR = 1.54, 95%CI: 1.04-2.30, P = 0.03).

CONCLUSION: The International Task Force on Nutrition and Aging reported that gait speed is a powerful predictor for older adults of adverse outcomes such as hospitalization. In our data, gait speed--apart from, as well as in conjunction with, recent fall history--was associated with HD patients' hospitalization for multiple causes. Gait speed may be a sensitive health indicator among HD patients across the age spectrum.

Key words: Falls; Gait speed; Hemodialysis; Hospitalization; Walking disability

Core tip: Walking places demands on the heart, lungs, circulatory, nervous, and musculoskeletal systems. Studies of older adults support the prognostic importance of slowed gait speed for the risk of poor health and function, including hospitalization. However, little is known about the association of gait speed with hemodialysis (HD) patient outcomes. The usual gait speed of 668 HD patients was measured in a United States Renal Data System special study. Slowed gait speed--apart from, as well as in conjunction with, recent fall history--was associated with HD patients' hospitalization for

multiple causes. Gait speed may be a useful monitoring tool in the HD clinical setting.

Kutner NG, Zhang R, Huang H, Wasse H. Gait speed and hospitalization among ambulatory hemodialysis patients: USRDS special study data. *World J Nephrol* 2014; 3(3): 101-106 Available from: URL: http://www.wjgnet.com/2220-6124/full/v3/i3/101.htm DOI: http://dx.doi.org/10.5527/wjn.v3.i3.101

INTRODUCTION

The rate of hospital admissions among dialysis patients is high[1]. Hospitalization consumes a large portion of healthcare expenditures and is associated with risk for the patient. Identifying and understanding factors that may lead up to hospitalization is important for multiple quality improvement objectives[2].

In the geriatric population, a deficit in gait speed is recognized as a relatively easily measured and consistent prognostic indicator of patient outcomes, including hospitalization risk[3-5]. Studenski *et al*[5] have provided compelling evidence of the prognostic importance of slow gait speed for multiple health outcomes among older adults and have identified 0.8 m/s as a cutpoint below which gait speed connotes significantly increased likelihood of poor health and function and even mortality. End-stage renal disease (ESRD) patients appear to represent a model of early aging in many ways. While it is recognized that average gait speed is slower among ESRD patients compared with age-adjusted norms[6,7], the association of gait speed with ESRD patient outcomes has received limited attention.

A recent study by Beaubrun *et al*[8] of 52408 hemodialysis (HD) patients who were followed 2000-2009 reported that over one-fifth of patients who incurred a non-fracture-related hospitalization, and more than half of those who were hospitalized following a fracture, were characterized by "walking disability/history of falls". Medicare claims diagnoses of "difficulty walking" and "abnormal gait" define walking disability in administrative data[9]. Walking disability and falls are frequently associated[9]. At the same time, "walking disability" does not specify a quantitative measure of gait speed, and the potential association of walking disability with hospitalization apart from falls remains unknown when walking disability and history of falls are treated as one combined variable.

In this study of a large contemporary cohort of patients aged 20-92 undergoing maintenance HD therapy, we measured patients' gait speed and investigated its association with hospitalization in conjunction with, and apart from, patients' recent fall history.

MATERIALS AND METHODS

ACTIVE/ADIPOSE (A Cohort Study to Investigate the Value of Exercise in ESRD/Analyses Designed to Investigate the Paradox of Obesity and Survival in ESRD) is a multi-center study of prevalent patients on HD coordinated by the United States Renal Data System (USRDS). An overview of the study design and measures is available in the 2011 USRDS Annual Data Report[10]. Institutional review boards at Emory University and the University of California-San Francisco approved the study. A total of 668 study participants had gait speed and fall history information and are the focus of the analyses reported in this paper.

Usual gait speed was measured two times over a 15-feet walkway, and the average speed of the two trials was determined. Coordinators observed whether the participant used an assistive device for walking and whether an assistive device was used to perform the walk. For the analyses reported in this paper, slow gait speed was defined as < 0.8 m/s. Hospitalization during the past 12 mo was identified in the patient's clinic records, and the first hospitalization after gait speed assessment (or the competing event of death) was identified in the 2013 USRDS Standard Analysis Files.

Statistical analysis

Participants' sociodemographic and clinical characteristics were described by percentage or mean (SD) and compared using chi-square or t-test. A four-level variable was used to summarize participants' gait speed and fall history status, i.e. slow gait speed + no fall history; slow gait speed + fall history; faster gait speed + no fall history; faster gait speed + fall history. The association of this variable with hospitalization during the past 12 mo was examined in a multivariable logistic regression model, and time to first hospitalization (or death) through December 31, 2011 was examined in a multivariable Cox proportional hazards analysis using the USRDS 2013 Hospitalization Standard Analysis File; patients were censored at the end of follow-up. Statistical analyses were conducted using SAS 9.3 (SAS Institute, Cary, NC, United States).

RESULTS

Overall mean (SD) age of study participants was 57.1 (14.3); the median age was 57.5. The primary cause of ESRD was diabetes or hypertension in 72% of the cohort, similar to the total United States in-center HD population[1]. Forty-one percent were women, and median time since ESRD treatment start (ESRD vintage) was 3 years. Consistent with the study sites, African-American patients were more heavily represented than in the overall United States HD population, and the average age of study participants was correspondingly younger.

There were 103 patients who could not be included in the analysis due to lack of gait speed assessment or fall information. Compared with patients who had gait speed and fall information, patients missing this information were more likely to be women, older, and white; to have diabetes, congestive heart failure (CHF), coronary artery disease/myocardial infarction (CAD/MI), cerebrobascular accident/transient ischemic attack

Table 1 Characteristics of patients included (n = 668) and not included (n = 103) in the analysis

	Measured walk and fall information (n = 668)	Missing measured walk or fall information (n = 103)[1]	P value
Male (%)	61.2	45.6	0.003
Age (yr), mean ± SD	56.3.(14.1)	62.2 (14.1)	< 0.001
Race (%)			< 0.001
White	22.2	31.1	
Black	63.6	45.6	
Native American	0.5	1	
Asian	10.9	12.6	
Other (Native Hawaiian, other Pacific Islander, other)	2.8	9.7	
ESRD vintage, yr, mean ± SD	5.0 (5.2)	4.4 (4.1)	0.24
Diabetes (%)	48.1	73.5	< 0.001
COPD (%)	7.5	11.8	0.14
Cancer (%)	7.7	9.8	0.46
CHF (%)	27.3	42.2	0.002
CAD/MI (%)	26	39.2	0.005
CVA/TIA (%)	9.3	15.7	0.047
PVD (%)	6.5	31.4	< 0.001
Other cardiac diseases (%)	23.9	43.1	< 0.001
KDQOL-CF score, mean ± SD	88.0 (16.3)	86.3 (18.2)	0.35
Hemoglobin, g/dL, mean ± SD	11.6 (1.3)	11.3 (1.3)	0.1
Assistive walking device (%)	19.5	91.7	< 0.001
History of recent fall(s) (%)	27.1	37.2	0.04

[1]One patient lacked fall history information only; no physical performance information was obtained for 19 patients; and study coordinators reported that 83 patients were not able to walk 15 feet to perform the walk test. CAD: Coronary artery disease; CHF: Congestive heart failure; COPD: Chronic obstructive pulmonary disease; CVA: Cerebrovascular accident; ESRD: End-stage renal disease; KDQOL-CF: Kidney Disease Quality of Life-Cognitive Function; MI: Myocardial infarction; m/s: Meters per second; Other cardiac disease: Cardiac dysrhythmia, atrial fibrillation, tachycardia, pericarditis, cardiac arrest; PVD: Peripheral vascular disease; TIA: Transient ischemic attack.

Table 2 Characteristics of patients with measured walk and fall information, by slow gait speed (< 0.8 m/s) and faster gait speed (⩾ 0.8 m/s)

	Gait speed < 0.8 m/s (n = 232)	Gait speed ⩾ 0.8 m/s (n = 436)	P value
Male (%)	47	68.8	< 0.001
Age, yr, mean ± SD	62.1 (13.4)	53.2 (13.5)	< 0.001
Race (%)			< 0.001
White	13.8	26.6	
Black	75	57.6	
Native American	0.4	0.5	
Asian	8.2	12.4	
Other (Native Hawaiian, other Pacific Islander, other)	2.6	3	
ESRD vintage, yr, mean ± SD	4.5 (4.9)	5.2 (5.3)	0.1
Diabetes (%)	59.9	41.7	< 0.001
COPD (%)	12.1	5.1	0.001
Cancer (%)	8.2	7.4	0.71
CHF (%)	31.5	25.1	0.08
CAD/MI (%)	33.2	22.1	0.002
CVA/TIA (%)	12.5	7.6	0.04
PVD (%)	9.5	4.8	0.02
Other cardiac diseases (%)	29.3	21	0.02
KDQOL-CF score, mean ± SD	84.3 (18.0)	90.0 (14.9)	< 0.001
Hemoglobin, g/dL, mean ± SD	11.5 (1.3)	11.6 (1.3)	0.67
Assistive walking device (%)	45	6	< 0.001
Assistive walking device used when gait speed measured (%)	26.8	2.3	< 0.001
History of recent fall(s) (%)	36.2	22.3	< 0.001
Gait speed, m/s, mean ± SD	0.61 (0.15)	1.04 (0.18)	< 0.001

CAD: Coronary artery disease; CHF: Congestive heart failure; COPD: Chronic obstructive pulmonary disease; CVA: Cerebrovascular accident; ESRD: End-stage renal disease; KDQOL-CF: Kidney Disease Quality of Life-Cognitive Function; MI: Myocardial infarction; m/s: Meters per second; Other cardiac disease: Cardiac dysrhythmia, atrial fibrillation, tachycardia, pericarditis, cardiac arrest; PVD: Peripheral vascular disease; TIA: Transient ischemic attack.

(CVA/TIA), peripheral vascular disease (PVD) and other cardiac diseases; to use an assistive device for walking; and to report having fallen in the past 12 mo. Patients who lacked gait speed or fall information and could not be included in the analyses reported in this paper did not differ from the included patients with respect to ESRD vintage, chronic obstructive pulmonary disease (COPD), cancer, Kidney Disease Quality of Life-Cognitive Function (KDQOL-CF) score, or hemoglobin level (Table 1).

Characteristics of the 668 study participants who had gait speed measured are shown in Table 2. Corresponding mean (SD) gait speed values were 0.61 (0.15) m/s for those with slow gait speed *vs* 1.04 (0.18) m/s for those with faster gait speed; $P < 0.001$. Participants with slow gait speed were more likely to be women, older, and black. They were more likely to have diabetes, COPD, CAD/MI, CVA/TIA, PVD, and other cardiac diseases. Their average score on the KDQOL-CF scale was lower, they were more likely to use an assistive device for walking and to have used such a device to perform the walk speed test, and they were more likely to report having fallen during the past 12 mo. Participants with slow gait speed and those with faster gait speed did not differ with respect to ESRD vintage, cancer, CHF, and average hemoglobin level.

Gait speed < 0.8 m/s characterized 34.7% of patients, and falling during the past 12 mo was reported by 27.1% of patients. Slow gait speed was more likely to characterize patients with a history of falls than patients without a history of falls (46% *vs* 30%, $P < 0.001$).

Almost half of the study cohort had been hospitalized in the past 12 mo, and 41% of those hospitalized had slow gait speed, compared with 29.3% of those who had not been hospitalized ($P = 0.001$). Patients with slow gait speed but no history of recent falls (n = 148) were 1.79 times more likely to have been hospitalized compared with patients with faster gait speed and no recent falls (n = 339) (OR = 1.79, 95%CI: 1.11, 2.88, $P = 0.02$). Patients with slow gait speed and a history of recent falls (n = 84) were over two times more likely to have been hospitalized compared with patients having faster gait speed and no recent fall history (OR = 2.10, 95%CI: 1.19, 3.73, $P = 0.01$). The risk of hospitalization among patients with faster gait speed and recent fall history (n =

Table 3 Characteristics associated with hospitalization in past 12 mo from multivariable logistic regression analysis

	[1]Adjusted odds ratios (95%CI) for Hospitalization	*P* value
Gait speed/fall history		
[2]Faster walk/no fall history (reference)	1	
[3]Slower walk/no fall history	1.79 (1.11, 2.88)	0.02
Slower walk/fall history	2.10 (1.19, 3.73)	0.01
Faster walk/fall history	1.46 (0.90, 2.39)	0.13
Female	0.55 (0.39, 0.78)	< 0.001
Age, yr	0.98 (0.97, 1.00)	0.01
ESRD vintage, yr	0.95 (0.92, 0.98)	0.003

[1]Adjusted also for race, diabetes, CHF, CAD, CVA, PVD, other cardiac diseases, COPD, cancer, hemoglobin level, KDQOL-CF score, assistive device use (variables not significantly associated in this logistic regression model with hospitalized/not hospitalized in past 12 mo); [2]Faster walk = gait speed ⩾ 0.8 m/s; [3]Slower walk = gait speed < 0.8 m/s. ESRD: End-stage renal disease.

Table 4 Multivariable Cox proportional hazards model predicting index hospitalization/death[1] from baseline gait speed assessment through December, 2011

	[2]Adjusted hazard ratios (95%CI)	*P* value
Gait speed/fall history		
[3]Faster walk/no fall history (reference)	1	
[4]Slower walk/no fall history	1.11 (0.79, 1.56)	0.53
Slower walk/fall history	1.54 (1.04, 2.30)	0.03
Faster walk/fall history	1.47 (1.05, 2.05)	0.02
CAD	1.34 (1.01, 1.78)	0.04
Black race	2.09 (1.48, 2.94)	< 0.001
ESRD vintage, yr	1.02 (1.00, 1.04)	0.03

[1]Hospitalization events = 271; deaths = 17; [2]Adjusted also for age, diabetes, CHF, CVA, PVD, other cardiac diseases, COPD, cancer, hemoglobin level, KDQOL-CF score, assistive device use; [3]Faster walk = gait speed ⩾ 0.8 m/s; [4]Slower walk = gait speed < 0.8 m/s. ESRD: End-stage renal disease.

97) was not significantly higher than the hospitalization risk among patients with faster walk and no recent fall history. Female sex, one-year increment in age, and one-year increment in ESRD vintage were associated with a lower risk for hospitalization (Table 3).

Patients with slower gait speed and a history of falls were more likely to incur subsequent hospitalization (or death) compared with the reference group of patients who had faster walk/no fall history (HR = 1.54, 95%CI: 1.04, 2.30, $P = 0.03$). In the Cox analysis, increased risk of these events was also evident for patients with faster walk and fall history, those with a history of CAD, and blacks, and a one-year increment in ESRD vintage was associated with increased likelihood of hospitalization/death (Table 4).

DISCUSSION

Slow gait speed, defined in this analysis as < 0.8 m/s, characterized one-third of the ambulatory HD patients whom we studied. Patients with gait speed < 0.8 m/s were more likely than those with gait speed ⩾ 0.8 m/s to have been hospitalized during the past 12 mo, even in the absence of a history of recent falls. When we prospectively examined gait speed/fall history status in relation to first hospitalization (or death), patients with gait speed < 0.8 m/s and a history of falls were more likely to incur hospitalization/death than those with faster gait speed and no history of falls.

The causes of hospitalization among patients with slow gait speed varied widely, *e.g.*, cardiac issues, respiratory distress, mini-stroke, neuropathy, leg pain. Studenski *et al*[5] have emphasized that gait speed is a sensitive marker of health because it may reflect known and unrecognized disturbances in multiple organ systems. Walking places demands on the heart, lungs, circulatory, nervous, and musculoskeletal systems, and slowed gait may reflect damaged systems as well as a high-energy cost of walking[5]. Mobility limitations can be early indicators of muscle weakness, pain or discomfort, and shortness of breath, as well as potential falls[7]. In addition, decreasing mobility may induce a cycle of reduced physical activity and deconditioning[5].

Slow gait speed may have contributed to "walking disability" in the Beaubrun *et al*[8] 2013 analysis of HD patients who were hospitalized following fracture. In their study, walking disability/history of falls characterized 55.6% of the study population[8]. The number of patients in our study cohort who were hospitalized following fracture was small ($n = 24$), but slow gait speed and fall history were prominent; 45.5% of patients hospitalized post-fracture had slow gait speed and 70.8% had recently fallen.

The International Task Force on Nutrition and Aging concluded that gait speed is strongly associated with adverse outcomes, including falls and hospitalization[11]. However, there has been little investigation of the association of gait speed with hospitalization in the dialysis population. As we have noted, the recent study by Beaubrun *et al*[8] links walking disability/history of falls with hospitalization risk, but that study did not have information about gait speed.

The frailty index developed by Fried *et al*[12] includes slow walk as one component. Several studies of chronic kidney disease patients, both dialysis-dependent and non-dialysis-dependent, have measured gait speed and used the Fried index to classify patients as frail[13-18]. One of these studies investigated HD patients' hospitalization in association with frailty. McAdams-DeMarco *et al*[17], in a study of 146 HD patients in one dialysis center, found that 42.6% of frail participants, compared with 28.2% of nonfrail participants, had two or more hospitalizations in the year following study enrollment, and frailty was associated with 1.4 times (95%CI: 1.00-2.03, $P = 0.049$) more hospitalizations independent of age, sex, comorbidity, and disability[17]. The Fried index classi-

fies individuals as frail who have three or more of the five criteria that comprise the index (recent weight loss, reported exhaustion, weak grip strength, slow walk, and low physical activity)[12], which does not necessarily include having slow walk.

Our study has several strengths. Data were supplied by a large multi-center study cohort. Performance-based gait speed was carefully assessed, along with a large number of patient characteristics and treatment-related factors. The fall prevalence that we observed was very similar to 12-mo fall prevalence estimates from other studies[19]. We acknowledge, however, that although the ACTIVE-ADIPOSE cohort shares many similarities with the general ESRD population, the rate of fracture-related hospitalization would be expected to be higher in a cohort with higher representation of whites and older patients[8]. In addition, having been hospitalized over the past 12 mo could have influenced participants' gait speed, and prospective examination of hospitalization events was truncated for study participants whose baseline gait speed assessments were obtained near the end of the study enrollment period in 2011. These also represent potential study limitations.

We used the cutoff of < 0.8 m/s to define slow gait speed, the cutoff that has been most often observed to predict adverse health outcomes among older adults[11]. However, 0.6 m/s and 1.0 m/s have been used as cut-offs in other studies[5,7]. Most of the existing evidence about gait speed and outcomes is derived from studies of older, community-dwelling populations. Investigation of other gait speed cut points may be useful in dialysis cohorts that have a different sociodemographic and/or clinical profile compared with the cohort we studied. Studenski *et al*[5] noted that further work is needed to examine associations of gait speed with outcomes such as disability and health care use, especially in populations based in clinical practice.

Gait speed provides a straightforward and informative indicator of health status. It may offer the clinician a tool for assessing expected outcomes and tailoring goals of care[5]. Compared with patient-reported mobility difficulty, a gait speed test provides a quantitative marker that facilitates tracking mobility changes[7]. Referral to a specialist (physical therapist, clinical exercise specialist, cardiac rehabilitation) for further evaluation and intervention may be indicated. Gait speed performance can be assessed relatively easily and quickly (less than two minutes) in the clinical setting, and poor performance may indicate that there are underlying health problems placing the patient at increased risk for hospitalization, as well as for other potential adverse outcomes.

ACKNOWLEDGMENTS

The interpretation and reporting of the data presented here are the responsibility of the authors and in no way should be seen as an official policy or interpretation of the United States government. The results presented in this paper have not been published previously in whole or part, except in abstract format.

COMMENTS

Background

Usual gait speed has been shown to be a powerful predictor of outcomes, including hospitalization, among older persons, and hemodialysis (HD) patients represent a model of early aging. Frailty, which may include slow gait speed, has been shown to be associated with HD patients' hospitalization risk, as has the syndrome of "walking disability/history of falls," but the utility of gait speed alone as a predictor of HD patients' hospitalization has not been studied.

Research frontiers

There is growing recognition of the significance of physical performance deficits among chronic kidney disease (CKD) patients, including increased mortality risk. Measures of lower extremity function may capture a complex set of skeletal muscle and neurologic impairments that develop in individuals with CKD and may substantially affect their survival as well as their independent physical functioning. Research frontiers include identifying mechanisms that underlie decreased physical performance and evaluating whether interventions that improve physical performance may also positively influence comorbidity and clinical outcomes in CKD.

Applications

Gait speed can be considered a vital sign, and measuring gait speed is simple, quick, reproducible, inexpensive, and feasible in clinical settings.

Terminology

Gait speed is typically measured as the time it takes an individual to walk at a usual pace over a measured distance (usually 4 to 6 meters). The time for that distance is then converted into a speed in m/s.

Peer review

Clearly stated study indicating that slow measured walking speed in dialysis patients can be used as a predictor of subsequent fracture and hospitalization for all causes.

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P- Reviewer: Friedman EA **S- Editor**: Ji FF **L- Editor**: A
E- Editor: Wu HL

World Journal of Nephrology

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Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.5527/wjn.v3.i3.107

World J Nephrol 2014 August 6; 3(3): 107-113
ISSN 2220-6124 (online)

RANDOMIZED CONTROLLED TRIAL

Long-term outcome of ketoconazole and tacrolimus co-administration in kidney transplant patients

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Received: April 4, 2014 Revised: June 25, 2014
Accepted: July 27, 2014
Published online: August 6, 2014

Abstract

AIM: To study the long-term outcome of ketoconazole and tacrolimus combination in kidney transplant recipients.

METHODS: From 2006 to 2010, ketoconazole was given in 199 patients and was continued for at least 1 year or until graft failure (Group 1), while 149 patients did not receive any ketoconazole (Group 2). A combination of tacrolimus, mycophenolate and steroid was used as maintenance therapy. High risk patients received basiliximab induction.

RESULTS: Basic demographic data was similar between the 2 groups. The 5-year cumulative incidence of biopsy-confirmed and clinically-treated acute rejection was significantly higher in Group 1 than in Group 2 (34% *vs* 18%, $P = 0.01$). The 5-year Kaplan-Meier estimated graft survival (74.3% *vs* 76.4%, $P = 0.58$) and patient survival (87.8% *vs* 87.5%, $P = 0.93$) were not different between the 2 groups. Multivariable analyses identified ketoconazole usage as an independent risk of acute rejection (HR = 2.33, 95%CI: 1.33-4.07; $P = 0.003$) while tacrolimus dose in the 2nd month was protective (HR = 0.89, 95%CI: 0.75-0.96; $P = 0.041$).

CONCLUSION: Co-administration of ketoconazole and tacrolimus is associated with significantly higher incidence of acute rejection in kidney transplant recipients.

Key words: Kidney transplant; Rejection; Survival; Tacrolimus Ketoconazole; Pharmacokinetics; Cytochrome P450

Core tip: Tacrolimus is mainly metabolized by cytochrome P450 enzymes and ketoconazole is a potent inhibitor of P450. Transplant programs often use ketoconazole to reduce the tacrolimus dose and financial cost. Small short-term studies had previously supported such practice, but the long-term outcome are still lacking. We hereby report our center's experience of this combination in kidney transplant recipients. Our study suggests that co-administration of ketoconazole and tacrolimus is associated with significantly higher incidence of acute rejection in kidney transplant recipients.

Khan E, Killackey M, Kumbala D, LaGuardia H, Liu YJ, Qin HZ, Alper B, Paramesh A, Buell J, Zhang R. Long-term outcome of ketoconazole and tacrolimus co-administration in kidney transplant patients. *World J Nephrol* 2014; 3(3): 107-113 Available from: URL: http://www.wjgnet.com/2220-6124/full/v3/i3/107.htm DOI: http://dx.doi.org/10.5527/wjn.v3.i3.107

INTRODUCTION

Tacrolimus is a macrolide antibiotic produced from

Streptomyces tsukubaensis. It binds to FK506-binding protein to form a complex that inhibits calcineurin phosphatase in Tlymphocytes [commonly referred as calcineurin inhibitor (CNI)]. Tacrolimus has been widely used as the primary immunosuppressive agent in kidney transplant patients[1]. Due to its narrow therapeutic index, optimal dosing with therapeutic monitoring is necessary. Tacrolimus is mainly metabolized by cytochrome P450-3A in the liver and a substrate of P-glycoprotein, an efflux pump, in both liver and intestine[2,3]. The inhibition of P450-3A and P-glycoprotein can slow down its metabolism and ketoconazole is such a potent inhibitor. Transplant programs often use ketoconazole to reduce the tacrolimus dose and cost. The financial benefit and short-term safety of such a practice had been reported previously by a few small studies in Egypt[4,5]. But the long-term outcomes of such a practice are still lacking.

Our interest in examining this issue comes from our participation in the National Institutes of Health funded pilot study of solid organ transplants in human immunodeficiency virus (HIV) infected patients[6-8]. There were unexpectedly higher incidences of acute rejection (by a factor of 2 to 3) in both kidney and liver recipients. About half of the rejections were early and aggressive[6,7]. In these patients, the targeted trough levels of CNIs were consistent with those in non-HIV recipients, but their required doses of CNI were significantly lower due to the necessary treatment with anti-retroviral protease inhibitor, which also inhibits P450-3A and P-glycoprotein. Due to the altered pharmacokinetics, their total exposure to CNI [area under curve (AUC)] likely was considerably low. This may be more plausible than other proposed viral and/or immunological factors to explain the higher-than-expected rejection rates in HIV infected patients[9-11]. In the current study, we analyze the long-term outcome of patients who received co-administration of ketoconazole and tacrolimus in non-HIV infected kidney transplant recipients.

MATERIALS AND METHODS

Study population

This is a retrospective review, and patients were identified using the transplant center database at Tulane University Hospital and Clinic. During the period between 2006 and 2010, there were consecutive 450 non-HIV infected adult patients who underwent a primary kidney transplant at our center. Among them, ketoconazole was given in 199 patients after transplant surgery and was continued for at least 1 year or until graft failure (Group 1), while 149 patients did not receive any ketoconazole (Group 2). A combination of tacrolimus, mycophenolate and steroid was used as maintenance therapy in all patients. Factors for excluding patients from this study included primary graft non-function (n = 3), death in first week of transplant surgery (n = 2), incomplete data due to lost fellow-up (n = 19), different combination of maintenance immunosuppressives (n = 48) or usage of other drug that inhibits (such as diltiazem, amiodarone, *etc.*) or reduces (such as phenytoin, isoniazid, *etc.*) P450 enzymes (n = 9). The patients, whose ketoconazole was stopped before 1 year of kidney transplant (n = 17) or before the graft failure (n = 4) were also excluded from analysis. All patients were followed up closely at our center, including scheduled routine labs and clinic visits. Patients with acute illness were usually directly admitted or transferred to our center, and the outside events were recorded in a timely manner.

Immunosuppressive therapy

High risk patients, defined as having peak panel reactive antibody > 10%, human leukocyte antigen mismatch > 4, donor cold ischemia time > 18 h or expanded criteria donor kidneys, received induction therapy with 2 doses of basiliximab (20 mg per dose). Corticosteroids were administrated as methylprednisolone 500 mg IV intraoperatively, then tapered on postoperative days 1 to 3, and changed to oral prednisone 60 mg on postoperative day 4. The patients typically continued oral prednisone 20 mg daily for the first month, then 10 mg daily for the 2nd month and 5 mg daily thereafter. Each patient was started mycophenolate, either mycophenolate mofetil at 1 g or enteric-coated sodium mycophenolate at 720 mg, twice daily after transplant. Oral tacrolimus was started immediately after transplant, and doses were adjusted to keep the 12-h trough levels at 8 to 12 ng/mL for the first year. The target of tacrolimus trough levels was then maintained at 4 to 6 ng/mL after the first year. Whole blood tacrolimus concentrations were measured with liquid chromatography-tandem mass spectrometry in our hospital. The decision of adding ketoconazole was typically made within the first week of transplant surgery in order for patients to achieve the targeted trough levels prior to discharge. The dose of ketoconazole was started at100mg per day in all patients, and further increased to 200 mg per day in 4 patients.

Infection prophylaxis

All patients received one tablet of sulfamethoxazole/trimethoprim DS three times per week for the first year as prophylaxis for pneumocystis pneumonia and bacterial infections. Three-month antifungal prophylaxis with oral nystatin was given to patients who did not receive ketoconazole. Cytomegalovirus (CMV) prophylaxis was given to CMV seronegative recipients who received organs from a CMV seropositive donor. The regimen included IV ganciclovir during the transplant hospitalization followed by oral ganciclovir or valganciclovir for 3 mo.

Rejection

Acute rejection was presumed when patients had a sudden increase of serum creatinine that could not be explained by other clinical causes. Kidney biopsy was performed before the treatment. The severity of rejection was defined according to Banff criteria. Acute cellular rejection of grade 1 or below was initially treated with IV methylprednisolone for 3 d. Thymoglobulin was used for steroid resistant cellular rejection, or as the initial therapy

Table 1 Demographic characteristics of transplant patients between Group 1 (with ketoconazole) and Group 2 (without ketoconazole)

	Group 1 (n = 199)	Group 2 (n = 149)	P value
Age, mean ± SD (yr)	47.2 ± 13.2	48.8 ± 14.4	0.21
Gender (%)			
Male	56	61	0.47
Female	44	39	
Race (%)			
Black	64	55	0.19
Non-black	36	45	
BMI (kg/m2)	28.3 ± 5.4	27.4 ± 5.7	0.34
Peak PRA (%)	15.5 ± 25.3	13.8 ± 27.0	0.27
HLA mismatch	4.1 ± 1.4	3.9 ± 1.6	0.52
Causes of ESRD (%)			0.75
Diabetes	25	31	
Hypertension	38	35	
Nephritis	19	15	
PCKD	8	6	
Others	10	13	
Induction (%)	55	51	0.51
Donors (%)			0.63
Living	26	29	
Deceased	74	71	
CIT (h)	17.8 ± 7.2	18.5 ± 6.4	0.24

SD: Standard deviation; BMI: Body mass index; PRA: Panel reactive antibodies; HLA: Human leukocyte antigen; ESRD: End stage renal disease; PCKD: Polycystic kidney disease.

for rejection of Banff grade 2 or higher. Acute antibody mediated rejection (AMR) was diagnosed with positive C4d staining in the peritubular capillaries and/or demonstration of donor specific antibody. AMR was treated with a course of 5 to 7 daily plasmapheresis and intravenous immunoglobulin (IVIG) (150 mg/kg) in addition to IV methylprednisolone and thymoglobulin.

Statistical analysis

The outcome measures included: (1) incidence of biopsy-confirmed and clinically-treated acute rejection; (2) patient and kidney graft survival; (3) quality of graft function; and (4) incidence of clinically treated infections. Statistical analyses were performed using Statistics Analysis System (SAS) version 9.3 software (SAS Institute Inc, Cary, NC, United Statas). Chi-squared or Fisher exact test was used for count data, t test was used for continuous measures. Product-limit estimates of survival curves were generated by the Kaplan-Meier method and the survival difference was analyzed by log-rank test. Multivariable logistic regression analysis with a stepwise variable selection was used for examining risk factors of acute rejection. A P value < 0.05 was considered statistically significant. If there was no data at or around the particular time point, the previous or next available measure was used for analysis.

RESULTS

From 2006 to 2010, a total of 450 adult patients received a primary kidney transplant in our center. All of them were transplanted 3 to 7.5 years ago as of July 31, 2013, which is the end of the study period. Table 1 summarizes the demographic characteristics at the time of kidney transplants, and shows that there was no significant difference between the 2 groups. The total daily tacrolimus dose, 12-h trough level and graft function (serum creatinine) are summarized in Table 2. Both groups achieved similar targeted trough levels at all times according to our immunosuppressive protocol. Compared to Group 2, Group 1 initially required higher dose of tacrolimus during the first week of transplant. With administration of ketoconazole, their daily tacrolimus dose decreased. Subsequently, Group 1 required significantly lower dose of tacrolimus in the first month and in all times after that. The graft function remained comparable between the 2 groups.

Table 2 Tacrolimus dose, trough level and kidney function in the two groups

	1 wk	1 mo	2 mo	1 yr	3 yr	5 yr
Tacrolimus dose (mg/d)						
Group 1	10.9 ± 5.6	7.5 ± 4.8	6.0 ± 3.6	5.6 ± 3.8	5.3 ± 3.1	4.9 ± 2.8
Group 2	8.6 ± 4.1	8.3 ± 3.7	8.1 ± 3.2	7.8 ± 3.0	7.0 ± 2.2	6.2 ± 2.5
P value	0.004	0.03	< 0.001	< 0.001	< 0.001	< 0.001
Tacrolimus trough level (× 10 mg/L)						
Group 1	11.3 ± 2.1	9.7 ± 1.9	9.3 ± 1.7	8.6 ± 2.1	6.4 ± 1.9	5.3 ± 1.4
Group 2	10.7 ± 1.8	10.2 ± 2.0	9.5 ± 1.6	9.0 ± 1.8	5.8 ± 1.7	4.8 ± 1.5
Serum Cr (× 10 mg/L)						
Group 1	2.2 ± 1.3	1.7 ± 1.1	1.5 ± 0.9	1.6 ± 1.2	1.6 ± 0.8	1.7 ± 0.9
Group 2	1.9 ± 1.1	1.7 ± 0.9	1.6 ± 0.7	1.5 ± 0.8	1.5 ± 0.7	1.6 ± 0.8

The key post transplant events are summarized in Table 3. The delayed graft function (DGF) was defined by an inadequate renal function that required dialysis support in the first week after transplant. In both groups, the percentage of patients who had DGF was similar. Acute rejections were the biopsy-confirmed and clinically-treated ones. The 5-year cumulative incidence of acute rejection was significantly higher in Group 1 than Group 2, but the types of rejection were not different. There was no significant difference in the incidence of CNI toxicity or infectious disease between the 2 groups. Here, the CNI toxicity was the renal toxicity confirmed by kidney biopsy and required CNI dose reduction.

There was no statistical difference in graft survival by Kaplan-Meier analysis between the two groups (Figure 1A). The estimated graft survivals at 1, 3 and 5 years were 92.4%, 82.4% and 74.3% in Group 1, and 94.6%, 83.8% and 76.4% in Group 2 (Log-Rank P = 0.58). There was no difference in patient survival between the 2 groups (Figure 1B). The Kaplan-Meier estimated 1, 3, and 5 years patient survivals were 96%, 91.4% and 87.8% in Group 1, and 96.6%, 90.5%, 87.5% in Group 2 (Log-Rank P = 0.93). The causes of graft loss and death were listed in Table 3. There was no statistical difference in the overall causes of graft loss or patient death between the 2 groups.

The risk factors for acute rejection were examined by multivariable logistic regression analyses. The identified significant factors are listed in Table 4. We found that ke-

Table 3 Post transplant events and causes of graft loss and patient death

	Group 1 (*n* = 199)	Group 2 (*n* = 149)	*P* value
Posttransplant events, *n* (%)			
Delayed graft function	56 (28)	39 (26)	0.77
Acute rejection	68 (34)	27 (18)	0.01
Type of rejection			0.49
Cellular rejection	49	17	
Antibody rejection	14	6	
Both rejections	5	4	
CNI toxicity	8 (4)	15 (10)	0.09
Infectious diseases	63 (32)	54 (36)	0.37
Type of infection			0.67
CMV	32	22	
BKV	14	13	
HSV	5	6	
Bacteria	7	10	
Fungus	5	3	
Total graft loss, *n* (%)	52 (26)	35 (23)	0.57
Causes of graft loss			0.88
DWFG	22	16	
CAN	17	10	
Rejection	9	5	
Infection	2	3	
Others	2	1	
Total patient death, *n* (%)	27 (14)	18 (12)	0.68
Causes of death			0.88
CVD	14	10	
Infections	6	5	
Malignancy	2	2	
Others	3	1	

CNI: Calcineurin inhibitor; CMV: Cytomegalovirus; HSV: Herpes simplex virus; CVD: Cerebrovascular disease.

Table 4 Multivariable analysis of risk factors for acute rejection

	Hazard ratio	95%CI	*P* value
Race (black *vs* non-black)	2.68	1.67-6.73	0.032
Donor (living *vs* deceased)	0.32	0.11-0.94	0.038
Ketoconazole (yes *vs* no)	2.33	1.33-4.07	0.003
Delayed graft function (yes *vs* no)	2.14	1.22-3.73	0.008
Infection (yes *vs* no)	1.89	1.04-3.48	0.038
Tacrolimus dose (mg/d) in 2nd month	0.89	0.75-0.96	0.041

toconazole usage was an independent risk of acute rejection ((HR = 2.33, 95%CI: 1.33-4.07; P = 0.003). Tacrolimus dose at each time point was also tested. The daily dose of tacrolimus in the 2nd month after transplant was a significant factor in determining the risk of rejection (HR = 0.89, 95%CI: 0.75-0.96; P = 0.041), *i.e.*, the higher the daily dose, the lower the risk of rejection. Other commonly described risk factors in literatures, such as black ethnicity, DGF and infectious complications were also demonstrated in our study, while live donor kidneys were associated with lower risk of rejection compared to the deceased donor kidneys.

DISCUSSION

Tacrolimus remains a backbone of modern immunosuppressive therapy in solid organ transplants. Due to the numerous adverse effects, narrow safety margin and large intra- and inter-individual variability in pharmacokinetics, therapeutic monitoring is mandatory[1-3]. The normal pharmacokinetic curve of tacrolimus has a peak-and-trough pattern. A rapid peak phase reflects the absorption after an oral dose while a slow slope towards trough level reflects the drug metabolism. Ideally, tacrolimus dosing should be based on a 12 h area under the curve (AUC) that indicates the extent of systemic exposure. In clinical practice, oral doing is usually guided by monitoring 12 h trough levels, because of the convenience for blood sampling and the assumed correlation between trough level and AUC[2,3,12,13]. However, this correlation varies considerably and the best sampling time for a spot tacrolimus level to predict its total body exposure remains controversial[2,3,12-14]. The advance in pharmacogenetics has led the discovery of several gene polymorphisms in P450 family, which explains the inter-individual variability of tacrolimus metabolism[13,15,16]. Transplant patients

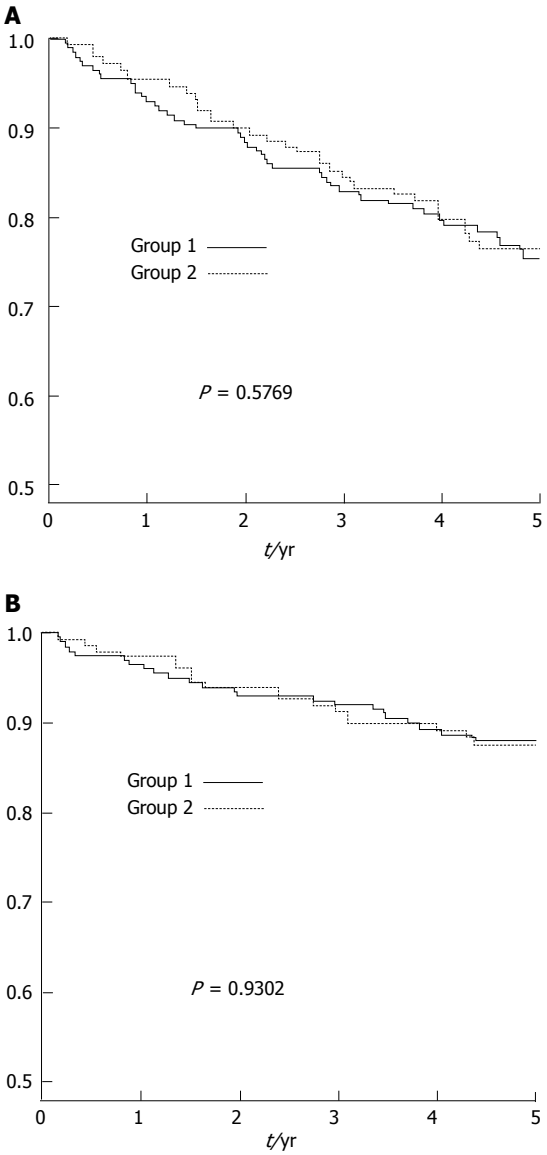

Figure 1 Kaplan-Meier estimated survival. A: Kidney graft survival; B: Patient survival.

expressing P450-3A5 (expressers) were shown to need higher doses of tacrolimus than non-expressers to reach similar trough levels[13,16].

Our study suggests that co-administration of ketoconazole with tacrolimus increase the risk of acute rejection. The 5-year cumulative incidence of acute rejection was significantly higher in Group 1 (34%) than Group 2 (18%), although the types of rejection were not different. Both groups achieved similar targeted trough levels at all time-points according to protocol. Compared to Group 2, Group 1 initially required higher dose of tacrolimus in the first week of transplant. As expected, their daily tacrolimus dose decreased with addition of ketoconazole. Subsequently, group 1 had significantly lower dose of tacrolimus in the first month and at all times after that. The risk factors for acute rejection were examined and we found that the use of ketoconazole was an independent risk of acute rejection (OR = 2.33, 95%CI: 1.33-4.07; P = 0.003). The daily dose of tacrolimus in the 2nd month after transplant was protective from rejection (HR = 0.89, 95%CI: 0.75-0.96; P = 0.041), *i.e.*, the higher the daily dose, the lower the risk of rejection. This suggests that higher incidence of rejection may be directly related to the reduced dose of tacrolimus from the co-administration of ketoconazole.

Previous studies from Egypt reported long-term safety and financial savings of coadministration of ketoconazole with cyclosporine in 51 patients after living-related kidney transplants[17,18]. The same group further studied the coadministration of ketoconazole with tacrolimus[4,5]. A total of 70 live donor kidney transplant recipients were randomized into ketoconazole group (100 mg/d) and control group (without ketoconazole). By six months, ketoconazole group experienced significant reduction of tacrolimus dose (by 58.7%) and cost (by 56.9%)[4]. After 2 years, ketoconazole group still had a remarkable reduction of tacrolimus dose (by 53.8%) and financial cost (by 52.9%). There was no adverse effect of ketoconazole throughout the 2 years[5]. None of the Egyptian studies noted higher incidence of acute rejection with ketoconazole. Our current study is different from theirs in many aspects. In addition to a larger population and longer follow-up, our transplant recipients were more heterogeneous where the majority of patients were African Americans (64% in Group 1). Deceased donor kidneys (74%) rather than living-donor kidneys were the dominant allografts and about 30% of patients experienced DGF after transplants. Many patients were also highly sensitized and/or poorly matched with donors. Therefore, our patients would be considered to have higher risk for acute rejection[19,20]. This may explain the difference in the results. Similar to their studies, we did not found any toxic side effect of using low dose of ketoconazole in kidney transplant patients.

In HIV-infected transplant patients, we have experienced difficulties in dosing CNI. As reported previously, the acute rejection rates were unexpectedly high (31% at 1 year, and 41% at 3 years) in HIV-infected kidney recipients despite the fact that their targeted trough levels of CNI were similar to non-HIV patients. More than half (52%) of acute rejection episodes did not respond to steroid therapy[6]. Even in HIV-infected liver transplant recipients, about half of the acute rejections occurred within the first 3 wk of transplant[7]. The protease inhibitor (also a potent inhibitor of P450-3A and P-glycoprotein) used to control HIV infection in these patients likely changed the normal pharmacokinetic curve of CNI. Jain *et al*[11] found that the pharmacokinetic curves of tacrolimus in these patients did not show a normal peak-and-trough pattern, but rather resembled a flat line. Recently, van Maarseveen *et al*[21] studied the pharmacokinetics of tacrolimus in patients receiving ritonavir. It was found that their pharmacokinetic curves lacked an absorption peak every 12 h. When similar trough level was targeted, their mean 12-h AUC was approximately 44% lower than the AUC in HIV-negative recipients. Therefore, the authors suggested that the trough levels of tacrolimus in the HIV-positive patients receiving ritonavir should be approximately 40% higher compared to HIV-negative recipients in order to achieve an equivalent exposure (AUCs) of tacrolimus. Indeed, the previous study also noted that a higher tacrolimus trough level was associated with a decreased risk of first rejection (HR = 0.90; 95%CI: 0.81-1.00; P = 0.04) in HIV-infected transplant patients[6].

Interestingly, a recent study found that HIV could infect the transplanted renal allografts despite undetectable viremia. The reinfection of HIV in tubular cells was hypothesized to stimulate immune responses and increase the risk of rejection[9]. A dysregulated immune response in HIV-infected host was also proposed by others[6,7]. However, a French report showed similar rejection rates (15%) after kidney transplants in HIV-infected patients *vs* non-infected patients[22]. They attributed the lower rejection rate to the use of raltegravir (an integrase inhibitor)-based antiviral therapy, which does not inhibit P450 system. Subsequently, their patients had "normal" exposures to CNI. Lack of higher incidence of rejection in the French study does not support the hypothesis of either viral infection or dysregulated immune response as the predominant mechanism for the higher rejection rates observed in the United States study. Taken together, a more plausible explanation appears to be lower exposure to CNI due to co-administration of protease inhibitor in these HIV-infected recipients.

We speculate that similarly altered pharmacokinetic phenomenon would exist in our patients, which could explain our result. Co-administration of ketoconazole lowered the dose of tacrolimus and flatted the normal peak-and-trough curve, therefore, decreased the AUC of tacrolimus and increased the risk of acute rejection.

Our study is limited by its retrospective nature, single center data, and lack of peak level monitoring and AUC data of tacrolimus. Nevertheless, it is the first report of high risk of rejection associated with coadministration of ketoconazole with tacrolimus in HIV-negative transplant recipients. This is consistent with the results in HIV-infected transplant patients. It is an important issue for car-

ing those patients with financial difficulty. This issue may be particularly relevant in the developing countries where co-administration of an inexpensive P450-3A inhibitor is a common practice to cut costs associated with expensive CNI. Our data suggests that high vigilance and careful monitoring is necessary, especially if other risk factors of rejection are present. Clearly, a prospective, randomized or a self-controlled study is needed to characterize the pharmacokinetic curves of tacrolimus with ketoconazole, so that a higher trough level can be proposed for clinical practice.

COMMENTS

Background

Tacrolimus is mainly metabolized by cytochrome P450 enzymes and ketoconazole is a potent inhibitor of P450. Transplant programs often use ketoconazole to reduce the tacrolimus dose and financial cost. But the long-term safety of this combination in kidney transplant recipients remains to be studied.

Research frontiers

This experience in human immunodeficiency virus (HIV) infected patients has noted unexpected high incidences of acute rejection after kidney and liver transplants. In these patients, the targeted trough levels of calcineurin inhibitors (CNIs) were consistent with those in non-HIV recipients, but their required doses of CNI were significantly lower due to the necessary treatment with antiretroviral protease inhibitor, which inhibits P450 enzymes.

Innovations and breakthroughs

Due to the altered pharmacokinetics of CNI by P450 inhibition from protease inhibitor, the total exposure to CNI (area under curve) is considerably low. This may explain the higher-than-expected rejection rates in HIV infected patients. In the current study, the authors have reported similar outcome from the coadministration of ketoconazole with tacrolimus in non-HIV infected kidney transplant recipients.

Applications

Coadministration of a P450 inhibitor to cut the dose and cost of tacrolimus may increase the risk of graft rejection. High vigilance and careful monitoring is necessary. A prospective, randomized or a self-controlled study is needed to characterize the pharmacokinetic curves of tacrolimus with ketoconazole, so that a higher trough level can be proposed for clinical practice.

Terminology

Kidney transplant: remove a kidney from a donor and put it into a patient with kidney failure; Rejection: recipient body attacks donor kidney as a foreign object; tacrolimus: a key drug to prevent rejection; ketoconazole: a cheap antibiotics that inhibits the breakdown of tacrolimus, therefore, saves the dose and cost of tacrolimus.

Peer review

This is a needed study exploring the outcome of attempting to reduce the dose of tacrolimus by adding ketoconazole to the regimen in first kidney transplant recipients to reduce the cost of immunosuppressive drugs. The interest of this work lies primarily in the observation of the authors regarding the five-year cumulative incidence of acute rejection in the group with ketoconazole and the consequently conclusion that the use of ketoconazole was an independent risk of acute rejection. The overall study is correct and the literature is to date.

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P- Reviewer: Cantarovich F, Friedman EA, Wagner KD
S- Editor: Song XX **L- Editor**: A **E- Editor**: Wu HL

World Journal of Nephrology

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Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.5527/wjn.v3.i3.114

World J Nephrol 2014 August 6; 3(3): 114-117
ISSN 2220-6124 (online)

CASE REPORT

Recurrent epiploic appendagitis and peritoneal dialysis: A case report and literature review

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Received: March 4, 2014 Revised: April 28, 2014
Accepted: May 28, 2014
Published online: August 6, 2014

Abstract

Epiploic appendagitis (EA) is rare cause of acute or subacute abdominal pain in patients on peritoneal dialysis (PD), where the diagnosis can be challenging as the clinical features, laboratory markers and imaging characteristics have not been described previously in this group of patients. Here, we present the management of a case of EA in a patient on PD and review published literature pertinent to the subject. The importance of establishing the diagnosis early by laparoscopy is emphasised.

Key words: Epiploic appendagitis; Peritoneal dialysis; Abdominal pain; Diagnosis; Laparoscopy

Core tip: The diagnosis and management of a patient with epiploic appendagitis (EA), who presents with acute abdominal pain, can be challenging. A high index of suspicion, exclusion of other causes of acute abdominal pain by computerised tomographic scan and an a low threshold for an early diagnostic laparoscopy is the way forward in establishing the diagnosis of EA and preservation of peritoneal dialysis catheter.

Shrestha B, Hampton J. Recurrent epiploic appendagitis and peritoneal dialysis: A case report and literature review. *World J Nephrol* 2014; 3(3): 114-117 Available from: URL: http://www.wjgnet.com/2220-6124/full/v3/i3/114.htm DOI: http://dx.doi.org/10.5527/wjn.v3.i3.114

INTRODUCTION

Epiploic appendagitis (EA) is a rare cause of acute or subacute abdominal pain, where the diagnosis can be challenging, particularly in a patient on peritoneal dialysis (PD)[1]. Lack of pathognomonic clinical, laboratory and radiological findings can pose significant difficulty in diagnosis and differentiation of EA from common acute abdominal pathologies. EA is a self-limiting condition with a benign clinical course, which can be managed conservatively, if the diagnosis can be made through exclusion. A Medline search on the management of EA on PD patients showed no publications on this subject. Here, we present the management of patient on PD presenting with left iliac fossa pain (LIF), where the diagnosis of EA was confirmed by laparoscopy and review the pertinent published literature.

CASE REPORT

A 61-year-old female, who was on peritoneal dialysis for 2 years for renal failure secondary to *Escherichia coli* sepsis in the past, had presented with sudden onset of continuous severe LIF in the early hours of the morning which was exacerbated by movements. There was no associated fever, vomiting, change in bowel habit or haemodynamic instability. There was marked tenderness and guarding in the LIF inferolateral to the exit site of the PD catheter. However, there was no clinical evidence of infection involving the exit site, catheter tunnel or peritonitis. The complete blood count was normal, but the C-reactive protein (CRP) was raised to 16 mg/L (normal range, 0-5

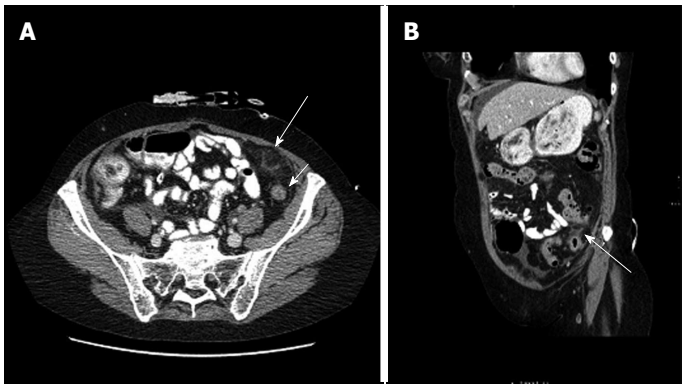

Figure 1 Computed tomography. A: Axial computed tomography (CT) scan of the abdomen showing a fat density area with some surrounding inflammation (large arrow) anterior to the descending colon (small arrow) in the left iliac fossa just below the abdominal wall at the site of epiploic appendagitis; B: A coronal oblique CT image showing the fat density area with surrounding inflammation (arrow) adjacent to the descending colon.

mg/L). The cytology of PD fluid showed a white blood cell count of 251/mm^3 with 94% polymorphonuclear leucocytes with negative culture. An ultrasound (US) scan and a non-contrast computerised tomographic (CT) scan of abdomen were unremarkable. She was treated with analgesics, intraperitoneal Vancomycin (2 gm) and Gentamicin (32 mg) on a presumed diagnosis of low-grade peritonitis. Five days later her abdominal pain had subsided significantly, hence she was discharged home.

Two weeks later, she presented with recurrent pain in the LIF, which on one occasion was very intense mimicking acute mesenteric ischaemia requiring repeated administration of morphine. Abdominal examination revealed tenderness and guarding in the LIF. The white cell count was normal, but the CRP was elevated to 67 mg/L. The culture of the PD fluid was sterile. Arterial blood gas and blood lactate were normal. A repeat contrast enhanced CT scan of abdomen showed patent coeliac axis, superior and inferior mesenteric arteries with normal perfusion of the intestine. However there was small amount of fluid in the pelvis and a fat density area with some surrounding inflammation anterior to the descending colon in the left iliac fossa just below the abdominal wall, which was suggestive of the possible diagnosis of epiploic appendagitis (Figure 1). A flexible sigmoidoscopy and transvaginal US scan were normal.

As the abdominal pain was intractable and persistent after 6 wk of treatment, under general anaesthesia, the PD catheter was removed and a laparoscopy was carried out using the PD catheter entry site. The greater omentum was adherent in the LIF where a small area on the anterior surface of the middle of the sigmoid colon was adherent to anterior abdominal wall over the site of maximum tenderness. There was evidence of inflammation on the anterior surface of the sigmoid colon where appendix epiploicae were present at the site of fibrinous adhesion, indicating epiploica appendagitis (Figure 2). Rest of the sigmoid colon and the abdominal organs were normal. The adhesion was released laparoscopically after inserting a 5 mm port in the right iliac fossa and a new PD catheter was replaced. This led to complete resolution of symptoms and restoration of PD.

DISCUSSION

Epiploic appendages are peritoneal outpouching of adipose tissue on the colonic surface, 50-100 in number, measuring 0.5-5 cm in length. The majority of them are situated on the sigmoid and descending colon, which explains the preponderance of EA in the left side of the abdomen, compatible with the finding in our case. Each appendage is supplied with one or two arteries from the colonic vasa recta and drained by a single vein[2]. These vascular structures are extremely mobile and susceptible to ischaemia, inflammation and necrosis if torsion, kink or stretching occurs[3].

Since the first report of EA in 1959 by T Case, several case series have been described in the normal population[4], but the clinical features and management of EA in a PD patient has not been reported previously. The primary EA is believed to occur spontaneously, whereas secondary EA occurs as a result of adjacent inflammatory diseases such as diverticulitis, appendicitis and cholecystitis[5,6].

The presenting symptom of EA is a localised non-migratory pain on the LIF, in the absence of severe illness, which is exacerbated by movements. Localised and rebound tenderness are usually present. There is lack of significant inflammatory response, particularly at the time of initial presentation. In PD patients, the nature of pain may be different if the inflamed appendage is adherent to the parietes and repeated distension of the abdominal wall during PD fluid exchanges may lead to stretching of the sensitive parietal peritoneum and severe pain. A high index of suspicion of EA after exclusion of acute diverticulitis, appendicitis, haemorrhagic ovarian cyst, torsion of an ovarian cyst, pelvic inflammatory diseases and mesenteric lymphadenitis, delayed perforation of bowel by the PD catheter or ischaemia of intra-abdominal organs, is necessary. Epiploic appendagitis adherent to the abdominal wall can lead to complications such as intestinal obstruction form torsion of the small intestine, strangulation, ileus and catheter drainage problems[7-10].

The patients on PD are more prone to serious intra-abdominal pathology than patients on haemodialysis due to an effect of PD to impair the normal physiological response of the peritoneal membrane[11]. An early diagnosis of EA by excluding all causes of surgical peritonitis is paramount in PD patients. An undiagnosed peritonitis in PD patients caused by perforation of intestine or intestinal ischaemia can prove fatal. The presence of pneumo-

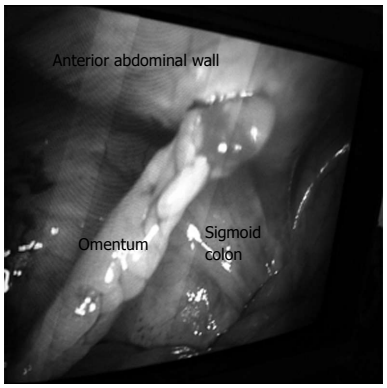

Figure 2 Laparoscopic appearance showing the omentum and anterior surface of the sigmoid colon adherent to the peritoneum lining the anterior abdominal wall in the left iliac fossa.

peritoneum visible in erect chest-X-ray and CT scan may not be sensitive or specific for a perforated hollow viscus since this may be present in 3.7% of healthy PD patients and in 0%-11% in patients with bowel perforation[12].

Laboratory findings specific to EA could be insignificant according to several studies. Mild leucocytosis and a low-grade increase in CRP is accepted as markers of mild inflammation[13]. The diagnostic yield of radiological investigations such as US, CT and magnetic resonance (MR) scan is considered to be high, although the findings are not specific for EA. Epiploic appendages cannot usually be seen during radiological imaging unless inflamed or associated with gross ascites or haemoperitoneum. EA may be diagnosed with US scan, with findings of solid, oval, non-compressible hyperechoic mass under the site of maximal tenderness, and lack central blood flow on Doppler US scan[14]. The CT features of EA are characteristic according to several studies, which show a pericolonic ovoid mass with hyperattenuating rim surrounded by fat stranding[15,16]. The anterior localisation of EA in relationship to the colonic lumen is mentioned as a usual finding in making a confident diagnosis. The other fatty lesions that can mimic US and CT findings of acute EA are chronic calcified EA, omental infarction, post-operative changes, and peritoneal carcinomatosis[17,18]. The MR scan findings include an oval shaped fat intensity mass with a central dot on T1- and T2-weighted images, which possessed an enhancing rim on post-gadolinium T1-weighted fat saturated images. The lesion is best visualized on post-contrast T1-weighted fat saturated images[19]. The only concern about MR scan is the risk of nephrogenic systemic sclerosis due to gadolinium.

The majority of patients with EA recover spontaneously within less than four weeks with conservative management[20]. Recurrence of symptoms have been reported in up to 40% of cases, where surgery in the form of laparoscopic or conventional open excision of the inflamed epiploic appendix may be needed[13]. Establishment of an early and definitive diagnosis of EA may not be straightforward, in PD patients, as it happened in our case. There was a delay of 6 wk before a diagnostic laparoscopy was performed. We believe that a low threshold for diagnostic laparoscopy should be maintained if the symptoms fail to resolve within a week or the diagnosis remains uncertain[21,22]. We did remove the original catheter and used the catheter entry site to introduce a port and telescope. In retrospect, laparoscopy without removal of catheter could have been performed, thereby avoiding the need of removal and replacement of PD catheter.

In a conclusion, establishment of the diagnosis of EA in patients on PD can be challenging, hence a diagnostic laparoscopy should be undertaken early to establish the diagnosis, exclude any other abdominal conditions and retain the PD catheter.

COMMENTS

Case characteristics

A 67-year-old female, on peritoneal dialysis (PD), presented with recurrent attacks severe left iliac fossa pain.

Clinical diagnosis

There was marked tenderness and guarding in the left iliac fossa.

Differential diagnosis

Consideration of acute diverticulitis, appendicitis, haemorrhagic ovarian cyst, torsion of an ovarian cyst, pelvic inflammatory diseases and mesenteric lymphadenitis, delayed perforation of bowel by the PD catheter or ischaemia of intra-abdominal organs, in the differential diagnosis is necessary.

Laboratory diagnosis

An elevated C-reactive protein was present.

Imaging diagnosis

Computed tomography scan of the abdomen showed inflammatory changes in the left iliac fossa (LIF) suggesting the possibility of epiploic appendagitis (EA).

Pathological diagnosis

Laparoscopy revealed adhesion of the sigmoid colon to the anterior abdominal wall with an appendix Epiploica was present.

Treatment

Laparoscopy and adhesiolysis led to complete resolution of symptoms.

Experiences and lessons

Recurrent pain over the LIF is patient on PD in the absence of classical clinical and radiological features should prompt a clinician to consider the diagnosis of EA and perform a diagnostic laparoscopy to establish the diagnosis.

Peer review

This case report is suitable for publication.

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P- Reviewer: Schuld J, Stavroulopoulos A **S- Editor**: Wen LL
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DOI: 10.5527/wjn.v3.i3.118

World J Nephrol 2014 August 6; 3(3): 118-121
ISSN 2220-6124 (online)

CASE REPORT

Innocuous-looking skin scab over an arteriovenous fistula: Case report and literature review

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Received: March 7, 2014 Revised: April 28, 2014
Accepted: June 10, 2014
Published online: August 6, 2014

Abstract

Little is written on the management of an innocuous-looking skin scab over an autogenous arteriovenous fistula (AVF) used for haemodialysis. The seriousness of the underlying pathology can be under-estimated, and this may lead to early loss of the AVF, and major-life-threatening haemorrhage. We describe the management of a 78-year-old patient presenting with an innocuous-looking scab over an AVF and review the pertinent literature on this subject.

Key words: Arteriovenous fistula; Scab; Duplex scan; Bleeding; Treatment

Core tip: An innocuous looking scab may develop over an arteriovenous fistula after repeated punctures. There may be serious underlying damage to the arterialised vein. This case report emphasizes the importance of an early diagnostic colour Doppler examination in these circumstances and timely surgical intervention, to prevent potential life-threatening haemorrhage or other complications.

Shrestha B, Boyes S, Brown P. Innocuous-looking skin scab over an arteriovenous fistula: Case report and literature review. *World J Nephrol* 2014; 3(3): 118-121 Available from: URL: http://www.wjgnet.com/2220-6124/full/v3/i3/118.htm DOI: http://dx.doi.org/10.5527/wjn.v3.i3.118

INTRODUCTION

An arteriovenous fistula (AVF) is a crucial life line for patients with end-stage renal failure, and its preservation is paramount. Complications related to AVF such as puncture haematoma, pseudoaneurysm formation, venous hypertension, arterial steal, high-output cardiac failure, and ischemic neuropathy, can not only lead to the early loss of an AVF but also can be life-threatening[1,2]. The prevalence of AVF compared to arteriovenous grafts, has risen steadily as a result of the National Kidney Foundation/Kidney Disease Outcome Quality Initiative (NKF/KDOQI) recommendations[3,4]. Little has been written on the pathological anatomy, natural history and management of visible scabs in the skin overlying AVF cannulation sites. We describe the management of a 78-year-old patient presenting with an innocuous-looking scab over the cannulation site of an AVF, emphasise the importance of early recognition and intervention, and review the pertinent literature related to the subject.

CASE REPORT

A 78-year-old male with renal failure secondary to obstructive uropathy had been on haemodialysis (HD) for two years using a brachiocephalic AVF. The AVF was cannulated using rope-ladder technique three times a week for 3 h in each session leading to excellent dialysis (pA: -71, pV: 73, blood flow: 360 mL/min, Kt/V: 1.4, pre-HD serum creatinine: 600 μmol/L and post-HD serum creatinine: 142 μmol/L). His haemoglobin was 12.2 gm/dL, white cell count of 7.5×10^6 / mL and C-reactive

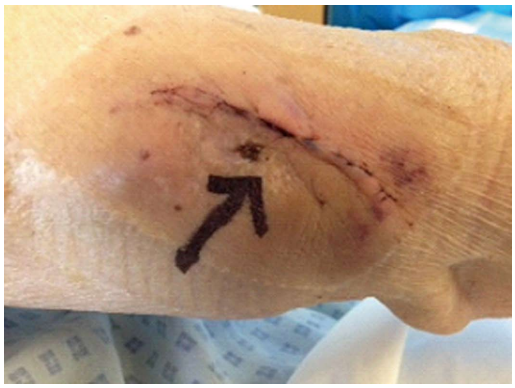

Figure 1 A 3 mm size scab over the arteriovenous fistula (black arrow).

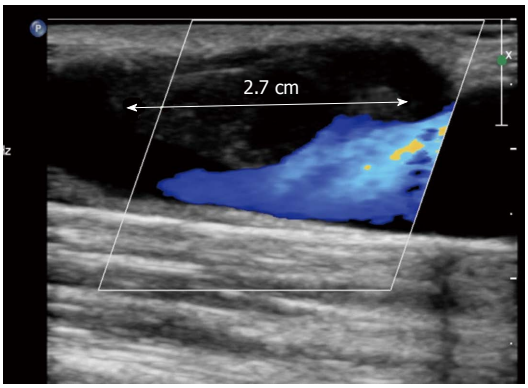

Figure 2 Colour Doppler scan showing a 2.7 cm long thrombus (white arrow) partially occluding the lumen of the arteriovenous fistula.

protein of 25 mg/L. His regular medication was erythropoietin, alphacalcidol and simvastatin.

The patient, who normally dialysed at a satellite unit, was referred to our tertiary referral teaching hospital because the dialysis staff were concerned about a visible black scab on the distal needling site which had developed over the previous 2 wk. There was no history of bleeding post-dialysis.

On examination, there was a 3 mm-sized black scab on the skin at the distal cannulation site without surrounding cellulitis or discharge (Figure 1). There was no clinical evidence of pseudo-aneurysm or subcutaneous haematoma. A palpable thrill and a low-pitched bruit were present along the course of the arterialised upper arm cephalic vein. The initial clinical impression was that the scab represented needle tract thrombus, which could be managed conservatively. However, based on previous experience of managing similar cases, a Doppler ultrasound scan was carried out. This showed a thrombus under the scab measuring 2.7 cm in length narrowing the vein significantly (Figure 2). It was suspected that although the size of the scab on the skin surface was about 3 mm in diameter, the defect in the cephalic vein wall could be more than 2 cm. A decision was made to explore the fistula and repair the defect.

Under general anaesthesia, through an incision parallel to the AVF, the arterialised cephalic vein was skeletonised and controlled proximal and distal to the scab. The skin containing the scab was dissected off the vein, and this revealed dark thrombus bulging out through a 2.5 cm long defect on the anterior wall of the vein. The thrombus was causing partial narrowing of the vein (Figures 3 and 4). The thrombus was removed and the vein filled proximally and distally with heparinised saline. The edge of the defect on the vessel wall was debrided and closed using continuous 4/0 prolene suture. The thrombus was sent for culture and remained sterile after 48 h of incubation. The AV fistula was used for HD after 24 h.

DISCUSSION

Dialysis access procedures and access complications are the leading causes of morbidity among HD patients, often resulting in an increased number of hospitalizations and missed dialysis sessions. These complications, such as erosion, infection, thrombosis, and pseudoaneurysm formation, burden the health care system and disrupt patient care. As such, limiting these complications can significantly improve the quality of life of dialysis patients whilst decreasing health care costs[5,6]. The trauma of recurrent cannulation of a functioning AVF through the same site can lead to a tear of the wall of the vein. Simultaneous heparinisation during HD allows blood to flow out through the tear leading to the formation of a small pseudoaneurysm. Thrombus can form within the pseudoaneurysm in hypotensive and hypercoagulable states. The size of the pseudoaneurysm may be difficult to estimate on physical examination. The incidence of pseudoaneurysms complicating AVF ranges from 2% to 10%[7]. Generally, investigation of an innocuous-looking scab over an AVF is seldom initiated unless there is bleeding post-HD from the cannulation site, an obvious pseudoaneurysm, recirculation or venous hypertension[8].

Our case illustrates the fact that although the scab was only 3 mm in diameter with no evidence of surrounding inflammation, it was associated with a 2.5 cm long defect in the vein wall. The defect was filled with a thrombus which was also narrowing the lumen of the vein. The appearances were consistent with a thrombosed pseudoaneurysm.

Repeated needling of thrombus within a pseudoananeurysm may cause infective complications which may lead to bacteraemia and systemic sepsis. There is also a risk of necrosis of the overlying skin, ulceration and a life-threatening haemorrhage[9,10]. Dislodgement of the thrombus can lead to fatal pulmonary embolism. Recognition of the potential danger of small skin scabs was important in the timely investigation and intervention in our patient. Not only was the AVF salvaged, but the potential hazard of massive bleeding was prevented.

When assessing patients with skin scabs, a history of bleeding post-dialysis, adequacy of dialysis and systemic sepsis should be obtained. Physical examination should include the inspection of skin overlying the AVF for the extent of skin necrosis, cellulitis, pulsatility and haematoma, as well as assessment of the nature of the bruit along the length of the AVF. Duplex ultrasound is the

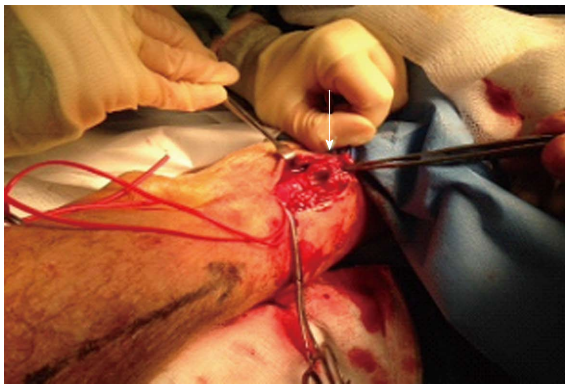

Figure 3 A 2.5 cm long defect on the anterior wall of the vein (white arrow).

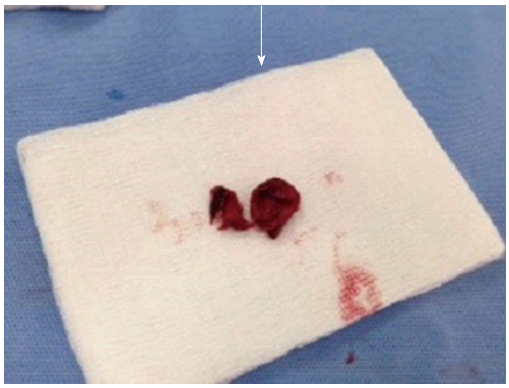

Figure 4 Thrombus removed from the defect in the vessel wall.

primary diagnostic modality, with a sensitivity of 94% in the assessment of the size of the defect, and complications such as the presence of thrombus, reduced blood flow, stenoses and pseudo-aneurysms[11]. Ultrasound-guided compression of pseudoaneurysms can be curative[12]. If a defect is detected in the wall of the AVF, the patient should be prepared for exploration of the AVF under brachial block or general anaesthesia. Broad-spectrum antibiotic cover should be provided at the time of surgery. Surgical repair of the defect can be done by direct suture, patch angioplasty using saphenous vein or a Dacron patch, excision followed by end-to-end anastomosis, excision and interposition of a Polytetrafluroethylene graft or insertion of a bypass graft[13]. A large skin defect due to necrosis may require a locally rotated full-thickness Limberg skin flap[14]. Any thrombus extracted from the AVF should be sent for microbiological assessment. In majority of the patients, the AVF can be salvaged and used for HD within 24 h. If a bypass graft is inserted, the reported primary-assisted patency at 12 mo is 50%[15].

Every effort should be made to prevent the development of pseudoaneurysms, venous stenoses, skin erosions, haemorrhage and reduced access patency. The cannulation technique of an AVF has been a subject of debate. The rope-ladder (RL) technique is the most common technique, although button-hole cannulation (BHC) or constant-site technique is recommended by the NKF/KDOQI vascular access guidelines. Previous studies have shown less haematoma formation and less intervention in BHC group compared to the RL group[16-19]. However, a recent study comparing access patency, intervention rate, bacteraemia and mean scores from kidney disease-specific health-related quality of life (KD HRQOL-36) survey between RL and BHC groups failed to show a significant difference in outcome between the two groups[20].

In conclusion, awareness of the possibility of a large defect in the vein wall under an innocuous-looking scab, with the potential for life-threatening haemorrhage and access failure, should be raised among patients and health care workers[21]. If there is clinical concern an urgent Doppler ultrasound should be arranged, with urgent surgical intervention if indicated. It is also essential that trained staff follow established cannulation techniques to reduce the risk of puncture site complications.

COMMENTS

Case characteristics

A 78-year-old male, who was on haemodialysis using a brachiocephalic arteriovenous fistula (AVF), was referred by the dialysis staff raising concern for a scab over the cannulation site.

Clinical diagnosis

On examination, there was a 3 mm-sized black scab on the skin on the distal cannulation site without surrounding cellulitis or discharge.

Differential diagnosis

A pseudoaneurysm and a haematoma underlying the scab were entertained in the differential diagnosis.

Laboratory diagnosis

The white cell count and C-reactive protein were within normal limits.

Imaging diagnosis

The Duplex ultrasound scan showed a 2.7 cm large thrombus and a 2 cm defect in the vessel wall.

Pathological diagnosis

A 2.5 cm defect in the wall of the cephalic vein was occupied by a thrombus.

Treatment

The vessel was explored and the defect was closed with 4/0 prolene suture.

Experiences and lessons

The size of the visible scab over an autogenous AVF is misleading as in our case where there was a 2.5 cm defect in the vessel wall filled with a thrombus although the size of the scab was of 3 mm diameter. A Duplex san should be performed early to assess the AVF for prevention of bleeding.

Peer review

The authors described the management of a 78-year-old patient who presented with an innocuous-looking scab over the cannulation site of an AVF which turned out to be a thrombus on the outer wall of the vein. This a good case report.

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P- Reviewer: Teo TKB, Olowu WA **S- Editor**: Wen LL
L- Editor: A **E- Editor**: Wu HL

ISSN 2220-6124 (online)

World Journal of *Nephrology*

World J Nephrol 2014 November 6; 3(4): 122-323

Volume End

Published by Baishideng Publishing Group Inc

WJN

World Journal of Nephrology

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INDEXING/ABSTRACTING

World Journal of Nephrology is now indexed in PubMed Central, PubMed, and Digital Object Identifier.

FLYLEAF I-III Editorial Board

NAME OF JOURNAL
World Journal of Nephrology

ISSN
ISSN 2220-6124 (online)

LAUNCH DATE
February 6, 2012

FREQUENCY
Quarterly

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World Journal of Nephrology
Room 903, Building D, Ocean International Center,
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PUBLICATION DATE
November 6, 2014

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DOI: 10.5527/wjn.v3.i4.122

World J Nephrol 2014 November 6; 3(4): 122-142
ISSN 2220-6124 (online)

REVIEW

Nephropathy in dietary hyperoxaluria: A potentially preventable acute or chronic kidney disease

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Received: April 26, 2014 Revised: June 12, 2014
Accepted: August 27, 2014
Published online: November 6, 2014

Abstract

Hyperoxaluria can cause not only nephrolithiasis and nephrocalcinosis, but also renal parenchymal disease histologically characterized by deposition of calcium oxalate crystals throughout the renal parenchyma, profound tubular damage and interstitial inflammation and fibrosis. Hyperoxaluric nephropathy presents clinically as acute or chronic renal failure that may progress to end-stage renal disease (ESRD). This sequence of events, well recognized in the past in primary and enteric hyperoxalurias, has also been documented in a few cases of dietary hyperoxaluria. Estimates of oxalate intake in patients with chronic dietary hyperoxaluria who developed chronic kidney disease or ESRD were comparable to the reported average oxalate content of the diets of certain populations worldwide, thus raising the question whether dietary hyperoxaluria is a primary cause of ESRD in these regions. Studies addressing this question have the potential of improving population health and should be undertaken, alongside ongoing studies which are yielding fresh insights into the mechanisms of intestinal absorption and renal excretion of oxalate, and into the mechanisms of development of oxalate-induced renal parenchymal disease. Novel preventive and therapeutic strategies for treating all types of hyperoxaluria are expected to develop from these studies.

Key words: Dietary hyperoxaluria; Chronic oxalate nephropathy; Acute oxalate nephropathy; Acute tubular necrosis; Interstitial nephritis; Nephrocalcinosis; Calcium oxalate nephrolithiasis; Oxalate transporters;

Inflammasomes

Core tip: Chronic nephropathy secondary to dietary hyperoxaluria has been reported in a limited number of patients. Dietary oxalate intake in these patients was lower than the average intake in certain parts of the world. This raises the question whether dietary hyperoxaluria has been a neglected cause of chronic kidney disease. This question along with recent findings elucidating the pathogenesis of oxalate nephropathy calls for further research in epidemiology, prevention and treatment of hyperoxaluria.

Glew RH, Sun Y, Horowitz BL, Konstantinov KN, Barry M, Fair JR, Massie L, Tzamaloukas AH. Nephropathy in dietary hyperoxaluria: A potentially preventable acute or chronic kidney disease. *World J Nephrol* 2014; 3(4): 122-142 Available from: URL: http://www.wjgnet.com/2220-6124/full/v3/i4/122.htm DOI: http://dx.doi.org/10.5527/wjn.v3.i4.122

INTRODUCTION

Oxaluria has been extensively studied in the context of nephrolithiasis[1-15]. While hyperoxaluria from various causes represents a definitive risk for calcium oxalate nephrolithiasis[1,2], lacking is convincing epidemiological evidence that oxaluria is a risk factor for idiopathic renal stone formation[9,10]. In addition to nephrolithiasis, hyperoxaluria can also cause nephrocalcinosis involving the renal cortex, the renal medulla, or both[16-21], acute kidney injury (AKI) and chronic kidney disease (CKD). Oxaluria has two sources: oxalate formed endogenously from metabolism of its precursors and oxalate absorbed from the gastrointestinal tract. Increased rate of formation or increased rate of absorption of oxalate can lead to hyperoxaluria. The principal aim of this review is to address various aspects of hyperoxaluric AKI and CKD with emphasis on nephropathy secondary to high dietary intake of oxalate. This topic was selected because of its potential epidemiologic importance. In addition, interest to the topic is enhanced by important recent developments in the pathogenesis of hyperoxaluric CKD and the relative paucity of published information on renal parenchymal disease from dietary hyperoxaluria.

This review will analyze in sequence the biochemistry of oxalate and oxalate stones, the pathways of hepatic synthesis of oxalate, the gastrointestinal absorption and renal excretion of oxalate, the various types of hyperoxaluria with emphasis on the dietary variety, and the histologic types of oxalate nephropathy and their pathogenesis. The final section focuses on future research avenues that may illuminate the topic of dietary hyperoxaluria. The potential benefit from this research could be a reduction of the incidence of end-stage renal disease (ESRD)[22].

CHEMISTRY AND PROPERTIES OF OXALIC ACID AND OXALATE STONES

Oxalic acid is a two-carbon dicarboxylic acid (HOOC-COOH). For a long time it was thought that oxalate stones were comprised of mono- and di-hydrates of calcium oxalate, with some contribution from trihydrates. However, recent studies have led to a picture in which some non-oxalate preformed particle such as a crystal of uric acid, phosphate salts, drugs or drug metabolite act as the heterogeneous nucleus for formation of the oxalate calculus[23].

Oxalic acid is a moderately strong acid with pKa values of 1.23 and 4.19. In its full ionic form it is called oxalate. Whereas oxalic acid is relatively soluble in water (8700 mg/dL; pH 7, 20 ℃), calcium oxalate is three to four orders of magnitude less soluble (0.67 mg/dL; pH 7.0, 20 ℃) and crystallizes readily. By way of comparison, calcium urate is about 400-fold more soluble than calcium oxalate[24]. Oxalate also forms crystals with other polyvalent ions, including magnesium, ferrous iron and zinc. The water solubility (expressed as mg/dL) of these complexes at 18 ℃ to 20 ℃ is as follows: magnesium oxalate 70.0, ferrous oxalate 22.0 and zinc oxalate 0.79, respectively. The solubility of calcium oxalate increases slightly with increasing pH; however, hydrogen ion changes in the physiological range have only a small effect on calcium oxalate solubility.

Oxalic acid is a toxic substance. It is not known whether oxalic acid and oxalate are themselves toxic before they react with calcium to form calcium oxalate. Under normal circumstances the concentration of oxalate in the blood and urine depends on the content of oxalic acid in foods and on metabolic conversion of endogenous oxalate precursors largely by oxidative reactions. Furthermore, dietary factors and substances other than oxalic acid per se can influence the tendency for oxalate crystals to form; these factors include: the amino acids 4-hydroxyproline, serine and glycine, calcium, and possibly ascorbic acid and fructose.

EXOGENOUS SOURCES OF OXALIC ACID

In Nature oxalic acid occurs in the free form but more commonly as the salt of sodium, potassium, calcium, magnesium or iron. The oxalate content of dietary items consumed by several populations has been analyzed[6,25-31]. Widely consumed foods that are rich in preformed oxalic acid include vegetables, nuts, cocoa, tea, and fruits high in vitamin C. Red meats, fish, poultry, eggs and dairy products contain relatively small amounts of oxalic acid. Items in Western diets that significantly increase urinary oxalate excretion include spinach, rhubarb, beets, nuts, chocolate, tea, wheat bran, and strawberries[6]. The bioavailability of ingested oxalate is influenced by other ingested items[32]. Oxalate content of various diets, its relation to nephrolithiasis, and guide-

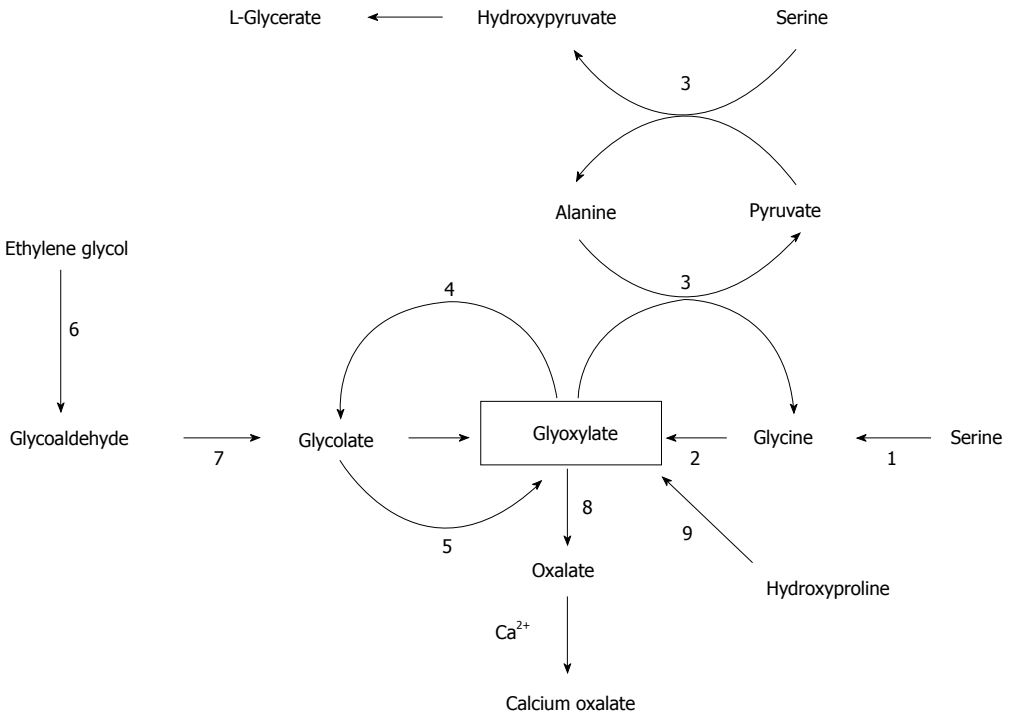

Figure 1 Biosynthesis of calcium oxalate. Glyoxylate is the main precursor of oxalate which combines spontaneously with calcium ions to form calcium oxalate. Names of enzymes: 1, serine hydroxymethyltransferase; 2, D-amino acid oxidase; 3, alanine:glyoxylate aminotransferase (AGT); 4, glyoxylate reductase-hydroxypyruvate reductase (GRHPR); 5, glycolate oxidase; 6, alcohol dehydrogenase; 7, aldehyde dehydrogenase; 8, lactate dehydrogenase; and 9, five enzyme-catalyzed reactions. PH1 results from mutations in AGT which is a hepatic peroxisomal enzyme. PH2 results from mutations in GRHPR which is a cytosolic enzyme found in several tissues, but primarily the liver. PH3 results from defects in the hepatic mitochondrial enzyme 4-hydroxy-2-oxoglutarate (HOG) aldolase which converts HOG and glyoxylate to pyruvate (reaction not shown), the last step in hydroxyproline catabolism. The reason why a deficiency of HOG aldolase activity increases oxalate production is obscure.

lines for oxalate intake have been reported[13-15,33-37]. One set of guidelines for prevention of nephrolithiasis proposed a maximal daily oxalate intake of 200 mg daily[33]. We found no epidemiological reports relating dietary oxalate intake to oxalate nephropathy and no guidelines for prevention of this nephropathy.

Table 1 shows estimates of dietary oxalate intake in six countries[13,35,36,38-42]. Oxalate intake varies greatly between countries and regions of the same country. For example, daily oxalate intake in Western diets ranges between 44 and 930 mg[13]. The seasonal variation of oxalate intake in a rural population in India is extreme (Table 1). Very high consumption of oxalate in the context of dietary intake can be comparable to some reported lethal doses of the compound. Although the average lethal dose (LD50) of oxalate was estimated at 375 mg/kg[43], or 26.3 g for a 70 kg person, much lower doses of oxalate can be lethal. An intravenous dose of 1.2 g of sodium oxalate, which is equivalent to 0.8 g of oxalate, was lethal in one reported case[44]. Of note also is that most studies cited in Table 1[13,35,36,39,40,42] as well as other large epidemiological studies[45] analyzed dietary oxalate intake to evaluate the risk of nephrolithiasis and no study addressed the risk of CKD from dietary hyperoxaluria.

SOURCES OF OXALIC ACID IN THE BODY

The body burden of oxalic acid has two sources, endogenous production in the liver and absorption from the gastrointestinal tract. The pathways of hepatic production and gastrointestinal absorption of oxalic acid are discussed below.

Hepatic production of oxalic acid

Oxalate is synthesized in the liver but is not metabolized further in humans. Oxalic acid produced by catabolism of ingested oxalate precursors by means of normal metabolic pathways contributes significantly to the body's burden of oxalate. Earlier reports estimated that only 10% of the urinary oxalate was derived from dietary oxalate, while the remaining 90% was derived equally from metabolism of other oxalate precursors, including ascorbic acid[46].

Figure 1 shows the metabolism of oxalate, with emphasis on the pathways of primary hyperoxaluria and of metabolism of ethylene glycol, which is a major cause of acute oxalate intoxication. The major precursors of oxalate under normal circumstances appear to be the amino acids hydroxyproline, glycine and serine (Figure 1). Glycine and serine are present in all food proteins. Oxalate is also the end-product of the metabolism of ingested ethylene glycol, the main component of antifreeze, which is encountered usually in the setting of attempted suicide. In order to facilitate understanding of the these endogenous pathways, it may be helpful to consult Figure 1 which relates the major two-and three-carbon compounds that are relevant to this discussion.

Table 1 Daily dietary oxalate intake in various countries and regions

Country-region	Subjects	Subject number	Oxalate intake (mg/24-h)	Ref.
Brazil, Sao Paolo	+Stones	70 (M:42, F:28)	98 ± 137[3]	[13]
	Healthy controls	41 (M:14, F:27)	108 ± 133[3]	
England	Hospital diet	Not reported	118	[38]
Germany	+Stones, ↑oxaluria	93 (M:73, F:20)	130 ± 181[3]	[39]
	+Stones, →oxaluria	93 (M:73, F:20)	101 ± 145[3]	
India, Rajasthan	Rural "common" diet	Not reported	78	[40]
	Rural rainy season	Not reported	2045	
	Urban, upper income	Not reported	606	
	Urban, lower income	Not reported	169	
	Hospital diet	Not reported	139	
India, Pune	Boys, upper income	100	193 (116-309)[4]	[41]
	Boys, lower income	100	169 (102-354)[4]	
	Girls, upper income	100	168 (115-209)[4]	
	Girls, lower income	100	133 (87-209)[4]	
Italy	Normal subjects[1]	12 (M:8, F:4)	335	[42]
	Normal subjects[2]	12 (M:8, F:4)	18	
United States, South	F, 50-79 yr, +Stones	1.179	330 ± 161[3]	[35]
	F, 50-79 yr. –Stones	1.179	345 ± 166[3]	
United States	M, +Stones	1.627	214 ± 117[3]	[36]
	M, -Stones	44.358	214 ± 121[3]	
	F, older, +Stones	1.414	184 ± 109[3]	
	F, older, -Stones	91.358	185 ± 112[3]	
	F, younger, +Stones	1.564	179 ± 121[3]	
	F, younger, -Stones	100.260	183 ± 121[3]	

[1]Diet containing fruits and vegetables; [2]Diet without fruits and vegetables; [3]Mean ± SD; [4]Mean (25^{th}-75^{th} percentile). +Stones: History of urinary stones; -Stones: Absence of history of urinary stones; M: Male; F: Female.

The key player in this story is glyoxylate: it is the nexus of pathways that lead to and away from oxalate.

Hydroxyproline is one of the most abundant amino acids in collagen. It is present in collagen-containing meat products, including gelatin, and is one of the most abundant proteins in the human body. In fact, collagen accounts for about 30% of total animal proteins and contains about 13% hydroxyproline[47]. Glyoxylate is the two-carbon end-product of hydroxyproline catabolism (pyruvate is the other product). The conversion of glyoxylate to oxalate is catalyzed by lactate dehydrogenase. Each day the human body turns over 2-3 g of collagen. In the process 240-420 mg of hydroxyproline are released with the concomitant production of 140-240 mg of glyoxylate[48].

Knight *et al*[48] demonstrated using healthy volunteers that daily ingestion of 30 g of collagen for three days increased glycolate and oxalate excretion by 43% and 5.3-fold, respectively. Glycolate is produced when glyoxylate is acted on by glyoxylate reductase which in the literature is also identified as hydroxypyruvate reductase and D-glycerate dehydrogenase. However, only 5% of the ingested hydroxyproline was recovered as glyoxylate plus oxalate, thereby indicating that most of the glyoxylate resulting from hydroxyproline catabolism was probably diverted to glycine synthesis in the reaction catalyzed by alanine:glyoxylate aminotransferase (AGT). The means of directing glyoxylate away from oxalate synthesis is the glyoxylate reductase reaction that converts glyoxylate into glycolate. Since oxalate is not oxidized to carbon dioxide and water or otherwise metabolized by humans, its only route of disposal is urinary excretion. Quantitatively, transamination of glycine and oxidation of glycine by D-amino acid oxidase are much less important than catabolism of hydroxyproline as sources of oxalate.

Since the metabolism of serine and glycine are so intimately linked in humans and because they are interconvertible, it is reasonable to expect that if one of these amino acids is metabolized to glyoxylate, the other too should be a precursor of glyoxylate, and that both should be sources of oxalate. Such is the case. The enzyme that catalyzes the serine-glycine interconversion is folate-dependent serine hydroxymethyl transferase. Another enzyme, namely D- amino acid oxidase, also converts glycine to glyoxylate.

Although the underlying metabolic link between ascorbic acid and oxalic acid is obscure, there is evidence that a high oral or intravenous intake of ascorbic acid can result in a moderate increase in urinary oxalic acid[8,39,49,50]. With regard to parenteral feeding, Robitaille *et al*[51] found that, on average, 80 mg of a 105 g infused dose of ascorbic acid was recovered as urinary oxalic acid in elderly adults with normal kidney function. Furthermore, intravenous ascorbic acid administration increased urinary oxalic acid excretion in a dose-dependent manner. These authors cautioned against high-dose infusions of ascorbic acid for individuals already at high risk of oxalate stones.

Epidemiologic studies that have addressed the relation between fructose intake and increased risk for oxalate stones have yielded conflicting results: however,

a large epidemiological study found a significant association between high consumption of fructose and risk of kidney stones[52]. On the other hand, studies of urinary oxalate excretion in humans administered high amounts of fructose orally[53] or intravenously[54] have produced equivalent results. A 2010 investigation of the relationship between fructose consumption and urinary oxalate in healthy subjects found that urinary excretion of oxalate and glyoxylate, which is a marker of oxalate synthesis, did not change when the fructose content of the diet was raised as high as 21% of calories[55]. A possible effect of fructose on the absorption of dietary oxalate or calcium excretion was not assessed in that study. Furthermore, lacking is evidence that humans metabolize fructose to oxalate. However, fructose could affect the serum oxalate level indirectly by affecting events in the gastrointestinal tract. For example, hyperabsorption of oxalate caused by a low intake of calcium for complexation with oxalate in the GI tract can exacerbate hyperoxaluria[39].

Gastrointestinal absorption of oxalate

The contribution of oxalate absorbed from the gastrointestinal tract to the total body burden of oxalate depends on the oxalate content of the diet. Recently, in a study of normal volunteers consuming diets with varying oxalate content, Holmes and associates[56] showed that oxalate excretion in urine depends significantly on the dietary oxalate intake. Dietary oxalate intake accounted for 24.4% of the urinary oxalate excretion when the diet contained 10 mg of oxalate per 2500 kcal. Urinary oxalate excretion and the percent of urinary oxalate derived from dietary oxalate increased progressively with progressive rises in dietary oxalate content, reached a value of 41.5% of the urine oxalate when the diet contained 250 mg of oxalate per 2500 kcal, and increased further to 52.6% of the urine oxalate when the diet contained both 250 mg of oxalate per 2500 kcal and a low calcium intake. In the same study, although urinary excretion of oxalate increased substantially with increasing oxalate intake, estimated fractional absorption of oxalate from the gastrointestinal tract decreased from 55.4% at the lowest oxalate intake to 5.8% at the highest intake and then increased to 9.7% at the highest oxalate intake combined with low calcium intake[56]. These findings are important in the context of dietary hyperoxaluria.

The functions involved in the disposition of dietary oxalate are exclusively absorption from the intestines and renal excretion[57]. In the intestines, oxalate is absorbed passively by means of a paracellular pathway. Whereas unbound oxalate is absorbable, oxalate salts of divalent cations such as calcium and magnesium are insoluble in water and therefore not absorbable. Oxalate transporters in the enteric[58,59] and renal epithelial cells have been identified and are discussed in some detail in the following subsection.

The magnitude of oxalate absorption is affected by various dietary substances and the gastrointestinal milieu. Dietary oxalate content is an important determinant of oxalate absorption that is particularly relevant to this review. The fact that urinary oxalate is derived from two sources, absorption of dietary oxalate and endogenously produced oxalate, complicates the study of oxalate absorption in the gastrointestinal tract. A reliable method for estimating oxalate absorption is by labelling oxalate with a stable carbon isotope (^{13}C), ingesting a known quantity of labelled oxalate, and measuring the fractional (or percent) excretion of the labelled oxalate in the urine[60,61]. The method assumes that absorbed oxalate is excreted exclusively in the urine. In one study conducted in normal subjects, oxalate absorption was evaluated by this method when total dietary intake of oxalate was low (63 mg daily) and high (600 mg daily). Mean daily urine oxalate was 25 mg at the low oxalate intake and 43 mg at the high intake, while the percent absorption of ingested oxalate increased from 7.9% at the lower intake to 14.7% at the higher oxalate intake[62].

The dietary content of certain divalent cations has clinically important effects on oxalate absorption. High dietary contents of calcium[63-65] and magnesium[66] inhibit oxalate absorption. The mechanism of this inhibition is formation of insoluble and poorly absorbable oxalate salts of these two divalent cations when they are in abundance in the enteric lumen. Fatty acids have an opposite effect from divalent cations on oxalate absorption. High intake of the 20-carbon polyunsaturated fatty acid arachidonic acid was shown to be associated with increased urinary excretion of oxalate[67]. Fatty acids bind bivalent cations, thereby decreasing the latter's availability for binding oxalate in the intestinal lumen. This effect of fatty acids on oxalate absorption also has clinical implications (see below).

Several anaerobic bacteria, including *Oxalobacter formigenes, Eubacterium lentum, Enterococcus faecalis* and *Lactobacillus acidofilus*, metabolize oxalate in the gut[68]. Administration of probiotics containing one or more of these bacteria to healthy subjects and, particularly, subjects with high baseline levels of oxalate absorption, decreases oxalate absorption[68,69]. Conditions that are known to affect oxalate absorption include the pH of the intestinal fluids and intestinal transit time[62]. Whether these conditions have clinical significance or not is unclear.

RENAL EXCRETION OF OXALATE

Oxalate is eliminated almost exclusively by the kidneys. In two studies involving subjects with normal renal function, more than 90% of injected radiolabelled oxalate was recovered in the urine[70,71]. Circulating oxalate is almost 100% ultrafilterable and it is filtered in the glomeruli[72] and excreted in the proximal tubules[73,74]. The basolateral membrane of proximal tubular cells contains a transporter, SLC26A1 that exchanges oxalate for bicarbonate or sulfate[75]. Exchangers of the SLC26 family, including SLC26A6, SLC26A7, SLC26A8, and SLC26A9, have been identified on the plasma membrane of cells

that transport oxalate[76,77]. The SLC26A6 transporter has also been localized to the brush border of proximal tubular cells[78]. Holmes and Assimos hypothesized that increases in plasma concentration of oxalate activate the basolateral SLC26A1 transporter which facilitates entry of oxalate into proximal tubular cells, which is then followed by oxalate efflux into the tubular lumen[79]. Tubular secretion of oxalate may have clinical significance. One study found enhanced tubular secretion of oxalate in hyperoxaluric patients compared to controls with normal oxalate excretion[4].

Oxalate transfer in the enteric epithelial cells gut is similar to that in the renal tubular cells. Oxalate transfer through the enteric tight junction is driven by a lumen-to blood concentration gradient and by water absorption. Soluble oxalate is secreted back into the enteric lumen through SLC26A1 and SLC26A6. SLC26A1 is located in the basolateral membrane and transfers oxalate from the paracellular space to the intracellular compartment. SLC26A6 is located in the apical membrane and returns oxalate to the enteric lumen. The transfer of oxalate through the anion transporters back into the enteric lumen modulates the absorption of this toxic compound[59].

In renal failure, oxalate excretion decreases roughly in proportion to the decrease in renal function and serum oxalate concentration increases[80]. As a compensatory mechanism, elimination of oxalate through the gastrointestinal tract is increased in renal failure[81,82]. A study by Hatch and colleagues provided evidence that the increased intestinal excretion of oxalate in renal failure is mediated, at least in part, by angiotensin Ⅱ[83]. Renal failure, therefore, is one condition in which oxalate is not eliminated in its entirety by the kidneys. Diuresis and body size are two factors that affect urinary oxalate excretion. In normal subjects, oxalate elimination in the urine increases in parallel to urinary flow rate[84,85]. The clinical significance of this finding is obscure because urinary oxalate concentration decreases in parallel as urinary flow increases[85]. Large body size is associated with a high urinary oxalate excretion rate[86,87]. This finding is clinically relevant because obesity is a risk factor for nephrolithiasis[88]. Finally, urinary oxalate excretion shows seasonal variations[89] that can have clinical importance.

CLINICAL TYPES OF HYPEROXALURIA

Hyperoxaluria can result from excessive endogenous production of oxalate, excessive absorption of dietary oxalate, excessive dietary or parenteral intake of oxalate, or a combination of these processes. Four main categories of hyperoxaluria are recognized: primary hyperoxaluria, absorptive or intestinal hyperoxaluria, idiopathic mild hyperoxaluria and dietary hyperoxaluria.

Primary hyperoxaluria

Primary hyperoxaluria (PH) consists of a family of autosomal recessive inherited disorders characterized by endogenous overproduction of oxalate[90-94]. Mutations in three enzymes involved in oxalate synthesis lead to three distinct PH subtypes, PH1[95], PH2[96,97] and PH3[98-100].

PH1 accounts for about 80% of all PH cases. PH1 results from mutations in the hepatic peroxisomal enzyme AGT[93-95]. The gene encoding AGT (AGTX) is located on chromosome 2q37.3[91]. AGT is pyridoxal-5-phosphate dependent[93,94] and catalyzes the transamination of glyoxylate to glycine[94,95]. PH1 mutations result in in accumulation of glyoxylate and excessive production of oxalate and glycolate[94]. Figure 1 illustrates these relationships.

As of 2013, 178 different AGT mutations had been discovered[94]. Phenotypes vary from nephrocalcinosis, failure to thrive and advanced renal failure in early childhood to recurrent or even occasional nephrolithiasis in adulthood[96,97]. As renal failure progresses, high plasma levels of oxalate result in supersaturation and precipitation of calcium oxalate crystals in various organs (oxalosis). Blood vessel walls, bones, joints, retinae, skin, bone marrow, cardiac tissue and the nervous system are sites affected in oxalosis[90-95]. Life-threatening clinical manifestations accompany the deposition of oxalate crystals in vital organs[97].

The diagnosis of PH1 is assisted by finding elevated levels of oxalate and glycolate in the urine. It should be noted, however, that approximately one quarter of subjects with PH1 do not have elevated glycolate levels in the urine[95]. Renal failure consistently decreases urinary oxalate excretion which can cause diagnostic problems[90]. In the past, liver biopsy for assessment of AGT activity was required for the diagnosis of PH1. Nowadays, however, the diagnosis relies on molecular genetic testing including DNA sequencing, deletion/duplication analysis and targeted mutation analysis[95].

The management of PH1 follows some of the same principles of management of urinary stones in general. Fluid intake to ensure large urinary volumes is recommended for patients without advanced renal failure. Calcium supplements and other measures to reduce gastrointestinal absorption of oxalate have limited effectiveness in treating PH. Potassium citrate or, in cases of advanced renal failure, sodium citrate may reduce the tendency to form stones[95]. Pyridoxine administration reduces oxalate formation in 10% to 30% of the patients with PH1[91]. Effectiveness of pyridoxine has been linked to the AGT genotypes Gly170R and Phe152Ile, which are associated with some residual activity of the enzyme[91,94]. Combined liver-kidney transplantation is the method of choice for PH1 patients with advanced renal failure[95].

PH2 is found in about 10% of the PH cases. PH2 results from mutations of the cytosolic enzyme glyoxylate reductase/hydroxypyruvate reductase (GRHPR)[94,96]. The gene for GRHPR is located on in chromosome 9p13.2[94]. GRHPR is present in tissues throughout the body and catalyzes the conversion of glyoxylate to glycolate and hydroxypyruvate to D-glycerate[94]. Reduced or absent GRHPR activity leads to increased availability of lactate and hydroxypyruvate for conversion to oxa-

Table 2 Surgical procedures and medical conditions associated with enteric hyperoxaluria

Surgical conditions	Medical gastrointestinal conditions	Other medical/surgical conditions	Drugs
Jejunoileal bypass[106,108,110]	Crohn's disease[109,119]	Morbid obesity[112]	Orlistat[130,131]
Roux-en-y gastric bypass[111,113]	Diabetic gastroenteropathy[115,116]	Cystic fibrosis[122,123]	Octreotide[132]
Small bowel resection[108,109]	Sprue[117]	Organ transplants[124-129]	
Partial gastrectomy[108]	Primary biliary cirrhosis[109]		
Pancreatectomy[109]	Chronic pancreatitis[118]		
External biliary drainage[114]	Intestinal lymphangiectasia[120]		
	Clostridium difficile colitis[121]		

late and L-glycerate. Urinary excretion of high levels of L-glycerate is a characteristic of PH2[96]. Nephrolithiasis, nephrocalcinosis, end-stage renal failure and oxalosis in advanced renal failure are the clinical hallmarks of PH2[92,96]. The severity of these manifestations is less than in PH1: nephrocalcinosis is rare and end-stage renal failure develops later in life[92]. The diagnosis can be made by assay of GRHPR in blood mononuclear cells[97]. The treatment of PH2 is similar to that of PH1, with two exceptions: pyridoxine is not effective in PH2; and renal transplantation has been used for treatment of end-stage renal failure, while combined liver-kidney transplantation has not been used in PH2[96].

PH3 accounts for 2.5% of PH cases. PH3 results from mutation of the hepatic mitochondrial enzyme 4-hydroxy-2-oxoglutarate aldolase (HOGA1)[98,99]. HOGA1 catalyzes the last step in the conversion of hydroxyproline to oxalate. The chromosomal location of the gene responsible for PH3 is in 10q242[94]. The mechanism by which non-functioning mutations of HOGA1 lead to hyperoxaluria is an enigma. Intuitively, decreased HOGA1 activity should lead to decreased production of oxalate through the hydroxyproline pathway. A hypothesis for the pathogenesis of hyperoxaluria in PH3 was recently proposed by Belostotsky and associates[100]. These investigators identified a cytosolic 4-hydroxy-2-oxoglutarate aldolase distinct from mitochondrial HOGA1 in human hepatocytes. They speculated that individuals with PH3 accumulate 4-hydroxy-2-oxoglutarate in mitochondria and that following transfer of this compound into the cytosol it is converted to glyoxylate by the cytosolic aldolase[100]. Oxaluria is less marked in PH3 than in PH1 or PH2 and the clinical manifestations are less severe. Urolithiasis is the main clinical manifestation in PH3. Furthermore, nephrocalcinosis and renal failure are uncommon, and oxalosis has not been described in PH3[94].

Surveys of primary hyperoxaluria in various countries[101-105] have identified prolonged delays in the diagnosis of PH. Delays in the diagnosis have been observed also in enteric hyperoxaluria and could be present also in dietary hyperoxaluria (see Figure 2 below).

Enteric hyperoxaluria

Table 2 lists some of the conditions and surgical interventions in the gastrointestinal tract, including medical diseases of the gastrointestinal tract, medical or surgical conditions outside the gastrointestinal tract, and medications, that are associated with hyperoxaluria secondary to excessive intestinal absorption of oxalate[106-132]. A common characteristic of the conditions listed in Table 2 is the presence of steatorrhea with excessive amounts of fatty acids in the enteric lumen which bind divalent cations, especially calcium, thereby increasing the availability of the unbound oxalate for absorption[109]. In certain morbid conditions, such as cystic fibrosis, solid organ transplants or octreotide administration, frequent use of antibiotics causing alterations in the intestinal flora and lack of colonization by oxalate-consuming bacteria increases the availability of oxalate for absorption[109,122-129]. In recipients of organ transplants, use of anti-rejection drugs (*e.g.,* mycofenolate) that cause diarrhea and steatorrhea can contribute to hyperoxaluria[127].

Studies conducted more than 30 years ago documented that the colon was the primary site of oxalate absorption and suggested that an intact colon is necessary for the development of enteric hyperoxaluria[133,134]. However, enteric hyperoxaluria has also been noted in patients with partial colon resection[119]. In patients with enteric hyperoxaluria, diarrhea causes volume depletion and metabolic acidosis leading to low urinary pH and hypocitraturia. In conjunction with hyperoxaluria, these conditions facilitate precipitation of calcium oxalate in renal tissues and promote the development of renal stones, nephrocalcinosis and oxalate nephropathy[109]. In patients with primary hyperoxaluria, the renal failure that follows the development of nephrolithiasis, hydronephrosis, nephrocalcinosis and particularly parenchymal oxalate nephropathy is chronic. Enteric hyperoxaluria can cause new-onset acute renal failure (acute oxalate nephropathy)[121,124-126,129,130,132,135-140], acute renal failure superimposed on pre-existing chronic kidney disease[116,118], or chronic oxalate nephropathy[110,113,119,120,128].

Idiopathic (mild) hyperoxaluria

Idiopathic hyperoxaluria is a condition characterized by hyperoxaluria that is much less severe than primary on enteric hyperoxaluria and recurrent calcium oxalate stone formation[5,141,142]. This entity is encountered in subjects without any of the known types of enteric or primary hyperoxaluria. Increased synthesis, increased gastrointestinal absorption, or increased renal tubular secretion of oxalate are the only known mechanisms of hyperoxaluria. All three mechanisms have been implicated in idiopathic hyperoxaluria. Increased absorption of oxalate by patients with idiopathic hyperoxaluria, es-

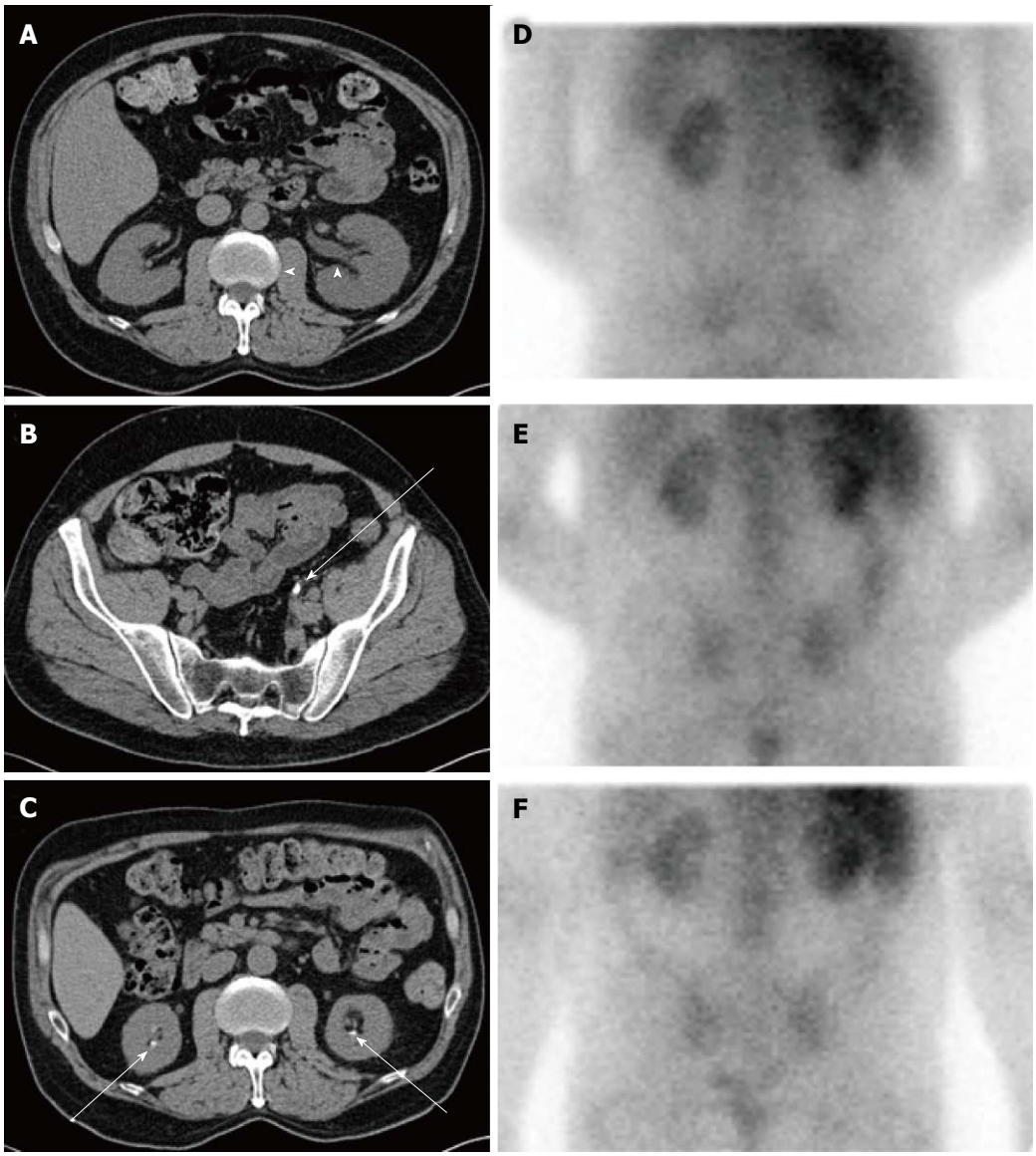

Figure 2 Sequential imaging studies of a not yet reported patient with chronic kidney disease from dietary hyperoxaluria. Axial computed tomography (CT) images obtained two years before the hyperoxaluria diagnosis show (A) mild left hydronephrosis (arrowheads) caused by (B) a left distal ureteral calculus (arrow). Axial CT image obtained around the time of the hyperoxaluria diagnosis shows (C) bilateral nephrolithiasis (arrows). Nuclear medicine gallium-67 citrate scan images were also obtained around the time of diagnosis, including (D) 4-, (E) 24-, and (F) 48 h after administration. These show abnormal, persistent bilateral renal activity at all time points, indicative of interstitial nephritis. Gallium scanning has classically been used to distinguish acute interstitial nephritis from acute tubular necrosis and other causes of acute renal failure[216-218]. In this patient chronic interstitial nephritis associated with hyperoxaluria led to this positive scan. The patient's diet for several years was based on nuts with estimated oxalate consumption $\geqslant$ 800 mg daily. During high oxalate intake, urine oxalate excretion was > 200 mg/24-h in several measurements obtained at serum creatinine levels > 3.5 mg/dL. After resumption of a diet low in oxalate and improvement of renal function to serum creatinine levels < 3.0 mg/dL, urine oxalate excretion decreased to normal levels.

pecially when the dietary content of calcium is low, has been reported[143-145]. In other studies in patients with the same entity, reduction of hyperoxaluria by large doses of pyridoxine was noted, suggesting that these subjects had excessive production of oxalate[146,147]. In another set of studies subjects with idiopathic hyperoxaluria developed higher levels of oxaluria than control subjects after ascorbate loads[148] or following meat ingestion[12,149]. This set of studies also pointed towards increased endogenous production of oxalate as the source of idiopathic hyperoxaluria. Finally, another study found enhanced tubular secretion in idiopathic hyperoxaluria[4]. Therefore, it is unclear whether idiopathic hyperoxaluria represents one or more types of hyperoxaluria. Further research is needed to clarify the mechanism(s) of hyperoxaluria in this particular condition.

Dietary hyperoxaluria

This section addresses dietary hyperoxaluria and hyperoxaluria secondary to medications or overdoses. The clinical and histological manifestations of these three categories of hyperoxaluria are similar. The reports of nephropathy from dietary hyperoxaluria, especially its chronic variety, are few and contain, in many instances,

Table 3 Reports of parenchymal renal disease induced by dietary hyperoxaluria

Ref.	Daily oxalate intake (mg), duration	Urine oxalate (mg/24 per hour)	Peak SCr (mg/dL)	Clinical diagnosis, course, outcome, final SCr (mg/dL)
150	310, many mo	16.6[1]	1.8	CKD with SCr 1.7-1.8
151	1880, 4 wk	34.2[2]		AKI on diabetic CKD. Progression to ESRD
152	2240-2800, 6 mo	-	8.08	CKD. Progression to ESRD
153a	9000, 4 d	60[3]	6.4	AKI, HDx10 days. SCr 0.9 in 6 wk
153b	4500, 5 d	-	9.3	AKI, HDx6 times. SCr 1.3 in 5 wk
153c	3600, NS	-	6	AKI, No HD. SCr 1.0 in 4 wk
153d	1800, NS	-	5.5	AKI, No HD. SCr 0.8 in 2 wk
153e	5400-6300, NS	-	12.3	AKI, HD. SCr 2.1 in 4 wk
153f	6300-7200, NS.	-	6.7	AKI, no HD. SCr 1.1 in 6 wk
153g	4500-5400, NS	-	9.8	AKI, HD. SCr 1.2 in 6 wk
153h	6300, NS	-	6.6	AKI, HD. SCr 1.1 in 4 wk
153i	2700-3600, NS	-	5.2	AKI, HD. SCr 0.8 in 2 wk
153j	7200 NS	-	10.4	AKI, HD. SCr 1.5 in 6 wk
154	1260, 6 wk	-	7.9	CKD on CKD from HTN. SCr 1.9 in 4 mo
155a	13120, once	7[4]	12	AKI, HDx2 times. SCr 1.3 in 1 yr
155b	9240, once	7[4]	11.7	AKI, no HD. SCr 1.3 in 4 mo
156	450-660, > 3 yr	-	6.9	CKD on other CKD, no HD. SCr 3.4 in 3 mo
157a	3725, once	-	-	AKI, no HD. Final SCr 1.1
157b	4360, once	-	6.3	AKI, no HD. Final SCr 1.1 NS
157c	7545, once	-	6.1	AKI, no HD. Final SCr 1.2
157d	1300, once	-	5.7	AKI, no HD. Final SCr 1.0
157e	2170, once	-	4.5	AKI, no HD. Final SCr 1.1
158	6830, once	-	16.4	AKI, no HD. SCr 0.9 mg/dL in 1 mo

[1]During recovery. SCr approximately 1.7-1.8 mg/dL; [2]Post-ingestion. SCr approximately 3.6 mg/dL; [3]During AKI. SCr approximately 6.4 mg/dL; [4]Post recovery. a,b,c,d,e,f,g,h,i,j,k in Ref. 153 and a,b,c,d,e in Ref. 157 represent the numerical sequence of the patients in these references (1st, 2nd, *etc*). SCr 1.3 mg/dL. SCr: Serum creatinine; AKI: Acute kidney injury; CKD: Chronic kidney disease; ESRD: End-stage renal disease; HD: Hemodialysis; NS: Not specified duration of intake.

incomplete information. Clinical and histological findings associated with the last two categories complete the picture of nephropathy in dietary hyperoxaluria.

Dietary hyperoxaluria should be differentiated from the other three categories of hyperoxaluria, since its treatment, which consists of reducing the dietary oxalate, is relatively simple. Elimination of the diagnostic option of primary hyperoxaluria may require genetic testing, but this is usually not required. A careful history should eliminate the possibility of enteric hyperoxaluria. Routine laboratory findings, such as normal serum albumin and electrolyte levels, may assist in eliminating this diagnosis. Differentiating between dietary and idiopathic hyperoxaluria can be difficult. Features establishing the diagnosis of dietary hyperoxaluria include: absence of primary or enteric hyperoxaluria; ingestion of large amounts of oxalate, usually found after the patient's oxalate-induced end organ damage has become manifest; documented hyperoxaluria associated with a high oxalate diet; and reduction of the oxaluria to within normal levels after normalization of the dietary oxalate. The evaluation of oxaluria is complicated in patients with impaired renal function, which, as noted earlier, decreases urinary oxalate excretion.

Dietary hyperoxaluria can cause renal disease and systemic oxalosis. Earlier studies focused mainly on the association between dietary hyperoxaluria and nephrolithiasis. A study by Neuhaus *et al*[11] established this association. More recently, several case reports of renal parenchymal disease manifested as either AKI or CKD[150-158] and oxalosis with primary neurological manifestations from dietary hyperoxaluria[159-163] have been published. Identified causes of dietary hyperoxaluria include ingestion of large amounts of the following: peanuts[150]; rhubarb[151]; Chaga mushroom powder[152]; *Irumban puli* (*Averrhoa bilimbi*), which is a fruit in the same family as star fruit[153]; juice made of celery, carrots, parsley, beets with greens, and spinach[154]; and, ingestion of star fruit (*Averrhoa carambola*), which has a very high content of oxalate[155-163]. Star fruit-induced oxalate nephropathy has also been investigated in experimental animals[164,165].

Table 3 shows estimates of oxalate intake and urinary excretion, type of clinical renal syndrome induced by oxalate (AKI *vs* CKD), peak serum creatinine concentration, whether dialysis was performed or not, and outcomes of patients with dietary hyperoxaluria-induced deterioration of renal function. The estimates of oxalate intake are approximations because estimates of the oxalate content of the same dietary item often vary widely[27,166-168]. We recorded in Table 3 either the oxalate intake reported in a study, or, if this intake was not reported directly, an estimate calculated from the amount of the dietary item consumed and the average oxalate content of this item.

Data regarding urinary oxalate excretion were missing from the majority of the published cases presented in Table 3. Even when urine oxalate excretion was reported, the findings were complicated by the presence of advanced renal failure, which, as noted above, decreases urinary oxalate excretion, or by the fact that oxalate excretion was measured in the recovery period after oxalate intake had been reduced. An elevation of urinary oxalate

excretion rate was reported only in one patient, who also had advanced renal failure[153]. Urinalysis findings varied: Proteinuria was absent in a few patients, modest in most patients, and as high as 3.7 gm/24 h in one patient who also had diabetes mellitus[151]. Hematuria and sterile pyuria were reported in several patients. Crystaluria was absent in several patients.

Oxalate nephropathy in subjects who briefly consumed food items containing very large amounts of oxalate tended to present as AKI, which was severe enough to require hemodialysis in some cases, but appeared to be reversible in all of them (Table 3). A few patients with chronic intake of oxalate at levels substantially lower than those causing AKI did develop CKD; their kidney function improved but did not normalize after reducing their dietary intake of oxalate[150,154,156].

The paucity of reported cases of chronic nephropathy secondary to dietary hyperoxaluria and of measurement of urinary oxalate in those cases led us to investigate other clinical states of temporary hyperoxaluria caused by excessive intake or formation of oxalate. These states include intake of ascorbic acid, drugs containing oxalate and intoxication with ethylene glycol.

As in dietary hyperoxaluria, excessive intake of ascorbate was initially linked to an increased risk of nephrolithiasis[169,170]. Recently, renal parenchymal disease from oxalate nephropathy causing AKI or CKD has been reported in patients with excessive oral[171-178] or parenteral[179-183] intake of ascorbate. An elevated urinary oxalate excretion rate at the time of ingestion of large amounts of ascorbate and decrease in oxaluria to within or close to its normal range was reported in several cases[171,172,175,179]. Severe AKI was present in most cases[171,173,174,179-183]. Several of these patients required hemodialysis for various periods of time and recovery of renal function was complete[171,174,179-183] or partial[173]. CKD was noted in four patients[175-178]. These patients were ingesting ascorbate chronically but usually in quantities substantially lower than the amounts of ascorbate that cause AKI. Two of these patients developed ESRD[176, 178] and one of them died[178].

Many cases of severe AKI after accidental or suicidal ingestion of oxalate[184,185] or ethylene glycol[186-198] have been reported. AKI had a protracted course in many of these patients and in most instances dialysis was required. Patients with severe ethylene glycol poisoning had significant mortality, especially in decades past[186]. Renal function did not return in several patients with AKI, although some did recover completely. Hyperoxaluria and calcium oxalate nephrolithiasis[199] or oxalate nephropathy with AKI or CKD were reported with the use of two medications used as vasodilators, namely pyridoxilate[200,201] and Praxilene[202-204]. Pyridoxilate is a combination of glyoxylate with pyridoxine. Pyridoxine was intended to redirect glycine formation away from glyoxylate. Nevertheless, at least a portion of the administered glyoxylate was still metabolized to oxalate. Praxilene's common name is naftidrofyryl oxalate. When this salt dissociates in the body oxalate is released. Finally, hyperoxaluria and oxalate nephropathy has been seen with the use of the anesthetic agent, methoxyfluorane[205]. The clinical and histologic features of drug-induced hyperoxaluria have been studied more extensively than those of dietary hyperoxaluria.

Urinary oxalate excretion rates differ between oxaluric states and can provide clues for the differential diagnosis between these states[95]. Table 4 summarizes reported daily rates of urinary excretion of oxalate in various clinical states. The table includes only representative studies for all types of hyperoxaluria, except dietary hyperoxaluria. For this last category of hyperoxaluria, we included in Table 4 all the reports providing measurements of oxalate excretion in patients with oxalate nephropathy that we could find. The degree of renal function has a major impact on urinary oxalate excretion. Primary hyperoxaluria, particularly PH1, is associated with very high rates of urine oxalate excretion[90,95,98,99]. However, even in primary hyperoxaluria, the renal oxalate excretion rate was within the normal range in patients with advanced renal failure[90,99]. Oxalate excretion rates in enteric hyperoxaluria depend on dietary oxalate content; the rate is generally less than in the primary variety, but can be within the range seen in primary hyperoxalurias[1,109,118,206].

Reported excretion rates of oxalate are comparable in idiopathic[35,39,95,206] and dietary[95,150,151,153] hyperoxaluria and substantially lower than in the primary varieties of hyperoxaluria. However, the degree of renal failure differs greatly between the reports of idiopathic and those of dietary hyperoxaluria. Determination of oxaluria in subjects with the dietary variety was usually performed in patients with AKI or advanced CKD whereas idiopathic hyperoxaluria was studied in the context of nephrolithiasis. The urinary oxalate excretion rate of patients with dietary hyperoxaluria may be in the range of subjects with the idiopathic variety (see the legend of Figure 2). Daily urinary oxalate excretion rates exceeding 90 mg (1 mmol) were considered primary or enteric hyperoxaluria[95]. We suggest that dietary hyperoxaluria can also cause oxalate excretion rates similar to those observed in primary hyperoxaluria.

RENAL PATHOLOGY AND PATHOPHYSIOLOGY IN HYPEROXALURIA

The chronic histologic lesions in the kidneys are indistinguishable between all categories of hyperoxaluria. Histologic lesions are also indistinguishable between AKI cases of enteric hyperoxaluria[115,121,125,126] and AKI cases of hyperoxaluria that have dietary, toxic or pharmacologic causes. Hyperoxaluric renal parenchymal disease is classified as a crystalline nephropathy[207], because it is widely acknowledged that oxalate injury to renal tissues begins with the deposition of abundant calcium oxalate crystals[208] in the lumen of renal tubules, the renal interstitium, and the walls of the renal vessels in all categories of hyperoxaluria[90,209-211].

Table 4 Daily urinary oxalate excretion in various hyperoxaluric states

Oxaluric state	Urinary oxalate, mg/24-h
Normal range	< 45, < 30[1]
PH1	> 90[95], > 63[94], 25-492[90], 26-530[99]
PH2	> 42[95], 44-520[99]
PH3	80-194[98], 35-120[99]
Enteric	> 90[95], 30-110[11], 63 ± 13[2], 130[109], 52-92[118], 77 ± 44[123], 48-90[206]
Oral ascorbic acid	98[171], 37[172], 84[175]
Parenteral ascorbic acid	76[179], 100[180], 176[181], 88[182]
Ethylene glycol	29[190], 10[195]
Methoxyfluorane	96-480[205]
Idiopathic	< 63[95], 56 ± 15[39], 38-50[206], 48[207]
Dietary	< 54[95], 16.6[150], 34.2[151], 60[153]

Oxalate excretion is presented as a single number representing the mean or median of the study (not specified in several studies), range (interquartile range in reference 120), or mean ± SD. For patients with two or more sequential measurements of urinary oxalate excretion rate, the Table reports the highest oxalate excretion. [1]Pediatric values. PH1: Primary hyperoxaluria, type 1; PH2: Primary hyperoxaluria type 2; PH3: Primary hyperoxaluria type 3.

Although finding calcium oxalate crystals in kidney biopsy specimens is necessary for the diagnosis of oxalate nephropathy, it is not a specific finding. Oxalate crystals are found in the kidneys in all conditions that elevate the plasma oxalate level. Principal among these conditions are all types of acute and chronic renal failure[212].

Extensive tubular damage with epithelial necrosis and tubular dilatation is the second cardinal characteristic of both acute and chronic oxalate nephropathy, while the involvement of glomeruli is inconsistent. The histologic features of renal tubules in hyperoxaluric AKI have the characteristics of acute tubular necrosis[115,121,151,153,158,159,164,171,174,195,213]. Changes in the renal interstitium are the other histologic characteristic of oxalate nephropathy. Profound interstitial fibrosis is present in chronic cases of oxalate nephropathy[90]. Tubulointerstitial nephritis with interstitial collection of mononuclear cells is a prominent characteristic of both chronic[90] and acute[175] cases of oxalate nephropathy. In some instances, interstitial nephritis takes the form of granuloma[150,214]. Oxalate-induced AKI may[157,164] or may not[153,155] exhibit interstitial nephritis in addition to acute tubular necrosis. Features of acute tubular injury, namely tubular simplification, flattening of tubular epithelial cells and dilatation of the tubular lumen are the earliest histological changes observed in kidneys of animals with experimental dietary acute oxalate nephropathy[165]. In addition to the kidneys, calcium oxalate crystals can be found in bone, skin, vessels and joints in patients with oxalosis[215]. Radiological and histologic features of nephropathy in a patient with dietary hyperoxaluria are shown in Figures 2 and 3 respectively.

The initial event in the development of oxalate nephropathy is the formation of calcium oxalate crystals in the lumen of proximal tubules[219]. Details of the mechanism of crystal formation, which have been reviewed extensively in the literature on stone formation, are outside the scope of this report. Randall's plaque (apatite collections in the interstitium of the papillae) was noticed in abundance in several hyperoxaluric states and may play a role in stone formation[220].

Adhesion of calcium oxalate crystals to the surface of tubular epithelial cells follows formation of the crystals. The mechanisms of adhesion have been extensively studied recently[221-227]. Coating with urine proteins, facilitated by low urinary pH, was shown to reduce the attachment of calcium oxalate crystals to renal inner medullary epithelial cells[221]. Calcium oxalate binding proteins that promote oxalate nephropathy have also been identified. Calcium oxalate monohydrate binding protein, one of these promoters, was shown to be upregulated by oxalate-induced oxidative stress[223]. A dual role was suggested for osteopontin, which inhibits calcium oxalate crystal formation and tubular retention[222], but also increases adhesion of these crystals to carboxylate ions that would promote oxalate-induced renal disease[225]. Prostaglandin E2 inhibits binding of calcium oxalate crystals to renal epithelial cells[224,226]. In a recent report, 26 oxalate-binding proteins were identified the kidney[227]. Further studies are needed to clarify the role of each of these proteins in oxalate-induced renal disease.

Evidence of the direct toxicity of supraphysiologic concentrations of oxalate to renal tubular cells was found in studies using cultured cells[228]. Both inhibition of cell proliferation and apoptosis have been identified as mechanisms of this nephrotoxicity. Studies in epithelial, endothelial and interstitial renal cell cultures found that exposure to sodium oxalate leads to reduced cell survival through inhibition of cell proliferation[229]. Evidence of oxalate-induced toxicity to renal cells was provided by finding increased levels of protein and mRNA of kidney injury molecule-1 in both human cell cultures and experimental animals[230]. In experimental animals hyperoxaluria increased production of TNF-α, FAS and FAS ligand, and apoptosis[231].

Research involving the mechanisms of innate immunity has shed considerable light on the molecular mediators and histologic features of oxalate nephropathy[232-243]. A role for toll-like receptors, NOD-like receptors and inflammasomes in AKI secondary to ischemia and sepsis has been documented[232]. A growing body of evidence has given inflammasomes a central place in our understanding of complex diseases (*e.g.*, metabolic syndromes, carcinogenesis) and physiological processes (*e.g.*, regulation of intestinal microbiome) and has identified them as important players of the intracellular surveillance system. Recent emphasis was also placed on the role of inflammasomes in various renal disease categories, including crystalline nephropathies[233].

Inflammasomes are part of the innate immune system. As their name suggests, inflammasomes represent large multimolecular cytosolic complexes that assemble into a platform for the activation of pro-inflammatory caspase 1[234-236]. Inflammasomes are important mediators

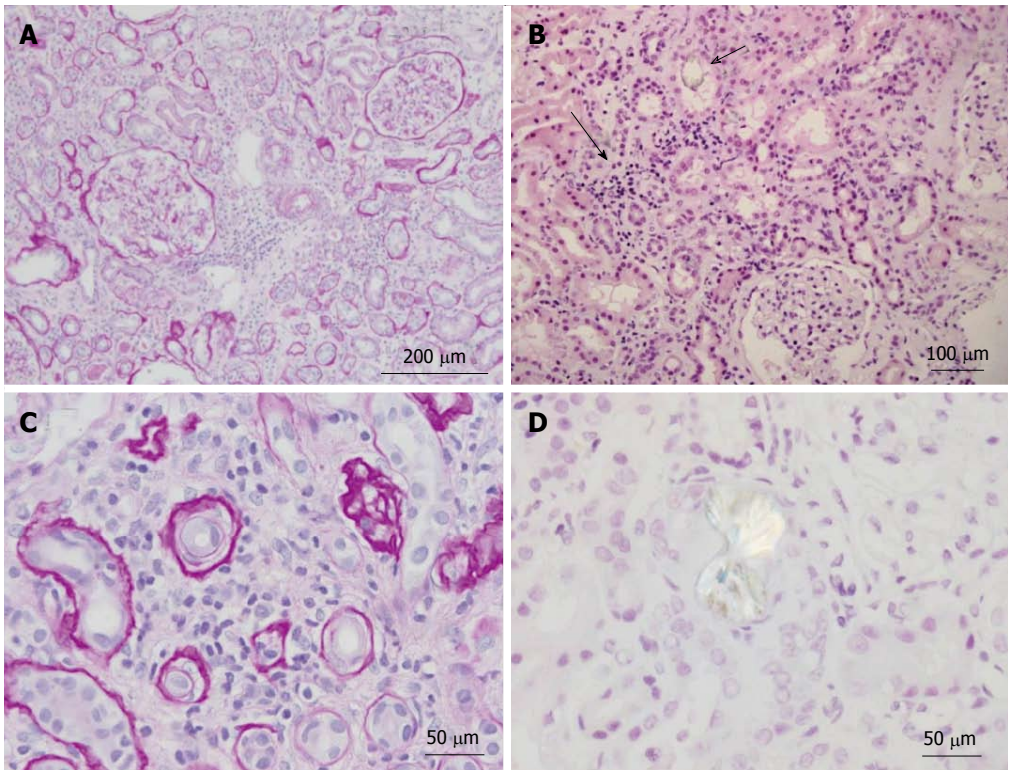

Figure 3 Renal histology in the patient depicted in Figure 2. A: Low power view of kidney showing two complete glomeruli and expansion of the interstitium by lymphocytes and edema. Periodic acid-Schiff (PAS) stain highlights the basement membranes of the tubules and Bowman's capsule. PAS stain; B: Low power view of renal parenchyma showing tubulointerstitial nephritis (solid arrow) and oxalate crystal within tubule (open arrow). H and E stain; C: High power view showing interstitium expanded by lymphocytic infiltrates and tubular atrophy. PAS stain; D: High power view of calcium oxalate crystal under polarized light. H and E stain.

of apoptosis, interstitial inflammation and fibrosis in various types of renal disease[237,238]. Of great importance in the context of oxalate nephropathy is the nucleotide-binding domain, leucine-rich repeat inflammasome (NALP3 or NLRP3). When activated, NALP3 proteins oligomerize and form a protein complex with caspase-1. This process activates caspase 1 which cleaves the inactive precursors of IL-1β and IL-18 to generate active cytokines that promote inflammation. The NALP3 inflammasome has been implicated in the molecular mechanism of nephropathy caused by urate crystals[239]. More recent studies detail the functional significance of the inflammasome and the IL-1β/IL-18 axis as an important factor in interstitial inflammation and fibrosis, as well as progression of renal failure, in oxalate nephropathy[240-242] and other kidney diseases[243]. In experimental models, genetic deletions of antagonists of the NALP3 inflammasome pathway have decreased the severity of oxalate nephropathy[240-242].

MANAGEMENT OF NEPHROPATHY IN ACQUIRED HYPEROXALURIAS

The general principles of management of oxalate-related nephropathies are the same in all categories of acquired hyperoxaluric nephropathy and include a diet low in oxalate and relatively high in calcium, fluid intake exceeding 1.5 L per m^2 body surface area per day, treatment with probiotics containing oxalate degrading bacteria, and medications to increase urinary solubility of crystals (*e.g.*, potassium citrate)[244]. Studies on the effect of probiotics on oxaluria have produced conflicting results. Intake of probiotics led to significant reduction of oxaluria in some studies[245,246], but had no effect on oxaluria in several other studies[247-249].

Specific measures targeted to the mechanism of hypercalciuria can be effective in patients with enteric hypercalciuria[244,250]. It is possible that probiotics may be useful in certain categories of patients with enteric hyperoxaluria, in particular, those who have altered enteric flora because of protracted courses of antibiotics, but this will require further study. A study by Toblli *et al*[251] reported that the angiotensin-converting enzyme inhibitor enalapril had a protective effect on the formation of tubulointerstitial lesions in rats fed ethylene glycol. Studies in humans with hyperoxaluria are needed to determine the effectiveness of this drug. Further studies are also needed to objectively assess the effectiveness of traditional herbal medications used for prevention or treatment of renal stones[252,253].

FUTURE RESEARCH

Our main reason for undertaking this review was to underscore the need for epidemiologic, biochemical and histologic studies of the effects of dietary hyperoxaluria on the development of CKD and end-stage renal disease (ESRD) across the globe. Occasional intake of nutrition-

al foods high in oxalate has been advocated[254]. While doing so may have merit, neither the highest "safe" dose of oxalate nor whether this dose differs between individuals has been determined. However, the main concern is not with brief ingestion of a relatively high dose of oxalate, but instead with the effects of chronic ingestion of high doses of oxalate on renal function, which is common in several parts of the world (Table 1). Interestingly, several patients with documented CKD due to chronic dietary hyperoxaluria had ingested amounts of oxalate comparable to or even lower than the average values reported in certain parts of the world (Tables 1 and 3). Difficulties and delays with the recognition of hyperoxaluria as the cause of CKD and ESRD have been documented, even for the primary hyperoxalurias[101,103,105,255], where early appearance of symptoms and renal failure, oxalosis and a family history of recurrent nephrolithiasis, renal failure and oxalosis should lead one to the diagnosis. That retention of oxalate in patients with CKD from any etiology may result in renal deposition of calcium oxalate, secondary deterioration of the renal function and systemic toxicities has been recognized[256]. However, in a recent comprehensive review excessive dietary oxalate intake was not listed among the primary risk factors for CKD[257]. Appropriate studies in populations with high dietary oxalate intake have the potential to reduce the rates of CKD and ESRD by simple dietetic interventions (*e.g.*, fluid intake, leaching of oxalate by soaking). Such studies should be encouraged.

Related to the need of studying the effects of oxalate intake on the development of CKD in various areas of the globe is the need to continue performing studies on genetic influences on oxalate absorption and excretion. Clinical and epidemiologic studies suggested that genetic influences can affect oxalate absorption and excretion[254,258-261]. Ongoing studies of genetic differences in intestinal and renal oxalate transporters[262-266] and of factors related to calcium metabolism[267] have the potential of leading to novel preventive and therapeutic modalities.

Future research should also include enzymologic and protein-structure studies aimed at identifying potential drugs that would either promote reductive metabolism of glyoxylate, the immediate precursor of oxalate, or inhibit oxidative enzyme-catalyzed reactions that increase oxalate production, for example the LDH reaction. Inhibiting LDH activity would reduce oxalate production and increase the levels of calcium glyoxylate and calcium glycolate which are 3 to 4 orders of magnitude more soluble in water than calcium oxalate. This approach is analogous to the treatment of gout where allopurinol inhibits xanthine oxidase activity, thereby reducing uric acid production and increasing the levels of much more water soluble xanthine oxidase substrates (*e.g.*, hypoxanthine). The inflammasome NLP3 is an emerging potential target for new drug development NLP3[268].

CONCLUSION

Hyperoxaluria, regardless of its mechanism, can cause not only nephrolithiasis and nephrocalcinosis, but also AKI, CKD and ESRD. Research to verify or reject the hypothesis that chronic dietary hyperoxaluria is underrecognized as a cause of CKD and ESRD, particularly in global areas with high dietary oxalate consumption, has the potential of improving health, well-being and economy in these areas. This research should be combined with research on the genetics of oxalate transport, oxalate-induced mechanisms of disease and development of medications affecting these processes.

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P- Reviewer: Lehtonen SH, Sakhaee K **S- Editor**: Wen LL
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World Journal of Nephrology

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DOI: 10.5527/wjn.v3.i4.143

World J Nephrol 2014 November 6; 3(4): 143-155
ISSN 2220-6124 (online)

REVIEW

Why do young people with chronic kidney disease die early?

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Received: June 29, 2014 Revised: September 19, 2014
Accepted: October 14, 2014
Published online: November 6, 2014

Abstract

Cardiovascular disease poses the greatest risk of premature death seen among patients with chronic kidney disease (CKD). Up to 50% of mortality risk in the dialysis population is attributable to cardiovascular disease and the largest relative excess mortality is observed in younger patients. In early CKD, occlusive thrombotic coronary disease is common, but those who survive to reach end-stage renal failure requiring dialysis are more prone to sudden death attributable mostly to sudden arrhythmic events and heart failure related to left ventricular hypertrophy, coronary vascular calcification and electrolyte disturbances. In this review, we discuss the basis of the interaction of traditional risk factors for cardiovascular disease with various pathological processes such as endothelial dysfunction, oxidative stress, low grade chronic inflammation, neurohormonal changes and vascular calcification and stiffness which account for the structural and functional cardiac changes that predispose to excess morbidity and mortality in young people with CKD.

Key words: Chronic kidney disease; Cardiovascular mortality; Cardiorenal syndrome; Endothelial dysfunction; Vascular calcification and stiffness

Core tip: In this review, we set out to summarise current opinion based on extensive scientific research that might explain the reasons for the disproportionately high death rate in chronic kidney disease and dialysis patients. The cardiovascular "phenotype" that poses increased risk to patients with chronic kidney disease (CKD) changes with progression of kidney dysfunction. Macrovascular disease is more important in early CKD whereas microvascular processes play an increasing role with worsening kidney disease.

Kumar S, Bogle R, Banerjee D. Why do young people with chronic kidney disease die early? *World J Nephrol* 2014; 3(4): 143-155 Available from: URL: http://www.wjgnet.com/2220-6124/full/v3/i4/143.htm DOI: http://dx.doi.org/10.5527/wjn.v3.i4.143

EPIDEMIOLOGY

People with chronic kidney disease (CKD) are at higher mortality risk compared with the general population[1,2]. End-stage renal disease is associated with highest mortality despite modern renal replacement therapy and pharmacological interventions. Mortality risk among individuals starting haemodialysis is greatest in the first 120 d, accounting for 27.5 deaths per 100 person-years and, thereafter, the annual mortality rate is around 20%[3,4]. Furthermore, around 9% of deaths in the first 3 years after commencing dialysis are in the 20-54 years age group[5]. Findings from the United Kingdom Renal Registry indicate that a person commencing dialysis aged 25-29 years has a median life expectancy of only 18.5 years, 33 years less than someone in the same age group in the general population without CKD[6]. Similarly,

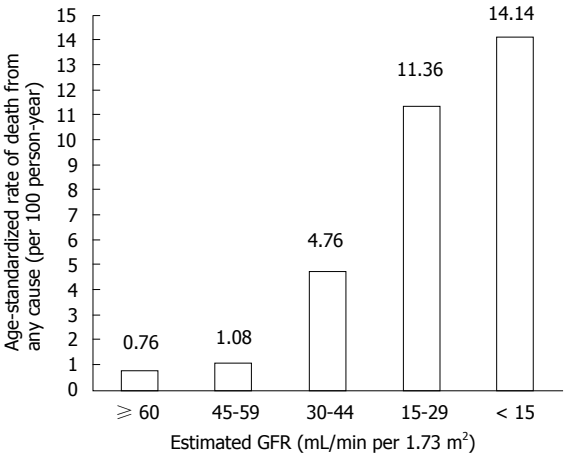

Figure 1 All-cause mortality and its relationship to worsening chronic kidney disease. After Go AS 2004[1].

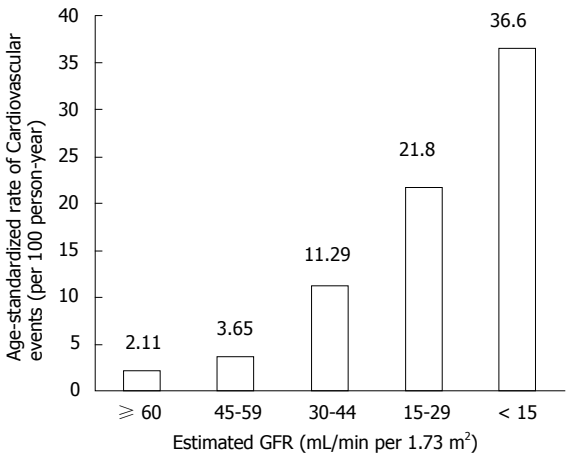

Figure 2 Cardiovascular event rates according to chronic kidney disease stage. After Go AS 2004[1].

patients aged 65-74 years, which is the commonest age group starting dialysis reported in the European Renal registry, can expect to live 5 years, about 50% less than those in the same age group in the general population[5].

The leading cause for this observed excess morbidity and mortality in end-stage renal disease is cardiovascular disease which accounts for 40%-50% of deaths[5,7-9]. In absolute terms, patients receiving dialysis have a 10-20 fold increased risk of cardiovascular death than do age and sex-matched controls in the general population. For younger dialysis patients, below 45 years of age, cardiac mortality is even higher, exceeding 100 times. After kidney transplantation, the risk is reduced but remains at 3-5 times that in the general population[7,10].

Of the non-cardiovascular causes of death in advanced renal failure, infection and malignancy are most important, accounting for approximately 15% and 8%, respectively, in the first 3 years of dialysis treatment[5,11]. Over this period of time, 10% of mortality due to infection was recorded in younger adults (20-54 years age group) and a similar percentage was attributed to malignancy. Vascular catheter-associated blood-borne infection often due to recurrent Staphylococcus aureus[12-15] and pneumonia[16] are the main causes. The high incidence of infections and inflammation in dialysis patients is related to disturbances in innate and adaptive immune mechanisms[17]. In particular, renal insufficiency is associated with down-regulation of toll-like receptors leading to sub-optimal stimulation of the innate immune system.

Non-adherence to haemodialysis and dietary restriction especially of high potassium-containing food, and excessive interdialytic fluid weight gain leading to congestive cardiomyopathy, are all contributory risk factors of premature death, especially in young dialysis patients[18]. The number refusing treatment or committing suicide is also not insignificant[5]. Treatment by peritoneal dialysis confers survival advantage over haemodialysis, at least in the first few years, before risk equalises[19,20]. Nevertheless, cardiovascular disease still poses the greatest risk in peritoneal dialysis (PD) patients[21,22]. This patient group shares similar cardiovascular risk factors to haemodialysis patients but they typically gain more weight (due to the high glucose load and insulin resistance) and demonstrate higher levels of chronic inflammation in response to exposure to non-physiological peritoneal dialysis fluid and episodes of peritonitis[21]. However, relatively little randomised trial data is available pertaining to cardiovascular risk factors/outcome in peritoneal dialysis patients and so much of the discussion below relates to haemodialysis patients.

CARDIORENAL SYNDROME

Large population studies have indicated that all stages of CKD predispose to premature death from cardiovascular and other causes, and is not restricted to those on dialysis[1]. Go and colleagues' landmark study demonstrated that the age standardised mortality rate from any cause increased in a step-wise manner, independent of known risk factors (Figure 1). Even in cases of moderate renal insufficiency (eGFR 45-59 mL/min), the risk of death was 8% higher per 100 person years than in the general population without kidney impairment. Thereafter, this study indicated that the risk increased exponentially with declining eGFR, exceeding 11 times risk in CKD stage 4. Similarly, when considering age-standardised rate of cardiovascular events, there was a significant increase in events with progressive renal insufficiency (Figure 2). With mild degrees of renal impairment (eGFR > 60 mL/min), the rate of coronary events was 2.11 per 100 person years greater than the general population with no kidney impairment, rising to 21.8 times the risk with CKD stage 4.

Most striking was the adjusted hazard ratio for death from any cause found to be 20% greater in CKD stage 3a and a 40% increase of having a coronary event, rising to 590% and 340% at CKD stage 5, respectively (Table 1). This study confirmed the findings of many longitudinal studies since the 1970s that had shown that patients with advanced CKD died from cardiovascular causes[23,24], and

Table 1 Adjusted hazard ratio for death from any cause and cardiovascular events in 1120295 adults according to estimated glomerular filtration rate

Estimated GFR (mL/min per 1.73 m^2)	Death from any cause	Any Cardiovascular event
⩾ 60	1	1
45-59	1.2 (1.1-1.2)	1.4 (1.4-1.5)
30-44	1.8 (1.7-1.9)	2.0 (1.9-2.1)
15-29	3.2 (3.1-3.4)	2.8 (2.6-2.9)
< 15	5.9 (5.4-6.5)	3.4 (3.1-3.8)

Adjusted hazard ratio with 95%confidence intervals given in parentheses. Data adjusted for age, gender, presence or absence of prior coronary heart disease, prior chronic heart failure, prior ischaemic stroke or ischaemic attack, prior peripheral arterial disease, diabetes mellitus, hypertension, dyslipidaemia, cancer, liver disease, chronic lung disease. After Go *et al*[1], 2004.

has been substantiated by a recent study[20]. Recognition that cardiovascular disease is a major threat to patients with CKD and, conversely, renal dysfunction is prevalent in patients with cardiac disease, indicating a poorer prognosis, led to adoption of the term, *cardio-renal syndrome*. Ronco *et al*[25] published their widely adopted classification to highlight this crucial interaction.

The bidirectional effect of heart failure and kidney failure is a key concept in the cardiorenal syndrome[26]. In the context of a failing heart due to pump failure, pressor systems (the sympathetic nervous system and renin-angiotensin-aldosterone axis) are activated to maintain the haemodynamic status quo[26,27]. Increased glomerular filtration pressure, achieved by efferent vasoconstriction helps to maintain glomerular filtration rate in low-output states, but the increased vascular resistance decreases kidney perfusion. Over time, this causes tubular hypoxic damage, renal cell apoptosis and replacement fibrosis, which leads to loss of nephron mass and to progressive renal dysfunction. Conversely, as chronic kidney disease progresses, the sympathetic nervous system is overactivated as a result of renal ischaemia, raised angiotensin Ⅱ levels and suppression of nitric oxide, causing hypertension, left ventricular hypertrophy and progressive left ventricular dilatation. Cardiac myocyte dysfunction and fibrosis, so-called "CKD cardiomyopathy", is believed to be the predominant pathophysiological mechanism. This may be compounded by salt and water overload caused by raised angiotensin Ⅱ levels leading to elevated central venous pressure and organ congestion.

TRADITIONAL AND NON-TRADITIONAL RISK FACTORS FOR CARDIOVASCULAR DISEASE

Patients with cardiovascular disease and those with CKD share many 'traditional' risk factors for atheromatous plaque formation, such as hypertension, dyslipidaemia, diabetes mellitus, obesity and smoking, but it is increasingly recognised that these factors fail to account fully for the disproportionate increase in cardiovascular mortality risk in CKD compared to the general population[8,23,24,28]. In addition, evidence based treatment strategies such as the use of statins for treating hyperlipidaemia in type 2 diabetic patients which have reduced morbidity and mortality significantly[29], appear less effective in diabetic patients requiring dialysis[30]. Changes unique to CKD are the progressive accumulation of uraemic toxins, electrolyte abnormalities, metabolic acidosis, sympathetic nervous system and renin angiotensin aldosterone system activation, and volume overload that result in structural and functional abnormalities of the heart, termed uraemic cardiomyopathy[31].

Non-traditional risk factors include endothelial dysfunction, elevated plasma homocysteine, increased levels of inflammatory factors and oxidative stress, abnormal apolipoprotein levels, enhanced coagulability, albuminuria, increased arterial calcification and arterial stiffness, anaemia, and left ventricular hypertrophy[8,23,24,32]. Whether and how these and other, as yet unidentified, factors contribute to morbidity and mortality is not entirely clear, but is the subject of on-going research. In this review, we discuss current knowledge about emerging non-traditional risk factors and their complex interaction.

ENDOTHELIAL DYSFUNCTION

Endothelial dysfunction is considered to be one of the first mechanisms involved in the development of atherosclerosis[33]. In CKD, endothelial dysfunction has been defined by increased levels of von Willebrand factor, reduced nitric oxide levels (NO) and over expression of inflammatory cytokines and C-reactive protein (CRP)[34]. An excess of pro-inflammatory over anti-inflammatory cytokines has been reported in early atherosclerotic lesions as lipid particles accumulate[33]. Microalbuminuria, a marker of early kidney disease, in both diabetic and non-diabetic patients, correlates with altered endothelial function. NO levels decline with progressive kidney disease so that reduced vasodilatory activity contributes to the increased risk of developing hypertension[34]. Schiffrin and colleagues suggested that the combination of impaired endothelial function, low grade inflammation, and dyslipidaemia may interact to promote accelerated atherogenesis and progressive renal disease[32].

In health, endothelium-derived nitric oxide is an important anti-atherogenic molecule. Circulating NO levels fall as asymmetric dimethylarginine (ADMA) levels rise[34,35]. This competitive inhibitor of nitric oxide synthase (NOS) also blocks the entry of L-arginine into cells to reduce NO synthesis[36]. ADMA is excreted by the kidneys or metabolized by dimethylarginine dimethylaminohydrolases (DDAH) and this pathway is attractive as a potential modulator of the NOS system in dialysis patients. Plasma ADMA concentrations increase with deteriorating kidney function, and the highest levels are found in dialysis patients[34]. ADMA levels are raised in elderly hypertensive patients and are strongly predictive of acute

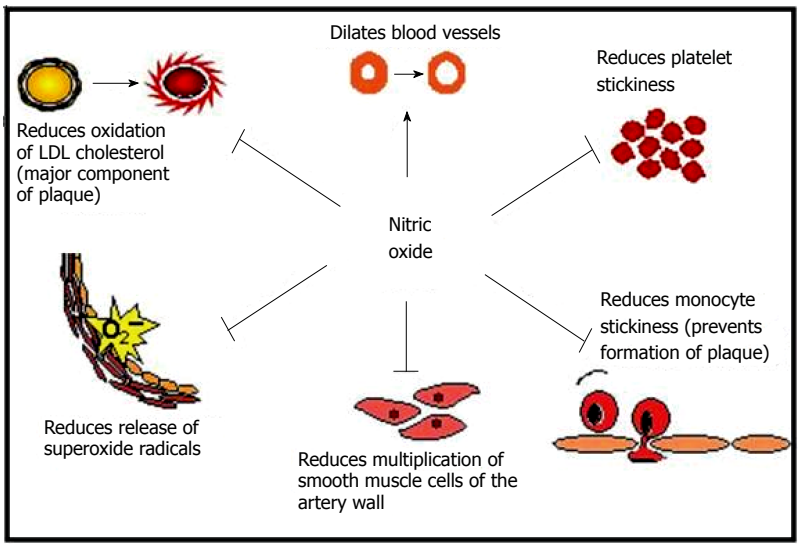

Figure 3 The physiological roles of nitric oxide on endothelial function.

coronary events and increased cardiovascular mortality risk in CKD[35,37-39].

The beneficial properties of NO are shown in Figure 3. Through NO inhibition, ADMA indirectly promotes vasoconstriction, raises blood pressure, impairs endothelium-dependent relaxation, and promotes platelet adhesion and aggregation and smooth muscle cell replication. It favours the release of superoxide radicals, formation of peroxynitrite and tyrosine nitration which cause vascular endothelial damage[40]. ADMA accumulation in CKD promotes atherogenesis and end-organ damage through sustained hypertension and other mechanisms[35]. In CKD, left ventricular hypertrophy, which is associated with premature death, may be a consequence of hypertension, partly triggered through prolonged inhibition of nitric oxide synthase. L-arginine supplementation should theoretically abrogate the effects of ADMA, but not all studies have found improvements in endothelial function[35]. One possible explanation is that chronically sustained high levels of ADMA induce irreversible vascular damage. There may be other mechanisms of action of ADMA other than NOS inhibition, yet to be identified, and not influenced by L-arginine supplementation. In an experimental model, DDAH activity of endothelial cells was decreased by almost half when incubated with oxidized LDL or TNF-α, and similar findings were observed in rabbits fed a high cholesterol diet[41]. These findings indicate that lipoproteins or cytokines may increase endothelial production of ADMA by reducing DDAH activity. This may be an important mechanism whereby local release and accumulation of intracellular ADMA inhibits NOS in hyperlipidaemia. The precise balance of pro and anti-inflammatory mediators present in the cellular milieu is likely to result in a variable overall effect which is directed towards or away from increased risk of atherosclerosis and atherothrombosis.

HYPERHOMOCYSTEINAEMIA

Many studies have shown an association between elevated levels of homocysteine and increased risk of cardiovascular events, stroke and peripheral vascular disease in the general population[42]. High levels of homocysteine contribute to endothelial dysfunction, oxidative damage and thrombosis[42-45]. Patients with declining kidney function accumulate homocysteine due to decreased metabolism by the kidneys[43,46]. In one study, the finding of elevated homocysteine and fibrinogen levels in CKD was thought to account for 38% of the attributable mortality risk, whereas individuals with CKD and low homocysteine levels (< 10 μmol/L) had mortality rates similar to those with normal renal function[43]. Stuhlinger *et al*[45] have proposed that homocysteine exerts its atherogenic activity by indirectly suppressing NO production, through inhibition of dimethylarginine dimethylaminohydrolases (DDAH) that metabolise ADMA. Homocysteine is not obtained from the diet, but is synthesized from the amino acid, methionine. Homocysteine can be recycled back into methionine and deficiencies of the vitamins folic acid, pyridoxine (B_6), or cyanocobalamin (B_{12}) can lead to high homocysteine levels. Supplementation with these vitamins lowers homocysteine levels but has not been associated with a significant reduction in cardiovascular events[42]. However, fortification of grain products with folic acid in the United States since 1998 has seen a decrease in stroke incidence which, at least in part, is thought to be due to lower homocysteine levels in the population[44]. A recent meta-analysis failed to demonstrate a significant decrease in the risk for cardiovascular events, stroke and all-cause mortality among a CKD population[47]. Hyperhomocysteinaemia may be a marker rather than a direct cause of CVD[46].

EPIDEMIOLOGICAL LINKS BETWEEN ALBUMINURIA AND CARDIOVASCULAR EVENTS

Microalbuminuria refers to albumin excretion of be-

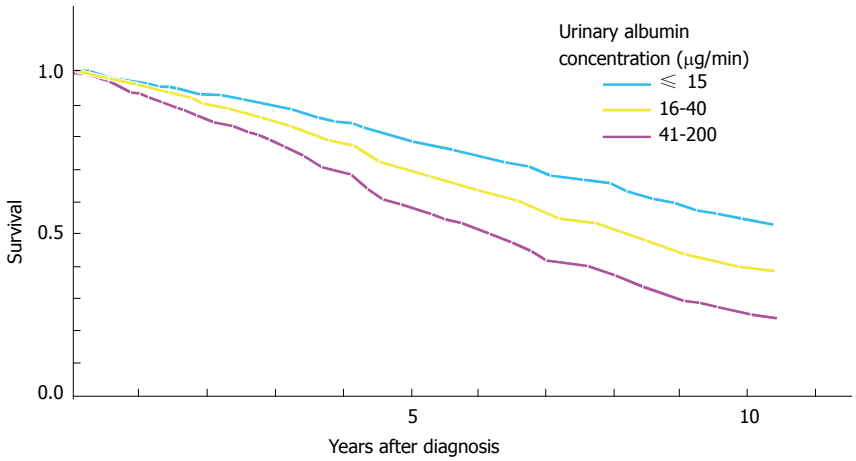

Figure 4 Microalbuminuria as a risk factor for death in type 2 diabetes. Reproduced from Schmitz *et al*[49], 1988.

tween 30 and 300 mg in the urine over 24 h (20-200 μg/min) that derives from a glomerular or tubular abnormality in primary kidney disorders, or it may reflect a generalised increase in vascular permeability due to vascular endothelial dysfunction[8]. Microalbuminuria, most commonly seen in diabetes mellitus and hypertension, signifies risk of progressive renal impairment and predates any fall in eGFR[52]. In a prospective study over 7 years, the degree of microalbuminuria in diabetic patients was found to correlate strongly with cardiovascular events and mortality[48]. Microalbuminuria was found to be a stronger predictor of cardiovascular outcomes than smoking, hypertension and raised serum cholesterol, in men who had no pre-existing cardiovascular disease. This was one of many studies that confirmed the early findings of Schmitz *et al*[49] (Figure 4). Diabetic patients were divided into 3 groups, entering the study with microalbuminuria of less than 15 μg/min, 16-40 μg/min and 41-200 μg/min. At 10 years, the overall mortality was 58%, caused by CVD or stroke with a further 3% dying from end-stage renal failure. 10-year survival was 55% and 25% in the < 15 μg/min and 41-200 μg/min microalbuminuria patient groups.

In diabetic patients, the development of microalbuminuria indicates microvascular disease which is associated with extracellular matrix expansion in the glomeruli of the kidney[50]. The natural history results in progressive glomerulosclerosis, increasing proteinuria, and chronic renal failure. But how does microalbuminuria, a marker of diabetic nephropathy, or a slightly reduced eGFR predict cardiovascular events and mortality before there is any evidence of overt coronary artery disease? Endothelial cell dysfunction seems to be the common pathophysiological pathway linking renal disease and CVD. Interplay between low-grade inflammation, elevated ADMA, increased circulating pro-inflammatory cytokines, dyslipidaemia, oxidative stress, sympathetic system over-activity and inappropriate activation of the renin angiotensin aldosterone system, is implicated for generalised endothelial cell dysfunction[51].

Large epidemiological studies have demonstrated that microalbuminuria in non-diabetic individuals is also associated with coronary, peripheral and cerebral vascular events[52]. Here again, the premise is that microalbuminuria is a marker of generalised endothelial cell dysfunction. Microalbuminuria is associated with increased transcapillary leakage of albumin and increased von Willebrand factor and other markers of endothelial dysfunction[50,51]. Inhibition of the renin-angiotensin-aldosterone system with either angiotensin converting enzyme inhibitors (ACE-I) or angiotensin receptor antagonists (AT1 receptor antagonists) can reduce albuminuria in both diabetic and non-diabetic individuals, to slow down the progression of renal disease and provide cardioprotection[53]. The underlying pathophysiology relates to the ability of ACE-I and AT1 receptor blockers to lower intra-glomerular capillary pressure by decreasing efferent arteriolar pressure and the reduction in transglomerular pressure decreases albuminuria. These drug classes have reduced progression of diabetic renal disease by reversing microalbuminuria to normoalbuminuria. Further long-term epidemiological studies are necessary to define its extent and enduring impact. The heart outcomes and prevention evaluation study (HOPE) is one of many studies showing that the ACE inhibitors reduce the progression of albuminuria and, at the same time, are effective in decreasing cardiovascular mortality in both diabetic and non-diabetic patients with normal blood pressure[54]. The Irbesartan Diabetic Nephropathy Trial demonstrated concomitant reductions in urinary protein excretion and cardiovascular endpoints in hypertensive type 2 diabetic participants[55].

Proteinuria is widely accepted as an independent risk factor for cardiovascular morbidity and mortality. Microalbuminuria progressing to overt proteinuria confers increasing cardiovascular mortality risk and concomitant CKD is associated with worsening cardiovascular and all-cause mortality, acting as risk multipliers across the CKD continuum[1,56,57]. Only a minority of the stage 3-5 CKD population progress to end-stage dialysis-requiring disease, most succumbing to premature cardiovascular death. Proteinuria surpasses blood pressure and cholesterol as a predictor of adverse clinical outcome[58]. Furthermore, the same group reported that the risk of acute myocardial infarction in subjects with CKD and

proteinuria was similar to or exceeded the cardiovascular risk associated with diabetes mellitus[59]. The PREVEND study found a significant independent association between microalbuminuria and myocardial ischaemia found on ECG[60]. Microalbuminuria is accompanied by raised inflammatory markers such as CRP and a fall in adiponectin levels[61]. Endothelial dysfunction has been implicated in the aetiopathology linking proteinuria with ADMA[62]. Since the detection of subclinical atherosclerosis is difficult and approximately 50% of cardiac events arise from the rupture of a vulnerable plaque in non-occlusive coronary disease, it may be that microalbuminuria associated with endothelial dysfunction is also acting as a marker of subclinical atherosclerosis. In this respect, studies have demonstrated that microalbuminuria independently predicts an increased carotid artery intima-media thickness which is a marker of subclinical atherosclerosis[63].

INFLAMMATION, DYSLIPIDAEMIA, ADVANCED GLYCATION END-PRODUCTS AND OXIDATIVE STRESS

Systemic inflammation is fundamental to the development of atherosclerosis[33,64]. Inflammatory markers including CRP, fibrinogen, soluble adhesion molecules and pro-inflammatory cytokines correlate with the future development of CVD and risk of sudden death in the general population[65].

Progressive renal insufficiency is characterised by a chronic inflammatory state represented by higher concentrations of CRP, interleukin-6 (IL-6), tumour necrosis factor α (TNF-α) and fibrinogen, and with lower levels of albumin[66]. But disorders commonly associated with CKD such as diabetes and hypertension exhibit low grade inflammation long before there is evidence of renal damage, and in the absence of any discernable reduction in serum albumin concentration. Irrespective of the mechanisms that trigger inflammation, there is no doubt that chronic inflammation induces vascular injury, initiating accelerated atherosclerosis[64], which is more pronounced in the younger patient with CKD. The causes of inflammation in renal disease can be partly attributed to reduced clearance of pro-inflammatory cytokines such as IL-6 and TNF-α, to the production of reactive oxygen species and the contribution of co-morbid conditions such as diabetes mellitus and heart failure[23,32,67]. Accumulation of advanced glycation end-products (AGE), normally cleared by the kidneys, may be important in the generalised chronic inflammatory process[68,69]. It has been established that AGEs are naturally formed in all tissues as part of ageing but at an increased rate in certain pathological processes such as diabetes and CKD[69]. Studies have demonstrated that up to 50% of patients with CKD have raised serum levels of CRP, fibrinogen, IL-1, IL-6, TNF-α, D-dimer and the soluble adhesion molecules E-selectin, VCAM-1 and ICAM-1[32,33], and research is focussed on the mechanisms of interaction that promote oxidative stress and atherosclerosis[64].

Correlation between raised serum CRP levels and future cardiovascular events in the general population is strong[65], but the association is more robust in CKD, in which it also closely predicts progression of kidney disease[67,70]. In a prospective study involving over 1000 dialysis patients with a median follow up of 2.5 years, 22% of patients died due to sudden cardiac death[71]. The highest third of high sensitivity CRP and IL-6 levels had twice the risk of sudden cardiac death. Moreover, those with a reduced serum albumin level had a 35% increased risk of suffering sudden cardiac death. IL-6 acts as a major stimulus for release of CRP by the liver. Ramkumar *et al*[72] identified increased production of IL-6 by intra-abdominal adipocytes, which provides a possible link between obesity and increased inflammation in CKD. In haemodialysis patients, other factors stimulating inflammation include repeated exposure to bio-incompatible dialysis membranes, exposure to foreign materials such as dialysis catheters and infection[23,32]. Infection in this group of patients is often subclinical being related to the presence of polytetrafluoroethylene (PTFE) central venous haemodialysis and peritoneal dialysis catheters.

Hyperfibrinogenaemia is observed with declining renal function and raised levels of this acute phase protein parallel those of other inflammatory markers. Hyperfibrinogenaemia predisposes to coronary thrombosis[72,73]. However, there is some uncertainty in defining the extent to which this factor contributes to overall mortality because fibrinogen is closely linked with other factors, such as dyslipidaemia[8].

Dyslipidaemia in CKD is a major factor in the inflammatory response and to atherosclerosis[74]. In health, high-density lipoprotein (HDL) cholesterol acts as an anti-atherogenic molecule in a number of ways. It reverses cholesterol transport, and has anti-thrombotic, anti-inflammatory and anti-oxidant properties, reducing oxidised LDL cholesterol[32,74]. It also promotes endothelial repair by decreasing the expression of adhesion molecules by vascular endothelial cells induced by cytokines[74]. HDL levels fall progressively as renal function declines, and the HDL cholesterol that is produced is dysfunctional. Figure 5 depicts the maturation of HDL and the protective effect of the apolipoprotein-A (apoA-I). In renal failure, apoA-I, synthesized by the liver, decreases so HDL cholesterol levels fall[32,74]. ApoA-I acts *via* ABCG1 taking up cholesterol from macrophages. The enzyme, lecithin-cholesterol acyltransferase (LCAT) esterifies cholesterol in the maturation of HDL cholesterol. The resulting HDL_3 isoform and, to a lesser extent HDL_2, are rich in anti-oxidative enzymes including paraoxonase 1 (PON 1). With progressive renal dysfunction, there is decreased LCAT activity and consequently less HDL. ApoA-I that normally comprises half of the proteins in HDL is replaced by serum amyloid A. This form of HDL cholesterol has a reduced ability to counteract the effects of oxidised LDL[75] and decreased

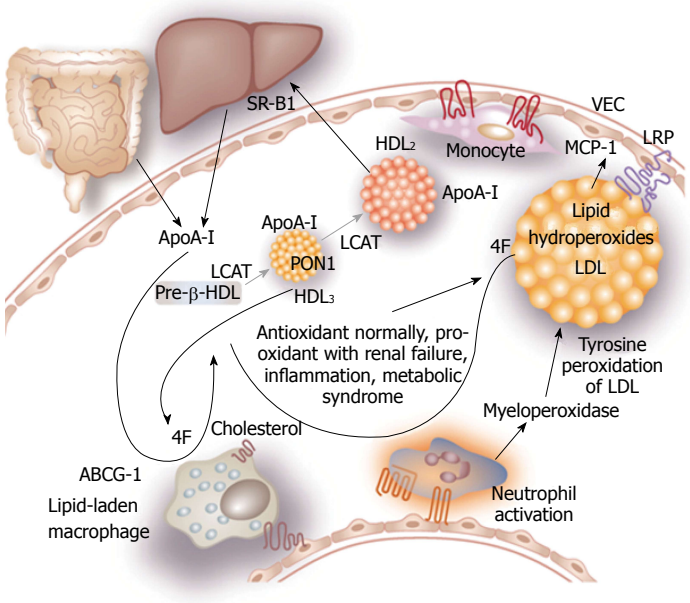

Figure 5 Maturation of high-density lipoprotein and the protective effect of apoA-I mimetic peptide 4F. (Apo)A-I: Apolipoprotein A-I; ABCG1: Adenosine triphosphate–binding cassette transporter G-1 protein; LCAT: Lecithin–cholesterol acyltransferase; SR-B1: Scavenger receptor B1; PON1: Paraoxonase-1; LRP: Lipoprotein-like receptor; VEC: Vascular endothelial cells; MCP-1: Monocyte chemoattractant protein-1; HDL: High-density lipoprotein. After Kaysen[74], 2009.

capacity to protect against cytokine action on vascular endothelium. Vaziri *et al*[76] showed that an apolipoprotein A-I mimetic peptide could reduce the effect of oxidised LDL on cultured aortic endothelial cells to produce the cytokine monocyte chemoattractant protein 1 (MCP-1), which may ultimately provide a therapeutic pathway to overcome dysfunctional HDL present in CKD (Figure 5).

Higher levels of LDL cholesterol and triglycerides are found with declining kidney function[8]. The impact on coronary artery disease is significantly greater than in the general population without renal impairment[8,74]. Accumulation of small dense atherogenic LDL cholesterol activates the renin-angiotensin-aldosterone system and also up-regulates the angiotensin type 1 receptor (AT_1). This increases the burden of oxidative stress and inflammation leading to endothelial dysfunction and atherosclerosis[32,33,75]. Not only does angiotensin Ⅱ cause hypertension (directly linked to atherosclerosis) but also activates vascular NADPH oxidase which induces superoxide anion generation (O_2^-). The superoxide anion inactivates nitric oxide which causes increased smooth muscle hypertrophy and proliferation, leading to hypertension and atherosclerosis[75].

Myeloperoxidase (MPO) is an enzyme present in leucocytes, particularly neutrophils, monocytes and tissue macrophages, which may play an important role in vascular injury and atherosclerosis in patients with advanced kidney disease[32]. Figure 5 shows how MPO promotes tyrosine peroxidation of LDL cholesterol. Leucocytes, which are activated in acute and chronic inflammation, secrete MPO into the blood which binds to vascular endothelium where it interferes with nitric oxide[74].

Lipoprotein a [Lp(a)] is a potent risk factor for CVD and serum levels increase progressively with declining renal function[8]. Lp(a) concentrations are highest in proteinuric states and fall after kidney transplantation.

Inflammation is a key component in the *malnutrition-inflammation-atherosclerosis* syndrome, observed across the spectrum of CKD, especially in young adults and is associated with substantial mortality[77]. Systemic inflammation, low serum HDL cholesterol and activation of angiotensin Ⅱ provide the rationale for use of anti-inflammatory agents such as aspirin, statins, angiotensin-converting enzyme inhibitors, angiotensin Ⅱ receptor blocking agents and antioxidants in combating endothelial dysfunction and atheroma[33,67,75].

SYMPATHETIC NERVOUS SYSTEM OVER-ACTIVITY, ANAEMIA AND LEFT VENTRICULAR HYPERTROPHY

Sympathetic nervous system over-activity in CKD is deleterious and results from renal ischaemia, raised angiotensin Ⅱ levels, and suppression of nitric oxide, causing hypertension, left ventricular hypertrophy and eventually left ventricular dilatation[24]. The prevalence of LVH was found to be 31% in those with GFR 25-49 mL/min and 45% in those with GFR < 25 mL/min[78]. There is evidence from epidemiological studies such as Framingham that LVH is independently associated with increased risk of fatal and non-fatal cardiovascular events[79]. ACE inhibitors and angiotensin Ⅱ receptor antagonists have been widely used to overcome sympathetic overdrive and to inhibit the renin angiotensin aldosterone system. However, a recent meta-analysis has demonstrated that although pharmacological intervention reduces LV mass it has no significant impact on reducing the risk of fatal and non-fatal cardiovascular events[80].

In the general population, LV dilatation and heart failure are generally the end result of end-organ damage sustained as a consequence of chronic hypertension and coronary atheroma. Although there is a higher prevalence of these conditions in young adult patients with CKD, cardiac myocyte death is accelerated by increased oxidative stress and anaemia[7,67,81]. Cardiac failure leads

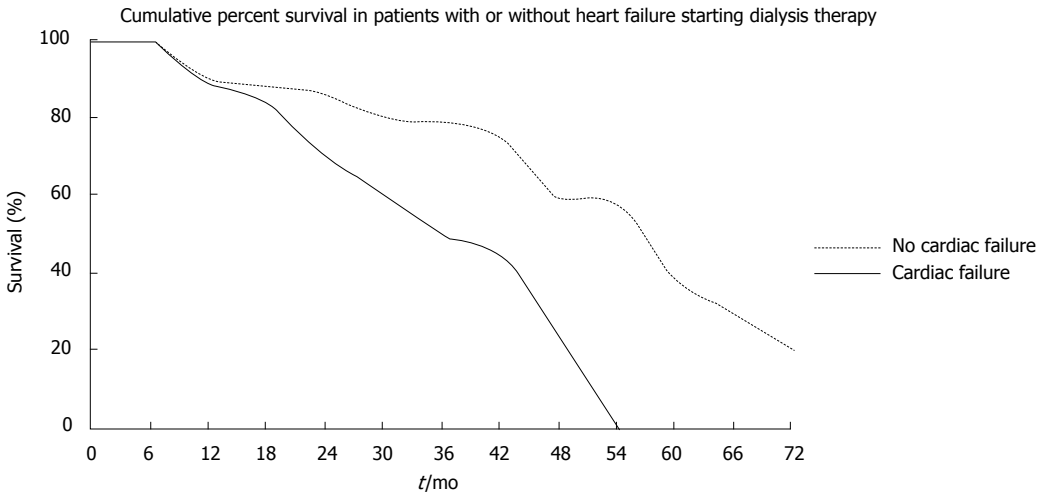

Figure 6 Premature death related to heart failure in haemodialysis. After Harnett *et al*[81], 1995.

to premature death (Figure 6)[81].

Anaemia appears in CKD stage 3 or 4 when erythropoietin production by the kidney diminishes[23]. If left untreated, the pathophysiological response is increased cardiac output, cardiomegaly, left ventricular structural abnormalities and, ultimately, congestive heart failure[24]. Correction of anaemia in CKD with erythropoietin results in regression of LVH and improved survival[23]. Timely intervention with iron and erythropoietin supplementation is important.

VASCULAR CALCIFICATION AND ARTERIAL STIFFNESS

Declining kidney function is associated with alterations in calcium and phosphorus metabolism that result in mineral bone disease (renal osteodystrophy), characterised by soft tissue and vascular calcification[82,83]. Secondary hyperparathyroidism, the physiological response aimed at correcting hyperphosphatemia by promoting phosphaturia, and vitamin D deficiency are both associated inflammation, vascular risk and cardiovascular mortality[83]. The situation is more complex with the discovery of fibroblast growth factor 23 (FGF-23) which is secreted by osteocytes. It acts as a phosphaturic hormone, inhibits production and secretion of parathyroid hormone, and reduces 1,25 vitamin D synthesis by interfering with vitamin D metabolism (Figure 7)[82]. α-Klotho is a hormone synthesised by the kidneys which seems to be essential for normal physiological functioning of FGF-23[84]. It is phosphaturic and may also have antioxidant and vasoprotective activity. As kidney function deteriorates, FGF-23 concentrations rise and early evidence indicates a strong association with left ventricular hypertrophy, independent of α-Klotho[85,86].

Coronary artery calcification is known to be important in the formation of atherosclerotic plaque[87]. Vascular calcification can take two forms, involving the tunica intima and media[82]. While it is not clear whether they follow a common pathogenesis, both are stimulated by CKD. Intimal calcifications develop in more than 80% of atherosclerotic plaques that occlude the vessel lumen causing ischaemia and myocardial necrosis[88]. Calcification of the tunica media involves smooth muscle cells and the elastic lamina[82,88,89], and is prevalent in more than 40% of patients with CKD stage 3B, who have accelerated coronary artery calcification[89]. Diabetic patients are particularly prone to calcific atheroma[32,67,89]. Calcification of the tunica media increases vascular rigidity and decreases compliance. Systolic hypertension occurs contributing to LVH. Increasing vascular calcification and stiffness, particularly of the aorta, measured by pulse-wave velocity has been shown to predict cardiovascular mortality in CKD patients[90,91].

Current theory suggests that tunica media calcification is an inflammatory process transforming vascular smooth muscle cells into osteoblast-like cells that express phosphorus transporter Pit-1 which concentrates calcium and phosphorus in an extracellular matrix that ultimately mineralises[87,92]. Vascular calcification is a complex process dependent upon the balance between promoters and inhibitors of calcification summarized in Table 2[87]. For example, a high calcium-phosphate product, raised parathyroid hormone levels and bone morphogenetic protein 2 (BMP-2) are pro calcific, whereas inhibitory factors include Fetuin-A, BMP-7, osteopontin, pyrophosphate and osteoprotegerin (OPG)[87,92].

Fetuin-A, produced by the liver, is a negative acute phase protein, its levels falling with rising CRP. Conversely, as CRP levels fall, fetuin-A levels may rise. This provides a link between inflammation and vascular calcification[93]. Matrix-Gla protein inhibits calcification of vascular smooth muscle and a negative correlation has been found with coronary artery calcification[32]. Osteoprotegerin regulates osteoclast activity and its deficiency has been associated with vascular calcification[82,87]. Osteoprotegerin deficiency is associated with increased mortality in dialysis patients[94]. Leptin is normally associated with nutritional state and satiety, but in the context of

Table 2 Promoters and inhibitors of vascular calcification

Promoters of vascular calcification	Inhibitors of vascular calcification
Traditional factors	Circulating inhibitors
Older age, male gender, hypertension, diabetes, smoking, high LDL cholesterol, low HDL cholesterol, genetic predisposition	Fetuin-A, bone morphogenetic protein-7, parathyroid hormone-related peptide, HDL cholesterol, magnesium,
Uraemia-related factors	
Uraemia, hyperphosphatemia, increased Ca x P product, exogenous vitamin D therapy, elevated parathyroid hormone level, duration of dialysis, calcium load and hypercalcaemia, chronic inflammation, warfarin, elevated leptin levels	Locally acting inhibitors Matrix Gla protein, α-klotho, osteopontin, pyrophosphate, osteoprotegerin, genetic predisposition

Adapted from Qunibi[87], 2005. HDL: High-density lipoprotein.

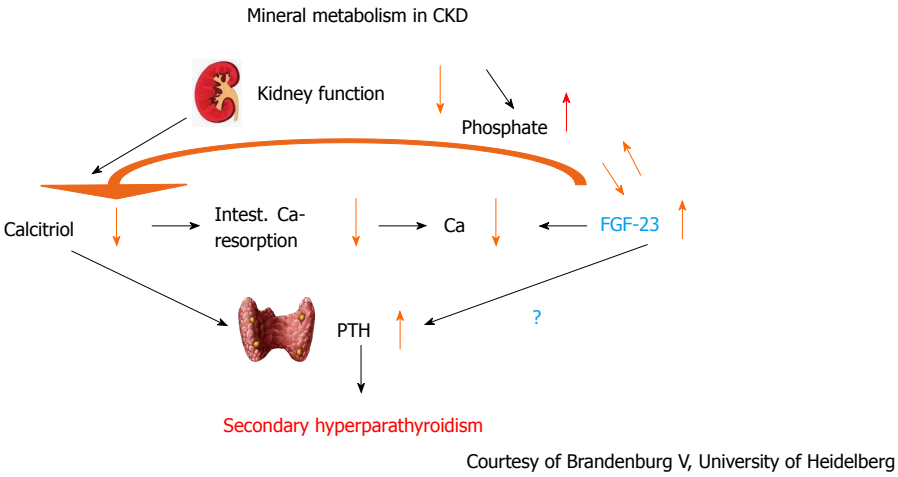

Figure 7 Interplay between calcium, phosphate, calcitriol and Fibroblast growth factor-23 in chronic kidney disease. FGF: Fibroblast growth factor; CKD: Chronic kidney disease.

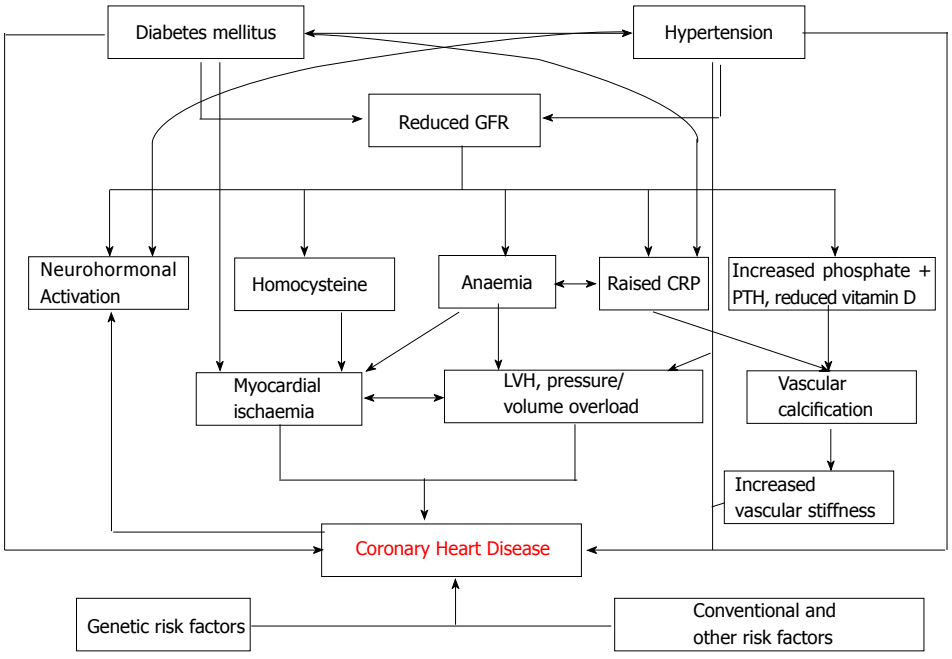

Figure 8 The association between chronic kidney disease and coronary heart disease. Adapted from Hage FG 2009[67].

renal failure, in which its levels are increased, it promotes calcification[87].

SUDDEN CARDIAC DEATH AND DIALYSIS

Dialysis patients have a 10-20 times increased risk of car-

diovascular death than do age and sex-matched controls in the general population[5,7-9], with the largest relative excess mortality seen in younger patients on dialysis. Sudden, unexpected death accounts for 25% of mortality on haemodialysis[95,96]. In most cases, acute coronary occlusion (the end-product of "accelerated atherosclerosis"), which was previously implicated[7], is not the underlying pathology. Instead, sudden cardiac death is explained by the composite effects of left ventricular hypertrophy caused by long-standing hypertension, left ventricular dilatation attributed to volume overload, chronic inflammation, over activation of the renin angiotensin aldosterone pathway, and increased propensity to ventricular dysrhythmias caused by electrolyte abnormalities[95-97].

The structural and functional abnormalities that occur through the interaction of numerous factors presented in this review, gives rise to stiff and non-compliant vasculature which has to negotiate myocardial ischaemia imposed by repeated haemodynamic stresses of haemodialysis therapy. Echocardiographic evaluation and positron emission tomography have shown that repeated haemodialysis adversely affects cardiac perfusion caused by transient myocardial stunning, but over time this leads to fixed regional myocardial wall abnormalities and fibrosis, culminating in heart failure and death[98,99]. In a recent pilot study, our group have demonstrated a possible relationship between endothelial dysfunction and the development of LVH in non-dialysis CKD patients[100]. The CRASH-ILR study is currently underway which is prospectively using implantable loop recorders in patients on haemodialysis to ascertain the frequency of cardiac arrhythmia. In an interim analysis of 18 patients monitored for 124 to 512 h there were 3 significant cardiac events including 1 bradyarrhythmia requiring pacing, 1 sudden cardiac death due to ventricular fibrillation and one onset of atrial tachycardia requiring anti-arrhythmic drug therapy. The final results of this study are awaited[101].

CONCLUSION

Cardiovascular disease is the most important cause for the high morbidity and mortality seen in patients with chronic kidney disease, especially younger people. Traditional risk factors play a major role, but coronary heart disease is somewhat different in CKD patients with increased contribution made by oxidative stress, low grade chronic inflammation and vascular calcification. The interaction of many of these pathophysiological processes is, if not unique to, most marked in CKD. Haemodialysis imparts the greatest mortality risk because of the challenges imposed on the heart by the repeated haemodynamic stresses that accelerate development of heart failure and dilated cardiomyopathy. Limited benefit of standard pharmacological interventions in the dialysis population emphasises the different pathophysiology which has evolved. The complexities of the association between chronic kidney disease and cardiovascular events are summarised in Figure 8. A better appreciation of the interplay and relative contribution of the various mechanisms discussed, along with new pathogenetic mechanisms yet to be discovered over the next few years, will enable new therapies to be developed. This will, hopefully, curb the excessive morbidity and mortality which seems to be particularly pronounced in young dialysis patients. Data from implantable loop recorders may give further insights to the precise events which result in sudden cardiac death and allow development of strategies to predict risk and reduce events.

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P- Reviewer: Friedman EA, Hatamizadeh P, Lopez-Hernandez FJ, Olowu WA **S- Editor**: Ji FF **L- Editor**: A **E- Editor**: Lu YJ

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DOI: 10.5527/wjn.v3.i4.156

World J Nephrol 2014 November 6; 3(4): 156-168
ISSN 2220-6124 (online)

REVIEW

Cardiovascular co-morbidity in chronic kidney disease: Current knowledge and future research needs

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Received: June 26, 2014 Revised: September 30, 2014
Accepted: October 14, 2014
Published online: November 6, 2014

Abstract

Chronic kidney disease (CKD) is recognised as a health concern globally and leads to high rates of morbidity, mortality and healthcare expenditure. CKD is itself an independent risk factor for unfavorable health outcomes that include cardiovascular disease (CVD). Coronary artery disease is the primary type of CVD in CKD patients and a significant cause of death among renal transplant patients. Traditional and non-traditional risk factors for CVD exist in patients with CKD. Traditional factors include smoking, hypertension, dyslipidemia and diabetes which are highly prevalent in CKD patients. Non-traditional risk factors of CKD are mainly uraemia-specific and increase in prevalence as kidney function declines. Some examples of uraemia-specific risk factors that have been well documented include low levels of haemoglobin, albuminuria, and abnormal bone and mineral metabolism. Therapeutic interventions targeted at more traditional risk factors which contribute to CVD, have not had the desired effect on lowering CVD events and mortality in those suffering with CKD. Future research is warranted to delineate clear evidence to the benefit of modifying non-traditional risk factors.

Key words: Cardiovascular disease; Chronic kidney disease; Risk factors; Inflammation

Core tip: Chronic kidney disease (CKD) has been recognised as a health concern globally and leads to high morbidity, mortality and healthcare expenditure. CKD is an independent risk factor for several different unfavourable outcomes including cardiovascular disease (CVD). Traditional and non-traditional risk factors for CVD exist in patients with CKD. Non-traditional risk factors of CKD are mainly uraemia-specific and include release of large levels of inflammatory and prothrombotic factors, low levels of haemoglobin, albuminuria, and abnormal bone and mineral metabolism. Future research is warranted to delineate clear evidence to the benefit of modifying non-traditional risk factors

Alani H, Tamimi A, Tamimi N. Cardiovascular co-morbidity in chronic kidney disease: Current knowledge and future research needs. *World J Nephrol* 2014; 3(4): 156-168 Available from: URL: http://www.wjgnet.com/2220-6124/full/v3/i4/156.htm DOI: http://dx.doi.org/10.5527/wjn.v3.i4.156

INTRODUCTION

CKD has become recognised as a key independent risk factor for several adverse health outcomes including cardiovascular disease (CVD). It is now increasingly apparent that individuals are more likely to die from cardiovascular disease than to develop end stage renal disease (ESRD)[1-3]. Initial evidence indicating a relationship between renal dysfunction and adverse cardiovascular events became apparent in those on dialysis, where the number of CVD deaths was found to be raised. Almost 50% of those suffering from established ESRD are un-

likely to survive a CVD event[2-5]. Compared to the age adjusted CVD mortality in the general populations this is approximately 15 to 30 times higher[4,6]. Although true across all ages it is particularly more profound in the 25-34 year age group, where a 500-fold increase in CVD mortality rate is found when comparing it to their counterparts in the general population[1].

Defining CKD

According to the Kidney Disease Improving Global Outcomes (KDIGO), CKD can be defined as either damage to kidneys or a glomerular filtration rate (GFR) of < 60 mL/min per 1.73 m^2 for a period of $\geqslant 3$ mo, with implications for health. Kidney damage can be defined by structural (detected by imaging) or functional abnormalities of the kidneys with or without a decrease in GFR. These may be apparent as either pathological irregularities or as indicators of kidney damage which include albuminuria > 30 mg/d, urine sediment abnormalities and electrolyte and other abnormalities secondary to tubular disorders.

Individuals with CKD are usually staged according to their GFR levels (Stage 1-5) and albuminuria category, with a higher stage representing lower GFR levels[7,8].

Traditional vs non-traditional risk factors

Traditional risk factors for atherosclerotic CVD are not enough to justify the significant upsurge in cardiovascular mortality seen amongst CKD patients and in particular ESRD. This has led to the suggestion that in patients with CKD two groups of CVD risk factors can be defined; traditional and non-traditional. Traditional factors are those described in the Framingham study[9] including hypertension, smoking, dyslipidemia and diabetes that are well known to contribute to the acceleration of the atherosclerotic process, and are highly prevalent in CKD patients[10]. Non-traditional risk factors of CKD increase in prevalence as kidney function declines. Some examples of such risk factors have been and includes, of large levels of inflammatory and prothrombotic factors, low levels of haemoglobin, albuminuria, and abnormal bone and mineral metabolism[4,11]. Some of these non-traditional risk factors will be discussed in more detail below (Figure 1).

CKD AND CARDIOVASCULAR OUTCOMES

Left ventricular hypertrophy and CKD

Echocardiographic studies report a high prevalence of left ventricular hypertrophy (LVH), systolic/diastolic dysfunction and ventricular dilatation (dilated cardiomyopathy) in patients with ESRD[12-15]. They are more strongly associated with an unfavourable prognosis than more established cardiovascular risk factors[14,16]. These structural and functional abnormalities can lead to sudden, presumed arrhythmic death and account for 50% of cardiovascular deaths in patients with ESRD[17-19].

LVH exists in 70% of patients starting dialysis and is an independent risk factor for cardiac death[12,13,20]. In a cross-sectional study by Stewart and colleagues, 296 non-diabetic renal disease patients underwent echocardiographic monitoring. The results showed that left ventricular mass was increased from even the earliest stages of renal disease (near-normal renal function). Eccentric LVH was found to be the prevalent pattern. The increase in LVH was progressive and 80% of those on renal replacement therapy were found to have LVH, with the concentric pattern being more dominant[19].

In an early study by Levin *et al*[21], 175 pre-dialysis patients underwent echocardiographic monitoring and had their left ventricular mass index (LVMI) assessed. The study demonstrated that the presence of LVH increases with progressive renal decline, reaching 45.2% in patients having severe renal impairment (CrCl < 25 mL/min)[21].

Paoletti *et al*[22] studied 244 non-diabetic pre-dialysis patients and found a greater prevalence, with LVH being associated with 51% in CKD stage 1 and 2 patients and 78% in CKD stages 3 to 5. In all studies - age, haemoglobin, systolic blood pressure and CrCl were found to be significantly different between those who either did or did not have LVH.

Coronary artery disease and CKD

Coronary artery disease (CAD) is one of the primary types of CVD in patients with CKD and is a major cause of death among renal transplant patients[23-27]. The prevalence varies from 24% in young patients without diabetes, to 85% in elderly haemodialysis patients with diabetes[28,29].

In earlier studies it was found that approximately 25%-40% of asymptomatic patients undergoing coronary angiography before their renal transplant exhibited evidence of significant stenosis (50%-70%) in one or more coronary arteries[30-32]. Furthermore, Liu and colleagues, showed that patients with CKD were found to have a 2.5 times higher chance of having 3-vessel disease compared with patients without CKD[33].

Kiyosue *et al*[27] studied the association found amongst renal dysfunction and the severity of CAD. It looked at 572 patients and graded severity according to how many stenotic coronary arteries were there and the estimated GFR (eGFR). More stenotic coronary arteries were present in the CKD group compared to other groups. Multivessel stenosis was also greater in the CKD group[27].

Whether or not CAD should be screened for and interventions offered for patients with advanced kidney disease remains a contentious issue. Coronary angiography remains the modality of choice for CAD investigation in CKD patients, however its invasiveness and associated risks in a group at risk of contrast-induced nephropathy, makes it unfavourable[34,35]. While other imaging modalities exist which are effective (CT coronary angiography, cardiovascular MRI), there are several factors including significant costs, adverse affects as well as technical difficulties that must be considered[36,37].

NON-TRADITIONAL RISK FACTORS

Anaemia

Anaemia is an anticipated consequence as renal function

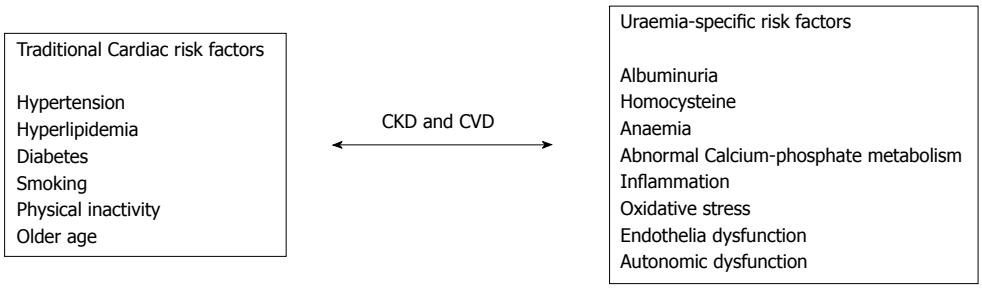

Figure 1 Traditional and non-traditional cardiovascular risk factors in chronic kidney disease[10,11]. CKD: Chronic kidney disease; CVD: Cardiovascular disease.

declines, and generally begins to develop before ESRD. The severity of anaemia however increases with declining kidney function[38,39]. There is a strong association between anaemia and cardiovascular complications. Specifically, anaemia is linked to LVH development, found in up to 74% of patients at the commencement of renal replacement therapy and is an independent predictor of consequent cardiac morbidity and mortality among patients with ESRD[12,13,21,40]. In an Observational study it was demonstrated that each 10 g/L drop in haemoglobin, leads to a 20%-40% increased risk of developing heart failure, LVH or mortality in those patients on long-term dialysis[41]. Interestingly sustained anaemia is often associated with eccentric hypertrophy, whereas hypertension is associated with concentric hypertrophy[21].

Physiologically, chronic anaemia leads to an increased cardiac output (CO) as a result of decreased afterload, an increase in preload and an increase in chronotropic/inotropic effects. This will eventually lead to ventricular dilation and LVH[42-44]. This chronic rise in CO eventually causes remodeling of the central elastic arteries of aorta or carotids. Subsequently it can result in enlargement of arteries and compensatory intima-media thickening, or arteriosclerosis. If either is present, they could be more directly correlated with future CVD risk (Figure 2)[44,45].

One of the first studies to demonstrate anaemia as an independent risk for CVD outcomes was carried out in the ARIC studies. It found anaemia to be associated with an adjusted hazard ratio of 1.41 for CVD in the entire cohort[46].

Jurkouitz *et al*[47] looked at 13329 patients and found a significant link between the haemoglobin (Hb) level and the serum creatinine (S-cr). In anaemic patients, a high S-cr level can result in the risk of coronary events rising by 2.7 fold when compared to a normal S-cr. This is independent of risk factors that include age, gender and race[47].

Levin *et al*[48] focused on the association between anaemia and LVH. 246 participants with a creatinine clearance of 25 to 75 mL/min (0.42 to 1.25 mL/s) underwent echocardiographic imaging done at baseline and 12 mo to specifically investigate LV growth. The results showed that each 0.5-g/dL (5-g/L) drop in Hb level led to a 32% increased odds of LV growth[48].

Further anaemia and echocardiographic studies carried out by Foley *et al*[41], demonstrated that each 1 g/dL drop in Hb was independently linked with the presence of left ventricular dilatation on repeat echocardiogram and the subsequent development of new/recurrent heart failure. Furthermore, every 1 g/dL drop in the haemoglobin level was independently linked with mortality when patients were on dialysis therapy[41].

Weiner *et al*[49] looked at the combination of both LVH and anaemia in patients at the earlier stages of renal dysfunction (eGFR 30-60), revealing that patients who had LVH as well as anaemia, had a risk of cardiac disease that increased by 4-fold compared with individuals who had neither anaemia nor LVH. However in those having LVH but no anaemia or anaemia but no LVH, the risk of CVD outcomes did not increase significantly[49].

The strong association between anaemia and cardiovascular complications has led to several studies investigating whether correction of haematocrit has any benefit on adverse cardiovascular outcomes. Besarab *et al*[50], looked at 1233 patients on haemodialysis with heart disease who were prescribed recombinant human erythropoietin (epoetin), looking at length of time till death or the first non-fatal myocardial infarction. Six hundred eighteen patients received sufficient doses to sustain a haematocrit of 42% and 615 to sustain a haematocrit of 30%. The study was halted due to the higher haematocrit target group having an almost significant mortality risk, and the fact that thrombotic vascular access events were also higher. No single unifying explanation was thought to be the cause[50].

The Cardiovascular Risk Reduction by Early Anaemia Treatment with Epoetin Beta (CREATE) trial looked at patients in the earlier stages of CKD (3 and 4) to try and achieve levels of normal; 130-150 g/L and low to normal; 105-115 g/L Hb. The normal Hb group was found to have an improved overall health and quality of life. LVMI was found in both groups to be stable, and thus treating the anaemia did not have an effect on the LVH progression[51]. This is supported by many other studies[52-54].

The Anaemia Working Group of European Renal Best Practice (ERBP) published a statement on its opinion of what Hb targets should be. Following results of the Trial to Reduce Cardiovascular Events with Aranesp Therapy (TREAT) Study they maintained that Hb values of 11-12 g/dL should be targeted in CKD patients,

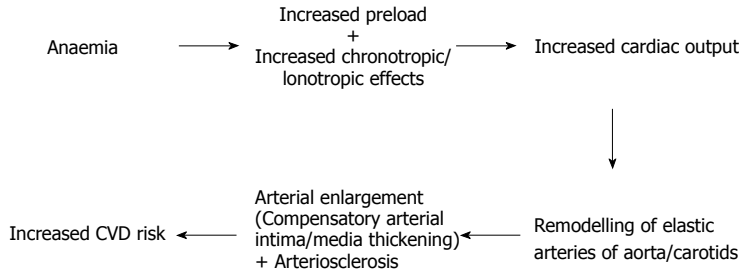

Figure 2 Physiological impact of chronic anaemia in chronic kidney disease. CVD: Cardiovascular disease.

and without deliberately aiming for targets above 13 g/dL[55,56].

Correcting the anaemia associated with ESRD with recombinant human erythropoietin (r-EPO)/ iron supplementation is essential, and results in significant improvements of Hb levels as well as a reduction in the need for blood transfusion requirements[57,58]. Recent literature however, suggests excess iron as well as contributing to arrhythmias and heart failure may also have a role to play in the development of vascular calcification[59,60].

A cross sectional study by Bagheri *et al*[61] looked at 337 patients to evaluate the importance of iron stores in the risk evaluation of atherosclerotic disease. All patients had an angiogram of the coronary artery, and the study revealed that the iron serum level was significantly more raised in the severe atherosclerosis group than in the normal group[61].

It is thought that one of the ways in which iron contributes to vascular calcification is by enhancing the oxidative stress process through the induction of reactive oxygen species. This subsequently activates molecular mechanisms that result in vascular calcification[60].

Drüeke *et al*[62] looked at the role of I.V. iron therapy in 79 dialysis patients. They concluded that iron therapy (at dose 1.5-2 g per year) can lead to arterial wall damage in the early stages of atherosclerosis[62]. This was later supported by Reis *et al*[63] who showed a significant relationship between ferritin, I.V. iron dosage and common carotid artery intima-media thickness in 60 dialysis patients[63].

Further research is required to assess the effect that iron has on arterial calcification, however the potential of iron to cause oxidant injury and CVD should not be disregarded.

Homocysteine

Homocysteine (HC) at high levels can be an independent risk factor for cardiovascular disease[64]. Levels of HC are elevated in renal failure and there is an inverse relationship between HC levels and GFR, such that more than 85% of patients on dialysis will experience a mild to moderate level of hyperhomocysteinemia[64,65].

The pathogenesis of hyperhomocysteinemia in renal dysfunction remains to be elucidated[64,66]. There is some evidence to suggest that high levels of homocysteine may be due to a reduced HC clearance and insufficient metabolism by less well functioning kidneys. However there is no direct evidence to support this[64].

Several epidemiological studies suggest that a high level of HC specifies a higher risk of CVD as well as stroke[67,68]. While the underlying mechanisms are yet to be defined, both *in vitro*/*vivo* studies suggest that production of potent reactive oxygen species (ROS) and decreased endothelial nitric oxide play a pivotal role. Thus high HC levels may facilitate oxidative damage at the vascular interface[69-71]. Other proposed mechanisms suggest that elevated HC causes proliferation of smooth muscle cells thus leading to increased oxidation of low-density lipoproteins[72]. Elevated HC is also associated with increased platelet aggregation and hence favouring a prothrombotic state[73]. This strongly links elevated levels of HC to the enhancement of atherosclerosis and other thrombotic events.

Several trials have looked at the efficacy of HC-lowering treatments on clinical outcomes. Two major studies, Homocysteine study (HOST)[74] and Heart Outcomes Prevention Evaluation-2 (HOPE-2)[75] looked for any benefits in certain vitamins including; folic acid, vitamin B6 and vitamin B12 supplements on overall CVD risk and mortality. Both studies found no significant benefit on CVD risk or all-cause mortality. Therefore based on these trials, there is not much to support using HC-lowering interventions for preventing cardiovascular outcomes.

Calcium and calcium-phosphorus product

Dysfunction in the metabolism of minerals occurs early in CKD. As GFR levels decline, there is a decrease in serum calcium (Ca) levels while parathyroid hormone (PTH) and phosphate levels become elevated[76]. An elevated level of serum phosphorus is highly prevalent in ESRD patients, and is a significant and independent risk factor of all cause and cardiovascular mortality[77]. A study by Block *et al*[77] found that phosphate levels greater than 6.5 mg/dL were associated with a far greater mortality risk (27%) when compared with levels of between 2.4-6.5 mg/dL[77].

Further studies by Kestenbaum *et al*[78] demonstrated that PO4 levels > 3.5 mg/dL are linked with an increased risk of death that is significant. Furthermore, for every 0.323 mmol/L serum phosphate increase, there

was an increased risk of death by 23%[78].

In a study by Dhingra *et al*[79] it was suggested that even phosphate levels within the normal range can contribute to CVD in patients who have kidney function within the normal, to near-normal range. Furthermore phosphate levels above 1.1 mmol/L can increase the risk of CVD events by 55%, following adjustment for any traditional cardiovascular risk factors. The cholesterol and recurrent events study (CARE) enlisted 4159 patients who had a background of previous myocardial infarction concluded that there was a graded, independent relationship between baseline fasting serum phosphate level and the subsequent risk for all-cause mortality, the development of new heart failure, and coronary events[80].

Calcium-phosphate products are also associated with increased risk of cardiovascular morbidity and mortality in CKD patients. Ganesh *et al*[81] demonstrated that for every rise in serum calcium-phosphate product by 0.8 mmol2/l2, there was an increased sudden death risk of approximately 7% in those on long-term haemodialysis[81].

The underlying mechanism through which hyperphosphatemia and an increase in calcium-phosphate product leads to cardiovascular disease is not well established. One theory is that high phosphate levels exacerbate the atherosclerosis process by increased calcification and proliferation of smooth muscle[82].

Raggi *et al*[83] carried out a cross-sectional study of 205 patients on hemodialysis who had baseline electron-beam tomography (EBT) testing to evaluate both vascular/valvular calcification. The incidence and degree of valvular calcification was found to be remarkable with 45% of subjects having calcification of the mitral valve, and 34% having calcification of the aortic valve, compared with 3%-5% prevalence in the general population. More than 70% of patients had coronary artery calcification significant enough to be linked with a high risk of future MI and coronary death in the general population[83].

Goodman *et al*[84] screened for calcification using EBT in 39 young patients (age range 7 to 30 years of age) with ESRD on dialysis. It found evidence of coronary calcification in 14 of the 16 patients aged 20-30 years[84].

Current KDOQI guidelines advise that serum phosphate levels should be maintained at 0.8 mmol/L in stage 3-4 CKD and between 1.1 and 1.8 mmol/L in stage 5 CKD[8]. Several phosphate binders exist in the treatment of hyperphosphataemia, however the choice of binders is controversial.

Calcium-based binders have been shown in observational studies to be associated with arterial calcification[84,85]. Sevelamer is a non-absorbable agent that does not contain calcium and has been shown in a significant number of trials to be effective in lowering serum phosphate levels. It has also been shown to have beneficial effects on vascular calcification progression and bone disease[86].

Two large studies have compared Sevelamer with calcium-based binders. In the Renagel in New Dialysis (RIND) trial it found that calcium-based phosphate binders resulted in higher cases of mortality than compared to Sevelamer[87]. The Dialysis Clinical Outcomes Revisited (DCOR) study however showed that the difference in mortality was not significant. Conflicting results can be possibly explained by difference in patient population[88].

Albuminuria

Not only is albuminuria a marker of renal damage, it is also an independent risk factor for CVD and leads to an increase in all cause mortality in diabetics, those with hypertension and in relatively unselected or general populations[89-93].

In the Heart Outcomes Evaluation (HOPE) trial results showed that in those with or without diabetes, albuminuria of any level can be a risk factor for CVD events. It also found microalbuminuria to result in an increased risk of future stroke, myocardial infarctions and death in both diabetic and non-diabetics without CKD.

Additionally, for every increase in the albumin:creatinine ratio (ACR) by 0.4 mg/mmol, there was a 5.9% increase in the HR hazard ratio (HR) of major CVD outcomes[91].

The Losartan Intervention for Endpoint Reduction in Hypertension (LIFE) trial studied 8206 patients in order to establish if the relationship between albuminuria and cardiovascular risk can be useful in predicting cardiovascular morbidity and mortality in hypertensive patients. It discovered that for every increase of the ACR by 10-fold, cardiovascular deaths increased up to 98% in non-diabetics without CKD[92].

These trials demonstrate that the relationship between albuminuria and experiencing a CV event is not entirely restricted to the microalbuminuria cut off range. The relationship between the ACR and CV disease can extend to at least as low as 0.5 mg/mmol and thus an ACR of 2.0 mg/mmol, the threshold for the screening of microalbuminuria may not be appropriate when considering the risk for CV outcomes[91,92].

Microalbuminuria has also been associated with several lipid abnormalities. In a study by Kahri *et al*[94], the lipid profiles in both those having microalbuminuria and normoalbuminuria were compared. It found that the high-density lipoproteins (HDL) that are known to be cardioprotective were 11.6-fold lower in microalbuminuric patients compared with normoalbuminuric patients[94].

The pathophysiology behind how microalbuminuria contributes to CVD remains to be fully understood, however studies suggest that microalbuminuria might reflect endothelial dysfunction[95,96]. As well as causing impaired arterial dilatory capacity[97], microalbuminuria has been shown to increase levels of several adhesion molecules including Von willeband factor (vWF), Vascu-

lar adhesion molecule-1, thrombomodulin, PAI-1, serum IV collagen and t-PA[87]. These all favour the formation of atherosclerosis[98].

ROLE OF INFLAMMATION, OXIDATIVE STRESS, HYPERTENSION AND URIC ACID

Inflammation

It is now well established that the incidence of acute-phase inflammation and oxidative stress in patients with ESRD is high, which are both significant contributors to a high degree of CVD morbidity and mortality[99-101]. Oberg *et al*[102] confirmed presence of increased oxidative stress and acute-phase inflammation in early and advanced stages of CKD (3-5) compared to healthy subjects. Renal insufficiency is associated with increased levels of several different inflammatory and pro-coagulant biomarkers, the main two being CRP and IL-6, which are strong predictors of all-cause mortality and cardiovascular outcomes in ESRD[103-105]. Elevation of these markers as well as fibrinogen, PAP, factor Ⅶ-Ⅷ, and D-dimer was apparent even in those who had no evidence of clinical or subclinical cardiovascular disease[106-108].

The extent to which GFR is related to biomarkers of inflammation is controversial. While one cross sectional study found that increased CRP was associated with decreased GFR, other studies have found serum CRP levels do not correlate with either GFR or disease progression[109].

A study by Friedman *et al*[110], which looked at both CRP and albumin in dialysis patients, found CRP to be a significant predictor of death and suggested that these patients need to have careful evaluation as well as monitoring irrespective of whether albumin concentration is in the normal range[111]. Evidence seems to suggest that CRP may also be responsible for numerous processes involved in propagating atherosclerosis, which includes plaque initiation, formation, and rupture[111].

Inflammatory and prothrombotic markers have been heavily linked with CVD and mortality in CKD patients. Shlipak *et al*[112] however, demonstrated that although CRP and IL-6 are linked with CVD, their collective impact on cardiovascular mortality is actually far less significant compared to the collective impact of the more traditional risk factors[112].

Oxidative stress

Numerous experimental studies have revealed that endothelium derived vasoactive mediator nitric oxide (NO) has a vital part to play in progressive kidney damage. Low levels of NO leads to endothelial cell injury and dysfunction, and plays a major role in potentiating atherosclerosis[113-115]. Animal studies in which NO synthase (NOS) was inhibited resulted in enhanced progression of the atherosclerotic process as well as causing impairment in the angiogenic response and loss of the capillary endothelium[116]. Endogenous NOS inhibitor, asymmetric dimethylarginine (ADMA) is thought to be significantly associated with the oxidative stress process through its inhibition of NO, and thus leading to endothelial dysfunction and vascular damage[117]. ADMA correlates with traditional and non-traditional risk factors, it is recognised as a strong indicator in atherosclerosis, and is a strong independent predictor of death and incident cardiovascular complications in both CKD and non-CKD patients[117-119].

In a cohort study of 131 patients with CKD, the correlation between levels of ADMA and the probability of progressing to ESRD and death was investigated. ADMA was found to be reliable in predicting event occurrence independently of other confounders, such as GFR, proteinuria and several others. Furthermore, ADMA was found to be inversely related to GFR and signifies an independent marker of risk for ESRD progression and mortality[117].

Several studies have looked at interventions in order to reduce the plasma levels of ADMA or its binding capability to NOS in an attempt to decrease any risk of CVD events in those suffering from CKD. Lerman *et al*[120] studied the effects of supplementing 26 patients with L-arginine, a precursor to NO in order determine its future therapeutic use. It found that following 6 mo of supplementation, it improved endothelium function in coronary vessels while also providing symptomatic relief and lowering levels of endothelin[120]. Further support of these results come from studies by Clarkson *et al*[121] who explained that L-arginine orally enhanced the peripheral endothelium-dependent dilation of hypercholesterolaemic patients, as well as Rector *et al*[122] who showed that L-arginine was helpful in heart failure subjects.

Other studies have looked at the antioxidant effects of acetylcysteine and Vitamin E. Both supplements have demonstrated a reduction in composite cardiovascular end points in haemodialysis patients[123,124].

Hypertension

Hypertension itself can act as a dominant risk factor for CVD in patients with CKD, and it is almost inevitable that CKD patients will have hypertension. The underlying mechanism considered most important in the elevation of blood pressure, is related to retention of sodium as well as stimulation of the renin-angiotensin system[125]. Sympathetic activation and elevated catecholamine release in CKD has also been linked[126,127]. Cardiac damage caused by hypertension in CKD patients is thought to be via LVH induction[128].

Two major studies have evaluated the relationship between renal function and mortality in hypertensive patients. In the Hypertension Detection and Follow-up Program Cooperative Group, 10490 patients where analysed to assess all-cause mortality. It demonstrated that in those who had a baseline creatinine of $\geqslant$ 1.7 mg/dL the mortality rate (8-year) was $\geqslant$ 3 times greater than that of all other patients[129].

The Hypertension Optimal Treatment (HOT) study supports these outcomes. They evaluated 18790 patients over 3.8 years, only 10% of whom had evidence of atherosclerotic plaques. It found that the relative risk for both mortality and CVD events was 1.65 and 1.58 respectively, in those with a GFR < 60 mL/min compared with those who had a GFR > 60 mL/min[130].

Despite strong evidence linking CVD mortality in hypertensive CKD patients, the ideal blood pressure (BP) targets in these patients remains a challenging area. The National Institute for Health and Clinical excellence (NICE) suggests that antihypertensive treatment be commenced in those < 80 years with declining renal function and hypertension-stage 1, to aim for a blood pressure of < 140 / 90 mmHg. The BP during haemodialysis/peritoneal dialysis period should not exceed > 160 mmHg[8,131]. However excessive BP lowering in these patients may prove to be detrimental due to the risk of exacerbating myocardial stunning[132-134]. There remains an insufficient number of RCT trials on optimum blood pressure control in CKD patients and it is important to address this issue.

Uric acid

While hyperuricemia is a well-recognised consequence of impaired renal function it is also linked with increasing hypertension risk, ESRD and unfavorable cardiovascular outcomes[135].

UA has been demonstrated as an independent risk factor for the onset of CKD, in a healthy population. Obermayer *et al*[136] reported that even with a modest rise in UA levels there was a two-fold increased risk of renal disease, whilst at 535 μmol/L or more the risk was threefold.

Sedaghat *et al*[137] further supported this theory. They analysed 2601 subjects aged > 55 years who where followed up over a 6.5 year period. They exhibited that for each 60 μmol/L rise of uric acid, there was a decline in the eGFR by 0.19 mL/min. In hypertensive patients the decline in eGFR was more profound[137].

The detrimental effects of hyperuricemia are also well documented in CVD. In the health professionals follow-up study hyperuricemia was found to increase CVD mortality even more than compared with those who already had established heart disease[138]. This was further supported by long-term data from the NHANES I study that demonstrated that there was a proportionate rise in CVD mortality with UA levels[139].

Studies have looked at the use of xanthine oxidase (XO) inhibitors (Allopurinol, Febuxostat), as potential treatment to prevent further renal deterioration and to provide a cardioprotective effect. In one meta-analyses, allopurinol was found to produce a small but yet significant reduction of both systolic/diastolic blood pressure. This is further supported by randomised controlled trials, where again a significant blood pressure drop is achieved with UA-lowering agents[140].

Rekhraj *et al*[141] demonstrated that treating patients with diagnosed LVH and ischaemic heart disease with high dose allopurinol (600 mg/d) for 9 mo, resulted in a reduction of the LVM and end systolic volume as well as improving endothelial function[141]. This modest reduction in LVM was shown in the LIFE study to reduce mortality and cardiovascular outcomes by 13%[142,143].

In the context of CKD, a study by Goicoechea *et al*[144] demonstrated that in those patients receiving allopurinol for 12 mo there was a diminution in the deterioration of kidney function or the need for dialysis compared to placebo. (143) In another study allopurinol reduced eGFR in patients with, established CKD (stage 3) independent of age, sex or diabetes. Adverse cardiovascular events were also found to be reduced[144].

FUTURE RESEARCH NEEDS

CKD is a major health concern globally and leads to high rates of morbidity, mortality and healthcare expenditure. Much of the morbidity and mortality associated with CKD is significantly attributable to cardiovascular outcomes. While we have tried to briefly analyse some of the current knowledge underlying the strong association between CKD and CV outcomes there still remains several non-traditional risk factors and pathophysiological mechanisms to be described.

Several markers have a clear association with current and subsequent CV outcomes including; reduced GFR, albuminuria, troponins, phosphate, vitamin D, FGF-23 and NT-proB-NP[145-152]. While these markers are present, there remains no routine screening for CVD in CKD patients, despite strong evidence supporting this. The screening and treatment of patients with abnormal markers cannot only reduce overall cardiovascular events and kidney failure, but could also prove to be cost effective.

Perhaps one reason for lack of implementation is due to the fact that these diagnostic screening tools are lacking in sensitivity and specificity to make them reliable, and are in need of more RCT-quality evidence in order to guide intervention.

Therapeutic interventions that aim at reducing traditional risk factors for CVD have not been shown to be effective at lowering the incidence of CVD events or mortality in those with CKD. More importantly, strategies in risk reduction have inadequately tackled non-traditional risk factors that have been exhibited as being essential in the progression of CVD. Furthermore there remains to be clear evidence with regards to the benefit of modifying these non-traditional risk factors.

In view of current knowledge it is perhaps favourable to investigate preventive strategies in early-stage chronic kidney disease and multifactorial interventions in late-stage chronic kidney disease.

Some of the excess CVD risk associated with CKD has been explained by a multifactorial range of myocardial and vascular insult including; uraemic cardiomyopathy, inflammation, oxidative stress, autonomic

dysfunction and endothelial dysfunction. However, it is very likely that there are as yet undiscovered factors, and inter-relationships, which are possibly of great significance.

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P- Reviewer: Chang CC, Chawla M, Fassett RG **S- Editor**: Ji FF
L- Editor: A **E- Editor**: Lu YJ

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Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.5527/wjn.v3.i4.169

World J Nephrol 2014 November 6; 3(4): 169-181
ISSN 2220-6124 (online)

REVIEW

Preeclampsia from a renal point of view: Insides into disease models, biomarkers and therapy

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Received: March 20, 2014 Revised: August 6, 2014
Accepted: September 4, 2014
Published online: November 6, 2014

Abstract

Proteinuria is a frequently detected symptom, found in 20% of pregnancies. A common reason for proteinuria in pregnancy is preeclampsia. To diagnose preeclampsia clinically and to get new insights into the pathophysiology of the disease it is at first essential to be familiar with conditions in normal pregnancy. Animal models and biomarkers can help to learn more about disease conditions and to find new treatment strategies. In this article we review the changes in kidney function during normal pregnancy and the differential diagnosis of proteinuria in pregnancy. We summarize different pathophysiological theories of preeclampsia with a special focus on the renal facets of the disease. We describe the current animal models and give a broad overview of different biomarkers that were reported to predict preeclampsia or have a prognostic value in preeclampsia cases. We end with a summary of treatment options for preeclampsia related symptoms including the use of plasmapheresis as a rescue therapy for so far refractory preeclampsia. Most of these novel biomarkers for preeclampsia are not yet implemented in clinical use. Therefore, we recommend using proteinuria (measured by UPC ratio) as a screening parameter for preeclampsia. Delivery is the only curative treatment for preeclampsia. In early preeclampsia the primary therapy goal is to prolong pregnancy until a state were the child has an acceptable chance of survival after delivery.

Key words: Preeclampsia; Pregnancy; Proteinuria; Biomarkers; Treatment

Core tip: This review summarises different pathophysiological theories of preeclampsia with a special focus on the renal facets of the disease. In this context current animal models are presented. The reader gets a broad overview about different biomarkers for preeclampsia. Furthermore, the article discusses treatment options for preeclampsia related symptoms including the use of plasmapheresis as a rescue therapy for so far refractory preeclampsia.

Müller-Deile J, Schiffer M. Preeclampsia from a renal point of view: Insides into disease models, biomarkers and therapy. *World J Nephrol* 2014; 3(4): 169-181 Available from: URL: http://www.wjgnet.com/2220-6124/full/v3/i4/169.htm DOI: http://dx.doi.org/10.5527/wjn.v3.i4.169

INTRODUCTION

There are many physiological changes in the function of different organs during normal pregnancy. Changes in kidney function and low grade proteinuria are common findings in pregnancy. However, new onset of proteinuria is also one of the primary symptoms for the clinical diagnosis of preeclampsia[1]. To understand pathological conditions in pregnancy it is important to know the normal changes and also typical complications that may occur during pregnancy. In pregnancy the physician always treats two patients. What is required for treatment of the mother is not always beneficial for the child. Many drugs

are contraindicated in pregnancy or there is no data on their safety. We review the development of kidney function and proteinuria in pregnancy in general and then discuss preeclampsia in particular. This is likely the first review where all prevailing animal models for preeclampsia and all currently suggested markers for early detection of the disease are presented with special focus on the kidney. Further more, we give treatment strategies for preeclampsia and discuss controversial new methods for therapy refractory preeclampsia.

KIDNEY FUNCTION IN PREGNANCY

During a normal pregnancy kidneys increase in size and the kidney volume can enlarge up to 30%[2]. In pregnant women the ureter and the renal pelvis are frequently dilated which can lead to an increased risk for urinary tract infections and pyelonephritis. There are no renal histological changes due to pregnancy but higher urinary frequency, nocturia, dysuria, urgency and stress incontinence are common[3]. The glomerular filtration rate (GFR) rises by approximately 40% to 50% above baseline levels in pregnancy. Thus, a normal serum creatinine can actually reflect significant renal insufficiency in a pregnant woman. Equations like Modification of Diet in Renal Disease (MDRD) formula, Cockcroft-Gault formula and the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula are usually used to estimate GFR but are not accurate during pregnancy. Smith *et al*[4] compared eGFR calculated using the MDRD formula with inulin clearance in 24 healthy women during and after pregnancy. They found that MDRD formula underestimated GFR by more than 40 mL/min during pregnancy. The Cockcroft-Gault formula, the MDRD formula and the CKD-EPI formulas were evaluated for their accuracy in preeclampsia by Alper *et al*[5] All of these equations were inaccurate in predicting GFR in preeclamptic women compared with creatinine clearance obtained from 24-h urine collections.

Cystatin-C does not seem to be a useful marker in pregnancy because it increases in the third trimester. This is thought to be due to changes in size and charge selectivity of the glomerular filtration barrier leading to decreased filtration of cystatin-C [6]. Increased placental production of cystatin-C may also play a role in the increased levels observed in late pregnancy[7]. In line with this Saxena *et al*[8] found that serum cystatin-C did not correlate with inulin clearance during pregnancy or postpartum. Therefore, the best method to determine the GFR in pregnancy is the mean of urea and creatinine clearance obtained from collected urine.

PROTEINURIA IN PREGNANCY

There are several methods used clinically to measure proteinuria. A dipstick test is the easiest method to quantify proteinuria, however, in a recent study the dipstick test in spot-urine over and underestimated proteinuria[9]. Another alternative to estimate proteinuria is the urine protein creatinine (UPC) ratio. A UPC-ratio above 700 mg/g creatinine predicts significant proteinuria while a UPC ratio of less than 150 mg/g creatinine is normal. Nevertheless, the UPC ratio has several disadvantages. It cannot detect changes in proteinuria over the course of the day or take into account orthostatic changes that can potentially cause relevant changes in proteinuria. Moreover, likely day-to-day biological variation of the UPC-ratio has to be considered and only relatively large changes indicate a reliable change in disease status[10]. Proteinuria is known to have a circadian rhythm so when samples for the calculation of UPC ratio are collected at a fixed time of the day UPC ratio can be an acceptable alternative for 24-h urine collections, especially in an outpatient setting[11]. UPC ratio was a reasonable "rule-out" test for detecting proteinuria of 0.3 g/d or more in hypertensive pregnancy[12], but normal UPC ratio cannot rule out mild preeclampsia[13].

Different cut-off values for UPC ratio have been suggested with different results. In one study a cut-off value of 220 mg/g creatinine predicted significant proteinuria with 87% sensitivity and 92.6% specificity[14]. According to another study random UPC ratio is helpful primarily when it is below 150 mg/g creatinine, in that proteinuria of more than 300 mg is unlikely below this threshold. The accuracy of this UPC ratio in predicting 300 mg of protein in 24-h urine collection in pregnant patients with suspected preeclampsia had a sensitivity ranged from 90%-99% and specificity ranged from 33%-65%[15]. In contrast to that UPC ratio and 24-h urine total protein level showed a poor correlation with negative predictive value of 47.5% and specificity of 55.8% in a study of 220 women by Durnwald *et al*[16] Nevertheless he same authors admit that UPD ratio can predict severe preeclampsia and thus can be used for rapid diagnosis of severe preeclampsia as the correlation of UPD ratio and 24-h proteinuria increases with the amount of proteinuria.

Therefore the gold standard for proteinuria is the 24-h urine collection. However, the 24-h urine collection is inconvenient for pregnant women, expensive and may be inaccurate due to insufficient collection. To address this problem and for validation of the results quantification of the urine creatinine excretion should be done. Urinary creatinine excretion should be between 15 and 20 mg/kg body weight if the collection was adequate.

Urinary protein excretion increases due to both increased glomerular filtration and increased permeability of the glomerular filtration barrier during normal pregnancy. Urinary protein excretion rises to about 180 to 200 mg/d in the third trimester of the pregnancy. In women with pre-existing proteinuria the rise in proteinuria is often higher and cannot be explained by increased GFR alone.

The majority of women with pre-existing glomerular disease have increased proteinuria during the course of their pregnancy and can develop nephrotic range

proteinuria in the third trimester. Nevertheless, the presence of nephrotic syndrome due to renal disease, in the absence of significant renal insufficiency or significant hypertension, does not seem to affect the natural course of renal disease or foetal survival[17]. *De novo* renal disease like lupus nephritis or renal diseases secondary to diabetes or hypertension are other possible causes of increased proteinuria in pregnant women. In addition a symptomatic urinary tract dilatation may also be associated with proteinuria in pregnancy[18]. Thus, the underlying reason for proteinuria in pregnancy is often clinically uncertain. Sometimes a definitive cause of renal disease can only be found histologically. The published evidence for the benefit of a kidney biopsy during pregnancy is heterogeneous and there are only a few reports of renal biopsies during pregnancy which were performed to determine the definite diagnosis of renal disease.

Packham *et al*[19] reported 111 renal biopsies performed before the 29th week of gestation where complications of the procedure were similar to those in the non-pregnant population. Day *et al*[20] showed that pregnancy itself does not increase the risk associated with a renal biopsy. In contrast to that, other investigators reported a significantly higher risk of complications for kidney biopsies in pregnancy, with a peak at around the 25th gestational week[21]. Some clinicians prescribe empirical therapy with steroids in nephrotic syndrome in pregnancy. However, diabetic nephropathy or amyloidosis may be exacerbated by steroid therapy. Lupus nephritis during pregnancy follows a variable course and the type and extent of renal lesions can only be assessed histologically. Patients with a biopsy-proven diagnosis of mesangial-proliferative lupus nephritis usually have a favourable prognosis. Diffuse proliferative lupus nephritis typically results in a decreased glomerular filtration rate, a poor prognosis and requires aggressive therapy. Renal biopsy for the diagnosis of glomerulonephritis or preeclampsia led to therapeutic changes in 66% of cases[21]. In general we would recommend waiting until postpartum before performing a renal biopsy unless an unexplained rapidly progressive loss of renal function or unexplained nephrotic range proteinuria occurs. Therapeutic options in pregnancy are given below.

PREECLAMPSIA

A common reason for increased proteinuria in pregnancy is preeclampsia. Preeclampsia affects 2%-8% of pregnancies and is defined as the combination of pregnancy induced hypertension and proteinuria[22]. Recently the American College of Obstetricians and Gynecologists removed proteinuria as an essential criterion for diagnosis of preeclampsia in 2013[23]. Therefore, it is possible that in recent studies 10% of women with clinical and/or histological manifestations of preeclampsia had no proteinuria[24].

It has been hypothesized that preeclampsia results from a reduction in uteroplacental perfusion which leads to uteroplacental ischemia. In the preeclamptic placenta trophoblasts do not develop normally and are unable to invade the myometrium effectively[25]. Specifically the placental tissue but not the foetus is involved in the development of preeclampsia, since preeclampsia also occurs in women with a hyaditiform mole[26-29]. Risk factors for preeclampsia include family history of preeclampsia, multiple gestation, nulliparity, obesity, older maternal age, molar pregnancies, diabetes mellitus, pre-existing hypertension, chronic renal disease and thrombotic vascular disease[30-33]. Paradoxically, smoking during pregnancy is associated with a reduced risk of preeclampsia[34,35]. Nicotine inhibition of thromboxane A2 production might explain this. However, it must be stated that smoking in general and especially during pregnancy has an increased health risk and is absolutely contraindicated.

Preeclampsia can cause small-for-gestational-age infancy, preterm delivery, hypoxic neurologic injury and foetal death. Perinatal mortality is approximately 10% and maternal mortality even occurs in 10% to 15%[36]. Maternal complications of preeclampsia include renal failure, eclampsia, HELLP syndrome (haemolysis, elevated liver enzymes, and thrombocytopenia), seizures, liver failure and stroke. In contrast to normal pregnancy where blood urea nitrogen (BUN) and creatinine decrease, preeclamptic women have BUN and creatinine levels similar to non-pregnant women due to reduced GFR and RPF.

Clinical signs of preeclampsia generally resolve spontaneously within 12 wk after delivery whereas proteinuria due to other renal disease does not. New-onset proteinuria after 20 wk of gestation together with new-onset hypertension is a strong indicator of preeclampsia. The severity of proteinuria does not correlate with the severity of preeclampsia and can even be absent in 10% of the cases[1,37,38]. However, a high UPC ratio in preeclamptic women is associated with a highly increased likelihood of adverse maternal outcomes[39].

In cases where information on the presence or absence of proteinuria in early pregnancy is lacking, the distinction between an underlying primary renal disease and preeclampsia can be very difficult. If *thrombotic thrombocytopenic purpura* occurs for the first time during pregnancy, it may mimic severe preeclampsia.

The timing of preeclampsia can also be atypical with onset before the 20th week of gestation or up to 4 wk postpartum. Thus, in some cases, the distinction between preeclampsia and other renal diseases in pregnancy can only be made in retrospect.

Postpartum preeclampsia is the occurrence of hypertension and proteinuria after delivery. Late postpartum eclampsia is an atypical form of eclampsia beginning between 48 h to 4 wk after delivery[40]. The incidence of postpartum preeclampsia is dependent on the population included in the study. In one 10-year retrospective case series it was 5.7%[41]. In the same analysis 15.9% of hypertensive or preeclamptic women in the postpartum period develop eclampsia.

Most patients with postpartum preeclampsia have no evidence of preeclampsia during pregnancy[42]. Hypertension is a common but not universal finding in postpartum preeclampsia. In postpartum preeclampsia proteinuria may occur less often than in preeclampsia during pregnancy[43]. Seizures are often more severe and refractory to treatment.

Persistence of trophoblasts is associated with the development of preeclampsia in gestational trophoblastic disease[28,29]. Even though found only on the microscopic level, trophoblastic, tissue was found in patients with postpartum preeclampsia and suggests that it causes the disease. Epstein *et al*[44] demonstrated that women with preeclampsia develop hypertension more often than their non-preeclamptic siblings.

ANIMAL MODELS FOR PREECLAMPSIA

A number of animal models have been proposed for preeclampsia but have some limitations. Mice have shallow trophoblast invasion and three trophoblast layers verses a single layer of trophoblasts of the human placenta in pregnancy. Therefore mice models are less useful for studying trophoblast invasion processes. In order to model reduced uterine perfusion pregnant rats undergo clipping of the aorta above the iliac bifurcation at day 14 of gestation[45]. There are also some mouse models of preeclampsia that employ manipulation of sFlt-1, VEGF 121[46], endothelin, endothelial nitric oxide synthase[47] or the renin-angiotensin system[48].

From a renal point of view the sFLT model is one of the most promising because it is the only one that shows glomerular endotheliosis as well as hypertension and proteinuria[49]. Karumanchi *et al*[50] created a rat model of preeclampsia by administration of a sFLT1-expressing adeno-virus. The administration of the sFLT1 by this vector resulted in a dose-dependent hypertension, proteinuria, and glomerular endotheliosis in pregnant rats[50]. As preeclampsia only occurs spontaneously in pregnant women, no animal model can completely mimic the entire pathogenesis of human preeclampsia and all animal models only reflect some limited aspects of the underlying disease. Thus, the definitive studies on preeclampsia must be clinical.

In the last few years many potentially useful biochemical markers have been proposed for the prediction and outcome of preeclampsia. The timeframe of diagnostic usefulness of these biomarkers to distinguish women at risk for preeclampsia from healthy pregnant women will be reviewed below.

BIOMARKERS IN PREECLAMPSIA

Autoantibodies

Gant *et al*[51] identified hypersensitivity to infused Angiotensin Ⅱ in preeclamptic patients. However, circulating levels of Angiotensin Ⅱ are not increased in preeclampsia[52]. Instead, immunoglobulins from preeclamptic women increased the beating rate of neonatal rat cardiomyocytes. These immunoglobulins contained Angiotensin Ⅱ type 1 (AT1) autoantibodies that stimulate the Angiotensin-receptor. The increased heartbeat rate could be blocked by treatment with losartan and it could be demonstrated that the autoantibodies bind to the second extracellular loop of the AT1 receptor[53]. AT1 agonistic autoantibodies are not only found in preeclampsia but also in antibody mediated kidney transplant rejection[54]. In kidney-transplant recipients who had severe allograft dysfunction without anti-HLA antibodies but detection of AT1 agonistic autoantibodies rejection was accompanied by accelerated hypertension and convulsions[55]. It is proposed that similar mechanisms might be involved in preeclampsia and refractory allograft rejection and it was found that one rejecting kidney-transplant recipient had had preeclampsia 16 years earlier[55].

Adrenomedullin

Pregnancy is associated with high concentrations of adrenomedullin in maternal and foetal blood and in the amniotic fluid[56]. Adrenomedullin has a potent and long-lasting hypotensive effect when injected intravenously in anaesthetised rats. Hata *et al*[57] measured circulating adrenomedullin concentrations in preeclampsia and normotensive pregnant women and showed that adrenomedullin concentrations are significantly lower in preeclamptic women.

Podocyturia

Renal involvement in preeclampsia can be at least partly explained by impaired podocyte function. Podocytes are the major source of VEGF in the glomerulus[58]. Podocyte-derived VEGF has paracrine functions on endothelial cells as well as autocrine functions on the podocytes themselves[58-60]. New data suggest that detection of podocyturia might serve as an early diagnostic marker for preeclampsia prior to the development of proteinuria and hypertension. Garovic *et al*[61] showed that podocyturia is present at delivery in women with preeclampsia. Podocyturia also had a significantly greater sensitivity and specificity for the subsequent diagnosis of preeclampsia than any single angiogenic marker or a combination thereof in the second trimester[62]. A strong correlation was found by Aita *et al*[63] between the number of podocytes lost in urine and blood pressure, but no correlation with proteinuria. Several markers have been used in different studies to detect podocyturia. Nevertheless, it is important to keep in mind that the expression of marker proteins does not allow a definite allocation of the involved glomerular cell types. De- or transdifferentiation and detachment of cells as well as changes in the urine milieu have a direct effect on marker protein expression. According to Skoberne *et al*[64], the urine markers most reliable for assessing disease activity of certain glomerular diseases are PDX- or CD68-positive cells.

mRNA

Recently, quantitative polymerase chain reaction for podocyte-specific markers was found to be a rapid meth-

od to detect preeclampsia. Significantly elevated mRNA levels of nephrin, podocin, and VEGF were detected in preeclamptic women compared with healthy controls[65].

Placental protein 13

Placental protein 13 (PP13) is a member of the galectin super family and is important for differentiation and proliferation. Than *et al*[66] found reduced PP13 mRNA levels in placentas obtained from patients with preeclampsia and HELLP syndrome in the first trimester compared to controls. Blood levels of PP13 mRNA were also significantly lower in preeclampsia compared to controls[67].

Pregnancy associated plasma protein-A

Pregnancy associated plasma protein-A (PAPP-A) is mainly produced by the placental trophoblasts. PAPP-A and PP13 serum levels were significantly lower in the first and second trimesters in women who developed preeclampsia[68]. First-trimester PAPP-A provided a prediction for preeclamspia when combined with uterine artery pulsatility measured by Doppler velocimetry[69].

Activin A and inhibin A

During the first trimester of pregnancy, the foeto-placental unit is the main source of circulating activin A and inhibin A. Activin A enhances *Follicle-stimulating hormone* (FSH) biosynthesis and secretion and is involved in the control of trophoblast cell differentiation in the first trimester. Inhibin A down regulates FSH synthesis and inhibits FSH secretion. Activin A seems to be a sensitive marker for the risk of later development of preeclampsia at 21-25 wk of gestation[70]. Inhibin A is thought to be more sensitive than activin A in predicting cases of early-onset preeclampsia at 15-19 wk of gestation[70].

P-selectin

P-selectin belongs to the group of cell adhesion molecules. It is expressed in granules of platelets and the Weibel-Palade bodies of endothelial cells and is involved in leukocyte-endothelial interactions. The P-selectin concentration was found to have a negative predictive value of almost 99% for preeclampsia. Mean plasma P-selectin concentrations were significantly elevated at 10-14 wk of gestation in women who later developed preeclampsia[71]. Wang *et al*[72] suggested that the increase in neutrophil-endothelial adhesion and activation seen in preeclampsia is at least in part due to up-regulation of P-selectin. This would be in line with the theory that preeclampsia reflects an excessive maternal inflammatory response to pregnancy[73].

Pentraxin 3

Another inflammatory molecule involved in preeclampsia is Pentraxin 3. It is expressed in response to inflammatory stimuli by endothelial cells, monocytes, macrophages and fibroblasts. Elevated maternal plasma levels of pentraxin 3 in preeclamptic in comparison to normal pregnancies could represent altered endothelial function[74,75]. The increase in maternal plasma develops from 11th to 13th week of gestation in women with subsequent preeclampsia[76].

Fibronectin

Maternal plasma fibronectin levels of patients with preeclampsia were significantly higher than those of healthy pregnant women[77]. Significant elevations in fibronectin levels with an extra type Ⅲ domain occurred in the first trimester before clinical evidence of preeclampsia. Fibronectin plays a major role in embryonic development, cell adhesion, growth, migration and differentiation.

Heat-shock proteins

Heat-shock proteins (Hsps) are highly conserved molecules that have chaperone functions. Circulating Hsps may also be cytoprotective, as exogenous Hsp70 increases the survival and protects from apoptosis in stressed arterial smooth muscle cells[78]. Fukushima *et al*[79] reported significantly higher Hsp70 serum levels in preeclampsia. Higher serum levels of Hsp70 were also found in patients with early onset of severe preeclampsia[80,81]. The difference in serum Hsp70 concentration between preeclamptic patients and the control group was statistically significant in each gestational age. Thus, Hsp70 might not only be a marker but also play a role in the pathogenesis of preeclampsia.

Fms-like tyrosine kinase 1/ placental growth factor

Gene expression profile studies identified the regulation of soluble fms-like tyrosine kinase 1 (sFlt-1) in preeclampsia. SFlt-1 binds and antagonises vascular endothelial growth factor (VEGF) and placental growth factor (PlGF).

The described functions of VEGF include induction of matrix metalloproteinases, regulation of angiogenesis, lymphangiogenesis and hematopoiesis and cell signalling. Serum concentration of sFlt-1 decreases from 8-12 wk to 16-20 wk of gestation, gradually increases at 26-30 wk of gestation and rapidly elevates at 35-39 wk of gestation in normal pregnancy[82].

SFlt1 concentrations increased gradually throughout pregnancy in women with preeclampsia and was significantly higher between 25 and 28 wk of gestation in women with preeclampsia than in women with normal pregnancies or isolated hypertension[83]. Of note, sFlt1 are high 5-6 wk prior to the onset of preeclampsia and correlate with the severity of disease[83-85]. In rats sFlt-1 infusion increased vascular and placental oxidative stress, decreased maternal circulating VEGF and NO and reduced foetal weight[85].

Serum concentration of PlGF increases gradually from 8 wk until 29-32 wk of gestation and then decreases at 33-40 wk of gestation in normal pregnancy[79]. PlGF levels in women who later developed preeclampsia were significant lower than those of controls from 13-16 wk of gestation until delivery[85]. As the change of PlGF occurs earlier than that of sFlt-1, it might be the better

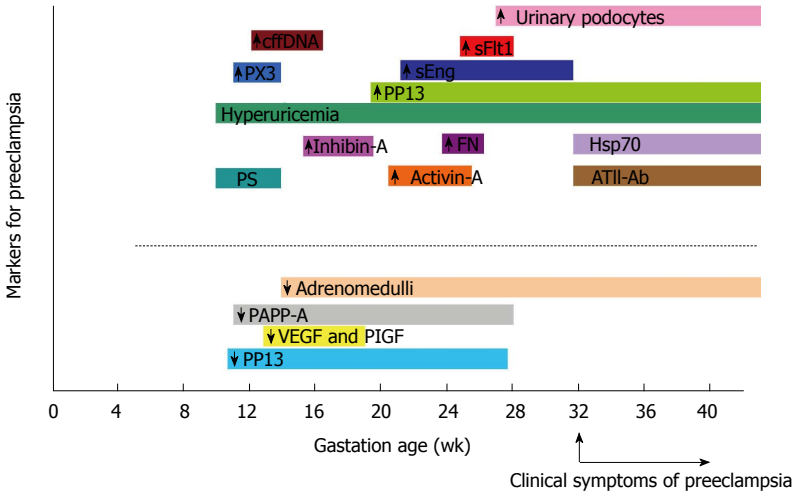

Figure 1 Treatment of preeclampsia. ATII-Ab: Angiotensin II type 1 autoantibodies; cffDNA: Cellfree fetal DNA; FN: Fibronectin; Hsp70: Heat-shock protein 70; PlGF: Placental growth factor; PAPP-A: Pregnancy associated plasma protein-A; PP13: Placental protein 13; sFlt-1: Fms-like tyrosine kinase 1; PS: Pselectin; PX3: Pentraxin-3; sEng: Soluble endoglin; VEGF: Vascular endothelial growth factor.

angiogenic factor for predicting preeclampsia. Serum sFlt-1 to PlGF ratio (sFLT-1/PIGF) was also suggested as screening parameter. An adenovirus-expressing sFlt-1 in rodents caused a clinical syndrome with glomerular endotheliosis, proteinuria, and hypertension[86]. Glomerular capillary endotheliosis is another typical lesion in preeclampsia.

Soluble endoglin

Serum levels of sEng in normal pregnancy are quite stable and slightly increase by 33-42 wk of gestation[87]. Placental endoglin is up-regulated in preeclampsia and released in the circulation. Rising levels of circulating soluble endoglin (sEng) herald the onset of preeclampsia. Women with higher sEng levels at 21 through 32 wk of gestation had an increased risk of preterm preeclampsia and an increased risk for a small-for-gestational-age infant[87].

Cellfree fetal DNA

Cellfree fetal DNA (cffDNA) is increased at 11-13 wk of gestation in pregnancies that experience preeclampsia[88]. Hypoxia within the intervillous space of the placenta leads to tissue oxidative stress and increases placental apoptosis and necrosis. This might be the cause of increased levels of cffDNA. Elevated cffDNA is not specific for preeclampsia and is also seen in other conditions associated with placental pathology[17].

Uric acid

Uric acid is the end product of purine metabolism in the liver. In normal pregnancy uric acid concentrations initially fall 25%-35% due to estrogens, expanded blood volume and increased glomerular filtration rate[89]. By term concentrations slowly rise to those observed in non-pregnant women. In contrast to that, uric acid levels increase at 10 wk of gestation and continue to rise until 48 h postpartum in preeclamptic women[90]. The increase in uric acid precedes the reduction in plasma volume in preeclampsia[91]. Uric acid may be protective during preeclampsia as an antioxidant, but is at the same time proinflammatory and contributes to endothelial dysfunction[92]. In a recent study the concentration of serum uric acid in preeclamptic women was associated with disease severity[93].

Nevertheless, lowering uric acid with probenacid had no effect on the degree of hypertension in preeclamptic women[94]. Another study with allopurinol showed no significant effects on the outcome of pregnancy in humans[95].

A summary of timed expression of these biomarkers in preeclampsia is given in Figure 1.

The optimal management of a pregnant woman with preeclampsia depends on gestational age and disease severity. Delivery is the only curative treatment for preeclampsia. Indicators for delivery in preeclampsia are given in Table 1 (modified from[22]).

The severity of the disease must always be weighed against the risks of infant prematurity. A mild preeclampsia at or beyond 37 wk should be delivered. In severe preeclampsia, induction of delivery should be considered after 34 wk of gestation. Prior to induction corticosteroids should be given to accelerate lung maturity. The prevention of seizures and adequate control of maternal blood pressure should also be of high priority. Maternal evaluation includes monitoring of blood pressure, urine output, cerebral status, epigastric status, tenderness or vaginal bleeding. Platelet count, liver enzymes and serum creatinine should be controlled closely. The target of blood pressure is between 140-160 mmHg systolic and 90-105 mmHg diastolic. The blood pressure should not be lowered under 140/90 mmHg to prevent insufficient utero-placental blood flow and reduced birth weight[96,97]. Foetal evaluation includes foetal heart rate monitoring, a biophysical profile, ultrasonographic assessment of foetal growth, amniotic fluid status and um-

Table 1 Indications for delivery in preeclampsia

Maternal	Foetal
Eclampsia	Severe foetal growth retardation
Shortness of breath, pulse oximetry of < 94% on room air, pulmonary oedema	severe oligohydramnios
AST or ALT > 2 times above normal	Foetal death
Uncontrolled severe hypertension	Repetitive late or variable foetal heart rate decelerations
Oliguria , serum creatinine level of ≥ 1.5 mg/d	Umbilical artery doppler imaging with reverse diastolic blood flow
Suspected abruptio placentae	
Persistent platelet count < 100000 /mm^3	

bilical artery doppler velocimetry.

Antihypertensives

Methyldopa, nifedipine, labetalol and hydralazine are the antihypertensives of choice for preeclampsia. Oral Methyldopa is suggested for mild to moderate hypertension in an outpatient with preeclampsia. Oral nifedipine is used for treatment of moderate or severe pregnancy hypertension in a dose of 10-20 mg every 4-6 h[98]. As a calcium channel blocker nifedipine acts on arteriolar smooth muscle cells and induces vasodilatation by blocking calcium entry into the cells. The side effects of nifedipine include tachycardia, palpitations and headaches. The calcium channel blockade with isradipine lowered the maternal mean arterial blood pressure in women with hypertension but not in women with proteinuria[99].

In hospital settings intravenous hydralazine (5-10 mg every 15-30 min) is commonly administered for hypertensive emergencies associated with pregnancies. Hydralazine is a direct peripheral arteriolar vasodilator. The most common adverse effect of hydralazine is the unpredictable hypotension. Other side effects are headache, nausea, maternal hypotension and vomiting. Labetalol is a selective alpha blocker and a nonselective beta blocker. The side effects of labetalol are dizziness, nausea and headaches. When the medications mentioned above have failed to lower blood pressure sodium nitroprusside may be given. Nitroprusside causes vasodilatation by the release of nitric oxide. Severe rebound hypertension may result. Therefore, nitroprusside should be reserved for use in postpartum care or just before the delivery because cyanide poisoning of the foetus is also a possible side effect.

Diuretics

Despite peripheral oedema, the intravascular volume is depleted in patients with preeclampsia. In contrast, pulmonary oedema can occur 48-72 h postpartum due to mobilization of extravascular fluid.

As preeclampsia is characterized by a reduction in circulating plasma volume diuretics are not generally recommended in preeclampsia. There are significant warnings against the use of thiazides during pregnancy like metabolic risks to the mother and fetus including hyponatremia, hypokalemia, thrombocytopenia, hyperglycemia.

Furthermore they may result in inhibition of labor and decrease placental perfusion in pregnany. Therefore we do not recommend the general use of diuretics in preeclampsia.

Nevertheless, the use of thiazide diuretics or loop diuretics is occasionally indicated for severe intractable or pulmonary oedema. The reduction of excessive oedema should be done slowly and under close supervision. There are no recommendations for dating, dosage or time for diuretics in this situation as it would be an individual symptom dependent and symptom guided therapy. We recommend to get written approval for the use of diuretics from the mother after detailed education on risks and side effects during pregnancy. Measures to respond to blood pressure drops must always be available and vital signs of the mother and foetus must be controlled continuously under diuretic treatment. This includes continuous electronic foetal heart rate monitoring and cardiovascular monitoring of the mother.

Antiepileptics

Magnesium sulfate is the drug of choice for the treatment and prevention of eclampcia. Magnesium sulphate more than halves the risk of eclampsia[100]. The Magpie-Trial compared magnesium sulphate with placebo for women with preeclampsia and found a preventive effect[101,102]. Therefore, prophylactic treatment with magnesium sulfate is indicated for all patients with severe preeclampsia. There is no consensus if patients with mild preeclampsia need magnesium prophylaxis. However, active seizures should be treated with an intravenous loading dose of 4 g magnesium sulphate over 5-10 min followed by an infusion of 1 g/h for 24 h. Seizures that are refractory to magnesium sulfate may be treated with lorazepam and phenytoin.

Antiplatelet therapy

There is an imbalance between thromboxane and prostacyclin production in preeclampsia. Thus, the use of low-dose aspirin in preeclampsia seems to be reasonable. Wallenburg *et al*[103] conducted the first prospective double-blind controlled trial using 60 mg aspirin per day for the treatment of women at risk for preeclampsia. Only two of the 23 treated women versus 12 of the 23 controls became preeclamptic. Supplementation of Aspirin at or before the 16th week of pregnancy reduced preterm preeclampsia without any effect on term preeclampsia[104]. In other studies low-dose aspirin had no

significant effect on the incidence of preeclampsia in the low-risk groups but was more beneficial in high-risk groups[105,106].

Plasmapheresis/ apheresis

The experience and safety of plasmapheresis (PE) in pregnancy is limited to case reports. In 1986 a successful use of plasmapheresis during pregnancy was reported in a patient with unusually fulminant, antibody-negative myasthenia gravis[107].

Another case report has suggested that PE may be a successful treatment for pregnant women with antiphospholipid syndrome[108]. PE was also successfully used in pregnant patients with acute fatty liver of pregnancy[109,110]. Additionally two cases of hypertriglyceridemia-induced acute pancreatitis during pregnancy and a case of a pregnant woman with Pemphigus vulgaris were successfully treated by PE[111,112]. Thrombocytopenia associated with microangiopathic disease in severe preeclampsia generally resolves within 3 to 4 d after delivery. It was suggested to use PE when thrombocytopenia persists beyond this time[113].

In preeclampsia a potentially useful approach of PE would be to subtract circulating autoantibodies from maternal circulation. In 1986 fourteen cases of PE with fresh frozen plasma for maternal indications in selected cases of preeclampsia and eclampsia were reviewed with promising results[114]. In contrast, PE did not prolong pregnancy in preterm preeclampsia in a report by Martin *et al*[115]. In another report, plasma exchange was commenced at 23, 26 and 29 wk of gestation in preeclamptic women and continued until delivery. Here preeclamptic signs regressed and renal function stabilised. One baby with severe hyaline membrane disease died but the others were delivered in good health[116].

Like PE with fresh frozen plasma heparin-mediated extracorporeal low-density lipoprotein precipitation has been attempted in preeclampsia[117]. Pregnancies were prolonged for 3 to 49 d in 9 very preterm preeclamptic women by the use of this apheresis.

Thadhani *et al*[118] hypothesized that a selective adsorption column would create a concentration gradient and augment the removal of sFlt1. They treated 5 women with very preterm preeclampsia with dextran sulfate cellulose apheresis. This treatment reduced circulating sFlt-1 levels and proteinuria in a dose-dependent manner and stabilized blood pressure without apparent adverse events. Dextran sulfate cellulose apheresis was able to reduce sFlt-1 plasma levels by 20%-30%. Pregnancy was continued for 15 d with the use of two apheresis and for 23 d with four apherese sessions[118].

Maternal blood pressure was stable and was not markedly decreased after apheresis. However, antihypertensive medications were stopped before treatments and saline infusions were given during treatments. Three patients with postpartum HELLP syndrome and persistent thrombocytopenia were treated with PE with prompt resolution of their diseas[113]. There is no animal model for plasmapheresis at the meantime. Therefore the only way to get more experience on this filed is the clinical use in selected patients.

Taken together single cases indicate that PE seems to be a treatment with low risk during pregnancy and could be a promising treatment option for otherwise refractory preeclampsia. We recommend only use this therapy in specialized centers with first class experience on the filed and with written consent of the mother after detailed education about the risks and experimental status of this therapy.

Albumin substitution

Serum albumin levels of preeclamptic women are often even below 10 g/L. Thus, one can suggest albumin substitution in preeclampsia. Albumin infusions increased serum albumin and colloid osmotic pressure values in preeclampsia[119].

However, daily albumin infusions did not lower blood pressure and was unable to stabilise renal function. Albumin substitution was also associated with higher foetal mortality[120]. Therefore, we do not recommend using albumin substitution in preeclampsia.

Uterine curettage

Uterine curettage immediately after delivery accelerates the recovery of severe preeclampsia[121]. This operation was also successfully used in single cases of postpartum preeclampsia[122,123]. Due to the fact that even microscopic level of trophoplastic tissue can perpetuate preeclampsia uterine curettage should be done after delivery.

CONCLUSION

We reviewed the literature with a focus on proteinuria during pregnancy and preeclampsia. Several new diagnostic markers for preeclampsia were presented. Most of these are not yet implemented in clinical use and several are only used in studies or experimental conditions. Therefore, we recommend using proteinuria as a screening parameter for preeclampsia. The first screening should be UPC ratio. If the UPC ratio is below 150 mg/g total proteinuria is unlikely to be more than 300 mg/d and needs no further investigation at that time. For a UPC ratio greater than 150 mg/g we suggest performing a full 24-h urine protein collection as a second diagnostic tool for confirming accurate results. If a new onset of proteinuria greater than 300 mg/d together with hypertension and/or general oedema occurs after the 20th week of gestation the diagnosis of preeclampsia can be made. Atypical presentation must always be kept in mind. We presented different therapeutic options for preeclampsia.

Delivery is the only curative treatment for preeclampsia. In early preeclampsia the primary therapy goal is to prolong pregnancy until a state were the child has an acceptable chance of survival after delivery. In the mean time close maternal and foetal monitoring and evaluation

is necessary. We presented therapeutic options to treat hypertention, oedema and seizures during this period.

Plasmapheresis is not a common treatment strategy in preeclampsia but could be considered as rescue therapy in otherwise therapy refractory cases.

When performing PE in preeclampsia measures to respond to blood pressure drops must always be available and vital signs must be controlled during and after the entire session. This includes continuous electronic foetal heart rate monitoring and cardiovascular monitoring of the mother. Eventually, antihypertensives must be paused before and/or after PE. In general after the 34th week of gestation delivery is the better choice of treatment for both mother and child.

ACKNOWLEDGMENTS

We searched PubMed for articles with the keywords "preeclampsia AND animal models", "treatment of preeclampsia", "proteinuria in pregnancy", "treatment of preeclampsia", "markers for preeclampsia", "podocyturia", "physiological changes in pregnancy", "plasmapheresis in pregnancy". We also searched the bibliographies of the articles retrieved for further relevant references.

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P- Reviewer: Khalil RA, Rasmussen S, Tsikouras P, Xiu QZ
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World Journal of Nephrology

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DOI: 10.5527/wjn.v3.i4.182

World J Nephrol 2014 November 6; 3(4): 182-192
ISSN 2220-6124 (online)

REVIEW

Acute kidney injury due to bilateral ureteral obstruction in children

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Received: June 29, 2014 Revised: July 28, 2014
Accepted: September 4, 2014
Published online: November 6, 2014

Abstract

Bilateral ureteral obstruction in children is a rare condition arising from several medical or surgical pictures. It needs to be promptly suspected in order to attempt a quick renal function recovery. In this paper we concentrated on uncommon causes of obstruction, with the aim of giving a summary of such multiple, rare and heterogeneous conditions joint together by the common denominator of sudden bilateral ureteral obstruction, difficult to be suspected at times. Conversely, typical and well-known diseases have been just run over. We considered pediatric cases of ureteral obstruction presenting as bilateral, along with some cases which truly appeared as single-sided, because of their potential bilateral presentation. We performed a review of the literature by a search on PubMed, CrossRef Metadata Search, internet and reference lists of single articles updated to May 2014, with no time limits in the past. Given that we deal with rare conditions, we decided to include also papers in non-English languages, published with an English abstract. For the sake of clearness, we divided our research results into 8 categories: (1) urolithiasis; (2) congenital urinary tract malformations; (3) immuno-rheumatologic causes of ureteral obstruction; (4) ureteral localization of infections; (5) other systemic infective causes of ureteral obstructions; (6) neoplastic intrinsic ureteral obstructions; (7) extrinsic ureteral obstructions; and (8) iatrogenic trigonal obstruction or inflammation. Of course, different pathogenic mechanisms underlay those clinical pictures, partly well-known and partly not completely understood.

Key words: Acute kidney injury; Bilateral ureteral obstruction; Hydronephrosis; Anuria; Pediatrics; Ureteral stenting; Henoch-Schönlein purpura; Tuberculosis; Masses; Congenital malformations

Core tip: Bilateral ureteral obstruction in children is a rare condition related to several medical or surgical pictures. It needs to be promptly suspected in order to attempt a quick renal function recovery. It is a rare event, but to be kept in mind. We identified many potential causes grouped as follows: (1) urolithiasis; (2) congenital urinary tract malformations; (3) immuno-rheumatologic causes of ureteral obstruction; (4) ureteral localization of infections; (5) other systemic infective causes of ureteral obstruction; (6) neoplastic intrinsic ureteral obstructions; (7) extrinsic ureteral obstructions; and (8) iatrogenic trigonal obstruction or inflammation.

Bianchi D, Vespasiani G, Bove P. Acute kidney injury due to bilateral ureteral obstruction in children. *World J Nephrol* 2014; 3(4): 182-192 Available from: URL: http://www.wjgnet.com/2220-6124/full/v3/i4/182.htm DOI: http://dx.doi.org/10.5527/wjn.v3.i4.182

INTRODUCTION

The definition of acute kidney injury (AKI) has been deeply changing over the last decade, starting off with adult patients[1] and followed by a still debated extension to children[2-4], with some new biomarkers for AKI early detection proposed[5,6].

In particular, AKI in children has been widely discussed in several reviews and meta-analysis[2,3,7], including

some papers focused on AKI in newborns[8] and very preterm infants[9], while some articles concentrated on kidney injury due to urinary tract obstruction[10-13], especially on nephrolithiasis, which accounts for up to 30% of AKI in pediatrics[10].

Aim

Aim of the present paper is to give a diagnostic overview of rare or very rare causes of pediatric AKI due to sudden bilateral ureteral obstruction, which can be related to lots of different conditions - either medical or surgical - and needs to be promptly suspected in order to attempt a quick renal function recovery.

We decided not to focus on typical and well-known diseases, which have been just run over in this paper, while we intended to concentrate on uncommon pictures, with the aim of giving a summary of such multiple, rare and heterogeneous conditions joint together by the common denominator of sudden bilateral ureteral obstruction, which appears difficult to be suspected at times.

Thus, our purpose was to provide a sort of companion to causes of bilateral or potentially bilateral ureteral obstruction in pediatrics, given the lack of such a paper in the literature. We did not address the issue of different imaging modalities - for example, see Riccabona[12] about - and we just outlined some therapeutic aspects, with no intention of a systematic analysis.

Research

We performed a review of the literature by a search on PubMed, CrossRef Metadata Search, internet and reference lists of single articles updated to May 2014, with no time limits in the past. Given that we deal with rare conditions, we decided to include also papers in non-English languages, published with an English abstract.

We considered pediatric cases of ureteral obstruction presenting as bilateral, along with some cases which truly appeared as unilateral, but still at risk of bilateral involvement.

For the sake of clearness, we divided our research results into 8 categories: (1) urolithiasis; (2) congenital urinary tract malformations; (3) immuno-rheumatologic causes of ureteral obstruction; (4) ureteral localization of infections; (5) other systemic infective causes of ureteral obstruction; (6) neoplastic intrinsic ureteral obstructions; (7) extrinsic ureteral obstructions; and (8) iatrogenic trigonal obstruction or inflammation. Categories are summarized in Table 1.

UROLITHIASIS

Urolithiasis is generally considered a relatively rare disease in children, with some peaks of incidence in Turkey, some South Asian, African and South American countries[14].

Overall, kidney stone disease is considered to affect boys and girls equally[15] accounting for 1:1000-1:7600 hospital admissions in the United States of America, even if some studies pointed out a male prevalence in the first decade and a female prevalence during the second decade of life[11,15].

Causes of urolithiasis in children

A systematic review of urolithiasis goes beyond the scope of this paper, but some aspects can be of interest in our topic.

It has been evaluated that in some European countries, 75% of stones in children are composed of organic matrix and struvite, mostly sustained by Proteus infection or urinary tract anomalies[14].

Anyway, according to the underlying condition, we could consider systemic/genetic diseases and medical treatment-related conditions, according to Valentini *et al*[11].

Among systemic diseases, cystic fibrosis and inflammatory bowel diseases are considered risky conditions for stone formation because they result in intestinal calcium chelation, thus freeing up an exceeding amount of unbounded oxalate, which can be absorbed from the intestinal tract, finally becoming available for stone formation[11].

As well, spinal cord injuries and spina bifida have been traditionally considered potential causes of stone disease because a neurogenic bladder - usually seen in these conditions - is associated with a higher risk of struvite stones, although more recently some metabolic studies showed that calcium phosphate stones are becoming more frequent, with a minor incidence of struvite calculi, probably due to the better bladder care achieved by clean self-catheterization[11].

Some genetic aspects concern primary hyperoxaluria, classified in type-1 and type-2, which can lead to an early onset of disease with nephrocalcinosis and kidney injury, more clinically relevant in type-1 form.

Other conditions include tubular disorders such as cystinuria, which has been postulated as responsible for 3% of renal stones in one pediatric study[16]. This autosomal recessive disorder finally causes an increased excretion of the amino-acids cystine, ornithine, lysine and arginine because of a proximal tubular defect.

Other genetic disorders include the X-linked Dent disease, the Lesch-Nyhan syndrome and the extremely rare 2,8-dihydroxyadeninuria[11].

Clinical presentation

It has been pointed out that stone disease in children may present as flank/abdominal pain or hematuria, similarly to adults[11], even if urolithiasis in infants may mimic an intestinal colic[14].

To our knowledge, no studies investigated the real incidence of bilateral ureteral obstruction in urolithiasis among pediatric population.

CONGENITAL URINARY TRACT MALFORMATIONS

Lots of congenital urinary tract malformations can lead

Table 1 Causes of bilateral or potentially bilateral ureteral obstruction in children

Categories of disease	Single entities or underlying causes
Urolithiasis	Idiopathic
	Neurologic disease
	Metabolic and genetic disorder
	Inflammatory bowel disease
Congenital urinary tract malformations	Obstructive megaureter
	Uretero-pelvic junction obstruction
	Duplicated collecting system
	Horseshoe kidney and other anomalies
Immuno-rheumatologic diseases	Necrotizing vasculitis
	Periarteritis nodosa
	Kawasaki disease
	Henoch-Schönlein purpura
	Eosinophilic ureteritis
Ureteral localization of infections	Fungal infections
	Viral infections
	Bacterial infections
	Tubercular infections
Other systemic infections	Rotavirus
Neoplastic intrinsic ureteral obstructions	Fibroepithelial polyps, ureteritis cystica, malignant neoplasms
Extrinsic ureteral obstructions	Abdomino-pelvic masses
	Familial adenomatous polyposis
	Retroperitoneal fibrosis
Iatrogenic trigonal obstruction/inflammation	Device-induced obstruction
	Bulking agents for vesico-ureteral reflux
	Obstruction after appendectomy

to kidney injury, including uretero-pelvic junction obstruction, obstructive megaureter, vesico-ureteral reflux and posterior urethral valves.

Anyway, functional impairment may be graded and slowly progressive[13]. With regard to the purpose of the present review, we remember that megaureter can be characterized by transient or permanent urine flow impairment, above all if secondary to structural or functional obstruction of the distal ureter[17].

In a retrospective paper about obstructed megaureters in early infancy, 5 infants out of 47 between years 1963-1987 had a solitary kidney and one of them presented with anuria, while 7 out of the 47 patients had bilateral megaureter[18].

In the literature, just one case of bilaterally duplicated collecting systems with obstructing ureteral stones has been described in an adult patient[19], along with one case of pediatric bilateral ureteral reflux at the distal part in a bilaterally duplicated collecting system in a 5-year-old girl presenting with repeated urinary tract infections[20].

By the way, also retrocaval ureter can lead to ureteral obstruction and it can often be associated with other major anomalies (see Lopez Gonzalez *et al*[21] for a bibliographic review).

Moreover, ureteropelvic obstruction can be related to horseshoe kidney, extrarenal pelvis, transverse valves of periureteral junction and other congenital anomalies[22,23], both isolated or in genetic syndromes[24,25].

As a general rule, congenital urinary tract malformations should always be considered in case of hydronephrosis, both in children and young adults, as they can be asymptomatic for many years, giving signs in adolescence or adulthood.

IMMUNO-RHEUMATOLOGIC CAUSES OF URETERAL OBSTRUCTION

Stenosing ureteritis secondary to rheumatologic diseases is a rare condition, difficult to be estimated exactly because of the lack of papers about.

We found the description about a case of necrotizing vasculitis with ureteral involvement in a 12-year-old girl[26] firstly admitted to the hospital when she was aged 2 years because of arthritis, fever and growth retardation, successfully treated by aspirin and penicillin and then being healthy until the age of 8, when she had a bronchial asthma episode. A subsequent arthritis manifestation occurred at the age of 11, treated by penicillin and naproxene. At the age of 12 she presented with fever, legs ulcers, abdominal crisis, bronchial asthma, sinusal arrhythmia. Radiologic findings showed bilateral ureteral strictures and a skin biopsy revealed necrotizing vasculitis of medium-sized arteries.

The girl was successfully treated by prednisone and azathioprine.

In the literature, we found a couple of descriptions of ureteral involvement during periarteritis nodosa in a 13-year-age girl[27] and a 6-year-age boy[28], the latter treated by steroids.

To our knowledge, just one case has been published reporting on left ureteral obstruction in a 7-year-age boy affected by Kawasaki disease, who finally underwent excision of a left upper third ureteral stricture, with left-

dismembered pyeloplasty[29].

Henoch-Schönlein purpura is a common systemic vasculitic condition of which the majority of cases occur in pediatrics[30].

Urinary tract involvement in Henoch-Schönlein purpura usually concerns the kidney, with a focal proliferative glomerulonephritis occurring in 20%-90% of cases[30].

Ureteral obstruction secondary to Henoch-Schönlein purpura is rare, with 14 cases described in the literature. Most patients were treated by medical therapy, while two by surgery.

One of them underwent total bilateral ureteral replacement using ileal segment, but progressed to end-stage renal disease because of reflux along the graft, thus radical excision of the ileal graft and both native kidneys was performed in order to eradicate any infectious process before immuno-suppression therapy[31].

The other one was a boy aged 7 years, who underwent multiple conservative surgical treatments for two years, including bilateral nephrostomic tubes and ureterocalycostomies along with a left dismembered pyeloplasty, which appeared to be successful at the beginning but were then complicated by infections and worsening of renal function[30]. Finally, a left nephrectomy was performed and two and a half years after the onset of disease, the boy remained tube-free without hydronephrosis recurrence on the right, with no further hospital readmissions required[30].

Eosinophilic ureteritis is a rare disease with imaging presentation similar to ureteral tumors[32], leading to ureteral stricture due to mural involvement, with secondary hydronephrosis[33].

Even though it may be associated with hypersensitivity to bacteria, parasites, food and drugs, the etiopathogenic mechanism is not completely clear[34] and peripheral eosinophilia is not a constant finding[35]. In a paper, filariasis has been proposed as a possible triggering etiology of bilateral upper ureteric strictures in a 54-year-old man, as the patient had a previous history of cellulitis with epididymitis and came from an endemic area[35].

Up to 1991 just one case of eosinophilic ureteritis in children had been published[33], describing a 3-year-old boy with bilateral ureteral obstruction.

To our knowledge, no further cases of pediatric eosinophilic ureteritis have been described in the literature, while cases of eosinophilic cystitis have been reported, with some pathological aspects still debated[33].

Some molecular details of murine ureteritis causing obstructive uropathy with hydronephrosis have been investigated[34], providing a novel molecular pathogenesis for elucidating causes of aseptic inflammation in human upper urinary tract.

URETERAL LOCALIZATION OF INFECTIONS

Fungal infections

Systemic candidiasis with possible renal localization is uncommon in neonates and infants[36], although it is a well-documented entity in several special conditions, such as intensive care in premature newborns[37], prolonged antibiotic therapy, intravenous lines and immunocompromised patients[36].

The management of renal obstructive candidiasis is challenging and not well summarized over the past decades[38]. In a review[39], the clinical course and management of 35 neonates and infants were considered, with prematurity, broad spectrum antibiotics, prolonged hospital stay and the use of intravascular catheters resulted as predisposing factors. Among the other ones, candidemia and withholding antifungal therapy were poor prognostic factors.

After year 2011, some more cases of candidiasis in newborns have been described in the literature[40-43], with no standardized treatment at the moment[44]. Of course, transplant recipients must be considered at high risk for opportunistic pathogens[45,46] and obstructive anuria due to fungal bezoars has been described[47].

Therapeutic options range between medical drugs such as amphotericine-B or fluconazole and surgical treatment, consisting of nephrostomy or retrograde stenting along with irrigation by streptokinase as required, until open surgery if needed[43].

Viral infections

Viral infections can represent severe complications in immunocompromised patients. Among them, BK-virus has been related to hemorrhagic cystitis in bone marrow transplant recipients[48-50] or to pyelonephritis and ureteral stenosis in renal transplant recipients[51].

Also adenovirus infections are postulated as causes of urologic complications in bone marrow transplantation, mainly consisting of hemorrhagic cystitis[52], moreover obstructive pyelonephritis treated by double-J ureteral stenting has also been described[53].

Management of viral infections, including Epstein-Barr, cytomegalovirus etc, is a challenging problem in both hematopoietic and solid organs transplantation[54-56].

As to the purpose of this paper, we cite a case of late onset hemorrhagic cystitis and ureteritis induced by cytomegalovirus after kidney transplantation[57].

Bacterial infections

Although syphilis, toxoplasmosis and candidiasis are recognized as causes of infections leading to kidney injury in newborns[8], in the literature we found just one case describing a *Pseudomonas aeruginosa* infection with bilateral ureteral involvement[58]. It concerned a 14-mo-old male diagnosed with an acute lymphocytic leukemia, who showed bilateral ureteral obstruction caused by purulent debris from *Pseudomonas*, with a subsequent anuria. The Authors reported it was not possible to insert a ureteric catheter on the left side, while a right retrograde pyelogram revealed a medial deviation of the right ureter with no chance of further upward progression. An irrigation was performed and the patient became polyuric after the procedure, with a renal function recovery two days after

the cystoscopy, along with ciprofloxacin administration[58].

Tubercular infection

Tubercular infection is endemic in some geographic areas and genitourinary tract involvement is quite common[59].

In a paper based on a retrospective study over 13 years in a single Indian centre, Singh *et al*[59] identified ureteral involvement in 27.35% out of 117 patients with genitourinary tubercular disease.

In a Russian paper[60], the Authors analyzed 158 patients with active nephrotuberculosis, identifying 24 without obstructive uropathy, 70 with upper ureter obstruction and 64 with lower ureter obstruction. Bilateral involvement was recorded in 75% of patients. Unfortunately, just an English abstract was available in our research, so we do not know the amount of pediatric patients involved.

Other isolated cases have been published, including major surgical reconstructive treatment in one of them[61,62] and one case of primary papillary mucinous adenocarcinoma of the ureter mimicking genitourinary tuberculosis in a 54-year-old man[63].

In our opinion, tubercular infections could be suspected according to the geographic origin of the patient, although actual worldwide travelling habits should invite physicians to be cautious anytime.

OTHER SYSTEMIC INFECTIVE CAUSES OF URETERAL OBSTRUCTION

Rotavirus infections are the most common cause of severe diarrhea in infants and young children worldwide[64]. In a clinical paper, Ashida *et al*[64] retrospectively described 21 cases of gastroenteritis in Japanese children with acute post-renal failure due to ureteral obstruction from bilateral stones.

The patients were 18 boys and 3 girls, with a median age of 1.3 years, ranging between 0.4 and 3 years, while the median duration between the onset of oliguria and that of *Rotavirus* gastroenteritis was 6.7 d, ranging from 3 to 16 d.

The Authors highlighted that all the children were under 3 years old, many of them had hyperuricemia and the stones mainly consisted of ammonium acid urate. Some causes have been considered as possibly responsible for such stones, including a laxative-like mechanism related to water loss or fluctuations in urinary acidity, which in another paper[65] has been recognized to play a role in ammonium acid uric stones formation.

In our opinion, more studies are advocated to clear this entity.

Moreover, we found a paper published in 1991[66] describing about 4 cases of children aged between 14 mo and 13 years, including a 3-year-old girl, who presented with oligo-anuria and either flank pain or fluid retention. Three of them had a profuse vomiting and diarrhea in the previous days, with the forth one revealing a familial history of renal calculi. All the patients showed an evidence of crystalline sludge in their lower ureters.

Dehydration was postulated as a primary predisposing factor, even if three of them had an underlying crystalluria, two had a raised excretion of uric acid and one of cystine.

NEOPLASTIC INTRINSIC URETERAL OBSTRUCTIONS

Benign neoplasms

Fibroepithelial polyps are the most common ureteral benign neoplasms[67], although this mesodermal tumor rarely occurs in children[68].

It usually arises along the proximal ureter and is more common in boys, with presenting signs consisting of hematuria or flank pain due to urinary obstruction. Nevertheless, cases of single polyp with mid-ureter[69] or distal ureter[70] localization have been described, with one case prolapsing into the bladder, thus mimicking a bladder tumor[71].

Overall, in the literature we found some 40 cases of pediatric patients affected by ureteral polyps[72,73], with bilateral obstruction described in at least 5 cases, the first one in 1990[67,74-77].

Surgical management of such cases is not standardized, with some polyps treated by ureteroscopic procedures and other ones by segmental resection of the ureter[76,67]. A concomitant ureteropelvic obstruction underwent pyeloplasty, even if more studies are advocated about multiple metachronous polyps recurring after laparoscopic or robotic pyeloplasty[72].

A differential diagnosis is required between ureteral polyps and ureteritis cystic[78], which has been reported as a cause of ureteral obstruction in some cases[79-81], bilaterally in one of them[80].

Malignant neoplasms

Among malignant neoplasms, we should rapidly consider a collection of rare cases which truly presented as a single-side ureteral involvement.

A paper investigated the extension of Wilms' tumor into the ureter[82] and 45 children out of the Wilms' Tumor Study Group database showed ureteral involvement, with hydronephrosis identified in 12 and non-function of the kidney in another 8. Tumor was right-sided in 26 and left-sided in 19.

In the literature, we found a case of 17-year-old girl[83] and of a 12 year-old boy[84] with Ewing's sarcoma/neuroectodermal tumor with unilateral ureteral localization, both presenting with nausea, vomiting, hematuria and abdominal pain.

One case has been described of a 4-year-old girl presenting with an embryonal rhabdomyosarcoma, botryoid variant, arising within the left ureter[85].

Ureteral localization of lymphoma was identified in one adult in a clinico-pathological study of 40 cases of

genitourinary tract lymphomas, with the two pediatric cases involving kidney and testis[86]. Another paper reported a case of penile lymphoma in a 4-year-old boy[87].

An isolated case of bilateral ureteral obstruction due to lymphoma has been described in an adult[88]. To our knowledge, no cases of pediatric lymphomas with ureteral localization have been published.

EXTRINSIC URETERAL OBSTRUCTIONS

Abdomino-pelvic masses

Urinary obstruction secondary to malignant pelvic tumors is a well-known condition in adult patients[89].

In a paper published in 2004, Meir *et al*[90] retrospectively investigated about the same condition among the records of two major children's hospitals, identifying 17 patients affected by upper urinary tract obstruction - 9 boys and 8 girls - with a median age of 5.7 years, ranging between 0 and 12. The most represented tumor was rhabdomyosarcoma, followed by lymphoma, and the urinary obstruction was bilateral in 11 cases. Most of them were treated by ureteral retrograde stenting or nephrostomy, with just some cases deferred to major surgery.

In another paper, Alexander *et al*[91] ascertained the incidence and outcome of hydronephrosis in children affected by abdominal (non-renal) or pelvic tumors. They reviewed 366 patients from a database between 1995 and 2009, finding out 66 cases - 39 female and 27 male - of upper urinary obstruction due to a compression by the tumor or by surgery/radiotherapy, with a median age of 5.1 years. Out of those 66 cases, 35 were bilateral. The most represented tumor was neuroblastoma, followed by immature teratoma and rhabdomyosarcoma. For further details about histotypes involved, see Meir *et al*[90] and Alexander *et al*[91].

Mucinous cistoadenoma of the ovary is a rare neoplasm in pediatric age[92], with 20 cases described in the literature. Most of them presented late as an abdominal mass, with urinary outflow obstruction due to bilateral distal ureter compression, eventually leading to renal failure[92].

Mesenteric and omental cysts are considered as rare intra-abdominal lesions, with an incidence of about 1 per 105000 admissions to general hospitals, ranging in age from in-utero to 18 years[93]. They can lead to hydronephrosis because of compression, as described about an abdominal cyst causing anuria in a newborn girl[94], for example.

A single case of mesenteric cyst in a neonate responsible for not only obstructive uropathy but also secondary type-1 hyperaldosteronism has been described in the literature[95]. It concerned a 9-d-old female neonate who presented with lethargy, refusal to feed and anuria over the previous 2 d. An ultrasound scan revealed a round mass in the inferior abdomen, compressing both ureters and leading to bilateral hydronephrosis. The case was treated by subtotal surgical excision of the cyst.

The autosomal-dominant inherited disorder neurofibromatosis type-1 rarely involves the genito-urinary tract, but some pediatric cases of obstruction and hydronephrosis or bladder involvement have been described[96-102], with at least one needing ureterocutaneostomy[103].

Cases of bilateral ureteral obstruction have been described both in adults and children due to traumatic pelvic hematoma and increased retroperitoneal pressure, in the so-called acute pelvic compartment syndrome[104].

Familial adenomatous polyposis

Intra-abdominal desmoid disease is one fearful condition related to familial adenomatous polyposis (FAP), potentially causing ureteric obstruction. Joyce *et al*[105] retrospectively investigated the incidence of ureteric obstruction among patients with desmoids disease from the FAP registry within the Sanford R. Weiss Center for Inherited Colorectal Neoplasia[105] and they sorted out that 30 patients out of the 107 with desmoids disease presented with ureteral obstruction, which was bilateral in 13. The median age of first colonic surgery was 21 years, ranging between 11 and 60. Most patients were treated by endoscopic retrograde ureteral stenting or percutaneous nephrostomy, while 4 cases underwent nephrectomy, 1 ureteric resection and reimplantation and 1 ureterolysis.

A preceding Asian paper reports on two patients affected by the same condition, with a review of 14 previous cases in the literature[106].

Retroperitoneal fibrosis

Retroperitoneal fibrosis is considered a rare entity in childhood[107], with 26 cases published[107-109], one of whom associated to lymphoma[110].

IATROGENIC TRIGONAL OBSTRUCTION OR INFLAMMATION

Device-induced obstruction

In the literature, we found 2 cases of anuria secondary to balloon catheters in children, described in a paper published in 1977[111].

The first one was a 17-mo-old girl affected by spastic neurogenic bladder, presenting with a 16-Fr Foley catheter with an 8-mL balloon, placed to manage a vesicocutaneous fistula developed after bilateral ureterovesical reimplantation. The catheter was patent and when it was removed the urine output restored.

The second case concerns a 7-d-old male newborn who underwent a transurethral resection of posterior urethral valves, presenting with an 8-Fr Foley catheter with a 3-mL balloon via a perineal urethrostomy. The catheter was found to be patent and by deflating the balloon diuresis was restored. Of course, those complications are less likely to occur with current pediatric devices.

Endoscopic procedures

Patients undergoing minimally invasive endoscopic peri-

ureteral injection of bulking agents for vesicoureteral reflux are potentially at risk of hydronephrosis[112,113], but those situations are well-known and such patients are deferred to a urologic follow-up after the procedure[114].

Bilateral ureteral obstruction after appendectomy

Known surgical complications leading to ureteral damage goes beyond the scope of this paper, while in the literature there are some cases reporting on bilateral ureteral obstruction as a rare complication after appendectomy in pediatrics, not related to direct surgical ureteral damage.

The last report found on PubMed, with a review of the literature, dates back to 2005[115] when the Authors described a case of anuria in a 11-year-old boy, 5 d after surgery for a perforated appendix. At ultrasound examination some echogenic "plugs" were found in the distal portion of both ureters, with no abscess at an abdomino-pelvic computed tomography.

During a cystoscopy a bladder base inflammation was revealed, so the patient was treated by bilateral ureteric stenting, with a prompt recover of diuresis and renal function.

In the review of the literature, the Authors found out 15 similar cases, curiously all boys aged 6-15 years[115].

An edematous process has been postulated as possibly triggered by a localized peritoneal reaction to intraoperative bacterial contamination, with boys more susceptible because their appendix is located closer to the bladder, while in girls internal genitals are situated between the appendix and the bladder[116].

In our opinion, such mechanism can be considered as an attractive pathogenesis explanation, although we do not know if an inadequate fluid replacement therapy could play a role, above all in cases with underlying predisposing factors to urolithiasis. More studies would be necessary, including a focus on metabolic disorders in patients presenting with such condition.

Personally, we observed a case of a 16-year-old boy with similar presentation after appendectomy, who was diagnosed with bilateral ureteric stones successfully drained during ureteric bilateral catheterization. He was suggested to undergo a metabolic panel but the patient was lost at follow-up.

CONCLUSION

Bilateral ureteral obstruction in pediatric population is a rare condition and can be related to either medical or surgical underlying causes, thus it is not possible to identify a common etiology.

With regard to pathogenetic mechanisms, some aspects remain unclear, in particular: (1) more studies are advocated to clarify ureteral obstruction secondary to severe diarrhea after *Rotavirus* infection; (2) ureteritis in immuno-rheumatologic diseases is not a completely clear event, although murine models elucidated some details; (3) multiple metachronous polyps recurring after laparoscopic or robotic pyeloplasty should be further investigated; and (4) sudden ureteral obstruction secondary to appendectomy could be related to dehydration, although in our opinion further studies would be necessary to highlight critical points and to evaluate metabolic aspects.

As a recommendation for clinical practice, a possible ureteral obstruction should be investigated in patients presenting with any picture described in this paper.

An abdominal ultrasound scan could be a simple, first-line diagnostic tool useful in the evaluation of hydronephrosis in most patients.

Treatment of ureteral obstruction deeply varies according to the underlying condition, with some cases successfully managed by drugs and other ones requiring surgery.

Surgical procedures often consist of ureteroscopy, ureteral stenting or nephrostomic tubes, with some cases deferred to major surgery for ureteral resection and reimplantation. Nephrectomy can be an option in patients presenting with advanced infections, particularly if recurrent, inveterate or in those needing an immunosuppression therapy for their underlying condition.

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P- Reviewer: Duan SB, Shou ZF **S- Editor**: Ji FF **L- Editor**: A
E- Editor: Lu YJ

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DOI: 10.5527/wjn.v3.i4.193

World J Nephrol 2014 November 6; 3(4): 193-197
ISSN 2220-6124 (online)

REVIEW

Retrograde intrarenal surgery in pediatric patients

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Received: April 1, 2014 Revised: May 23, 2014
Accepted: August 27, 2014
Published online: November 6, 2014

Abstract

Urinary tract stone disease is seen at a level of 1%-2% in childhood (< 18 years). In recent years, however, there has been a marked increased in pediatric stone disease, particularly in adolescence. A carbohydrate- and salt-heavy diet and a more sedentary lifestyle are implicated in this increase. Although stone disease is rare in childhood, its presence is frequently associated with metabolic or anatomical disorders or infectious conditions, for which reason there is a high possibility of post-therapeutic recurrence. Factors such as a high possibility of recurrence and increasing incidence further enhance the importance of minimally invasive therapeutic options in children, with their expectations of a long life. In children in whom active stone removal is decided on, the way to achieve the highest level of success with the least morbidity is to select the most appropriate treatment modality. Thanks to today's advanced technology, renal stones that were once treated only by surgery can now be treated with minimally invasive techniques, from invasion of the urinary system in an antegrade (percutaneous nephrolithotomy) or retrograde (retrograde intrarenal surgery) manner or shock wave lithotripsy to laparoscopic stone surgery. This compilation study examined studies involving the RIRS procedure, the latest minimally invasive technique, in children and compared the results of those studies with those from other techniques.

Key words: Percutaneous nephrolithotomy; Pediatric; Renal stone; Retrograde intrarenal surgery; Shockwave lithotripsy

Core tip: In the last two decades, technological advancement of instruments have changed the treatment options of renal stone disease. Today retrograde intrarenal surgery may represent an alternative treatment modality to shock wave lithotripsy and percutaneous nephrolithotomy, with acceptable efficacy and low morbidity in pediatric patients.

Resorlu B, Sancak EB, Resorlu M, Gulpinar MT, Adam G, Akbas A, Ozdemir H. Retrograde intrarenal surgery in pediatric patients. *World J Nephrol* 2014; 3(4): 193-197 Available from: URL: http://www.wjgnet.com/2220-6124/full/v3/i4/193.htm DOI: http://dx.doi.org/10.5527/wjn.v3.i4.193

RETROGRADE INTRARENAL SURGERY IN CHILDREN

Treatment of urinary stone disease in pediatric patients is a challenging problem[1-5]. Although the indications employed in treatment selection in children are regarded as the same as those for adults, children respond particularly well to shock wave lithotripsy (SWL)[6]. The fact that developing kidney tissue transmits shock waves better and that spontaneous passage is comparatively

easier in children than in adults both play a role in this rapid response. SWL, which began being applied in the 1980s with the principle of the use of high-energy shock waves, represents a milestone in the treatment of stone disease in children[7].

Gofrit *et al*[8] compared the results of pediatric and adult patients administered SWL for renal stones larger than 10 mm, and reported stone-free status levels of 95% in children and 78.9% in adults. Similar results were obtained from many subsequent studies. In a recent randomized prospective study Mokhles *et al*[9] compared the outcome of retrograde intrarenal surgery (RIRS) and SWL for stones 10 to 20 mm in preschool age children. They found that the overall stone-free rate was 93% and 96% for SWL and RIRS groups, respectively. SWL is therefore recommended as the first treatment option in children with stones of up to 20 mm (approximately 300 mm^2) in modern guidelines[10]. However, the fact that the procedure usually requires general anesthesia in children, the need for general anesthesia in repeat sessions, concerns over the possibility of long-term renal scarring, hypercalciuria, hypertension or chronic renal insufficiency and some stones (cysteine stones, *etc*.) not responding to the technique represent concerns over its use in children[10,11].

Technological advances in recent years has permitted the miniaturization of endoscopic devices, as a result of which percutaneous nephrolithotomy (PNL) has become the first treatment option for stones larger than 2 cm in children[11]. Although the procedure was initially performed with adult-type devices, Jackman *et al*[12] described a "mini-perc" technique using a 7 Fr rigid cystoscope and 11 Fr vascular access. They emphasized that a smaller tract will lead to less tissue and nephron injury and that this is more significant in pediatric patients with small and delicate kidneys, citing the example of a 24 Fr access sheath used in an infant being equivalent to 72 Fr in an adult.

Desai *et al*[13] reported that intraoperative hemorrhage occurring during PNL is related to the number and diameter of tracts, for which reason tract diameter should not exceed 22 Fr. In the majority of subsequent pediatric PNL series, the risk of intraoperative complications has been shown to decrease with use of small-size instruments[11,14]. Indeed, new PNL modifications aimed at reducing complication levels still further, such as tubeless PNL, ultramini-PNL and micro-perc, have been described[15-17]. However, despite all these modifications and high success rates, major complications such as neighboring organ injury, severe hemorrhage and urosepsis are still reported at levels of up to 10%, and the debate over whether the procedure is truly non-invasive continues[18,19].

RIRS is a comparatively new concept in pediatric patients. Before embarking on the details of this method in children, it will be useful to briefly review the stages by which it arrived at its present-day position. Use of this technique for treating renal stones was first described in 1983, by Huffman *et al*[20], when a large stone located in the renal pelvis was broken with the help of a ureteroscope with a rigid rod-lens structure and an ultrasonic lithotripter. Although the authors maintain that stones in the upper ureter and renal pelvis can be effectively and safely treated using small caliber rigid devices, the technique as it stands has not achieved popularity, due to its low success rate and high level of complications. Retrograde treatment of renal stones has been able to enter into widespread use only with the development years later of flexible ureteroscopes (f-URS) possessing fiberoptic technology and retrieval instruments with a nitinol structure and the simultaneous entry into use of Ho:YAG laser in intracorporeal lithotripsy[21].

Following the first description of the pediatric ureteroscopy (URS) by Ritchey *et al*[22] in 1998, the development of URS decelerated due to concerns over existing instruments not being of suitable sizes for children, the inadequacy of optic imaging systems and development of complications post-URS in child patients, such as ischemia, injury, perforation, stricture and vesicoureteral reflux, and this delayed the use of RIRS in this patient population[22,23]. However, the development in subsequent years of more resistant and finer (< 8 Fr) ureteroscopes and auxiliary nitinol instruments, the improvement of optic system quality, the entry into use of Ho:YAG laser and, parallel, to all these technological advances, an increase in surgeon experience with flexible URS led to the technique also starting to be used in child patients.

The first wide series on the subject of pediatric RIRS was published by Cannon *et al*[24] in 2007. Twenty-one child patients (13 girls, 8 boys) administered RIRS due to lower pole renal stone and with a mean stone size of 12 mm were included in that study. After a mean 11 mo of follow-up, stone-free status was achieved at a level of 76%, and no intra- or postoperative complications were reported in any patient. Passive dilatation was applied using preoperative stent in 38% of patients, while a ureteral access sheath was used in 43% (Table 1). However, the upper age limit was set at 20 (mean 15.1) in that publication reporting a pediatric series and a great many cases were postpubertal (67%) patients.

A 100-case series was published by Smaldone *et al*[25] in that same year. Although 37% of the stones in that series were intrarenal (renal pelvis 6%, upper pole 10% and lower pole 17%). Mean stone size was 8.3 mm and mean patient age was 13.2 years, with 49% of cases being prepubertal children. Passive dilation was applied in 54% of cases, ureteral active dilatation with a coaxial dilator to 70% and ureteral access sheath to 24%. Stone-free status was achieved in 91% of patients, while ureteral perforation developed in 5 and ureteral reimplantation was required due to stricture in the late period in one. However, no correlation was reported in that study between the complications that developed and use of ureteral access sheath or ureteral dilation.

In a study from 2008, Tanaka *et al*[26] published the

Table 1 Outcomes of pediatric retrograde intrarenal surgery procedures in published series

Ref.	Patient No.	Mean age, yr	Mean stone size (mm)	Passive dilation	Active dilation	Ureteral access sheat	Success	Complications
Cannon *et al*[24]	21	15.2 (1-20)	12 (± 5.9)	38%	81%	43%	76%	0%
Smaldone *et al*[25]	100	13.2 (± 5.4)	8.3 (± 5.3)	54%	70%	24%	91%	Ureteral stricture (1%) Ureteral perforation (5%)
Tanaka *et al*[26]	50	7.9 (1.2-13)	8 (1-16)	56%	35%	48%	58%	0%
Kim *et al*[23]	167	5.2 (1-18)	6.1 (3-24)	57%	-	?	99%	0%
Unsal *et al*[27]	16	4.2 (0-7)	11.5 (8-17)	37.50%	29.40%	17.60%	88%	Ureteral perforation (n = 1)
Erkut *et al*[28]	65	4.3 (0-7)	14 (7-30)	-	100%	100%	93%	27% complication rate
Abu Ghazaleh *et al*[29]	56	8.2 (6-14)	12 (9-15)	100%	-	-	100%	Urinary infection (n = 3) Hematuria (n = 1)
Resorlu *et al*[30]	95	9.4 (0-17)	18 (10-30)	?	18.90%	63.10%	85%	% 8.4 complications

results from 50 pediatric patients with a mean age of 7.9 (1.2-13.6 years) and receiving RIRS due to renal stone. Mean stone size was 8 mm (1-16) mm; 58% of cases remained stone-free at long-term follow-up with a single procedure, while an additional procedure was required in 36%. Success rate was correlated with stone size (P = 0.005), while additional procedure requirement was correlated with both stone dimension (P = 0.002) and patient age (P = 0.04). However, the text refers to procedures being performed for stones as small as 1 mm.

Kim *et al*[23] reported the experience with flexible URS of the Philadelphia Children's Hospital, announcing the results of 170 procedures performed on 167 pediatric patients with a mean age of 62.4 mo (range, 3-218). Mean stone dimension was 6.1 mm (range, 3-24), with stones in 60% of cases being intrarenally located (28% upper ureter stone, 12% upper ureter stone). Access to the ureter could not be established in 57% of patients, for which reason a stent was inserted and left to passive dilatation. Ureteral access sheath was only used in cases with a heavy stone burden or receiving passive dilatation, although no level of use was cited. Following surgery lasting a mean 107 min (range, 72-196), 100% of patients with stones smaller than 10 mm achieved stone-free status, and 97% of those with stones larger than 10 mm. No intra- or postoperative complications were reported in this series.

Unsal *et al*[27] examined the reliability of this procedure in pre-school children, evaluating 16 child patients with a mean age of 4.2 years (range, 10 mo-7 years). Mean stone dimension was 11.5 mm (range, 8-17); 37.5% of patients received double-j stent (passive dilatation), active dilatation was performed on 29.4%, and ureteral access sheath was used in 17.6%. One hundred percent of patients with stones smaller than 10 mm and 81% of those with larger stones achieved stone-free status. Ureteral perforation developed during ureteral dilatation in one case. That study showed that RIRS can successfully be used in infants aged under 1 year, describing the youngest (10 mo) case treated using the procedure in the literature. Subsequently, Erkurt *et al*[28] showed with a wider case series that the procedure can be safely used in pre-school age children. In that study, a ureteral access sheath was used in each case, and complication rates of 27% and stone-free status of 93% were reported.

In a study evaluating the efficacy of RIRS in prepubertal children Abu Ghazaleh *et al*[29] reported the results from 56 children (age 6-14) with stones less than 15 mm in size. Pre-procedural passive dilatation was performed in all cases, and electrohydraulic lithotripsy was used for stone breaking. At the end of 34-mo follow up, 100% stone-free status was reported and no intraoperative complication developed, although urinary infection was reported in 3 patients in the postoperative period and macroscopic hematuria in one. The use of a lithotripsy technique that has been abandoned due to high complication levels, each patient being subjected twice to anesthesia with the application of passive dilatation and stones inside the renal pelvis being broken with rigid URS represent question marks in that study, despite such high success rates.

In a multi-center comparative analysis (Table 2), Resorlu *et al*[30] compared the outcomes of patients with renal stones 10-30 mm in size treated with mini-perc (n = 106) or RIRS (n = 95). Stone-free status levels were 84% for RIRS and 86% for mini-perc, while complication levels were 8.4% for RIRS and 17% for mini-perc. All complications in both groups were minor (Clavien Ⅰ-Ⅱ), and no major complications (Clavien Ⅲ-Ⅳ) were observed. However, transfusion requirement at a level of 6% was reported in the mini-perc group. In addition, exposure to fluoroscopy, length of surgery and length of hospital stay were all lower in the RIRS group. Although RIRS appears to offer more advantages than mini-perc, when preoperative factors were assessed, there was a significant difference between the two groups in terms of stone size (23.7 mm *vs* 14.3 mm), and this was cited as a significant limitation in the text. When the groups

Table 2 Comparison of percutaneous nephrolithotomy and retrograde intrarenal surgery data in a recent study by Resorlu *et al*[30] *n* (%)

	PNL	RIRS
No. patients	106 (52.7)	95 (47.3)
Mean fluoroscopy time ± SD (s)	113.7 ± 36.6	33.2 ± 14.6
Mean operative time ± SD (min)	76.3 ± 21.2	42.1 ± 15.3
Mean hospitalization time ± SD (d)	3.1 ± 1.2	1.7 ± 0.6
Initial stone-free rate	91 (85.8)	80 (84.2)
Stones ⩾ 20 mm	78/93 (83.9)	4/8 (50.0)
Stones < 20 mm	13/13 (100)	76/87 (87.3)
Final stone-free rate	100 (94.3)	88 (92.6)
Minor (Clavien Ⅰ-Ⅱ) complications	18 (17.0)	8 (8.4)
Major (Clavien Ⅲ-Ⅳ) complications	-	-
Blood transfusion rate	7 (6.6)	-

PNL: Percutaneous nephrolithotomy; RIRS: Retrograde intrarenal surgery.

were compared again in terms of stone size, success rates of 87% in the RIRS group and 100% in the mini-perc group were obtained in stones of 1-2 cm, and 50% in the RIRS group and 84% in the mini-perc group in stones of 2-3 cm. The success rate of RIRS falls markedly when stone size exceeds 2 cm. In the light of these results, the authors reported that RIRS is superior to mini-perc in stones less than 2 cm in size, but that mini-perc has a better success rate with larger stones, and that RIRS can represent an alternative to it.

As technology has advanced, thinner and more resistant ureteroscopes and lithotripters with a greater deflection capacity and image quality have been developed[31]. This has made it easier to break stones at all points in the kidney. In the light of all these advances and increasing experience, the success rate of RIRS has increased and indications for use have widened, and it has now assumed a place together with SWL and PNL methods among treatment options for renal stones in children.

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P- Reviewer: Li BK, Lai S, Mohaupt M, Tepeler A
S- Editor: Wen LL **L- Editor**: A **E- Editor**: Lu YJ

World Journal of Nephrology

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Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.5527/wjn.v3.i4.198

World J Nephrol 2014 November 6; 3(4): 198-209
ISSN 2220-6124 (online)

REVIEW

Prostatic surgery associated acute kidney injury

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Received: May 28, 2014 Revised: August 7, 2014
Accepted: October 14, 2014
Published online: November 6, 2014

Abstract

Acute kidney injury (AKI) is associated with extended hospital stays, high risks of in-hospital and long-term mortality, and increased risk of incident and progressive chronic kidney disease. Patients with urological diseases are a high-risk group for AKI owing to the coexistence of obstructive uropathy, older age, and preexistent chronic kidney disease. Nonetheless, precise data on the incidence and outcomes of postoperative AKI in urological procedures are lacking. Benign prostatic hyperplasia and prostate cancer are common diagnoses in older men and are frequently treated with surgical procedures. Whereas severe AKI after prostate surgery in general appears to be unusual, AKI associated with transurethral resection of the prostate (TURP) syndrome and with rhabdomyolysis (RM) after radical prostatectomy have been frequently described. The purpose of this review is to discuss the current knowledge regarding the epidemiology, risk factors, outcomes, prevention, and treatment of AKI associated with prostatic surgery. The mechanisms of TURP syndrome and RM following prostatic surgeries will be emphasized.

Key words: Acute kidney injury; Prostatic hyperplasia; Prostate cancer; Transurethral resection of the prostate; Prostatectomy; Rhabdomyolysis

Core tip: Postoperative acute kidney injury has a significant effect on patient outcomes and has been associated with longer hospital stays, high risks of in-hospital and long-term mortality. Urology patients are a high-risk group for acute kidney injury (AKI) because of the common occurrences of obstructive uropathy, older age, and chronic kidney disease, as well as postoperative complications. The purpose of this review is to discuss the current knowledge regarding the epidemiology, risk factors, outcomes, prevention, and treatment of AKI associated with prostatic surgery.

Costalonga EC, Costa e Silva VT, Caires R, Hung J, Yu L, Burdmann EA. Prostatic surgery associated acute kidney injury. *World J Nephrol* 2014; 3(4): 198-209 Available from: URL: http://www.wjgnet.com/2220-6124/full/v3/i4/198.htm DOI: http://dx.doi.org/10.5527/wjn.v3.i4.198

INTRODUCTION

Prostatic diseases are associated with morbidity and mortality in elderly men. Benign prostatic hyperplasia (BPH) is the fourth most common diagnosis in older men[1]. Meanwhile, in developing countries, prostate cancer (PCa) is the most common solid neoplasm, and it is the currently second-leading cause of cancer mortality for men. Beyond conservative medical therapy, the surgical approach remains an important step for the treatment of these diseases[2].

A number of surgical techniques have been devel-

Table 1 Surgical approach to the treatment of benign prostatic hyperplasia and prostate cancer

Benign prostatic hyperplasia
Transurethral resection
Open simple prostatectomy
Electrovaporization
Laser prostatectomy
Holmium laser enucleation
GreenLight™ laser vaporization
Transurethral incision
Transurethral needle ablation
Prostate cancer
Radical Prostatectomy
Open (retropubic or perineal)
Minimally invasive
Laparoscopic
Robot-assisted

oped over the years to treat prostate diseases (Table 1). In recent decades, new surgical methods for treating BPH and PCa have been developed, such as laser- and robot-assisted prostatectomy. Although these procedures have been associated with lower postoperative complication rates in some studies, their efficacy and long-term robustness remain to be proven. At present, the gold-standard treatments for PCa and BPH are still open radical prostatectomy (ORP) and transurethral resection of the prostate (TURP), respectively[2].

In surgical patients, outcomes are strictly dependent on the occurrence of complications. Urology patients are a high-risk group for acute kidney injury (AKI) because of the common occurrences of obstructive uropathy, older age, and CKD, as well as bleeding and urinary obstruction, that sometimes follow the surgery. However, precise data on the incidence and outcomes of postoperative AKI in urological procedures are lacking[3].

Observational studies that compared different surgical approaches to treating prostatic diseases rarely monitored AKI as a relevant early postoperative complication. For instance, in a prospective multicenter analysis of the postoperative complications of 10654 patients subjected to transurethral prostatic surgery for BPH, no AKI case was reported[4]. Similarly, in a prospective observational study of 280 patients subjected to laparoscopic (LSP) or open simple prostatectomy (OSP) for BPH, three patients developed AKI (defined as a 50% rise above the patient's baseline serum creatinine level)[5]. Moreover, Marmiroli *et al*[6] studied the postoperative outcomes of 100 patients ⩾ 75 years old who had undergone TURP or OSP for BPH and found an incidence of 1% of AKI that required dialysis in this high-risk population. As is the case with the literature on BPH, AKI seems to be infrequently or underreported in patients undergoing ORP for PCa. Recently, one large retrospective analysis, including more than 77000 patients, examined outcomes after robot-assisted radical prostatectomy (RARP) and ORP, and AKI was not cited as a major complication[7].

Based on the available literature, episodes of severe AKI after prostate surgery appear to be unusual. Because the current recommended Kidney Disease Improving Global Outcomes (KDIGO) AKI definitions[8] (Table 2) have never been employed in this situation, acute subclinical serum creatinine (SCr) increases have never been systematically monitored across prostatic surgery outcome studies, and the exact incidence of AKI cannot be determined. However, AKI secondary to TURP syndrome has been consistently described[9,10]. Furthermore, a number of small case studies and case reports of rhabdomyolysis (RM)-associated-AKI after radical prostatectomy have also been published[11-13].

Table 2 Kidney disease improving global outcomes acute kidney injury definitions

AKI is defined as any of the following
Increase in serum creatinine by ⩾ 0.3 mg/dL within 48 h; or
Increase in serum creatinine to ⩾ 1.5 times baseline, which is known or presumed to have occurred within the prior 7 d; or
Urine volume < 0.5 mL/kg per hour for 6 h

AKI: Acute kidney injury.

This review outlines AKI associated with prostatic surgery, emphasizing the mechanisms of TURP syndrome and RM following prostatic surgeries. The surveillance, prevention and treatment of these complications will also be addressed.

TURP AND AKI

TURP requires the use of an irrigating fluid to expand the operating field and to wash away debris and blood. TURP syndrome is a clinical complication caused by the systemic absorption of the irrigating fluid and is characterized by a combination of hyponatremia and fluid overload, causing potential damage to the cardiovascular, renal and nervous systems[14,15].

The incidence of TURP syndrome ranges from 1.0% to 8% of reported TURPs and appears to be decreasing in recent years[16,17]. Mortality rates are generally between 0.2% to 0.8%, but rates as high as 25% can occur if severe TURP syndrome develops[10,18].

TURP syndrome can be defined as sodium of 125 mEq/L or less after TURP with two or more circulatory and/or neurological symptoms[19]. However, no universal defining criteria have been adopted by all centers, and not all studies have used clear definitions of TURP syndrome. Of note, the definition and severity of kidney dysfunction are not always detailed in TURP syndrome, and no studies have used the definition proposed by the most recently updated Kidney Disease Improving Global Outcomes (KDIGO) AKI guidelines. Similarly, there are scant data on late prognoses because few studies have reported on outcomes more than three to six months after the event. In particular, the incidence of CKD resulting from TURP syndrome is unknown. Many aspects of TURP syndrome are still unclear, and its overall burden is not completely determined.

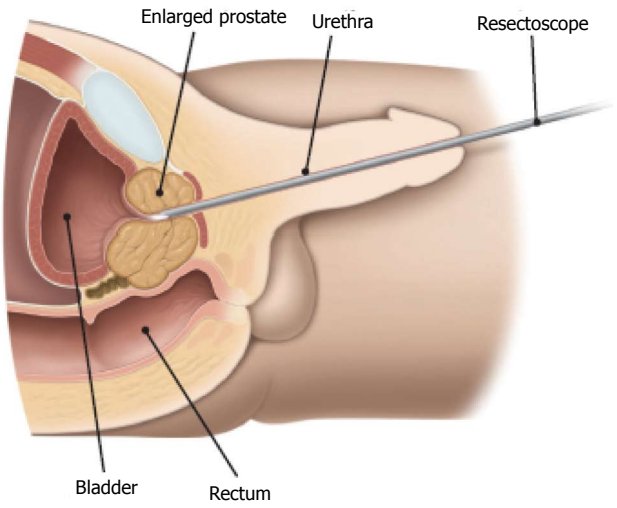

Figure 1 Surgery through the urethra. Courtesy of the European Association of Urology.

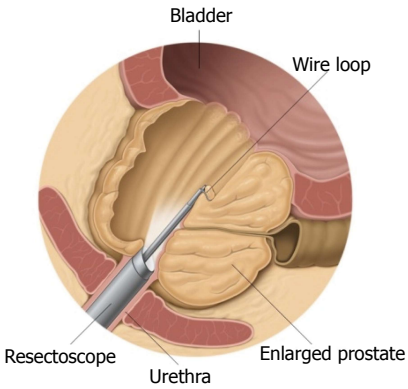

Figure 2 The resectoscope removes parts of the prostate tissue during transurethral resection of the prostate. Courtesy of the European Association of Urology.

RISK FACTORS

Although important differences appears according to the compositions of the irrigation fluids, the most important risk factor for TURP syndrome is the amount of fluid absorbed, which can vary from < 300 to 3000 mL[20]. After the absorption of 1.0 mL of fluid, serum sodium reduction is approximately 6 to 8 mEq/L, and it can achieve 20 mEq/L after absorption of 3.0 L[21]. The rate of fluid absorption is most likely also an important risk factor, and absorption in excess of 200 to 300 mL per 10 min is more frequently related to hyponatremia[22]. The risk of the TURP syndrome is higher in the presence of bleeding, longer resection times, higher absorption of irrigating fluid and prostate size larger than 45 g[23]. Smoking is a factor known to be associated with the increased risk of TURP syndrome , but the malignancy does not seem to be associated with the increased risk[24].

FLUID ABSORPTION MECHANISM

In TURP, a resectoscope loaded with a diathermy loop is introduced into the bladder to resect prostatic tissue (Figures 1 and 2). TURP typically takes 60 min, and approximately 10 to 20 liters of irrigating fluid are generally required. During TURP, irrigation pressure is regulated to achieve 60 mmHg, a much higher threshold than that for physiological intravesical pressure, which peaks at 25 mmHg during micturition[25]. When prostate tissue is resected, veins may be severed, and irrigating fluid can be rapidly absorbed into the vascular system. Intravenous fluid absorption begins when the fluid pressure exceeds the prostate venous pressure by approximately 12.5 mmHg[26], and it rarely ceases once it begins[20]. Most of the fluid absorption takes place during the second half of the resection when the resectoscope approaches the vein plexus, reaching larger vessels, and an extended area for fluid influx is opened[20]. Herein, small amounts of fluid are always absorbed during TURP, and absorption in excess of one liter has been reported to occur in 5%-20% of procedures[27,28]. Extravasation can occur after instrumental damage of the prostatic capsule or the bladder wall during TURP, which occurs in at least 10% of surgeries (Figure 3). The fluid pressure must only exceed an intra-abdominal pressure of approximately 3.72 mmHg for extravasation to occur, and several liters of irrigating fluid will be rapidly deposited in the periprostatic, retroperitoneal or intraperitoneal spaces[29]. The fluid is absorbed by lymphatic drainage, a slower process than vascular intake, which can translate into a delayed reduction in serum sodium compared with classic intravascular absorption. Extravasation contributes to TURP syndrome development in approximately 20% of cases[30].

IRRIGATION FLUID PHYSIOPATHOLOGY

An ideal irrigating fluid should be isotonic, nonhemolytic, electrically inert, nontoxic when absorbed, not metabolized, and transparent; should not influence blood osmolality; should be rapidly excreted; and should not cause significant expansion of extracellular fluid volume. However, no such solution exists; each irrigating fluid has particular physicochemical and pharmacokinetic characteristics and each comes with its own potential complications.

Distilled water

Distilled water (D.W.) was the first irrigating fluid used in TURP surgeries and is still often used because of its advantages: clear visual field, absence of electric conductivity, volume of distribution equal to the body's full water content, and lower cost[31,32]. After the description of cases of hemolytic AKI[33,34], iso- or hypo-osmolar nonhemolytic solutions other than D.W. were introduced to overcome this issue. D.W. continues to be used, but it should be restricted to laser TURP or to procedures that are expected to be short and performed by very skilled surgeons with all precautions taken to avoid the risk of

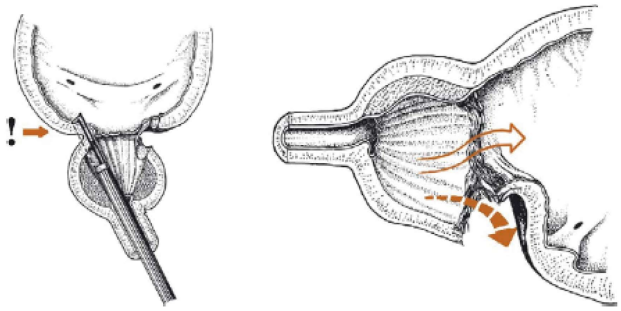

Figure 3 Division of the bladder neck with subsequent extraperitoneal extravasation. From Rassweiler *et al*[28].

TURP syndrome[32].

Glycine

Glycine (Gly) is a neutral, nonessential amino acid, used as an irrigating fluid solute since 1949. A 1.5% Gly solution is commonly used in TURP because it is nonconductive, hypo-osmotic (osmolality of approximately 200 mOsm/L), and nonhemolytic and it provides good optical visibility[35]. This amino acid spreads through intracellular compartments, creating osmotic water movement into cells, which gradually increases serum sodium and minimizes the risk of severe hyponatremia. However, Gly presents some drawbacks and is commonly associated with TURP syndrome. It has cardiotoxic properties, and can cause serious visual disturbances due to retina damage[36]. Hyper-ammoniemic encephalopathy[37] and hyperoxaluria have been associated with Gly metabolites[38].

Sorbitol solutions

Solutions based in sorbitol are frequently used in the United States of America as irrigating fluids, typically in the concentration range of 2.2% to 3% and frequently in association with mannitol[39]. Sorbitol is non-electrolytic, has an osmolality of approximately 180 mOsm/L, and clears rapidly from the plasma after its transformation to fructose and glucose by liver cells. Sorbitol is considered a safe irrigating fluid and is associated with low rates of TURP syndrome.

Mannitol

Mannitol is frequently used as an irrigating fluid in concentrations of 3% or 5% solution (osmolalities of 175 and 275 mmOsm/L, respectively)[40]. Mannitol is nonconductive and nonhemolytic and gives a satisfactory visual operating field. Mannitol is excreted unchanged in the urine, promoting osmotic, electrolyte-free diuresis, which could help to increase serum sodium concentration. Mannitol is considered a suitable irrigating fluid that is associated with low rates of TURP syndrome.

Physiologic saline

Saline solution cannot be used as an irrigating fluid during the standard prostatic resection because its dissipates the electrical current of bipolar resectoscope, therefore preventing both cutting and coagulation. Normal saline (0.9%) osmolality is approximately 300 mOsm/L, which makes it the most suitable irrigation fluid for TURP. Very few cases of TURP syndrome have been described with this solution. Fluid overload is more likely during the absorption of normal saline solution due to the higher volume expansion[41]. In addition, the excessive sodium chloride infusion can cause hyperchloremic acidosis.

CLINICAL AND LABORATORY PRESENTATION

TURP syndrome occurs from 15 min to 24 h after prostate resection. The incidence and severity of TURP syndrome symptoms increase progressively as more solution is absorbed. When the threshold of 3.0 L is exceeded[10], the symptoms are severely impaired.

Some symptoms may be noticed in the intraoperative period. Vague, nonspecific symptoms may occur such as the sense of being unwell[27], transitory feeling of burning, accompanied by nausea, restlessness and headache. Neurologic events are more frequently observed when glycine solutions are used and in patients with decreases in serum sodium of 10 mEq/L or more[42]. Focal or generalized seizures and altered mental states can occur. This is generally associated with irrigant absorption levels as high as 2.0 to 3.0 L. Brain stem herniation, persistent brain injury and death have also been reported[43]. Visual disturbances, including transitory blindness have been observed, mostly related to glycine solution[36].

Patients may develop both hypervolemic and hypovolemic complications. Bradycardia and hypotension at the end of the operation, or immediately after, are often early signs suggesting TURP syndrome[44]. Shortness of breath and pulmonary edema can occur in surgeries where mild/less severe bleeding is observed[45]. Chest pain and hypertension have also been observed in 5% of the patients, particularly when more than 1 L is absorbed[46]. Small elevations in cardiac enzymes can occur, especially when Gly solution is used[47].

Most patients subjected to TURP are elderly with coexisting diseases, reduced functional heart and kidney reserves and less capacity to endure stress[48]. CKD patients are also at exacerbated risk for TURP syndrome[15]. AKI has been reported in TURP syndrome patients; it is typically oliguric and can be observed as early as the first postoperative day. Bilen *et al*[9] assessed a group of 439 patients who had undergone TURP using mostly distilled water as the irrigating fluid. AKI defined as an increase in postoperative SCr > 1 mg/dL occurred in 16 (3.64%) of the patients.

Severe TURP syndrome, defined by a drop in serum sodium concentration to < 120 mEq/L, is a rare but well-described event in the specialized literature, characteristically reported when more than 3.0 L of irrigating fluid are absorbed[49]. A review of 24 severe

cases in which Gly 1.5% was used as the irrigating fluid demonstrated neurological complaints in 92%, cardiovascular signs in 54%, visual disturbances in 42%, and gastrointestinal symptoms in 25% of these patients, with a mortality rate of 25%. AKI is observed in more than 50% of cases of severe TURP syndrome, sometimes requiring renal replacement therapy (RRT)[50].

Hyponatremia (< 135 mEq/L), a hallmark of the syndrome, is seen in nearly all patients, and it is more frequently observed at the end of surgery (or one to two hours subsequent)[51]. Hyponatremia might be transitory and could go undetected if serum sodium is assessed more than three hours after surgery completion. Although most irrigating fluids are hypo-osmolar (approximately 200 mOsmol/L), compared with normal serum osmolality (approximately 290 mOsm/L), hypo-osmolality in TURP syndrome is less pronounced than that observed in other hyponatremia etiologies because the solute contents of irrigating fluids (Gly, sorbitol, mannitol) prevent large osmolality reductions[52]. In TURP syndrome, serum osmolar gaps reflect the concentrations of the infused/absorbed irrigants and can achieve 30 to 60 mOsm/kg[53]. Herein, serum osmolality should be measured in all TURP syndrome patients.

PATHOPHYSIOLOGY

Hemodynamics

The rapid volume expansion which can reach up to 200 mL/min can cause hypertension and reflex bradycardia. Hypertension coupled with hyponatremia can trigger pulmonary edema and hypovolemia due to net water flux from the intravascular space into the pulmonary interstitium[54]. In sequence, a major hypokinetic hemodynamic phase ensues, distinguished by low cardiac output, hypovolemia and hypotension[44,55].

Natriuresis has been highlighted as a key element in promoting dilutional hyponatremic shock and explains why hypovolemic hypotension persists despite the administration of large amounts of fluid. The osmotic diuresis leads to sodium losses and occurs when the renal reabsorption mechanisms are either overwhelmed (Gly) or absent (mannitol)[56]. The capacity of the kidneys to control the urine's composition is then undermined, and a number of small solutes, including amino acids and sodium, are ultimately lost from the body.

Other factors that contribute to the hemodynamic changeover include metabolic acidosis[57], acute hypothermia[58], release of endotoxins into the bloodstream[59], and depression of the heart conductivity system[47].

Central nervous system

Even moderate osmolality reduction could result in a fluid influx into the cerebral space, leading to brain edema[59,60]. Other factors that contribute to central nervous system impairment in TURP syndrome, such as the very low serum sodium concentration itself, are Gly toxicity and the accumulation of its metabolic derivates (ammonia, serine, and/or glyoxylate)[37,61].

Renal disease

AKI following TURP has been reported since 1947, and a variety of mechanisms have been proposed for its development[62,63]. When sterile water was used for irrigation, intravascular hemolysis was thought to be the principal insult[64]. Hemolysis takes place in the blood as well as in the bladder, where hemolyzed blood is absorbed. In both cases, hemoglobinuria develops and renal injury occurs through a number of pathways. Heme proteins have powerful oxidant effect, it can trigger renal vasoconstriction[65] and under acidic conditions, precipitate with Tamm-Horsfall proteins contributing to tubule obstruction. Hemolysis should be investigated in all AKI events after TURP[34] although these events have become rare with the observed change to other irrigating fluids, and other pathogenic mechanisms have been described.

Hyponatremia-associated RM resulting in AKI has been reported as a complication following TURP in a small number of cases[66]. After a number of hours, muscle cellular swelling induced by hyponatremia will peak because of the potassium outflux from the muscle cells into the extracellular fluid. Hyponatremia also reduces the concentration gradient for sodium entry into the muscle cells, resulting in a decreased outward flux of calcium, which leads to increased intracellular calcium[67] destroying the cell structure[68]. At this point, all patients with AKI-related TURP syndrome should be screened for RM.

It has been suggested that "hemodynamic" acute tubular necrosis is an important cause of AKI in some patients after TURP. Hypotension coupled with osmotic diuresis results in ischemic kidney episodes[69]. Another possible mechanism is sudden kidney cell swelling as a result of acute hypo-osmolality, similar to the development of central nervous system edema[70].

More recently, Kim *et al*[71] described three cases of AKI after laser vaporization of the prostate using distilled water as the irrigating fluid. All patients developed significant hyponatremia, and two of them required RRT[71]. Histological findings were tubular cell necrosis and Tamm-Horsfall protein stasis with regurgitation into the Bowman capsule accompanied by an amount of eosinophilic interstitial infiltrate. Special staining for hemoglobin and myoglobin had negative results, and there was no histologic evidence of ischemic damage. Hyponatremia and hemodynamic mechanisms could not be ruled out, but this scenario strongly suggested direct damage to the tubular epithelium by urinary stasis and the backflow of the irrigating fluid, hemoglobin, and prostate secretions resulting from high intravesical pressure. This report suggests transient vesicoureteral reflux as a new pathogenic mechanism of kidney injury in TURP syndrome, although this has yet to be confirmed by other studies[71].

The physiopathology of renal injury in TURP syndrome is complex, multifactorial and not completely un-

derstood. It is most likely that one or more of a number of mechanisms are implicated.

PREVENTION

Prostate gland size and operative time

There are no definitive data establishing an operative time threshold beyond which excessive fluid is absorbed, but after one hour of surgery, the risk increases significantly[72], and after that point, the patient´s overall status, the volume of fluid absorbed, and the anticipated time to completion should be reassessed[73]. For patients with large glands and expected long procedures, it is advised that bipolar TURP or other low-risk techniques be used.

Fluid bag height

Fluid is infused using the force of gravity (elevating the infusion bag to different heights) or by inflating a large blood pressure cuff around the infusion bag. Placing the irrigating fluid bag at 60 cm above the operating table has been advised to avoid fluid absorption. Nevertheless, two studies including almost 600 patients did not demonstrate conclusive benefits in higher fluid bag[74,75].

Intraprostatic vasopressin injection

Transrectal intraprostatic vasopressin (IPVP) injected at the operating site is considered to vasoconstrict intraprostatic vessels and reduce blood loss and fluid absorption during TURP[76]. IPVP appears to be effective and could be used in patients with large prostates or when fluids associated with higher incidence of TURP syndrome such as Gly and D.W. are used.

Low-pressure irrigation

Irrigating fluid absorption is less pronounced when TURP is performed under low pressure. A number of measures to maintaining low intra-bladder pressure has been used, such as suprapubic catheterization, intermittent drainage of the irrigating fluid and continuous flow resection[77].

Bipolar TURP

Bipolar resection of the prostate utilizes a specialized resectoscope loop that incorporates both the active and the return electrodes. The bipolar loop resects, coagulates, vaporizes and transects the tissue. Because the bipolar resectoscope uses a 0.9% sodium chloride solution as the irrigation fluid, the risk of TURP syndrome is eliminated, allowing for longer and safer resections[78]. Omar *et al*[79] recently published a systematic review and meta-analysis comparing bipolar and monopolar TURP. The study comprised 24 trials, and no case of TURP syndrome was observed in the bipolar group. Therefore, bipolar TURP is a safe procedure that is suitable for high-risk patients such as CKD patients and those with large glands.

Laser and vaporization prostatectomy vs TURP syndrome

Photoselective vaporization of the prostate (PVP) and other laser techniques are novel procedures that promote effective hemostasis with nearly bloodless removal of prostate tissue and minimal absorption of irrigating fluid[80]. PVP can use normal saline as the irrigating fluid, and laser therapies have been reported to successfully treat patients with very large prostates (> 100 g) and those with ongoing oral anticoagulation[71].

Trans-operatory monitoring of fluid absorption

A key aspect to preventing the development of TURP syndrome is monitoring the fluid absorption during the endoscopic surgery. A number of alternatives have been attempted to achieve this goal. Volumetric fluid balance, the difference between the amount of irrigating fluid used and the output volume, is the most commonly used technique to estimate fluid absorption. However, other variables such as bleeding, irrigant leakage, urinary output (diuresis), and blood dilution make this a comparatively unreliable tool[81]. Although it has limitations, volumetric fluid balance is simple, noninvasive, and inexpensive, and it should be performed in every surgery.

The gravimetric method is often used and requires that the surgery take place on a bed-scale. The method relies on the supposition that increases in the body weight are generated by fluid absorption. Bleeding and intravenous infusions must be considered in recordings, that must be carried out when the bladder is empty[82].

To minimize the risk of hyponatremia, an intraoperative approach based on the amount of absorbed fluid is suggested[27]. If more than 1.0 L of fluid is estimated to be absorbed, the surgical team should temporarily halt the procedure, fluid inflow should cease and serum sodium should be measured. If mental and cardiovascular status are maintained, surgery can be resumed for as short a period as possible. If more than 2.0 L of fluid were absorbed, hemorrhage points should be coagulated and the procedure should be terminated. Serum sodium concentration and mental status should be closely monitored.

MANAGEMENT AND TREATMENT OF TURP SYNDROME

The urologic surgeon and the anesthetist should be aware of the development of TURP syndrome. Asymptomatic and stable patients should be kept under observation. Specific treatment is not required, particularly if sodium reduction is below 5 mEq/L. In these cases, if renal function is adequate, excretion of the excess water and metabolism of the infused solute will rapidly correct the hyponatremia[83]. There is no specific treatment for the visual symptoms of Gly intoxication, and even blindness is typically resolved in 24 h without the need for specific treatment[84].

Hypertonic saline is indicated to replace the excreted sodium in symptomatic patients with marked hyponatremia, particularly those who have substantially reduced serum osmolality or a cerebral edema[85]. Hypertonic

saline in a 3% solution can be given as a 100 mL bolus at 10-min intervals or continuously infused (approximately 1.0 L in 12 h). Rapid correction of hyponatremia is most likely safe following TURP because of the extremely short duration of hyponatremia and the restricted time for cerebral adaptations. A reasonable and safe strategy is to increase the serum sodium concentration to up to 12 mEq/L in the first 24 h. Furosemide may be used to reverse the fluid overload, although furosemide increases natriuresis and hyponatremia, further reducing plasma volume and increasing the cellular edema[86,87]. Furosemide should not be routinely given in TURP patients in the absence of fluid overload.

Hemodialysis will rapidly correct hyponatremia, osmotic derangements, and volume expansion and remove the non-electrolyte solute and its toxic metabolites (Gly, sorbitol, mannitol). It has been used in symptomatic patients with severe renal disease and in patients with severe neurologic symptoms and marked hyponatremia[53].

In the case of important fluid extravasation and large fluid collections, it might be necessary to carry out a open surgical drainage by percutaneous drainage[88].

ANESTHESIA AND TURP SURGERY

For years, spinal anesthesia was considered the anesthetic technique of choice for TURP. Spinal anesthesia is considered to reduce the risk of pulmonary edema, to decrease bleeding risk and to allow a prompt diagnosis of neurologic symptoms[89]. However, spinal anesthesia reduces central venous pressure, affecting prostate venous pressure, which could result in greater absorption of the irrigating fluid[89]. During general anesthesia, the detection of TURP syndrome may be more difficult, based on afterward changes in blood pressure and electrocardiographic abnormalities[89]. In fact, the best anesthetic technique for TURP procedures has not yet determined.

RADICAL PROSTATECTOMY AND RHABDOMYOLYSIS

Radical prostatectomy is the main surgical treatment for PCa. Radical retropubic prostatectomy (RRP) is performed with patients in the supine position, while in radical perineal prostatectomy (RPP), patients are placed in an exaggerated lithotomy position[90]. More recently LSP and RARP have been developed, and these minimally invasive procedures are replacing open radical prostatectomy in some countries[2].

The first description of position-induced RM with subsequent AKI after a knee-chest position was in 1953 by Gordon[91]. Renal failure following radical prostatectomy is uncommon, and the incidence of subclinical RM following this procedure is currently unknown[90]. In a recent retrospective study of 175699 patients subjected to robotic or non-robotic radical prostatectomy, the incidence of RM was 0.08%[92]. In a prospective study of 60 patients undergoing RARP and lymph node dissection with prolonged positioning in a steep Trendelenburg position, Mattei *et al*[93] demonstrated that ten patients developed RM (serum creatine kinase > 5000 IU/L). Although AKI following radical prostatectomy appears to be rare, there are a number of case reports that suggest RM secondary to an exaggerated lithotomy position as a cause of AKI in this setting[90,94-96].

AKI associated with myoglobinuria is an important complication of RM[97]. After glomerular filtration, myoglobin is uptake by the tubule epithelial cell through endocytic pathways and is metabolized. The precise mechanisms leading to the glomerular filtration rate impairment are unclear, however some evidence suggests that hypovolemia, vasoconstriction, intraluminal cast formation, oxidative stress and direct heme-induced cytotoxicity are all responsible for kidney injury.

RM after the exaggerated lithotomy position during surgery is usually due the intraoperative development of lower extremity compartment syndrome or from muscle breakdown in the back and gluteal regions[90]. Improperly positioned or inadequately padded patients are prone to ischemia-reperfusion injury from excessive compression. Compartment syndrome was reported in 93% of 46 patients with position-related RM associated with AKI[96]. The lower extremities were most often involved (50%), with muscle swelling and ache being the presenting symptoms[96]. Lithotomy and chest-knee position were the most frequent postures in these patients[96]. Fortunately, compartment syndrome is a rare complication of radical prostatectomy surgery[92].

Rhabdomyolysis associated with prostatectomy prevention and management

To prevent RM associated with surgical position, all pressure points should be protected, including paying special attention to the shoulders, back, and sacrum[90]. The vascular status of the patient's lower extremity should be evaluated with a preoperative vascular examination, and repositioning the lower extremities every two hours could improve perfusion and avoid the occurrence of injury[90]. Some investigators have suggested noninvasive or invasive intraoperative monitoring to assess for impending limb compartment syndrome[94]. Others have recommended obtaining preoperative CPK levels, levels every two hours intraoperatively and levels six to eighteen hours postoperatively if the procedure is expected to be prolonged[11], especially in high-risk groups[93].

An approach to the extensive management of RM was published elsewhere[97]. The approach to RM after prostatectomy is not different from that in other settings. Early and aggressive intravascular volume expansion with crystalloids to restore kidney blood flow and increase urine flow is the cornerstone intervention for preventing and treating AKI. Intravenous fluids should be initiated ideally within the first 6 h after muscle injury, at a rate that maintains a urine output of 300 mL/h or more in adults, for at least the first 24 h[98]. There

Table 3 Suggestions for the prevention and management of transurethral resection of prostate syndrome

Preoperative
Estimate GFR using the CKD-EPI equation
Identify patient risk factors: large prostate gland (> 45 g), heart disease, CKD, and smoking
Advise bipolar TURP or laser techniques for high-risk patients
Intraoperative
Avoid D.W. and glycine as irrigating fluids. Sorbitol and mannitol are good options. Physiologic saline is a safe choice when feasible
Maintain low-pressure irrigation
Consider the use of intra-prostatic vasopressin injection in high-risk patients
Alert surgical team when surgery exceeds one hour
Monitor the volume of absorbed fluid. Consider aborting the procedure if the absorbed volume exceeds 1.0 L and suspend surgery if absorbed volume exceeds 2000 mL
Both spinal and general anesthesia are adequate
Avoid hypotension and central venous pressure reduction and closely monitor the vital signs
Post-operative
Assess serum sodium and serum creatinine in all patients in the immediate postoperative period
Apply KDIGO AKI definitions to AKI diagnosis
If TURP syndrome is diagnosed, initiate medical treatment:
Assess serum osmolality
Maintain asymptomatic and mildly symptomatic patients under close observation
Initiate hypertonic saline 3% infusion in symptomatic patients with marked hyponatremia, reduced osmolality and cerebral edema
Restrict diuretic use to treat fluid overload
If AKI occurs, test for hemolysis and rhabdomyolysis
Consider hemodialysis in symptomatic patients with severe renal disease
Patients that developed AKI should be followed and eGFR equations must be used to identify CKD

AKI: Acute kidney injury; CKD: Chronic kidney disease; CKD-EPI: Chronic Kidney Disease Epidemiology Collaboration; D.W.: Distilled water; GFR: Glomerular filtration rate; KDIGO: Kidney disease improving global outcomes; TURP: Transurethral resection of prostate.

is insufficient evidence to support the routine use of bicarbonate-containing fluids, mannitol, or loop diuretics. Hyperkalemia and compartment syndrome are other complications that should be closely surveyed, early diagnosed, and effectively treated.

NEPHROLOGY CONSULTATION

Preoperative nephrology consultation might identify clinical risk factors such as CKD or other comorbidities. To accurate diagnosis and stage CKD we suggest the CKD Epidemiology Collaboration (CKD-EPI) equation. The CKD-EPI equation was published in 2009 and intended to be more generalizable across various clinical settings[99].

TURP syndrome and RM after prostatectomy are generally diagnosed and treated by anesthesiologists and urologists. Nephrologists are typically called only in the most severe cases, which require RRT. Nephrologists should be aware of the risk factors, physiopathology, clinical picture and treatment strategies of TURP syndrome and RM after prostatectomy. An active role of nephrologists in the whole procedure could improve the care of those patients. Nephrologists would be an important add for early identification and treatment of AKI, electrolytes abnormalities, fluid overload, and previous chronic kidney dysfunction.

Although the limitations in the quality of published evidence preclude firm recommendations in this field, some suggestions on preventive and management strategies are depicted in Tables 3 and 4.

CONCLUSION

Severe AKI appears to be a rare event after prostate surgery. However, it is a hazardous surgical complication that increases the risk of permanent kidney damage or death. Because mild SCr elevations were not systematically monitored across the majority of the available studies, the exact incidence of AKI is underdetermined. Studies using the current definitions of AKI are very necessary for providing a better understanding of AKI risk factors and the influence of AKI on patient outcomes after prostate surgeries. Preoperative nephrology consultation might be helpful to better assess kidney function and the presence of other risk factors for AKI, allowing for adequately planning the surgical technique and reinforcing preventive strategies. Affected patients should be followed to assess long-term prognosis and CKD development.

In the last years, several studies about urinary and serum biomarkers for the diagnosis and prognostication of AKI have been published[100,101]. The question that arises is which biomarker is a reliable differential diagnostic tool under which circumstances. As hematuria and need of bladder irrigation are common after prostatic surgeries, the urinary biomarkers might be less suitable in this setting. Further research in this field is warranted before biomarkers can be introduced in the clinical practice.

The available data suggest TURP syndrome as the main mechanism for AKI following prostatic surgery. The absorption of 1-2 L of irrigating fluid occurs in 5%-10% of patients and results in easily overlooked mild TURP syndrome. Fortunately, TURP syndrome inci-

Table 4 Suggestions for the prevention and management of surgical position-related rhabdomyolysis

Preoperative
Identify patient risk factors: obesity, hypovolemia, diabetes mellitus, hypertension, chronic kidney disease, peripheral vascular disease, expected surgery time longer than 5 h
The vascular status of the patient's lower extremity should be carefully assessed with a well-documented preoperative vascular examination
The patient´s volume status should be evaluated
Intraoperative
Ensure correct patient positioning and protect all pressure points
Monitor lower extremities and vascular status
Reposition lower extremities every two hours
Adequate fluid reposition, avoiding hypovolemia
Monitor serum potassium levels
Appropriate operative time, completing the procedure as quickly as possible
Post-operative
Assess serum-CK and SCr 6 h and 18 h postoperatively in high-risk patients
Closely check serum creatinine, potassium levels, and acid-base disorders
Apply KDIGO AKI definitions to AKI diagnosis
Monitor signs of compartmental syndrome and consider fasciotomy if present
If RM syndrome is diagnosed, initiate medical treatment:
Initiate aggressive early fluid repletion;
Treat acid-base and electrolyte abnormalities;
Consider early RRT

CK: Creatine kinase; KDIGO: Kidney disease improving global outcomes; RM: Rhabdomyolysis; RRT: Renal replacement therapy; SCr: Serum creatinine. AKI: Acute kidney injury.

dence appears to be declining because of the use of laser surgery techniques and bipolar circuitry, together with the systematic institution of routine precautions to minimize the risk of TURP syndrome development (*e.g.*, low-pressure irrigation, monitoring the extent of absorption and surgery length). TURP syndrome pathophysiology is complex, multifactorial and not completely understood. The pathogenic mechanisms postulated for AKI development include acute hemolysis, renal interstitial edema, ischemic tubular injury, RM and reflux nephropathy resulting from the absorption of irrigating fluid, dilutional hyponatremia and high intra-bladder pressure. A variety of different irrigating fluids are available, but studies in animals, volunteers and patients show that glycine solution should be avoided. Treatment of symptomatic hyponatremia should be based on the administration of hypertonic saline rather than of diuretics. RRT might be necessary in severe AKI.

RM-induced AKI after radical prostatectomy has also been described in a small number of case reports. These reports identified a number of risk factors, such as exaggerated lithotomy position, preexisting CKD, obesity, and surgery longer than 5 h. In patients at high risk for AKI, every effort should be made to ensure correct positioning during surgery. Early diagnosis of RM and aggressive volemic expansion are the keys to the patient's successful recovery.

It is important for nephrologists to know the main aspects of the physiopathology, clinical presentation, treatment and particular characteristics of AKI in the context of prostate surgery. Close collaboration with the urologist and anesthesiology staff is extremely important to allow for the adoption of preventive measures and to detect any earlier, elusive clinical presentations of AKI.

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P- Reviewer: Prakash J, Passadakis PS, Wang F **S- Editor**: Ji FF
L- Editor: A **E- Editor**: Lu YJ

World Journal of Nephrology

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DOI: 10.5527/wjn.v3.i4.210

World J Nephrol 2014 November 6; 3(4): 210-219
ISSN 2220-6124 (online)

REVIEW

Metabolic syndrome and chronic kidney disease: Current status and future directions

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Received: June 25, 2014 Revised: August 26, 2014
Accepted: September 23, 2014
Published online: November 6, 2014

Abstract

Metabolic syndrome (MetS) is a term used to denote a combination of selected, widely prevalent cardiovascular disease (CVD)-related risk factors. Despite the ambiguous definition of MetS, it has been clearly associated with chronic kidney disease markers including reduced glomerular filtration rate, proteinuria and/or microalbuminuria, and histopathological markers such as tubular atrophy and interstitial fibrosis. However, the etiological role of MetS in chronic kidney disease (CKD) is less clear. The relationship between MetS and CKD is complex and bidirectional, and so is best understood when CKD is viewed as a common progressive illness along the course of which MetS, another common disease, may intervene and contribute. Possible mechanisms of renal injury include insulin resistance and oxidative stress, increased proinflammatory cytokine production, increased connective tissue growth and profibrotic factor production, increased microvascular injury, and renal ischemia. MetS also portends a higher CVD risk at all stages of CKD from early renal insufficiency to end-stage renal disease. Clinical interventions for MetS in the presence of CKD should include a combination of weight reduction, appropriate dietary modification and increase physical activity, plus targeting of individual CVD-related risk factors such as dysglycemia, hypertension, and dyslipidemia while conforming to relevant national societal guidelines.

Key words: Metabolic syndrome; Cardiovascular disease; Diabetes; Dialysis; Hyperlipidemia; Hypertension; Microalbuminuria; Obesity; Progression

Core tip: Metabolic syndrome is associated with chronic kidney disease but its role in chronic kidney disease incidence and progression has not been established. When both these conditions are present, management should be targeted to individual risk factors for kidney disease progression and cardiovascular disease.

Prasad GVR. Metabolic syndrome and chronic kidney disease: Current status and future directions. *World J Nephrol* 2014; 3(4): 210-219 Available from: URL: http://www.wjgnet.com/2220-6124/full/v3/i4/210.htm DOI: http://dx.doi.org/10.5527/wjn.v3.i4.210

INTRODUCTION

Metabolic syndrome (MetS), previously called "syndrome X" is a term in popular use for the past quarter century, having first been described in 1988 by Reaven[1] to denote a combination of selected, widely prevalent cardiovascular disease (CVD)-related risk factors. Although the general principle behind using the MetS concept is to denote an increased CVD or diabetes risk, the MetS definition itself is ambiguous through the inclusion of different criteria and assigning them different levels of importance[2-6]. Furthermore, some MetS definitions have been revised over the years, leading to the multiple definitions in current use[2-6]. The four most common definitions are summarized in Table 1. Nonetheless, the general concept is that a MetS patient will have some combination of conditions, among which are insulin resistance or hyperglycemia, dyslipidemia, hypertension, and obesity. Identifying such combinations should add meaningfully to the long-term clinical management of

Table 1 Criteria in definitions of the metabolic syndrome[2-6]

Definition and its criteria	Values
World Health Organization 1998	
Insulin resistance	Type 2 diabetes mellitus or impaired fasting glucose (> 100 mg/dL per 5.6 mmol/L) or impaired glucose tolerance
Plus two of the following:	
Abdominal obesity	Waist-to-hip ratio > 0.9 in men or > 0.85 in women or BMI > 30 kg/m^2
Triglycerides and/or HDL cholesterol	> 150 mg/dL (1.7 mmol/L) and/or < 35 mg/dL (0.9 mmol/L) in men and < 39 mg/dL (1.0 mmol/L) in women respectively
Blood pressure	⩾ 140 mmHg systolic; ⩾ 90 mmHg diastolic
Microalbuminuria	Urine albumin ⩾ 20 μg/min or albumin-to-creatinine ratio ⩾ 30 mg/g
American Heart Association/National Heart, Lung, and Blood Institute (2004)	
Any three of the following:	
Waist circumference	> 102 cm in men and > 88 cm in women
Triglycerides	⩾ 150 mg/dL (1.7 mmol/L)
HDL cholesterol	< 40 mg/dL (1.03 mmol/L) in men and < 50 mg/dL (1.29 mmol/L) in women
Blood pressure	≥ 130 mmHg systolic; ≥ 85 mmHg diastolic
Fasting glucose	⩾ 100 mg/dL (5.6 mmol/L)
International Diabetes Federation 2005	
Central obesity based on ethnicity	Waist circumference for Europeans > 94 cm in men and 80 cm in women; South Asians, Chinese, and Japanese > 90 cm in men and > 80 cm in women; ethnic South and Central Americans use South Asian data; for sub-Saharan Africans and Eastern Mediterranean and Middle East (Arab) populations use European data. Can be assumed if BMI > 30 kg/m^2
Plus two of the following:	
Triglycerides	⩾ 150 mg/dL (1.7 mmol/L)
HDL cholesterol	< 40 mg/dL (1.03 mmol/L) in men and < 50 mg/dL (1.29 mmol/L) in women
Blood pressure	⩾ 130 mmHg systolic; ⩾ 85 mmHg diastolic; treatment of previously diagnosed hypertension
Fasting glucose	⩾ 100 mg/dL (5.6 mmol/L), in which case oral glucose tolerance test is recommended
Harmonized (Consensus) Definition incorporating IDF and AHA/NHLBI definitions (2009)	
Any three of the following:	
Waist circumference	According to population and country-specific definitions
Triglycerides	⩾ 150 mg/dL (1.7 mmol/L)
HDL cholesterol	< 40 mg/dL (1.03 mmol/L) in men and < 50 mg/dL (1.29 mmol/L) in women
Blood pressure	⩾ 130 mmHg systolic; ⩾ 85 mmHg diastolic
Fasting glucose	⩾ 100 mg/dL (5.6 mmol/L) or use of medication

IDF: International Diabetes Federation; AHA/NHLBI: American Heart Association/National Heart, Lung, and Blood Institute.

MetS.

MetS is known to be with increased CVD risk in the general population[7,8]. It has also been associated with incident overt type 2 diabetes[9,10], in those previously without diabetes contributing to their MetS definition. Other MetS associations include non-alcoholic fatty liver disease[11], and hyperuricemia[12]. In addition, the association of MetS with chronic kidney disease (CKD) is receiving increased attention in selected populations[13-15]. CKD however is also a long-term illness, just like MetS, and often progresses over many years from mild reductions in glomerular filtration rate to more advanced preuremic states and eventual renal replacement therapy. CKD is asymptomatic until very late in its course, when symptoms such as fatigue, nausea and anorexia, itching, cramping and muscle twitching, and edema occur. The relationship between MetS and CKD is typically approached as snapshots in isolation during each CKD stage. The major purpose of this review is to approach the MetS-CKD relationship from the standpoint of each stage as points along the CKD spectrum where the diagnosis of MetS may be raised. This may help to better determine whether MetS is either in fact part of the etiology of CKD, the end result of risk factors common to both MetS and CKD, or a completely unrelated entity.

METABOLIC SYNDROME AS AN ASSOCIATION WITH CKD

Many studies associate MetS with CKD. This is perhaps the lowest level of evidence for causation. Each component of MetS has been associated with both CKD incidence and progression. MetS and CKD share a complex, bidirectional relationship. Obesity is associated with CKD[16-18]. Both obesity and CKD are increasing in prevalence, at least in the United States[19]. The earliest stages of CKD are typically missed because of its asymptomatic nature and lack of screening in annual physical examinations. It is therefore difficult to assemble sufficiently large cohorts known to be without CKD based on the appropriate baseline data and then follow them over a sufficient length of time in order to determine whether they have developed early (*i.e.*, stages I or II) CKD or not. This will require measurements of renal function in the normal range that tools such as the Modification

Table 2 Renal associations of metabolic syndrome

Renal outcome	Ref.
eGFR < 60/mL/min per 1.73 m^2	[15,19]
Proteinuria and/or microalbuminuria	[13,22,23,29,30]
Histopathological abnormalities (tubular atrophy, interstitial fibrosis, arterial sclerosis)	[31]
Ultrasound abnormalities (increased intra-renal resistive indices)	[32]

of Diet in Renal Disease (MDRD) equations[20] were not designed to handle.

A meta-analysis of eleven studies[13,14,21-29] of 30146 subjects reported that MetS was associated with development of an estimated GFR (eGFR) < 60 mL/min per 1.73 m^2, (Stage Ⅲ CKD) with odds ratio (OR) 1.55 (95%CI: 1.34-1.80)[19]. Many of these studies specifically excluded those with diabetes[21,22,26-28], which is not only a potential component of MetS, but a major cause of CKD. Not included also was a study[15] from the National Health and Nutrition Examination Survey (NHANES Ⅲ) database of 7800 subjects followed for 21 years, who having had normal renal function at baseline, were found to have an OR of 2.6 (95%CI: 1.68-4.03) for CKD if MetS was present. These authors[15] also determined a relationship between the number of MetS components present and risk. Sometimes, surrogate markers of CKD such as microalbuminuria or proteinuria are used instead[13,22-23,29] using varying definitions for both protein loss and MetS[19]. A small number of studies showed an increase in albumin or protein excretion associated with MetS[19]. Another study from the NHANES database also showed an increase in microalbuminuria with MetS[30].

Despite the large number of such associative studies between MetS and CKD, causality remains unproven[19]. The time-to-onset of both MetS and CKD are equally difficult to determine. Since individual components of MetS are prone to fluctuating values and are sensitive to unmeasured lifestyle modifications, medication effects, or acute illness, it is possible that some proportion of subjects experience change with respect to their MetS status overall during follow-up. Similar changes may occur with eGFR and a CKD diagnosis that is based on arbitrary eGFR cut-offs. Over-simplification of MetS criteria (such as using only body mass index (BMI) while ignoring waist and hip circumference, or systematically ignoring ethnicity), further limits making firm conclusions about associations. Obesity and CKD are increasing in prevalence[19] and this could obfuscate the relationship between two common disease entities. Investigation at the level of the individual subject may help shed light on the association of MetS with CKD.

A histopathology-based cross-sectional report of 146 patients undergoing nephrectomy showed a higher prevalence of CKD features, including global as well as segmental glomerulosclerosis in those with MetS. Other features noted included a higher prevalence of tubular atrophy, interstitial fibrosis, and arterial sclerosis[31]. Loss of renal function post-nephrectomy was more pronounced[31], but sequential biopsy studies are of course not feasible. Another approach is to study intra-renal hemodynamics by ultrasound, wherein renal parenchymal damage in MetS may be reflected by increased intra-renal resistive indices[32]. These novel studies may help us progress beyond making simple associations, but will need prospective evaluation in larger numbers of patients for validation. A summary of important renal associations with MetS is provided in Table 2.

METABOLIC SYNDROME AS AN ETIOLOGY OF CKD

More convincing than association alone would be a mechanistic explanation for MetS as a cause for CKD. The search for mechanisms is essential to remove the "black boxes" that exist along any proposed causal pathway between MetS and CKD. It may be all one linear mechanism that leads from MetS to CKD, or it may equally likely be a number of distinct but inter-dependent mechanisms set in motion by MetS and operating simultaneously to result in significant renal impairment. The mechanisms leading to MetS may also be the same ones causing CKD. In this context, there may be a "perfect storm" of multiple risk factors including insulin resistance, inflammation, abnormal lipid metabolism, and hypertension leading to increased expression of profibrotic factors[33]. Finally, we still cannot exclude chance associations between two otherwise common diseases.

Insulin resistance may be the most important MetS-related etiological factor for CKD. Insulin is an anti-inflammatory hormone. Insulin resistance, which is typical of type 2 diabetes, leads to inflammation, leading to oxidative stress and renal insufficiency[34]. Raised insulin levels stimulate insulin-like growth factor 1 (IGF-1) production, which increases connective tissue growth factor, thus causing fibrosis in the diabetic state[35]. Furthermore, and possibly independently, obesity may lead to increased secretion by adipose tissue of pro-inflammatory cytokines such as leptin, interleukin-6, and tumor necrosis factor-alpha (TNF-α)[36]. Leptin may lead to increased intra-renal expression of transforming growth factor-beta (TGF-β), leading to glomerulosclerosis[37]. It may also promote type IV collagen production[38,39]. TNF-α may lead to the production of reactive oxygen species (ROS) that can in turn lead to renal endothelial cell dysfunction, mesangial expansion and fibrosis[40]. Anti-inflammatory hormones like adiponectin may be reduced[36,41], contributing to insulin resistance as well. Adiponectin deficiency is associated with vascular intima thickening and smooth muscle cell proliferation[42]. Its vascular effects may even be independent of insulin sensitivity[43], and so may extend to CKD. Obesity also leads to increased glomerular volume, podocyte hypertrophy, and mesangial matrix expansion preceding CKD[44]. Triglycerides and free fatty acids may themselves be nephrotoxic by

Table 3 Potential mechanisms of chronic kidney disease in metabolic syndrome

Mechanism	Ref.
Oxidative stress	[34,40]
Increased pro-inflammatory cytokines (leptin, interleukin 6, tumor necrosis factor α)	[36]
Increased connective tissue growth and/or fibrosis factors (connective tissue growth factor, transforming growth factor β, type Ⅳ collagen)	[35,37-39]
Increased glomerular volume and podocyte hypertrophy	[44]
Triglyceride- and free-fatty acid induced injury	45
Increased ischemia and microvascular injury (angiotensin Ⅱ)	[46,47]
Hyperuricemia	[48,49]

promoting pro-inflammatory cytokine production[45]. In association with hypertension, another MetS component, angiotensin Ⅱ stimulates ROS production, in turn decreasing nitric oxide synthase production and causing renal microvascular injury, ischemia, and tubulointerstitial damage[46,47]. Dissecting out the relative contribution of insulin resistance, obesity, and hypertension to these findings versus the composite of MetS however is difficult. In this regard, the presence of early arterial hyalinosis[31] which is more typical of diabetes but not MetS, may point towards MetS being a distinct risk factor for CKD independent of its individual components. One more somewhat provocative hypothesis is that hyperuricemia, not a "traditional" MetS component but associated with MetS[12], is a promoter of CKD through the inhibition of nitric oxide production[48] or even recurrent nephrolithiasis[49]. Another limitation to be pointed is that most mechanistic explanations have been derived from animal models, and so their importance in human patients with MetS and CKD, with their different lifespans and disease profiles remains to be demonstrated. A summary list of possible mechanisms for CKD in MetS is shown in Table 3. Studies mostly support the direction of the relationship to be from MetS to CKD and not vice versa, but this is unconfirmed.

METABOLIC SYNDROME AND PROGRESSIVE CKD

Once CKD is identified, with the understanding that the definition is somewhat arbitrary, monitoring progression becomes more straightforward. Several population-based studies have identified MetS with CKD progression. Once stage Ⅲ or Ⅳ CKD has been reached, the presence of MetS has been associated with a hazard ratio of 1.33 (95%CI: 1.08-1.64) for end-stage renal disease (ESRD) over a follow-up period of just 2-3 years in a cohort of over 15000 patients[50]. In particular, impaired glucose metabolism, hypertriglyceridemia, and hypertension were associated with an increased risk of ESRD. Similarly, an incremental increase in insulin resistance was associated with a greater rate of decline in renal function in a cohort of elderly patients with CKD[51]. On the other hand, it was demonstrated that the relationship of MetS to CKD may not be constant over the progression through CKD stages. In the later stages of CKD, MetS as a risk factor for progression may become less important[52], perhaps because CKD itself leads to rapid progression in a form of vicious circularity. Also, greater attention may be paid to MetS in the later stages of CKD, so that its impact becomes less prominent. Another study showed that even though MetS was associated with albuminuria, the effect of MetS on CKD progression was independent of this[53]. This is controversial however, since proteinuria is a known risk factor for CKD progression to ESRD and is also a component of some MetS definitions. Despite the greater than 30% risk with MetS, adjustment for proteinuria attenuated the risk for development of a composite endpoint of significant decrease in GFR, ESRD, or death in the African American Study of Kidney Disease and Hypertension trial[54].

The distinction between CKD incidence and progression may be arbitrary since renal insufficiency must first progress to the point where CKD is diagnosed at some threshold level of renal function. If the underlying progression is left unchecked however and the new pathophysiology of CKD becomes established, then the risk factors common to CKD and MetS combine to accelerate CKD progression. First, obesity-related glomerular hyperfiltration could combine with that induced by CKD itself, leading to accelerated glomerulosclerosis. Second, inflammation and oxidative stress are worsened in CKD[55]. Hypertension and hypertriglyceridemia are worsened[55], and insulin resistance may be promoted by the undernourished state that can be caused by CKD as well as lead to CKD[56,57]. This relationship is thus bidirectional. Third, this insulin resistance may combine with inflammation to cause "endoplasmic reticulum stress". According to this theory, misfolded proteins accumulate in the lumen of the endoplasmic reticulum, suppressing insulin secretion through phosphorylation of the insulin receptor substrate (IRS-1)[58]. Finally, insulin resistance also worsens renal hemodynamics through increasing sodium retention, and affecting the transport of other cations and anions[59]. Hypertension is worsened, leading to further renal damage. Similarly, the sympathetic nervous system is activated[60], leading to unfavorable renal hemodynamics, proteinuria, and ischemia. Proteinuria itself may lead to podocyte injury, and eventually lead to chronic tubulointerstitial injury, thereby worsening CKD[61]. Unless cardiovascular mortality intervenes, progression to ESRD may occur.

Role of cardiovascular disease in progression of metabolic syndrome-related CKD

Even mild CKD has been associated with increased CVD risk[62]. CVD mortality also increases with increasing serum creatinine concentrations[62]. Advanced CKD is also a high risk situation for cardiovascular events and mortality. In one study of stage Ⅳ or Ⅴ CKD patients, MetS was predictive of a composite of CVD mortality, acute coronary syndrome (ACS), revascularization, non-

fatal stroke, and amputation (hazard ratio 2.46, 95%CI: 1.17-5.18)[63]. In this study of 200 patients, intensive risk factor modification was not effective[63]. Coronary heart disease is promoted by the components of MetS. Patients with both MetS and CKD exhibit greater coronary artery plaque burden with higher lipid content, as demonstrated by intravascular ultrasound[64]. With renal insufficiency, myocardial infarction (MI) in the context of MetS is associated with higher mortality at one year[65]. In over 900 patients undergoing carotid revascularization where 14% had some degree of CKD, MetS increased the risk for stroke, MI, and death[66].

The patient with MetS and progressive CKD treads a dangerous path towards ESRD. Besides being simply associated with CKD, MetS may also lead to CKD through a variety of pathophysiological mechanisms. MetS may also lead to more rapid CKD once it is established. Both MetS and CKD in turn are associated with increased risk for CVD events, and when both occur together the effect may be additive. It stands to reason that ACS and MI are major contributors to all-cause mortality seen when these two common conditions are combined, regardless of whether MetS leads to CKD or both are independently acquired. Furthermore, it is likely that ACS and MI themselves lead to acute kidney injury and acceleration of CKD. This could happen as a result of acute shifts in effective intravascular volume or contrast exposure, for example. Increased mortality effectively prevents progression of CKD to ESRD, so it is reasonable to speculate that fewer patients with MetS will actually reach ESRD.

METABOLIC SYNDROME AND RENAL REPLACEMENT THERAPY

Many patients receiving hemodialysis (HD) also have MetS. The mortality rate is very high in the initial few months after HD initiation both in the United States[67] and worldwide[68]. A significant proportion of this mortality is attributable to CVD, related to existing cardiovascular comorbidities[69]. Therefore, patients with MetS starting chronic HD are at increased risk for major cardiovascular events and mortality. The prevalence of MetS is quite high (often exceeding 50%) in HD cohorts worldwide[70-72]. Interestingly, the presence of MetS has even been extended to their relatives[73]. Longer-term follow-up also indicates a higher incidence of cardiovascular events[74] and high rate of hospitalization[75]. Other co-morbid conditions may co-exist with MetS in HD patients. A higher prevalence of moderate-to-severe periodontal disease has been reported[76]. However, significant correlations were not noted in dialysis patients with reduced bone mineral density[77], quality of life[78], or mood[78].

Several pathophysiological mechanisms may be operational during hemodialysis that could further exacerbate the effects of MetS. Besides insulin resistance, hyperlipidemia, and hypertension carried over from the pre-dialysis phase of CKD, further inflammation and oxidative stress may result from dialysis treatment itself. There is a loss of antioxidants and increase in leukocyte activation during dialysis[79-82]. This may occur through loss of vital antioxidants through the hemodialysate, or reactions to semi-synthetic dialysis membranes, or both. Dialysis patients are also prone to infections further promoting inflammatory stress. Logistical difficulties in hemodialysis in obese patients, such as vascular access difficulties leading to suboptimal renal functional replacement may promote inflammation. Chronic volume expansion promotes worsening hypertension, further adding to morbidity. Insulin resistance, even in those without diabetes, may also lead to chronic malnutrition as part of an overall catabolic condition[83].

MetS has an impact on patients on peritoneal dialysis (PD) as well. When glucose-containing solutions are used there is systemic glucose absorption via the peritoneal membrane, leading to increased intra-abdominal fat[84] consistent with MetS. The increased glucose load increases serum LDL cholesterol and triglyceride concentrations, which when combined with hypertension and volume overload, may increase CVD[85]. MetS increases patient mortality on PD[86,87] and also decreases PD technique survival in patients on PD for at least three months[87]. Technique failure and subsequent conversion to HD is also a stressful state that can cause inflammation. MetS has been associated with an elevated white blood cell count and C-reactive protein level, independently of infection but consistent with inflammation[88]. MetS is also associated with lower circulating adiponectin levels in PD[89], and this may increase CVD risk.

TREATING THE METABOLIC SYNDROME IN THE PRESENCE OF CKD

There is an obvious clinical need to reduce CKD morbidity and mortality, and MetS seems to be an easily identified target for intervention. However, the approach is far from precise. Randomized clinical trials in CKD patients are few and are limited by small sample sizes. Renal outcomes are often not described as the primary outcomes. Studies of CKD prevention or progression require large numbers of patients followed over long periods of time to gather sufficient CVD or ESRD outcomes, and if MetS is added as an inclusion criterion, recruitment difficulties are exacerbated. There is a shortage of high quality randomized, controlled trials in nephrology generally[90]. Nonetheless, targeting MetS as a risk factor for CVD, for which CKD patients are at risk is certainly reasonable.

An initial approach should include some combination among weight reduction, dietary modification, and increased physical activity[91], preferably all three. A small clinical trial of 38 patients with MetS but without CKD, randomized to dietary weight loss, weight loss plus aerobic exercise, or no treatment was able to dem-

Table 4 Possible clinical interventions for metabolic syndrome in chronic kidney disease[a,b]

Clinical intervention	Ref.
Lifestyle modification: weight reduction, dietary adjustment (calorie and phosphate reduction), increased physical activity, and/or smoking cessation	[91-94]
Weight loss medication (orlistat) or surgery	[95,97]
Lipid-lowering medication (statins, fibrates)	[96,104,105]
Blood pressure-lowering medication (renin-angiotensin system antagonists)	[100]
Blood glucose-lowering medication (metformin, thiazolidinediones)	[102,103]

[a]It is recommended that individual national society guidelines be followed in the management of individual metabolic syndrome components; [b]Interventions are individualized and used in combination.

onstrate a relationship between weight loss, albuminuria reduction, and improvement in eGFR, augmented by exercise[92]. An observational analysis of PREMIER, a randomized trial of blood pressure lowering in obese subjects but again without CKD, showed a relationship between reduction in waist circumference and urinary albumin excretion[93]. A decrease in phosphate intake may be beneficial as well[93]. It is unclear if these results indicate an improvement in incipient renal disease, or an improvement in systemic endothelial dysfunction manifest as microalbuminuria. Achieving weight loss without a corresponding loss in muscle mass may be difficult to achieve in CKD, especially ESRD. In a sample of 2288 participants with CKD from the NHANES Ⅲ survey, regular physical activity but not diet was associated with decreased mortality[94]. Although unproven, it is likely that patients with both CKD and MetS will especially benefit. A multi-disciplinary approach that involves an exercise specialist to ensure regular physical activity and a dietician to achieve the goal of weight loss through reduced calorie intake, while avoiding malnutrition at the same time is preferable. Hospitalizations are likely to lead to setbacks. Smoking cessation has been associated with reduced mortality in CKD[94]. Measurement of waist circumference or the waist-to-hip ratio may allow for better compliance with existing MetS definitions[2-6] both for diagnosis and follow-up.

Both pharmacotherapy and surgical procedures for weight loss in patients with MetS have been explored. It is unclear if drugs prescribed for weight loss have significant adverse effects on renal function. Orlistat may be beneficial for MetS in the general population[95]. Fibrates on the other hand may worsen renal function[96], and could be harmful in patients with MetS and CKD. Pharmacotherapy needs to be combined with lifestyle modification in order to have significant effect, and would not be recommended in the absence of clinical trial data pertinent to CKD. Bariatric surgery has been shown to improve MetS parameters and also decrease mortality in the general population[97], and also reduce albuminuria[98]. However, the ability of CKD patients to recover from major surgery needs to be considered. Clinical trials in CKD patients are needed before this can be recommended.

Blood pressure control reduces CVD risk and CKD progression, and so relevant national guidelines for blood pressure targets and therapeutic agents should be followed depending on the presence or absence of CKD and/or diabetes, in the absence of specific guidelines for MetS patients. Thiazides may worsen MetS, perhaps through hyperuricemia, hypokalemia, and diabetes[99]. Renin-angiotensin system antagonists may prevent new-onset diabetes[100]. This may be considered for those with MetS who have not yet developed diabetes. MetS is associated with increased sympathetic activity, and so renal denervation has been considered when hypertension is part of MetS[101]. However, the value of this procedure for achieving sustained blood pressure reduction is controversial.

Management of dysglycemia requires special attention in the context of MetS. Metformin is associated with improved insulin resistance and endothelial function[102]. However, metformin is not used in more advanced CKD due to concerns surrounding lactic acidosis. Thiazolidinediones may also be considered[103], but their side effect profile deserves special attention. They may improve endothelial function as well as have anti-inflammatory effects[103]. Weight loss may also help improve glycemic control, but dietary conflicts among diabetes-related restrictions (such as carbohydrates) and CKD-related restrictions (potassium and phosphorus) may be especially problematic in MetS where total caloric intake must also be reduced and protein intake maintained. Specialized dietician input is again required.

Finally, the use of statins in MetS requires consideration. Statins may reduce proteinuria[104], either through improved endothelial function or reduction in systemic inflammation[104,105]. Success with statins in reducing cardiovascular events in ESRD[106] has been variable.

A summary of possible therapeutic interventions for MetS in the context of CKD is provided in Table 4. In the absence of firm data, relevant national guidelines should be followed for each individual cardiovascular or CKD-related risk factor.

CONCLUSION

At this point, it remains unclear whether MetS adds further cardiovascular risk to that conferred by CKD alone. Further research is first required to firmly establish the link between MetS and incidence of CKD in the first instance, and then between MetS and the progression of CKD. A single large, prospective clinical trial in human subjects that addresses both CKD incidence and progression in established CKD will help to provide the necessary justification for MetS intervention. However, the major clinical concern surrounding MetS is its association with CVD. A prospective clinical trial of intervention targeted to multiple MetS parameters in CKD would help address whether MetS is more than the sum of its parts in the context of CKD. Until then, we are

left with using surrogate markers such as proteinuria or microalbuminuria for both CKD and CVD. Statins, fibrates, and renin-angiotensin system antagonists allow for targeting specific MetS components including diabetes, hyperlipidemia, hypertension, and microalbuminuria. In combination with aggressive lifestyle modification, there is potential in the meantime for reducing MetS, CKD, and CVD mortality. Beneficial snapshot effects may be found in the literature for a particular intervention in one CKD sub-population and not another, such as in the case of statins. However, viewing CKD as a longitudinal construct allows for better understanding of the pathophysiology of CVD and CKD progression with MetS, and may thus allow for more rational therapeutic choices.

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P- Reviewer: Faienza MF, Gagliardi G, Khattab MA, Masaki T
S- Editor: Ji FF **L- Editor**: A **E- Editor**: Lu YJ

World Journal of Nephrology

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DOI: 10.5527/wjn.v3.i4.220

World J Nephrol 2014 November 6; 3(4): 220-229
ISSN 2220-6124 (online)

REVIEW

Chronic kidney disease and erectile dysfunction

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Received: May 20, 2014 Revised: June 22, 2014
Accepted: September 6, 2014
Published online: November 6, 2014

Abstract

Erectile dysfunction (ED) is a common condition among male chronic kidney disease (CKD) patients. Its prevalence is estimated to be approximately 80% among these patients. It has been well established that the production of nitric oxide from the cavernous nerve and vascular endothelium and the subsequent production of cyclic GMP are critically important in initiating and maintaining erection. Factors affecting these pathways can induce ED. The etiology of ED in CKD patients is multifactorial. Factors including abnormalities in gonadal-pituitary system, disturbance in autonomic nervous system, endothelial dysfunction, anemia (and erythropoietin deficiency), secondary hyperparathyroidism, drugs, zinc deficiency, and psychological problems are implicated in the occurrence of ED. An improvement of general conditions is the first step of treatment. Sufficient dialysis and adequate nutritional intake are necessary. In addition, control of anemia and secondary hyperparathyroidism is required. Changes of drugs that potentially affect erectile function may be necessary. Further, zinc supplementation may be necessary when zinc deficiency is suspected. Phosphodiesterase type 5 inhibitors (PDE5Is) are commonly used for treating ED in CKD patients, and their efficacy was confirmed by many studies. Testosterone replacement therapy in addition to PDE5Is may be useful, particularly for CKD patients with hypogonadism. Renal transplantation may restore erectile function. ED is an early marker of cardiovascular disease (CVD), which it frequently precedes; therefore, it is crucial to examine the presence of ED in CKD patients not only for the improvement of the quality of life but also for the prevention of CVD attack.

Key words: Erectile dysfunction; Chronic kidney disease; Nitric oxide; Phosphodiesterase type 5; Testosterone

Core tip: Erectile dysfunction (ED) is a common condition in chronic kidney disease (CKD) patients. The etiology is multifactorial. Phosphodiesterase type 5 inhibitors are commonly used for the initial treatment. ED has gained attention as an early marker for cardiovascular disease (CVD), which it frequently precedes. Therefore, it is pivotal to examine the presence of ED in CKD patients not only for the improvement of quality of life but also for the prevention of CVD attack. The pathophysiology of erection, which most nephrologists are not familiar with, is also discussed.

Suzuki E, Nishimatsu H, Oba S, Takahashi M, Homma Y. Chronic kidney disease and erectile dysfunction. *World J Nephrol* 2014; 3(4): 220-229 Available from: URL: http://www.wjgnet.com/2220-6124/full/v3/i4/220.htm DOI: http://dx.doi.org/10.5527/wjn.v3.i4.220

INTRODUCTION

Erectile dysfunction (ED) is defined as an inability to

attain and/or maintain penile erection sufficient for satisfactory sexual performance. It is now a common condition and approximately 150 million males worldwide are estimated to suffer from ED[1]. The prevalence of ED in 2025 is projected to be approximately 300 million worldwide[2]. It is well known that age, metabolic disorders (hypertension, diabetes, and hyperlipidemia), and smoking are major risk factors for ED. Recently, chronic kidney disease (CKD) has also gained attention as a risk factor for ED. Although CKD causes sexual dysfunction in both genders, this review article focuses on the role of CKD in the development of ED. We discuss the etiology and treatment of ED in CKD patients.

PREVALENCE OF ED IN CKD PATIENTS

The prevalence of ED in the United States male population aged > 50 years (Participants: 31,742 men, age 53-90 years) was reported to be 33%[3], whereas that in the Turkish male population aged > 40 years (Participants: 2158 men) was 69.2%[4]. However, the prevalence was 36% when mild ED cases were excluded. Navaneethan *et al*[5] reported in their meta-analysis study that the prevalence of ED in CKD patients was 70% on average. Furthermore, Mesquita *et al*[6] reported that the prevalence of ED in CKD outpatients with stages 3, 4, and 5 was 72.3%, 81.5%, and 85.7%, respectively. Nassir reported that the prevalence of ED in patients just entering dialysis programs was 82.7%[7]. Thus, it is observed that ED frequently occurs in CKD patients.

BLOOD SUPPLY TO THE PENIS

The blood supply to the penis originates predominantly from the internal pudendal artery, which branches into the penile artery. The penile artery then branches into the cavernous arteries. The cavernous artery enters the cavernous body and subsequently divides into many branches called the helicine arteries, which open into the cavernous sinuses. Blood in the cavernous sinuses is drained by the subtunical veins that form the venous plexuses just beneath the tunica albuginea and then returns to the circulation *via* 3 sets of veins; the superficial, intermediate and deep veins.

PATHOPHYSIOLOGY OF PENILE ERECTION

Penile erection and detumescence are regulated by relaxation and contraction, respectively, of the smooth muscle located in the arteries and the cavernous body. In the flaccid state, the sympathetic nervous system is dominant, and the arterial and corporal smooth muscle is tonically contracted. As a result, only a minimal amount of blood flows through the cavernous artery into the cavernous body. After sexual stimulation, parasympathetic activity causes a decrease in the peripheral resistance due to vasodilatation, and the blood flow through the cavernous and helicine arteries increases. The intracavernous pressure increases without any increase in the systemic pressure. In the full erectile state, increased blood volume in the cavernous body and the following compression of the subtunical drainage veins against the rigid tunica albuginea lead to a reduction in the venous outflow (referred to as the veno-occlusive mechanism), and therefore, high intracavernous pressure is maintained. However, when the corporal smooth muscle is unable to relax sufficiently and/or the corporal tissue loses its normal compliance, the increased intracavernous pressure during erection cannot adequately compress the subtunical veins, resulting in the leakage of blood out of the cavernous body during erection. This is a major cause of ED and is referred to as the corporal veno-occlusive dysfunction (CVOD). CVOD occurs when the smooth muscle content decreases and/or when the collagen content increases in the cavernous body[8]. Therefore, the ratio of the smooth muscle content to the collagen content in the cavernous body decreases in CVOD.

REGULATION OF PENILE SMOOTH MUSCLE CONTRACTION

Detumescence of the penis is predominantly mediated by adrenergic nerve terminals whose neurotransmitter, norepinephrine, activates adrenergic receptors on the penile smooth muscle. The contraction of penile arteries and trabecular smooth muscle is largely mediated by α-1 adrenergic receptors[9,10]. Other vasoconstrictors including endothelin-1, prostaglandin F2α, thromboxane A2 and angiotensin II are also implicated in the contraction of smooth muscle in the penis[11-13].

REGULATION OF PENILE SMOOTH MUSCLE RELAXATION

Dilatation of the cavernous artery and helicine arteries is the first event in the development of an erection. The blood flow and pressure increase in the cavernous sinuses, and subsequently, smooth muscles surrounding the trabeculae relax, resulting in further expansion and accumulation of blood in the cavernous body. It is now well established that nitric oxide (NO) plays a pivotal role in the initiation and maintenance of erection. NO acts through the stimulation of the soluble guanylate cyclase, which mediates the subsequent formation of cyclic-GMP (cGMP). cGMP activates protein kinase G (PKG), and PKG is implicated in the relaxation of smooth muscle. cGMP is inactivated by phosphodiesterase type 5 (PDE5), which is predominantly located in the cavernous smooth muscle and is the target of PDE5 inhibitors (PDE5Is) such as sildenafil and vardenafil. NO synthase (NOS) uses the amino acid L-arginine and molecular oxygen to produce NO. Three distinct isoforms of NOS have been identified. Two constitutive forms, neuronal NOS (nNOS) and endothelial NOS (eNOS), are present in the

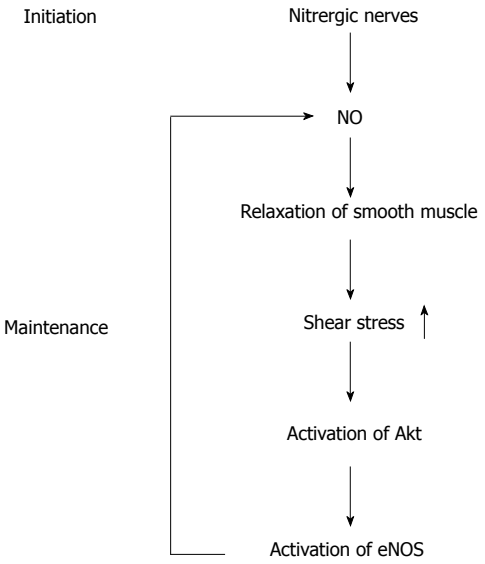

Figure 1 Nitric oxide is critically implicated in the initiation and maintenance of penile erection. NO: Nitric oxide; eNOS: Endothelial nitric oxide synthase.

nervous system and vascular endothelial cells, respectively. A third isoform, inducible NOS (iNOS) is expressed in a variety of cells in response to inflammatory mediators and bacterial products. The isoforms nNOS and eNOS are expressed in the autonomic nerves and endothelium of the penis, respectively[14-17]. Under physiological conditions, iNOS is not expressed in the penis. Postganglionic parasympathetic nerves, which express nNOS and release NO as a cotransmitter with acetylcholine, are now termed nitrergic nerves[17,18]. The stimulation of the cavernous nerve activates nitrergic nerve fibers and elicits NO release at the nerve terminals, which causes relaxation of penile smooth muscle. The functional role of NO released from the nitrergic nerve termini during the relaxation of penile smooth muscle has been demonstrated in many studies in which penile erection induced by stimulation of the cavernous nerves or the spinal cord can be inhibited by NOS inhibitors[14,19-21]. The role of eNOS in erection has also been studied. One possibility was that acetylcholine released from postganglionic cholinergic nerves evoked the release of NO from the endothelium to induce endothelium-dependent relaxation of the penile smooth muscle. However, atropine, a competitive inhibitor of the muscarinic effect of acetylcholine, did not inhibit cavernous nerve-induced penile erection[14]. Furthermore, neurogenic relaxation of the cavernous body does not require a functional endothelium[22,23], suggesting that acetylcholine-induced endothelium-dependent relaxation of the smooth muscle is not required for cavernous nerve-induced penile erection. A second possibility was the activation of eNOS by shear stress. During erection, an increased blood flow on the luminal surface of the penile artery and cavernous sinuses can cause shear stress, which may lead to the activation of protein kinase Akt (also known as Protein kinase B) and subsequent phosphorylation and activation of eNOS, facilitating NO release from the endothelium. Hurt *et al*[24] demonstrated that both electrical stimulation of the cavernous nerve and direct intracavernosal injection of a vasorelaxant drug, papaverine, caused a rapid increase in the phosphorylation and activation of Akt and eNOS. The authors also showed that penile erection elicited by papaverine is significantly reduced in eNOS gene knockout mice. They proposed a model in which the rapid, brief activation of nNOS initiates the erectile response, whereas Akt-dependent phosphorylation and activation of eNOS are necessary for sustained NO production and maximal erection (Figure 1).

POSSIBLE CAUSES OF ED IN CKD PATIENTS

Most studies in this field have been performed using dialysis patients and renal transplant recipients. Little data exist on the etiology and treatment of ED in CKD patients before entering a dialysis program.

Hormonal abnormalities

Chronic renal failure (CRF) is associated with impaired spermatogenesis, and it often results in infertility[25]. In addition, testes develop endocrine dysfunction. Total and free testosterone levels are typically reduced, although the binding capacity and concentration of sex hormone-binding globulin are normal[26-28]. Serum luteinizing hormone (LH) level increases in CRF patients, and testosterone secretion in response to acute administration of human chorionic gonadotropin (HCG), a compound with LH-like actions, shows a blunted response, suggesting that the testosterone-producing Leydig cells have low responsiveness to LH and that this is the primary cause of low testosterone levels in CRF[29]. Interestingly, a factor capable of blocking the LH receptor *in vitro* has been identified in uremic serum, providing an explanation for the blunted response of Leydig cells to infusion of HCG. This blocking activity is inversely correlated with GFR and almost disappears after renal transplantation[30]. In addition, follicle-stimulating hormone (FSH) secretion increases in men with CRF. FSH release from the pituitary gland is negatively regulated by inhibin, a peptide product of Sertoli cells that are located in the convoluted seminiferous tubules. FSH concentration appears to increase in uremic patients because of the damage to seminiferous tubules, resulting in the suppression of inhibit production[31].

Testosterone is required not only for libido but also for the maintenance of the normal morphology and function of the penis. Testosterone deficiency leads to the loss of smooth muscle in the cavernous body and its replacement with collagen fibers[32,33]. This may result in CVOD. It has also been demonstrated that the activity of nNOS and PDE5 are positively regulated by testosterone[32].

Elevated plasma prolactin levels are commonly found in CRF[34]. Increased production is the main cause because the kidney plays little, if any, role in its catabolism. Secondary hyperparathyroidism may be implicated in the increased prolactin secretion in CRF because an infusion

of parathyroid hormone (PTH) in healthy men enhances prolactin release[35]. Depletion of zinc reserves may also play a role in uremic hyperprolactinemia[36]. Hyperprolactinemia induces the loss of libido and low serum testosterone levels[37], which may cause ED.

Endothelial dysfunction

It is now well known that CKD is a risk factor for cardiovascular disease (CVD)[38,39]. Endothelial dysfunction is an early marker of CVD, and has also been reported to occur in CKD patients[40-42]. In addition, endothelial dysfunction is a cause of ED, because NO production from the endothelium decreases in this state. Therefore, it is not surprising that ED frequently occurs in CKD patients. Furthermore, CKD patients often suffer from metabolic diseases such as hypertension, hyperlipidemia, and diabetes. Diabetes is a major cause of CKD. These metabolic diseases also cause endothelial dysfunction and are risk factors for ED. Therefore, in addition to the concomitant metabolic diseases, CKD *per se* appears, at least in some part, to cause ED via the induction of endothelial dysfunction.

Disturbance in the autonomic nervous system

Autonomic neuropathy occurs in end-stage renal disease and can be a cause of ED[43,44]. It is well known that autonomic neuropathy is a common complication of diabetes, and it can be a cause of ED in CKD patients.

Anemia and erythropoietin deficiency

Erythropoietin (Epo) has been widely used to treat anemia in uremic patients. Several reports have demonstrated that treatment with Epo improved erectile function in dialysis patients[45-47], suggesting that anemia and/or Epo deficiency are implicated in ED. The mechanism by which Epo restores erectile function remains unclear. Epo normalized the increased serum prolactin level in early studies[45,48], but this finding was not confirmed by other studies[49-51]. Moreover, Epo increased serum testosterone levels in some studies[51,52]; however, this finding was again not confirmed by other studies[45,46,49,50]. Allaf *et al*[53] examined the effects of Epo on the recovery of erectile function in a rat model of cavernous nerve injury and found that Epo restored erectile function. They also found that Epo stimulated axonal regeneration of the injured cavernous nerve. Therefore, Epo may stimulate the regeneration of the cavernous nerve. Epo reportedly has protective effects against ischemic damages *via* its anti-apoptotic activity[54-59]. Therefore, Epo may protect the cavernous body against injuries *via* its anti-apoptotic activity. Furthermore, the receptor for Epo is expressed on vascular endothelial cells (VECs) and Epo stimulates the proliferation and migration of VECs[60,61]. Epo is also capable of mobilizing endothelial progenitor cells (EPCs) from the bone marrow[62,63]. EPCs were originally isolated from human peripheral blood[64]. EPCs are progenitor cells whose differentiation potential is restricted to VECs. They were incorporated in the capillaries and small arteries of ischemic tissues *in vivo* and expressed markers for VECs such as CD31 when introduced into the circulation using a hindlimb ischemia model[64], suggesting their involvement in the stimulation of angiogenesis. Several studies have reported that the number of circulating EPCs decreased in ED patients[65-67]. These data suggest that Epo may restore erectile function *via* its proangiogenic activity. In summary, Epo has nerve-protective, anti-apoptotic, and proangiogenic activities, at least in animal models, and these activities may be implicated in Epo-induced restoration of erectile function. It is likely that Epo restores erectile function via interaction with its receptors on cells such as nerves and VECs rather than on red blood cells with a resultant improvement in anemia.

Vitamin D deficiency and secondary hyperparathyroidism

Although no conclusive data have been published, Massry *et al*[68] reported that a decline in serum PTH concentration by treatment with 1,25(OH)$_2$ vitamin D$_3$ correlated with the recovery of erectile function in dialysis patients. It was also reported that PTH administration increased serum prolactin concentration[35]. Therefore, it is possible that secondary hyperparathyroidism is implicated in erectile dysfunction in dialysis patients.

Drugs

Many drugs used for CKD patients potentially cause ED. Common examples are anti-hypertensive drugs including diuretics, agonists for α-2 adrenergic receptors, and beta-blockers. Other examples are cimetidine, tricyclic antidepressants, and metoclopramide.

Depression

The prevalence of depression among dialysis patients has been estimated to be 20%-30%[69-71]. Several studies demonstrated that depression is an independent risk factor for ED[72,73].

Zinc deficiency

Several reports demonstrated that oral zinc supplementation restored erectile function, which was associated with an increase in serum testosterone concentration[74,75]; however, some negative effects of zinc supplementation on erectile function were also reported[76]. Possible causes of ED in CKD patients are summarized in Table 1.

ED AS AN EARLY MARKER FOR CVD

Because of the high prevalence of ED among CVD patients, ED was traditionally regarded as a secondary complication of CVD. Recently, ED has gained attention as an early marker of CVD, because ED often precedes the occurrence of CVD. The Prostate Cancer Prevention Trial was a prospective, randomized, and placebo-controlled trial to assess whether finasteride decreased the prevalence of prostate cancer[77]. Finasteride is an

Table 1 Possible causes of erectile dysfunction in chronic kidney disease patients

Abnormalities in the gonadal and pituitary systems
Testosterone↓
LH↑, FSH↑
Prolactin↑
Endothelial dysfunction
Hypertension, diabetes, hyperlipidemia
Autonomic neuropathy
Anemia (Erythropoietin↓)
Secondary hyperparathyroidism
Drugs
Diuretics
Agonists for α-2 adrenergic receptors and β-blockers
Cimetidine
Tricyclic antidepressants
Depression
Zinc deficiency

LH: Luteinizing hormone; FSH: Follicle-stimulating hormone.

inhibitor of 5α-reductase, and inhibits the conversion of testosterone to dihydrotestosterone, which is the primary androgen in the prostate. Participants were regularly monitored for overall health, including cardiovascular events and sexual function. Data from 9457 men randomized to the placebo group in this trial were analyzed to assess the hypothesis that ED is an early marker of patients with occult CVD[78]. At entry to the study, 8063 (85%) men had no CVD; of these men, 3816 (47%) patients reported some level of ED. Among the 4247 men without ED at study entry, 2420 men (57%) reported an incident ED after 5 years, and this incidence increased to 65% at 7 years. Incidents of ED were significantly associated with subsequent angina, myocardial infarction, or stroke; hazard ratio after adjustment was 1.25. Several other studies also confirmed this finding that ED often precedes the onset of CVD[79-81]. Furthermore, ED has been recognized as an early marker for silent coronary artery disease (CAD). Gazzaruso *et al*[82] examined the prevalence of ED in 133 uncomplicated type 2 diabetic men with angiographically verified silent CAD and in 127 diabetic men without myocardial ischemia[82]. The groups were comparable for age and diabetes duration. The prevalence of ED was significantly higher in patients with silent CAD than in those without silent CAD (33.8% *vs* 4.7%, $P = 0.000$). Significant risk factors for silent CAD were identified using multiple logistic regression analysis. These risk factors included ED, apolipoprotein (a) polymorphism, smoking, microalbuminuria, HDL, and LDL. Interestingly, among these risk factors, ED was the strongest predictor of silent CAD (odds ratio 14.8). García-Malpartida *et al*[83] also examined the association between ED and silent myocardial ischemia (SMI) in 154 type 2 diabetic patients without a clinical evidence of CVD and demonstrated that ED was significantly associated with SMI (18.1% in patients with ED *vs* 4.1% in patients without ED, $P = 0.018$). Therefore, ED should be examined carefully in CKD patients not only for the improvement of their quality of life but also for the prevention of CVD.

TREATMENT

Sufficient dialysis and adequate nutritional intake are necessary to improve the general condition of uremic patients. In addition, control of anemia using Epo and control of secondary hyperparathyroidism using phosphate binders, an active form of vitamin D and/or cinacalcet hydrochloride are required. Zinc supplementation may be necessary when zinc deficiency is suspected. If a psychological problem is suspected, psychotherapy and/or antidepressant medications may be necessary.

PDE5Is

PDE5Is are inhibitors of PDE5 and suppress the degradation of cGMP, thereby stimulating the relaxation of smooth muscle in the cavernous body. Many studies have demonstrated the efficacy of PDE5Is for the treatment of ED in dialysis patients and in renal transplant recipients[84-90]. Although headache, flushing, and dyspepsia are the most common adverse effects[91], PDE5Is were well tolerated among dialysis patients in these studies. Among PDE5Is, sildenafil without dose adjustment has been used to treat ED in dialysis patients in several studies. However, it may be safer to start with half the dose (25 mg) and subsequently increase it up to 100 mg, depending on the patients' responses. Special care should be taken when PDE5Is are administered to patients with cardiovascular or hepatic diseases.

Testosterone replacement therapy

Although testosterone replacement therapy is generally effective for patients with low circulating levels of testosterone when causes of ED are other than CKD, the administration of testosterone to uremic men usually fails to restore libido or potency, despite increased testosterone levels[92,93]. However, one pilot study demonstrated that treatment with testosterone gel improved erectile function in hypogonadal hemodialysis patients[94]. Testosterone stimulates an increase in NO production and degradation of cGMP, because it reportedly increases the activities of nNOS and PDE5 simultaneously[32,95,96]. Thus, the stimulatory effect of testosterone on NO production may be negated by its stimulatory effect on PDE5 activity. In this regard, combination therapy of testosterone and PDE5Is may be more effective than treatment with either testosterone or PDE5Is alone. Indeed, several reports demonstrated the efficacy of combination therapy on erectile function in hypogonadal men who did not respond to PDE5Is[97-100]. The efficacy of the combination therapy was also reported in dialysis patients and renal transplant recipients[101]. However, a recent randomized, double-blind, placebo-controlled trial did not show a significant effect of the addition of testosterone to sildenafil therapy on erectile function[102]. Therefore, the efficacy of the combination therapy is still controversial.

Other treatments for ED

Other options for the treatment of ED include injecting prostaglandin E1 into the shaft of the penis, vacuum constriction devices and constriction bands, and penile prostheses. These treatments are beyond the scope of this review, and have not been discussed in detail.

EFFECT OF RENAL TRANSPLANTATION ON ERECTILE FUNCTION

It is well recognized that dialysis therapy does not improve sexual function[103,104]. Several reports demonstrated the improvement of erectile function after renal transplantation[104-106]. Nassir performed a prospective study in which the erectile function of 52 patients undergoing dialysis therapy was analyzed before and after renal transplantation[104]. No improvement of erectile function was observed in patients during dialysis therapy, whereas renal transplantation significantly improved erectile function. Akbari *et al*[107] examined the effect of renal transplantation on sperm quality and sex hormone levels. The authors found that sperm motility significantly improved, although morphology and sperm count did not change significantly. They also found that the level of testosterone significantly increased, whereas levels of FSH, LH and prolactin significantly decreased after renal transplantation. Furthermore, erectile function was compared between patients on dialysis therapy and renal transplant recipients in several studies, and erectile function was reportedly better in renal transplant recipients[108-110]. However, ED is still common in renal transplant recipients (approximately 50%)[111,112], and the prevention of the occurrence of CVD seems necessary in these patients to maintain erectile function[113,114].

STUDY LIMITATIONS

Most studies on this topic collect information from patients on dialysis therapy and renal transplant recipients. Little reliable data exist with regard to the prevalence, etiology, and treatment of ED in CKD patients before starting dialysis therapy. Future studies are required to elucidate these points.

CONCLUSION

ED is a very common disease in CKD patients, and it is a multifactorial disease whose causes include hormonal, metabolic, nutritional, and psychological factors. PDE5Is are commonly used during treatment. Testosterone replacement therapy together with PDE5Is may be useful, particularly for CKD patients with hypogonadism. Renal transplantation may restore erectile function, particularly for young patients. ED is an early marker for CVD and it precedes the occurrence of CVD; therefore, ED should be examined carefully in CKD patients to avoid occurrence of CVD.

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P- Reviewer: Bernieh B, Chung FT **S- Editor**: Song XX
L- Editor: A **E- Editor**: Lu YJ

World Journal of Nephrology

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DOI: 10.5527/wjn.v3.i4.230

World J Nephrol 2014 November 6; 3(4): 230-236
ISSN 2220-6124 (online)

REVIEW

Searching for a treatment for Alport syndrome using mouse models

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Received: June 16, 2014 Revised: July 15, 2014
Accepted: September 16, 2014
Published online: November 6, 2014

Abstract

Alport syndrome (AS) is a hereditary nephritis caused by mutations in COL4A3, COL4A4 or COL4A5 encoding the type IV collagen α3, α4, and α5 chains, which are major components of the glomerular basement membrane. About 20 years have passed since COL4A3, COL4A4, and COL4A5 were identified and the first Alport mouse model was developed using a knockout approach. The phenotype of Alport mice is similar to that of Alport patients, including characteristic thickening and splitting of the glomerular basement membrane. Alport mice have been widely used to study the pathogenesis of AS and to develop effective therapies. In this review, the newer therapies for AS, such as pharmacological interventions, genetic approaches and stem cell therapies, are discussed. Although some stem cell therapies have been demonstrated to slow the renal disease progression in Alport mice, these therapies demand continual refinement as research advances. In terms of the pharmacological drugs, angiotensin-converting enzyme inhibitors have been shown to be effective in Alport mice. Novel therapies that can provide a better outcome or lead to a cure are still awaited.

Key words: Alport syndrome; Angiotensin-converting enzyme; Genetic; Hereditary nephritis; Pharmacological; Renal injury; Stem cell therapy

Core tip: There is currently no curative treatment for Alport syndrome, a progressive hereditary nephritis. However, many drugs have been demonstrated to slow the progression of renal injury in Alport mouse models. Alport mice treated with vasopeptidase inhibitors or angiotensin-converting enzyme inhibitors showed a more than two-fold longer survival than untreated Alport mice. A human clinical trial of an angiotensin-converting enzyme inhibitor is currently in progress. Genetic approaches have been used to elucidate the pathogenesis of this progressive renal disease. Stem cell therapies were also attempted, with some beneficial effects; however, they need to be improved before being tested in clinical trials.

Katayama K, Nomura S, Tryggvason K, Ito M. Searching for a treatment for Alport syndrome using mouse models. *World J Nephrol* 2014; 3(4): 230-236 Available from: URL: http://www.wjgnet.com/2220-6124/full/v3/i4/230.htm DOI: http://dx.doi.org/10.5527/wjn.v3.i4.230

INTRODUCTION

Alport syndrome (AS) is characterized by a classic triad of renal injury, sensorineural deafness and ocular abnormalities[1]. The disease frequency of AS is about 1:5000[2]. AS begins with asymptomatic microscopic hematuria, progresses to characteristic thinning, thickening and splitting of the glomerular basement membrane (GBM), and finally leads to end-stage renal failure[3]. The causative genes of this syndrome are COL4A3, COL4A4 and COL4A5, which are associated with two types of

disease: X-linked and autosomal. The X-linked type of AS is caused by mutations in COL4A5[4], while the autosomal type of AS is caused by mutations in COL4A3 or COL4A4[5,6]. COL4A3, COL4A4 and COL4A5 encode the type Ⅳ collagen α3, α4 and α5 chains, respectively. Since the type Ⅳ collagen α3, α4 and α5 chains are major structural components of the GBM, AS is a type Ⅳ collagen disease.

The purpose of this review is to summarize the current knowledge that has been obtained using mouse models of Alport syndrome.

PATHOGENESIS

At the molecular level, there are only three triple-helical protomers, α1.α1.α2, α3.α4.α5 and α5.α5.α6, in type Ⅳ collagens[7]. The non-collagenous domain (NC1) at the carboxyl terminus of these protomers joins them to each other to make the suprastructure of the GBM. The α1/α1/α2, α1/α2/α5/α6 and α3/α4/α5 heterohexamers were identified by digesting the NC1 hexamer from human glomeruli with bacterial collagenase[7]. Interestingly, the α3/α4/α5 heterohexamer consists of one α4-α4 homodimer and two α3-α5 heterodimers, while the α1/α1/α2 heterohexamer consists of two α1-α1 homodimers and one α2-α2 homodimer, and the α1/α2/α5/α6 heterohexamer consists of two α1-α5 heterodimers and one α2-α6 heterodimer[7]. The α3 (Ⅳ) and α4 (Ⅳ) chains have to accompany the α5 (Ⅳ) chain, and the α3/α4/α5 heterohexamer consists of compositions of $(\alpha3)_2(\alpha4)_2(\alpha5)_2$[7]. NC1 domains were also demonstrated to contain recognition sequences to form α1.α1.α2 (Ⅳ) and α3.α4.α5 (Ⅳ) networks[8].

There is a developmental switch from α1 and α2 (Ⅳ) chains to α3, α4 and α5 (Ⅳ) chains; the GBM from capillary loop stage contains α3, α4 and α5 (Ⅳ) chains, as well as α1 and α2 (Ⅳ) chains, while the GBM at the comma- and S-shaped stages contains only α1 and α2 (Ⅳ) chains[9,10]. In mature glomeruli, the GBM is mainly composed of α3, α4, and α5 (Ⅳ) chains. While only the distal tubular basement membranes (TBMs) were positive for the α3, α4 and α5 (Ⅳ) chains in humans, nearly the full range of TBMs in the mouse are positive for the α3, α4 and α5 (Ⅳ) chains[9].

GBM in X-linked AS patients consists of only α1 and α2 (Ⅳ) chains because the developmental switch does not occur[10]. The loss of the α5 (Ⅳ) chain leads to the loss of all three chains (α3, α4 and α5 (Ⅳ) chains) in the GBM because of the defective assembly of triple-helical α3.α4.α5 (Ⅳ) protomers[11]. This abnormal GBM in X-linked AS patients is more susceptible to proteolysis by bacterial collagenase, cathepsin B, cathepsin G and *Pseudomonas* elastase than that in normal humans[10], because the collagenous domain of α1.α1.α2 (Ⅳ) protomers contains fewer disulfide cross-links than do α3.α4.α5 (Ⅳ) protomers[11].

Interestingly, AS patients with 5' glycine mutations have a later onset of end-stage renal failure than those with 3' glycine mutations, which is compatible with the fact that type Ⅳ collagen assembly starts from the NC1 domain at the carboxyl terminus[12,13].

By generating two hybrid kidneys that contained wild endothelial cells and COL4A3 -/- podocytes or COL4A3 -/- endothelial cells and wild podocytes, type Ⅳ collagen α3, α4 and α5 chains proved to be originally produced specifically by podocytes in the kidney[14], thus suggesting that AS is podocyte-associated disease.

Table 1 Mouse models of Alport syndrome

Gene	Mutation	Ref.
ARAS		
COL4A3	exon 48	[15]
COL4A3	exon 48-50	[16]
COL4A3-COL4A4	COL4A3 exon 2-COL4A4 exon 12	[17]
COL4A4	exon 30	[18]
XLAS		
COL4A5	exon 1	[19]

ARAS: Autosomal recessive Alport syndrome; XLAS: X-linked Alport syndrome.

MOUSE MODELS OF ALPORT SYNDROME

There were two COL4A3 knockout models reported in 1996. One model was generated by cloning a neomycin cassette into exon 48 of COL4A3[15]. The other model was generated by deleting three exons between exons 48 and 50 of COL4A3[16]. Both models aimed to disrupt exons in the NC1 domain, and the resulting phenotypes resembled those of autosomal recessive AS in human. The COL4DELTA3-4 model, which has a large deletion between exon 2 of COL4A3 and exon 12 of COL4A4, was also reported[17]. This mouse model was found because of the observation that there was unexpected renal disease in a transgenic line, and this model had a more severe type of AS than the above COL4A3 knockout models, because the expression of COL4A3 and COL4A4 mRNAs were not detected due to a lack of the intergene region of COL4A3-COL4A4. A new COL4A4 mouse model, which has a splice site mutation and skips exon 30 of Col4a4, was also recently reported[18]. Since this mutation does not cause a frame shift, this mouse model retains a mutant α4 (Ⅳ) chain in the GBM and represents a good new AS model.

Regarding the X-linked type, a COL4A5 knockout model was generated by making a nonsense mutation in exon 1 of COL4A5, and this has made the analysis of female carriers easier[19]. These five mouse models are summarized in Table 1.

The COL4A3 -/- mice have been the most commonly used as a mouse model of AS in experimental studies. This is partly because the survival of COL4A3 -/- mice is less variable than that of COL4A5 -/- mice[15,16,19]. Interestingly, the survival of COL4A3 -/- mice is influenced by the genetic background; being 66 d on a 129X1/SvJ background compared to 194 d on a

Table 2 The efficacy of pharmacological drugs in COL4A3 -/- mice

Drug	Survival (d)	Efficacy
Vasopeptidase inhibitor[22]	172	(+++)
ACE inhibitor[23]	150	(+++)
ARB[24]	98	(++)
HMG-CoA reductase inhibitor[26]	91	(++)
CCR1 inhibitor[27]	86	(+)
TNF-alpha antagonist[29]	81	(+)
Renin inhibitor[30]	78	(+)
Vitamin D analog[31]	75	(+)
Untreated (129SvJ background)	71	

ACE: Angiotensin converting enzyme; ARB: Angiotensin-II receptor blocker; CCR1: Chemokine (CC motif) receptor 1; HMG-CoA: 3-hydroxy-3-methylglutaryl-coenzyme A; TNF: Tumor necrosis factor.

C57BL/6J background[20]. A linkage analysis of quantitative trait loci identified three markers on chromosome 9 and one marker on chromosome 16 that were suggested to be modifier genes. In this regard, it is important to use appropriate control littermates for all experiments. Although the 129 genetic background is good enough to assess the efficacy of new therapies in AS, the C57 genetic background might be better for assessing the long-term effects of new therapies.

The big difference between COL4A3 -/- mice and COL4A5 -/- mice is the existence of the α5 (IV) chain in the GBM of COL4A3 -/- mice[21]. Of note, the expression level of the α5 (IV) chain is more prominent in mice with a C57 genetic background than in those with a 129 genetic background[21]. To assess the efficacy of regeneration therapy in COL4A3 -/- mice, it is recommended that the α3 and α4 (IV) chains, not the α5 (IV) chain, should be used.

PHARMACOLOGICAL INTERVENTIONS

A vasopeptidase inhibitor, AVE7688, extended the lifespan of COL4A3 -/- mice dramatically, and it is the most effective drug against COL4A3 -/- mice identified so far[22]. The various drugs that have shown efficacy in treating COL4A3 -/- mice are summarized in Table 2.

An angiotensin-converting enzyme (ACE) inhibitor, Ramipril, was demonstrated to be effective for treating COL4A3 -/- mice[23]. Notably, early initiation of ACE inhibitor treatment was associated with a longer survival time, and this indicated that the ACE inhibitor had a renoprotective effect in the COL4A3 -/- mice, regardless of its impact on the blood pressure.

Moreover, Gross *et al*[24] compared the antifibrotic effects between an ACE inhibitor and an angiotensin receptor blocker (ARB), which was also known to be an angiotensin receptor 1 antagonist. Although both drugs prolonged the survival of COL4A3 -/- mice, the ACE inhibitor was much more effective than the ARB. Treatment with an ACE inhibitor reduced the transforming growth factor-beta 1 (TGF-β1) and connective tissue growth factor (CTGF) levels more effectively than did treatment with an ARB, which might explain the different effects between ACE inhibitors and ARBs, because TGF-β1 was demonstrated to be associated with renal disease progression in COL4A3 -/- mice[25].

A 3-hydroxy-3-methylglutaryl-coenzyme (HMG-CoA) reductase inhibitor, which was originally used for the treatment of hypercholesterolemia, showed an antifibrotic effect in COL4A3 -/- mice, because it prolonged the survival by inhibiting the activation of fibrotic markers[26]. Interestingly, late initiation of treatment with the HMG-CoA reductase inhibitor at week 7 prolonged the survival of the mice from 71.3 to 90.5 d, while late initiation of ACE inhibitor treatment did not[23].

A chemokine receptor 1 antagonist, BX471, prolonged the survival of COL4A3 -/- mice by preventing interstitial macrophage recruitment[27]. That study showed the involvement of chemokines in the renal fibrosis of COL4A3 -/- mice. However, Ccl2 blockade did not prolong the survival of COL4A3 -/- mice even though it reduced the number of renal macrophages[28].

A tumor necrosis factor alpha antagonist prolonged the survival of COL4A3 -/- mice by decreasing podocyte apoptosis[29]. Aliskiren, a direct renin inhibitor, prolonged the survival of COL4A3 -/- mice by 18% by downregulating both TGF-β1 and CTGF in the kidney[30]. The combination of paricalcitol with an ACE inhibitor led to longer survival than the combination of calcitriol with the ACE inhibitor, which indicated that the different analogs of the active form of vitamin D exert different effects[31].

A matrix metalloproteinase (MMP) -2, -3, and -9 inhibitor cocktail prolonged the survival of COL4A3 -/- mice if it was administered before the onset of proteinuria[32]. In contrast, late administration of the inhibitor cocktail after the onset of proteinuria aggravated the renal disease of COL4A3 -/- mice, which was associated with increased interstitial fibrosis. This dual effect might explain why MMPs played a pathogenic role in the early stage, although they played a protective role in the late stage of disease in COL4A3 -/- mice[32]. MMP-12, also known as macrophage metalloelastase, was upregulated in the podocytes of Alport mice, and a MMP inhibitor, MMI270, which blocks MMP-2, -3, -9, -12 and -14, prolonged the survival of COL4A3 -/- mice from eight to 10 wk, while treatment with a MMP inhibitor that blocked MMP-2, -3 and -9 did not[33]. The authors of that study also showed that a CC chemokine receptor 2 antagonist, propagermanium, also prolonged the survival of COL4A3 -/- mice from eight to 11 wk.

At present, an ACE inhibitor has been reported to be the most effective treatment in humans[34]. A vasopeptidase inhibitor might be considered as the next candidate, since this drug led to the longest survival in COL4A3 -/- mice (Table 2).

GENETIC APPROACHES

TGF-β1 is involved in the progression of renal disease in COL4A3 -/- mice[25]. TGF-β1 was found to be sig-

nificantly upregulated after the onset of proteinuria. TGF-β1 and integrin α1β1 were found to affect distinct pathways in the pathogenesis of COL4A3 -/- mice[35]. While TGF-β1 inhibition prevented the thickening of the GBM, the deletion of integrin α1β1 diminished the foot process effacement of podocytes. Treatment with a combination of these approaches prolonged the survival of Alport mice. Recently, the same group showed that integrin α1 deletion in COL4A3 -/- mice decreased the mesangial invasion into the capillary loops of glomeruli[36]. Integrin α2 deletion in COL4A3 -/- mice prolonged the survival by 20% on a C57Bl6 background[37].

The deletion of discoidin domain receptor 1 (DDR1) in COL4A3 -/- mice prolonged the survival from 64.3 to 94.2 d[38]. Since DDR1 is expressed in podocytes, these results again showed the importance of podocyte involvement in the pathogenesis of AS.

Uterine sensitization-associated gene-1 (USAG-1) deletion in COL4A3 -/- mice improved the renal phenotype and improved the survival[39]. This result was compatible with the finding that recombinant human bone morphogenetic protein-7 (BMP-7) had a protective effect in COL4A3 -/- mice[40], because USAG-1 is known to counteract BMP-7 and is normally expressed in the distal tubules of the kidney[41]. Interestingly, they found that USAG-1 was also expressed in the macula densa, and showed the possibility of crosstalk between the macula densa and extraglomerular mesangial cells[39].

Although MMPs had been thought to be involved in the damage to the GBM in COL4A3 -/- mice, MMP-9 deletion did not affect the progression of renal disease in these mice[42]. Three MMPs; MMP-2, -3, and -9, were genetically ablated in COL4A3 -/- mice, and compensatory upregulation was shown among these MMPs[32]. Therefore, broad-spectrum MMP inhibition is likely required for any effects associated with the MMPs.

A mouse line which had a yeast artificial chromosome including COL4A3 and COL4A4 was generated, and this transgene could rescue the phenotype of COL4A3 -/- mice[43]. Although the expression level of the COL4A3 and COL4A4 transgenes were about 20% of the levels of COL4A3 and COL4A4 in a wild type mouse, the human α3 and α4 (IV) chains could assemble with the mouse α5 (IV) chain. This finding is very interesting, because the amino acid sequence homology of the α3 and α4 (IV) chains between the human and mouse, which are 79% and 78%, respectively, still allows for the formation of triple-helical α3.α4.α5 (IV) protomers.

The expression of an inducible human/mouse chimeric COL4A3 transgene after birth prolonged the lifespan of COL4A3 -/- mice by expressing α3, α4 and α5 (IV) chains in the GBM[44]. Notably, expression of the inducible transgene after three weeks of age could still rescue the phenotype of COL4A3 -/- mice, and the α3.α4.α5 (IV) protomers could integrate into the damaged GBM that was comprised by mainly a α1.α1.α2 network.

STEM CELL THERAPIES

There have been two reports that showed the efficacy of wild-type bone marrow transplantation (BMT) against the renal injury in COL4A3 -/- mice[45,46]. Prodromidi *et al*[45] reported that the blood urea nitrogen (BUN) and serum creatinine (Cr) levels were significantly improved in COL4A3 -/- mice that received wild-type (WT) bone marrow compared to those that received COL4A3 knockout (KO) mouse bone marrow (Table 3). The renal histopathology showed significant improvement of the glomerular injury and tubulointerstitial fibrosis in the WT to KO transplanted mice than in the KO to KO transplanted mice. Moreover, the α3 (IV) chain could be detected partially by immunofluorescence, but not in a Western blot analysis. Sugimoto *et al*[46] reported similar results (Table 3). They also showed that the BUN, Cr, and renal histopathology were significantly improved in the COL4A3 -/- mice that received 21-wk WT bone marrow than did the mice that received KO mouse bone marrow. An immunofluorescence study showed patchy staining of the α3 (IV) chain in the GBM of WT to KO transplanted mice. These two reports shared a common findings that BMT after irradiation from WT to COL4A3 -/- mice dramatically improved the renal injury even though the expression level of the α3 (IV) chain was very low. Neither group examined the survival after BMT as an absolute evaluation marker, so it is unclear whether the BMT could prolong the survival of the mice.

We also reported the results of BMT after irradiation in COL4A3 -/- mice[47]. In contrast to the previous two reports, the BUN, Cr, renal histopathology and survival were significantly improved in both WT to KO and KO to KO mice compared to the untreated KO mice, but there were no significant differences between the WT to KO and KO to KO mice (Table 3). The de novo expression of the α3 (IV) chain could not be detected in the WT to KO mice by immunofluorescence and Western blot analyses. However, wild type COL4A3 mRNA could be identified in the WT to KO, not in the KO to KO, mice by reverse transcription polymerase chain reaction. In fact, fewer than 1% of the podocytes were donor-derived when BMT was performed in a mouse model of mesangial sclerosis[48]. Since KO bone marrow had similar effects as WT bone marrow in the COL4A3 -/- mice, the effect of irradiation itself was examined at sublethal doses. Surprisingly, a sublethal dose of irradiation without subsequent BMT improved the survival of COL4A3 -/- mice. This suggests that the renal injury of COL4A3 -/- mice was improved by the irradiation, not by the BMT. The mechanism by which irradiation improved the survival remains to be clarified, since radiation exposure induces numerous effects.

Another group reported that multipotent mesenchymal stromal cells (MSCs) could not prolong the survival of COL4A3 -/- mice although they improved the interstitial fibrosis by producing vascular endothelial growth

Table 3 The effects of bone marrow transplantation therapy in COL4A3 -/- mice

	Prodromidi *et al*[45]		Sugimot *et al*[46]		Katayama *et al*[47]	
	WT -> KO	KO -> KO	WT -> KO	KO -> KO	WT -> KO	KO -> KO
α3 (IV) IF	+	-	+	-	-	-
α3 (IV) WB	no data	no data	+	-	-	-
Col4a3 mRNA	+	-	+	-	+	-
BUN and Cr	improved	no change	improved	no change	improved	improved
Renal pathology	improved	no change	improved	no change	improved	improved
Survival (d)	no data	no data	no data	no data	125	135

WT: Wild-type; KO: Knockout; IF: Immunofluorescence; WB: Western blot; BUN: Blood urea nitrogen; Cr: Creatinine.

factor[49]. MSCs in the kidney that transdifferentiated into renal cells could not be identified.

However, wild-type bone marrow cells were also shown to prolong the survival of unirradiated COL4A3 -/- mice[50]. Surprisingly, wild-type blood transfusion, as well as the injection of undifferentiated mouse embryonic stem cells, improved the renal function of unirradiated COL4A3 -/- mice, with the appearance of the *de novo* expression of the α3 (IV) chain in the GBM. Although these data confirmed that cell-based therapies could be effective, there was a large discrepancy between the expression patterns of the α3 and α5 (IV) chains: the expression of the α3 (IV) chain was patchy, while that of the α5 (IV) chain was linear. There might be an unknown association between the small amount of *de novo* α3 (IV) chains and the renal improvement of COL4A3 -/- mice that received WT bone marrow. Of interest, a single injection of amniotic fluid stem cells was recently shown to prolong the survival of COL4A5 -/- mice without *de novo* expression of α5 (IV) chains[51].

CONCLUSION

At present, there is no treatment available that can cure AS, and symptomatic renal protective therapies are currently the mainstay of treatment for AS. During the search for a treatment in Alport mice, ACE inhibitors were found to be the most promising therapeutic drugs as first-line therapy. This is a good example of the benefits of mouse studies, because this has led to a double-blind, randomized, placebo-controlled, multicenter EARLY PRO-TECT Alport trial[52]. BMT therapy is also promising, but is still controversial, given the fact that BMT itself is invasive[53]. Other therapeutic agents that have been proven effective in AS mouse models should be considered as the next options for clinical trials in patients with AS.

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P- Reviewer: Fujigaki Y, Pedersen EB, Watanabe T
S- Editor: Gong XM **L- Editor**: A **E- Editor**: Lu YJ

World Journal of Nephrology

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Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.5527/wjn.v3.i4.237

World J Nephrol 2014 November 6; 3(4): 237-242
ISSN 2220-6124 (online)

MINIREVIEWS

Role of insulin resistance in uric acid nephrolithiasis

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Received: June 25, 2014 Revised: July 21, 2014
Accepted: September 4, 2014
Published online: November 6, 2014

Abstract

Metabolic syndrome has been implicated in the pathogenesis of uric acid stones. Although not completely understood, its role is supported by many studies demonstrating increased prevalence of uric acid stones in patients with metabolic syndrome and in particular insulin resistance, a major component of metabolic syndrome. This review presents epidemiologic studies demonstrating the association between metabolic syndrome and nephrolithiasis in general as well as the relationship between insulin resistance and uric acid stone formation, in particular. We also review studies that explore the pathophysiologic relationship between insulin resistance and uric acid nephrolithiasis.

Key words: Nephrolithiasis; Kidney calculi; Uric acid; Insulin resistance; Metabolic syndrome

Core tip: Increasing awareness of the association between prevalence of metabolic syndrome and uric acid nephrolithiasis has caused a closer examination into modifiable risk factors for stone recurrence. The mechanism behind this association is thought to be due to decreased ammoniagenesis as caused by insulin resistance in the proximal tubule of the kidney. The presence or recurrence of uric acid stones should prompt the physician to look for traits of metabolic syndrome. Further studies into this causal relationship may provide additional medical interventions to decrease incident stones.

Li H, Klett DE, Littleton R, Elder JS, Sammon JD. Role of insulin resistance in uric acid nephrolithiasis. *World J Nephrol* 2014; 3(4): 237-242 Available from: URL: http://www.wjgnet.com/2220-6124/full/v3/i4/237.htm DOI: http://dx.doi.org/10.5527/wjn.v3.i4.237

INTRODUCTION

In the United States, the prevalence of kidney stones has risen since 1976 and was estimated to be 8.8% in 2010[1,2]. The magnitude of this problem is exacerbated by a recurrence rate as high as 50% within 5 years[3]. The prevalence of kidney stones is largely dependent on many un-modifiable patient factors including gender, ethnicity, and geography[1]. However, a growing interest in the relationship between modifiable risk factors such as obesity, diabetes mellitus (DM) and metabolic syndrome (MetS) has developed in light of the increasing prevalence of these conditions[4].

The majority of kidney stones are calcium-based with uric acid (UA) nephrolithiasis comprising only 10% of calculi in the overall stone-forming population[5]. However, UA stones disproportionally affect certain cohorts. Among obese patients, UA nephrolithiasis accounts for up to 63% of the stone burden[6].

The central role insulin resistance appears to play in UA stone formation has been the subject of much research and debate. The exploration of this important relationship is the purpose of the current review. We first present epidemiologic studies that demonstrate the link between MetS and nephrolithiasis in general. We then

Table 1 Insulin resistance and kidney stone formation

Ref.	Type	Year	*n*	Study population	Relevant variables	Conclusion
Taylor *et al*[8]	Prospective	2005	241623	Health professionals from 3 different study cohorts starting as early as 1980	Patient reported BMI, waist circumference, and incidence of nephrolithiasis	Obesity, weight gain, and waist circumference are positively associated with renal stone disease
Taylor *et al*[7]	Cross-sectional	2005	220478	Health professionals	Patient reported incidence of diabetes and kidney stones	Patients with DM have higher relative risk of having stones. Patients with kidney stones were more likely to develop DM
Rendina *et al*[27]	Cross-Sectional, single institution	2009	2132	Consecutive Caucasian inpatients in a single Italian hospital	AHA/NHLBI criteria for MetS diagnosis, kidney stones diagnosed on US	MetS, specifically HTN and obesity (in females) is significantly associated with US evidence of kidney stones
Chang *et al*[28]	Prospective, single institution	2011	3872	South Korean workers participating in comprehensive health exam from 2002-2009	National Cholesterol Education Program's Third Adult Treatment Panel criteria for MetS diagnosis, kidney stone diagnosed on US	MetS is significantly associated with acidified urine and increased risk of kidney stones MetS over time as well as each additional MetS trait predicted development of kidney stones
Kabeya *et al*[9]	Cross-Sectional, single institution	2012	2717	Japanese patients undergoing MetS screening	Fasting serum insulin, FPG, HbA1c, US for diagnosis of kidney stone	Glycemic control may be an independent risk factor for kidney stones. The number of MetS traits is positively associated with kidney stone risk; specifically, patients with all 5 traits are at a 2.7 x increased risk of kidney stones compared to those with 2 traits
Kohjimoto *et al*[29]	Cross-Sectional	2013	11555	Japanese survey	MetS traits, incident kidney stones – multiple and recurrent	Increasing number of MetS traits increased stone burden

IR: Insulin resistance; AHA/NHLBI: American Heart Association/National Heart, Lung, and Blood Institute; FPG: Fasting plasma glucose; MetS: Metabolic syndrome; DM: Diabetes mellitus; UA: Uric acid.

highlight studies examining the increased prevalence of UA stones in patients with insulin resistance. Finally, we review currently accepted pathophysiologic mechanisms that support the role of insulin resistance in UA stone formation.

METABOLIC SYNDROME AND NEPHROLITHIASIS

MetS comprises traits of insulin resistance (IR), obesity, hypertension (HTN), and hyperlipidemia. Multiple studies demonstrate that MetS and its constituent components are associated with increased risk of kidney stones (Table 1).

One of the largest studies to examine the link between components of MetS and nephrolithiasis was by Taylor *et al*[7] who reported a cross-sectional analysis of three large national patient surveys: Nurses Health Study I, Nurses Health Study Ⅱ, and Health Professionals Follow-Up Study. This investigation included over 200000 health professional males and female nurses responding to surveys administered every 2 years with an age range of 25 to 75 years of age. The study concluded that DM type Ⅱ was significantly associated with kidney stone formation with a relative risk of 1.38 in older women, 1.67 in younger women, and 1.31 in men as compared to non-diabetic patients after controlling for age, body-mass index (BMI), thiazide use, and diet. Additionally, they reported that among patients with kidney stones, the relative risk of developing diabetes was 1.33 in older women, 1.48 in younger women, and 1.49 in men as compared to patients without nephrolithiasis. In a separate analysis of these data, Taylor *et al*[8] reported that obesity, weight gain, and waist circumference were risk factors for incident kidney stones. Together, these studies support the underlying connection between the main components of MetS and kidney stones. While the conclusions of these studies are strengthened by the very large size of the study cohort, the analysis is likely biased by the use of self-reported outcomes in these datasets.

In another large study, Kabeya *et al*[9] showed a significant association between certain traits of MetS and kidney stone formation in 2717 healthy Japanese individuals. Traits significantly associated with increased risk of kidney stone formation included glucose intolerance (fasting plasma glucose ⩾ 100 mg/dL, OR 1.53) and HTN (Systolic ⩾ 130 mmHg, Diastolic ⩾ 85 mmHg, OR 1.42). In addition, they demonstrated a dose-dependent relationship between metabolic syndrome traits and kidney stone formation. The odds of patients with three or more traits of MetS (abdominal obesity, glucose intolerance, HTN, hypertriglyceridemia, and/or low high density lipoprotein) developing kidney stones was 1.48 times higher than those without these traits. This association was not shown for patients with two or fewer traits of MetS. This finding is especially important, as a dose dependent response suggests a causal link between MetS and kidney stones. Although this study provides robust evidence for this association, it was limited by inclusion of a single Japanese population, reducing generalizability to other at risk populations. In addition, the cross-sectional study design confounds the temporal relationship between kidney stone formation and MetS.

Table 2 Insulin resistance and uric acid stone formation

Ref.	Type	Year	*n*	Study population	Relevant variables	Conclusion
Lieske *et al*[30]	Retrospective, Case Control, single county in Minnesota	2006	7122	Known stone former *vs* Control	Stone analysis, metabolic evaluation	DM, obesity, and HTN are associated with the development of kidney stones. DM is significantly associated with UA stone formation
Daudon *et al*[10]	Cross-sectional	2006	2464	DM *vs* Non-DM stone formers	Stone analysis, BMI, clinical and lab data in a subset of stone formers	DM is associated with a higher overall frequency of kidney stones, specifically, UA. UA stone formation can reflect IR and patients should be evaluated for MetS and/or DM if UA stones are diagnosed.
Akman *et al*[11]	Retrospective, single institution	2012	146	MetS *vs* Non-MetS undergoing PCNL	Kidney stone analysis, imaging for initial/recurrent kidney stone diagnosis, baseline blood chemistry and urinalysis	Patients with MetS have a higher frequency of UA stones (21.9% *vs* 4.1%) and a higher rate of all stone recurrence following PCNL
Cho *et al*[12]	Retrospective, three institutions	2012	712	MetS *vs* Non-MetS undergoing endourologic intervention for stones	Stone analysis, metabolic data, International Diabetes Federation definition for MetS	MetS, specifically the traits of impaired fasting glucose and hypertriglyceridemia, is significantly associated with UA stone formation, but calcium based stones remain most common in this group
Kadlec *et al*[31]	Retrospective, single institution	2012	590	All stone formers undergoing endourologic intervention	Stone analysis, MetS factors (presence of obesity, DM, HTN, and HL)	DM and HTN, components of MetS, are significantly associated with UA containing stones
Stansbridge *et al*[32]	Retrospective, single institution	2013	1504	UA stone formers *vs* Non-UA	24H urine, stone analysis, relevant underlying diagnoses, including DM	UA containing stones are increased in DM, but calcium containing stones are still the most common in DM
Inci *et al*[33]	Case-control, single institution	2012	99	Control *vs* Stone formers (substratified by stone type)	Stone analysis, metabolic evaluation	BMI and Hyperlipidemia, two major traits of IR/MetS, are significantly associated with calcium and UA stone formation
Zhou *et al*[34]	Retrospective, single institution	2013	269	UA stone formers *vs* Non-UA stone formers undergoing PCNL	CT for visceral fat area measurement, stone analysis, metabolic evaluation	HTN and visceral fat area, two traits highly associated with IR/MetS, are independent risk factors associated with UA stone formation

IR: Insulin resistance; MetS: Metabolic syndrome; DM: Diabetes Mellitus; UA: Uric acid; HTN: Hypertension; PCNL: Percutaneous nephrolithotomy.

INSULIN RESISTANCE INCREASES RISK OF URIC ACID NEPHROLITHIASIS

Because different types of kidney stones have a tendency to form in different urine milieus, there has been substantial interest in studying the link between insulin resistance and UA stone formation. Low urine pH is a factor of both insulin resistance and UA stone formation; it has therefore been hypothesized that MetS should favor the formation of UA stones[10]. Multiple studies performed stone analyses in order to query the relationship between insulin resistance and specific kidney stone type (Table 2). In a study of 2464 kidney stone formers, Daudon *et al*[10] found that in patients with DM, UA stones accounted for 35.7% of all stones while only 11% in non-diabetic patients, $P < 0.0001$. The authors recommended that patients with UA stones should be evaluated for insulin resistance or MetS as the prevalence of DM in the UA stone population (27.8%) was significantly higher than the prevalence of DM in the population forming other stone types (6.9%).

Other studies have also demonstrated increased odds of UA stones in patients with MetS. In particular, Akman *et al*[11] found UA stones to be significantly more common in patients with MetS compared to patients without MetS (21.9% *vs* 4.1%, $P < 0.001$) in a group of 146 stone formers. Furthermore, the authors suggested that patients with MetS may be more susceptible to UA stone recurrence. In their study, a trend toward higher recurrence of UA stone formation was demonstrated in patients with MetS as compared to patients without MetS (42.9% *vs* 0%, $P = 0.51$). Although a statistically significant association was not found, the study may have been underpowered to detect a difference. Therefore, a relationship between MetS and UA stone recurrence may exist, and further study is required.

In a separate study of UA stone formation in MetS, Cho *et al*[12] showed that MetS was an independent risk factor for UA stone. In an analysis of individual MetS traits, a direct relationship between UA stone and MetS traits was uncovered: as the number of MetS traits increased, the risk for UA stones increased (10.2% in patients with one MetS trait and as high as 30.4% with four components). These studies are limited by their use of cross-sectional or retrospective designs. Nevertheless, the relationship between UA stones and MetS established by these studies should prompt physicians to evaluate patients presenting with UA stones for underlying insulin resistance and related comorbidities.

Table 3 Pathophysiologic relationship between insulin resistance and uric acid stone formation

Ref.	Study Type	Year	*N*	Study population	Outcomes	Conclusion
Facchini *et al*[13]	Cross-sectional, single institution	1991	36	Healthy volunteers with varying degrees of IR	24H urine (pH, UA), UA clearance, steady-state plasma glucose, metabolic evaluation	As IR increases serum UA increases and urinary UA clearance decreases. Thus, increased serum UA concentration may be considered an additional trait of MetS
Cappuccio *et al*[14]	Cross-sectional, single institution	1993	568	Factory volunteers	Fasting spot urine (UA), fractional excretion of Na+, fasting blood analysis	The higher the serum UA level, the greater the amount of renal Na+ reabsorption. This phenomenon is consistent with hyperinsulinemia, and possibly IR, as insulin is known to increase renal sodium reabsorption
Pak *et al*[15]	Retrospective, single institution	2001	56	UA stone formers *vs* matched control with diet control	24H urine	UA stone formers have increased serum UA, decreased fractional excretion of urinary UA, and decreased urinary pH
Sakhaee *et al*[16]	Prospective, single institution	2002	70	Healthy *vs* stone formers (UA *vs* Calcium *vs* Mixed) with diet control	24H urine (pH, NH4+), fasting glucose	UA stone formers are more likely to have IR/DM. UA stone formation occurs due to impaired NH4+ excretion and urine acidification. Acid loading further decreases urinary pH in these patients as compared to non-UA stone formers/Controls
Abate *et al*[17]	Prospective, single institution	2004	68	Stone free patients *vs* UA stone formers with diet control	24H urine (pH, NH4+), glucose disposal rate	Acute hyperinsulinemia leads to elevated urinary pH and NH4+ excretion in normal insulin-sensitive subjects. Alternatively, IR is associated with low urinary pH and impaired NH4+ excretion and could be renal manifestations of IR causing UA stone formation
Maalouf *et al*[23]	Cross-sectional, single institution	2007	148	MetS *vs* No MetS (all stone free)	24H urine (pH, NH4+), Homeostasis model for IR, metabolic evaluation	Acidic urine is a feature of MetS and is associated with the degree of IR. As MetS traits increase, urine pH decreases
Bobulescu *et al*[24]	Prospective, single institution	2013	35	Matched patients with and without UA stones, matched non-stone forming diabetic controls	24H urine, urinary ammonium excretion	Both uric acid non-diabetic patients as well as DM non-stone forming patients had lower urinary pH as compared to matched non-stone forming non-diabetic controls
Cameron *et al*[25]	Prospective, single institution	2011	19	UA stone formers *vs* normal controls with diet control	24H urine, diurnal urinary pH	UA stone formers had decreased urinary pH with increased undissociated UA secretion compared to normal controls

IR: Insulin resistance; DM: Diabetes mellitus; UA: Uric acid; MetS: Metabolic syndrome.

PATHOPHYSIOLOGY OF URIC ACID STONE FORMATION IN PATIENTS WITH INSULIN RESISTANCE

The pathophysiologic basis for UA stone formation in patients with insulin resistance has been widely studied and a summary of important articles on this subject can be found in Table 3. Surprisingly, UA stone formation in insulin resistance does not depend on the presence of more UA in the urine. In fact, several studies revealed that insulin resistance decreases UA clearance[13,14]. This finding suggests that another causal mechanism may be responsible for UA nephrolithiasis in patients with insulin resistance. Clinically, UA stone formers have low urinary pH. Pak *et al*[15] studied 56 pure and mixed UA stone formers and 68 control subjects. Patients were instructed to consume a calorie restricted diet and maintain high fluid intake. They showed that UA stone formers had higher serum UA levels but lower urinary UA levels. Urinary pH was 5.34 in UA stone formers compared to 6.17 in control subjects. This study suggests that it may be low urine pH rather than elevated urine UA levels that plays a critical role in UA stone formation.

In 2002, Sakhaee *et al*[16] published a key study revealing a defect in urinary ammoniagenesis among UA stone formers. After equilibrating to a control diet, UA stone formers demonstrated lower urinary pH and decreased urinary ammonium excretion as compared to normal controls and calcium stone formers. Furthermore, after patients were given an acidic load, pure and mixed UA stone formers experienced a greater degree of urine acidification when compared to both normal controls and calcium stone formers. These findings suggest that although diet has a strong impact on stone formation, patients forming UA stones may be at a particular disadvantage relative to their calcium stone forming peers at any level of diet acidity.

Given the above findings, it is reasonable to ask what is unique about UA stone formers that could cause this defect in urinary acid handling. Abate *et al*[17] revealed that insulin resistance is a driver of low urinary ammonium and pH. UA stone formers and healthy volunteers underwent a study in which they were maintained at a steady state diet and were given controlled doses of insulin (hyperinsulinemic-euglycemic procedure). Baseline 24-h urine collection revealed evidence of lower urinary pH, lower citrate excretion, higher net acid excretion and lower ammonium excretion in the UA stone formers. This suggests that the acid that is secreted is not being buffered adequately by ammonium. They also noted (though not statistically significant) that UA stone form-

ers with progressively lower urine pH tended to have lower glucose disposal rates (insulin resistance).

The specific mechanism for urinary acidification has been suggested by several novel *in vitro* studies. Insulin receptors are expressed in the renal tubular epithelium, and insulin stimulates the renal tubular sodium-hydrogen exchanger (Na^+/H^+ exchanger) to increase reabsorption of hydrogen[18,19]. The activation and up-regulation of the Na^+/H^+ exchanger by insulin promotes ionic trapping of ammonia in the renal tubule; hydrogen ions become bound to ammonia, which is converted to ammonium and is unable to exit the lumen of the renal tubule[20-22]. Resistance to insulin thereby results in decreased buffering capacity for urinary acidification due to decreased ammonia secretion.

The critical relationship between MetS and urine acidification has been supported by Maalouf *et al*[23] who showed that non-stone formers with MetS had decreasing urinary pH with increasing number of MetS traits. Their work supports the theory that insulin resistance plays a role in renal acid handling causing decreased ammoniagenesis and thereby increasing risk of UA stone formation.

Though insulin resistance appears to be playing a significant role in UA stone formation, not all DM patients go on to develop UA stones. This principle was explored by Bobulescu *et al*[24], who prospectively studied BMI-matched non-diabetic pure UA stones formers, diabetic non-stone formers and non-stone forming non-diabetic control patients. Their results demonstrated that both non-diabetic UA stone formers as well as diabetic non-stone forming patients have decreased urinary pH as compared to matched non-diabetic non-stone forming controls. However, non-diabetic patients with UA stones have impaired ability to secrete ammonium after acid loading as compared to diabetic and non-diabetic control patients without nephrolithiasis[24]. This suggests that while insulin resistance plays a role in UA stone formation, additional derangements may occur in these UA stone formers as compared to non-stone formers with DM.

Another salient and surprising feature of studies examining 24-h urine chemistries in UA stone formers *vs* non-stone formers is a frequent absence of difference in urine chemistries. Cameron *et al*[25] discovered a significant diurnal variation in urine acidification occurring in UA stone formers. This intermittent elevation in urinary acid levels leads to transiently lower urine pH, allowing for the precipitation of UA, despite a relatively normal 24-h urine chemistry.

MANAGEMENT RECOMMENDATIONS

Currently, the American Urologic Association guidelines recommend metabolic testing for recurrent stone formers and high-risk stone patients[26]. This work-up includes an initial 24-h urine chemistry followed by repeat testing if stones recur or after initiation of therapy. For patients with UA stones, fluid intake should be sufficient for 2.5 liters of urine output and dietary changes aimed at limiting animal protein, a key driver of urinary acid levels. Additionally, potassium citrate should be recommended to alkalinize the urine (increase urine pH) in an effort to decrease recurrence of UA stones. Nevertheless, these management guidelines do not address the underlying mechanism responsible for UA stone formation in insulin resistance. Further research targeting the defects in ammoniagenesis in insulin resistance may yield novel therapies for this challenging clinical problem.

CONCLUSION

This review explores the relationship between UA nephrolithiasis and insulin resistance. Several epidemiologic studies identify the association between insulin resistance and kidney stones, specifically UA stones. The mechanism underlying this association relates to the importance of renal insulin receptors in acid handling. Insulin resistance results in impaired excretion of urinary ammonia leading to lower urinary pH. Ultimately, these conditions induce UA precipitation out of the urine, leading to the formation of UA stones. As one of the key components of metabolic syndrome, insulin resistance should be suspected in patients with recurrent UA nephrolithiasis, and attention should be directed to the other components of metabolic syndrome, including hypertension, dyslipidemia, and obesity.

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P- Reviewer: Bellomo G, Wagner CA **S- Editor**: Ji FF
L- Editor: A **E- Editor**: Lu YJ

World Journal of Nephrology

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DOI: 10.5527/wjn.v3.i4.243

World J Nephrol 2014 November 6; 3(4): 243-248
ISSN 2220-6124 (online)

MINIREVIEWS

Ureteroscopy and stones: Current status and future expectations

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Received: June 16, 2014 Revised: August 5, 2014
Accepted: August 27, 2014
Published online: November 6, 2014

Abstract

Urolithaisis is becoming an ever increasing urological, nephrological and primary care problem. With a lifetime prevalence approaching 10% and increasing morbidity due to stone disease, the role of ureteroscopy and stone removal is becoming more important. We discuss the current status of stone disease and review the ever increasing role that ureteroscopy has to play in its management. We discuss technological advances that have been made in stone management and give you an overview of when, how and why ureteroscopy is the most common treatment option for stone management. We touch on the role of robotic ureteroscopy and the future of ureteroscopy in the next 10 years.

Key words: Ureteroscopy; Techniques; Ureteral stones; Calculi; Treatment; Advances

Core tip: This manuscript demonstrates the advent, technical progression and modern use of ureteroscopy for stone disease. It begins with a brief epidemiology of renal stone disease, technological advances in flexible ureteroscope, use of laser for stone disease and the different types of surgical options available. We also share the current evidence of ureteroscopy for stone treatment in obesity, pregnancy, pediatrics and patients with bleeding diathesis and large renal stones. In the end we discuss what the future holds for ureteroscopy including an insight into robotic ureteroscopy.

Wright AE, Rukin NJ, Somani BK. Ureteroscopy and stones: Current status and future expectations. *World J Nephrol* 2014; 3(4): 243-248 Available from: URL: http://www.wjgnet.com/2220-6124/full/v3/i4/243.htm DOI: http://dx.doi.org/10.5527/wjn.v3.i4.243

INTRODUCTION

With an increasingly ageing population, rising obesity, poor dietary habits and lack of adequate fluid intake we are seeing a rise in the incidence of renal and ureteric calculi[1-9]. This directly effects patient morbidity and places an ever increasing demand on healthcare resources. The concept of urinary stones is not new, indeed "cutting for the stone" was one of the classic three operations described more than 2000 years ago. It is somewhat ironic now, that endourological surgeons rarely "cut for the stone", but more "fish out" the stone with ureteroscopy (URS). Without doubt, the technological advances over the last 30 years has revolutionised our current management of urinary tract stone disease. We aim to highlight the importance of stone disease and take you through the important technological changes, discuss current concepts in stone management, explain what is new in ureteroscopy and touch on the future of ureteroscopy in the management of stone disease.

EPIDEMIOLOGY OF STONE DISEASE

Urolithiasis is a major clinical and economic burden for modern healthcare systems[10]. International epidemiological data suggest that the prevalence of stone disease is increasing[11]; with a rise in lifetime prevalence between 7%-12%. The mean age of patients with upper tract stones has remained constant at 49 years, although there has been an alarming increase of 19% in the number of children diagnosed[11]. The ever increasing prevalence of stone disease has a direct effect on healthcare resources, with the number of URS performed for stone disease increasing by 127% over the last 10 year period 2000-2010[11].

The rising prevalence of stone disease is multifactorial, but poor dietary habits and fluid intake, increasing levels of obesity and "metabolic syndrome" may further increase stone-related clinical episodes[12,13]. This emphasises the importance of education and lifestyle adaptations in attempting to prevent stone formation for at risk groups and the critical role of secondary prevention for those who have already suffered with stones.

TECHNOLOGICAL ADVANCES IN URETEROSCOPY

The use of URS has dramatically increased over the last 30 years mainly due to the rapid speed of technological advances. Since the advent of the first recorded URS in 1912[14]; the past century has seen a continued development of the ureteroscope alongside diversification of its use. Evaluation of the urinary tract was initially explored with specula, next came urethroscopy with dilatations of the urethra using knives and wax instruments[15]. The prototype endoscope, the "Lichtleiter", was introduced back in 1806 by Phillip Bozzini, and consisted of a hollow tube transmitting candlelight *via* a mirror[15]. This enabled the first true endoscopic operation in 1853 when Desormeaux extracted a urethral papilloma through the endoscope[15]. Further modifications to the endoscope were introduced by the dermatologist Grunfield of Vienna, who developed an endoscopic loop threader and scissor forceps allowing the first endoscopic bladder papilloma excision in 1881. The step from idea to realisation of endoscopic surgery was difficult and protracted. Bozzini *et al* ideas from the early 1800's were well ahead of their time. They were considerably hindered by the technical capabilities of the nineteenth century engineering, which resulted in clumsy and heavy instruments. In parallel with the development of the cystoscope there was continuing advancements in the endoscopic light source. A system of mirrors and lens' were introduced alongside candlelight to transmit light through a hollow tube; this idea was superseded by fibre-optic technology utilising the principle of internal reflection permitting the "bending" of light within flexible glass[16]. These principle and understanding lead onto the development of the first rigid ureteroscope in 1980. This was developed by Perez-Castro in collaboration with Karl Storz, incorporating a separate working and optic channel. These developments allowed the art of ureteroscopy to flourish and develop over the last 35 years[17].

The development of electrohydraulic and ultrasonic lithotripsy soon followed, enabling the fragmentation of ureteric stones[17]. Flexible tip ureteroscopes were introduced in 1983[16], and the modern digital scopes soon followed. Modern digital flexible ureteroscopes consists of a fiberoptic lens, with a single cable electronically transferring the image detected at the tip of a scope to the image display on a monitor ("Chip to tip" technology). Digital and conventional (fibre-optic) flexible ureteroscopes have seen a dramatic improvement in ergonomics, with lighter scopes and improved manoeuvrability[18]. The advent of digital images has resulted in improved resolution and colour discrimination, as well as significantly reduced operative times[16,19-21]. Figure 1 demonstrates the modern flexible ureterorenoscopes that we use in clinical practice today.

Despite improvements in scope technology, one still needs to fragment and/or remove the stone once visualised. Stones are commonly fragmented with a holmium laser (Light Amplification by Stimulated Emission of Radiation). Albert Einstein and Satyendranath Bose proposed the concept of lasers, but lasers were initially seen as a great invention with no obvious use. With time and hard work by laser pioneers, we now cannot imagine a world in which we don't use lasers. Indeed, the role of the Holmium laser in the management of renal tract stones has resulted in many stones in the urinary tract have been accessible to treatment in a minimally invasive fashion. Laser offers the surgeon a safe, effective method of stone fragmentation. One real benefit is the fact that laser can be manoeuvred around bends, enabling it to be used throughout the kidney. The lithotripter, although a useful adjuvant for ureteroscopy, has its limitations including stone retropulsion back into the kidney. The lithotripter is still commonly used for percutaneous nephrolithotomy surgery (PCNL), where larger stones can be fragmented quickly, without the need to manoeuvre around each calyx.

SURGICAL MANAGEMENT OF STONE DISEASE

Traditionally ureteric and renal stones were managed by open surgical techniques, and it was not until the 1980s and the advent of the Dormier H3 lithotriptor that shock wave lithotripsy (SWL) became common place[16]. SWL offered a relatively minimally invasive treatment option for patients, with acceptable outcomes in terms of stone free rates (SFR)[22]. With the advent of minimally invasive surgery, particularly URS, SWL treatment numbers are falling. Recent United Kingdom, American and Australian data clearly demonstrate dramatically rising rates of ureteroscopy, which far exceed small rises in

Figure 1 Flexible ureterorenoscope.

the use of SWL[1,11,23].

Current American and European Urology Association Stone guidelines summarise the current evidence based treatment for stone management based on stone size and location[24]. The size and location of the stone are the most important factors in determining which treatment options are most suitable, but individual surgeon's treatment preference is important in making treatment decisions for each treated stone.

The position of the stone in the ureter directly reflects in the success of the procedure. More distal stone have higher success rates when treated with rigid ureteroscopy, compared to the more proximal stones[24]. Indeed proximal stones can fall back into the kidney, therefore they often require a concurrent flexible ureteroscopy to achieve good stone free rates. Current guidelines recommend ureteroscopy, over other treatments including SWL, for the majority of ureteric stones[24].

In terms of stone size conservative management may be appropriate for smaller stones; 95% of stones up to 4 mm pass within 40 d[25]. Current recommendations advise the use of PCNL over URS and laser for larger more complex stones. The recommended size of stone treated by URS is increasing with each new update of stone guidelines, with the current size value of 20 mm and above favouring a percutaneous approach to treatment (PCNL)[24]. Despite this there is very good clinical evidence[26] for using URS for stones greater than 20 mm in size, with 94% deemed stone free after a mean number of 1.6 URS treatments. This data is comparable, and arguably better, than standard PCNL treatment with reduced morbidity and shorter length of hospital stay[27].

Stones greater than 2 cm often require planned two stage URS procedures to achieve complete stone clearance[28]. Although this necessitates staged procedures, it may be a worthwhile sacrifice in view of nephron preservation and the low complication rate[29]. This is not an insignificant consideration when treating an ever-increasing co-morbid patient. A comparison of the available treatment modalities, in terms of advantages, disadvantages and contraindications is summarised in Table 1.

URETEROSCOPY IN THE CURRENT ERA

Technological advances in the design and size of the ureteroscopes has enabled easier access to the kidney and ureters *via* the urethra, removing the need for any surgical incision. With rigid and flexible URS nearly all areas in the urinary tract can be readily accessed, with stunning high quality digital optics providing very accurate assessment of stones and mucosal lesions. One of the main benefits of URS is that there are minimal contra-indications for the procedure. A general anaesthetic is often required, but upper tract access with spinal or local anaesthetic can be achieved[30]. The only real contraindication would be a ureteric stricture preventing successful ureteric access and scope passage[24]. Fluoroscopy is required during URS, but radiation exposure can be reduced with careful consideration of when and how much fluoroscopy is needed. The benefits of URS are clearly evident in the literature, with low complication rates, high SFR, and short length of stay[26,28].

As with any procedure complications can happen, but the reported complication rates are relatively low[29,31]. The overall complication rate for URS is approximately 3.5%; which are mostly minor. Probably the most feared complication of ureteroscopy is ureteral avulsion, however it is rare (< 1%). Common complication include mucosal or ureteric injury (1.5%-1.7%), post-operative fever (1.8%), urosepsis, haematuria, ureteral stricture (0.1%) and persistent vesicoureteric reflux (0.1%)[29,32]. Due to its minimally invasive nature, URS can be performed as a day case procedure. This has obvious benefits for hospital finances, as well as patient satisfaction levels[11].

In recent years the role of URS has expanded, particularly with reference to an increasingly obese population, during pregnancy, bleeding diathesis and paediatric stone disease. With obesity rates at an all-time high[12,13] and the association of kidney stones in such patients, these groups can often be difficult to manage. The anaesthetic risk can be significantly increased and other treatment such as SWL or PCNL are often less successful[33]. Ureteroscopy is often ideal for such patients, as their renal tract can be readily be accessed[34]. Indeed, currently guidelines recommend URS as the most promising therapeutic option in obese patients[24].

Pregnancy offers a unique situation in terms of urinary stones disease. A cascade of metabolic changes occurs during pregnancy that may be associated with an increased likelihood of stone formation, particularly in the second and third trimester[35,36]. Whenever possible, conservative treatment of stones are encouraged. If complications do develop, URS can offer a minimally invasive treatment option for patients and hopefully avoid the need for long term urinary diversion with either a stent or nephrostomy tube[37,38]. A recent systematic review suggests that URS is a safe and effective procedure that can be used as the first line surgical management of

Table 1 Advantages and disadvantages of different techniques[24]

	Contra-Indications	Advantages	Disadvantages
Percutaneous nephrolithotomy	Pregnancy, potential malignant kidney tumour, tumour in access tract area, atypical bowel interposition	Large renal and staghorn stones Able to remove large fragments Quicker large stone fragmentation and removal	Needs renal puncture plus dilatation Renal bleeding +/- embolisation Patient positioning (often prone) Requires a general anaesthetic (with risk in prone ventilation) Multiple days inpatient stay
Shock wave lithotripsy	Infection, pregnancy, arterial aneurysm, bleeding diatheses, distal ureteric obstruction	Non-invasive treatment Out-patient treatment No anaesthetic needed	Lower success rates Renal colic (secondary stone fragments) Steinstrasse May need multiple treatments Success rates less for lower calyx stones
Ureteroscopy	None	No incisions Day case procedure Can be used in pregnancy, obese and patients not suitable for prone position	Might require 2 operations for stone clearance May need a ureteric stent post op Ureteric avulsion/strictures Requires a general anaesthetic

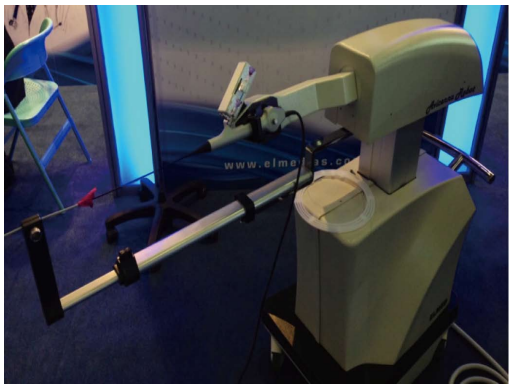

Figure 2 Robotic ureteroscopy.

symptomatic stones during pregnancy[36].

Patient with bleeding diathesis are at significantly increased risk of complications with treatments including SWL, PCNL, laparoscopic or open surgery[39-42]. For such patients, URS offers a safe and effective treatment modality. With ever increasing use of anticoagulation, based on risk assessment, these patients are an at-risk group and can be very difficult to manage surgically[24,43]. In term of URS and anticoagulation the literature is limited. A critical analysis of the published literature has shown good SFR with minimal complications when performing URS whilst the patient remains on anticoagulation. One worries about the rate of bleeding, but the combined data on URS reports a relatively low figure of 4% minor bleeding whilst on anticoagulation[44].

Childhood urolithiasis is becoming more prevalent, with a significant number of patients experiencing their first stone episode in childhood[24]. Such patients present diagnostic and treatment dilemmas, particularly their suitability for treatment due to their organ size. Traditionally the majority of these patients were treated with SWL, with reported SFR of approximately 80%[45]. With smaller calibre scopes and improved scope instrumentation such as smaller baskets and laser fibres, the role for URS has slowly increased. A recent systematic review has demonstrated SFR of up to 93% can be achieved with URS in a paediatric population[45].

FUTURE ADVANCES IN URETEROSCOPY

The future of URS is one of massive technological advances. With ever decreasing scope size, better optics and new device coming to market no corner of the urinary tract is inaccessible or unsuitable for access with URS. Ever more complex patients, with a plethora of medical problem are now becoming increasingly appropriate for URS.

Robotic surgery has recently entered the field of urology, particularly with reference to prostate, bladder and renal cancer treatment. URS has also had the robotic treatment, with the introduction of robotic flexible ureteroscopy. This "Robot" offers the surgeon the ability to control their flexible ureteroscope and laser fibre via the comfort of a robotic console. Figure 2 demonstrates this robotic device. The main robotic station holds the flexible ureteroscope whilst the surgeon controls the URS via a console and joystick devices. With only a few prototypes in clinical use and the procedure in its infancy this is a large area for future clinical development. Initial results are interesting; with the biggest benefit seeming to favour surgeon ergonomics rather than SFR[46]. Long term outcome data is awaited with anticipation.

Another area of future interest is the use of peptide-coated iron oxide-based microparticles[47]. These microparticles selectively adhere to calcium stone fragments enabling quicker retrieval of intraoperative stone fragments with the aid of a magnetic device, when compared to standard stone removal[47]. URS is without doubt an attractive area for technical innovation; where new advances have a huge potential to improve outcome and SFR.

CONCLUSION

With an ever-increasing prevalence of stone disease

careful consideration needs to be given to meet future demand. A large area of attention needs to be placed on primary and secondary stone prevention, with simple but effective patient education and lifestyle interventions.

In terms of URS, the future is one of great excitement. Larger stones, more complex patients, paediatric patients, pregnancy, bleeding diathesis and the obese are becoming more suitable than ever for minimally invasive URS. With the advent of future technological advances, the boundaries of what is achievable will be further expanded. Robotic is entering the playing field and is potentially the next big development in URS. The next 10 years is one of great excitement in URS and is likely to further transform of our current treatment strategies for the management of stone disease.

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P- Reviewer: Gunlusoy B, Sakhaee K **S- Editor**: Wen LL
L- Editor: A **E- Editor**: Lu YJ

World Journal of Nephrology

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Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.5527/wjn.v3.i4.249

World J Nephrol 2014 November 6; 3(4): 249-255
ISSN 2220-6124 (online)

MINIREVIEWS

Clinical audit, a valuable tool to improve quality of care: General methodology and applications in nephrology

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Received: June 13, 2014 Revised: August 1, 2014
Accepted: September 4, 2014
Published online: November 6, 2014

Abstract

Evaluation and improvement of quality of care provided to the patients are of crucial importance in the daily clinical practice and in the health policy planning and financing. Different tools have been developed, including incident analysis, health technology assessment and clinical audit. The clinical audit consist of measuring a clinical outcome or a process, against well-defined standards set on the principles of evidence-based medicine in order to identify the changes needed to improve the quality of care. In particular, patients suffering from chronic renal diseases, present many problems that have been set as topics for clinical audit projects, such as hypertension, anaemia and mineral metabolism management. Although the results of these studies have been encouraging, demonstrating the effectiveness of audit, overall the present evidence is not clearly in favour of clinical audit. These findings call attention to the need to further studies to validate this methodology in different operating scenarios. This review examines the principle of clinical audit, focusing on experiences performed in nephrology settings.

Key words: Clinical audit; Evidence-based medicine; Quality improvement; Nephrology; Hemodialysis

Core tip: Clinical audit is a part of the continuous quality improvement process. It consists in measuring a clinical outcome or a process against well-defined standards, established using the principles of evidence-based medicine. The comparison between clinical practice and standards leads to the formulation of strategies, in order to improve daily care quality. This review examines the basis of clinical audit and the data about the efficacy of this methodology, focusing on nephrology issues. We think that clinical audit could offer to the modern Nephrologists a useful tool to monitor and advance their clinical practice.

Esposito P, Dal Canton A. Clinical audit, a valuable tool to improve quality of care: General methodology and applications in nephrology. *World J Nephrol* 2014; 3(4): 249-255 Available from: URL: http://www.wjgnet.com/2220-6124/full/v3/i4/249.htm DOI: http://dx.doi.org/10.5527/wjn.v3.i4.249

INTRODUCTION

"Audit" is a Latin word, and the verb *audio* ('hear') indicates both active listening and the action of investigation and interrogation of the judiciary. Transferred to the English vocabulary "audit" takes on a meaning of "an official inspection of an organization's accounts, typically by an independent body"[1].

The term is nowadays widely used in different settings (economic, business, *etc.*) referring to procedures aiming to ensure that the activities carried out for a purpose are consistent and effective for the achievement of objectives. Clinical (or medical) audits are part of the continuous quality improvement process that focus on

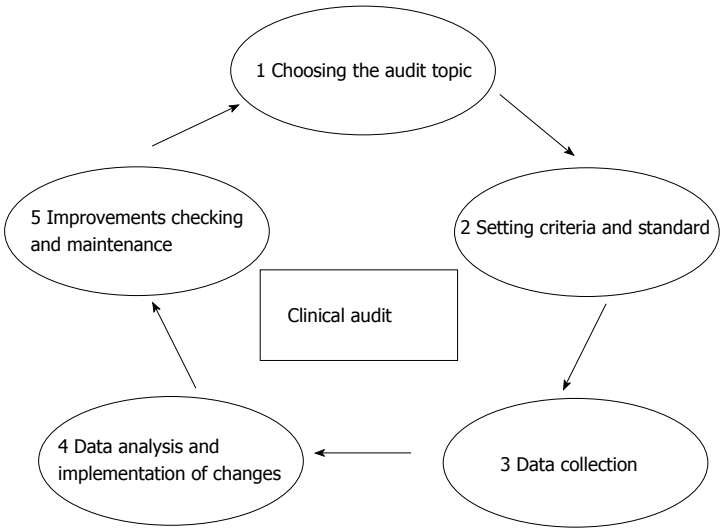

Figure 1 Clinical audit cycle.

specific issues or aspects of health care and clinical practice.

They consist of measuring a clinical outcome or a process, against well-defined standards set on the principles of evidence-based medicine. Aim of the audit is to highlight the discrepancies between actual practice and standard in order to identify the changes needed to improve the quality of care. A peculiar characteristic of the clinical audit is the "professionalism" of the initiative, which is expressed by some typical ingredients: clinical specific competence of the participants, the confidentiality of the results, the object strongly connected to the "quality" of professionals. From a methodological point of view, clinical audit consists of a "quality loop" (Figure 1): once chosen a topic and set shared and measurable criteria and standards, current clinical practice is evaluated, especially in terms of process or outcome, and suggestions for improvement are developed and applied, and then the cycle can begin again[2]. The audit should not be confused with data collection activities (*i.e.*, benchmarking) or clinical research: the latter, in fact, aims to define the characteristics of good practice on a unknown land, while the audit compares the current practice against well-defined and established standards[3]. The final aim of the clinical audit is always improving the care provided to the patient.

This achievement may be reach through different actions: (1) Increase the culture of clinicians; (2) Solve a problem; (3) Reduce the variability of professional conduct (standardize); and (4) Reduce the gap between theoretical standards and real life.

PRINCIPLES OF THE CLINICAL AUDIT

Step 1: Preparing for the audit

Good preparation is crucial for the success of an audit project.

The key elements to design valuable clinical audits are: choosing the topic, defining a clear purpose and providing the necessary organisation in terms of audit staff and resources.

The first step that must be accomplished in designing a clinical audit is to identify the topic (Table 1). The topic of the audit can be loosely identified in clinical practice and may relate to the adequacy of a care process or that of the results[4]. An audited theme should have specific characteristics: it should be of great clinical importance, of easy collection and analysis, and source of important consequences. The personnel involved in the audit have a key role in setting priorities among clinical problems to deal with. By choosing a suitable theme various aspects should be considered.

In particular, it would be a good choice to face a problem that involves the clinician in terms of: (1) High volumes of work; (2) High costs in terms of health and/or economic; (3) High risk; (4) High variability; (5) High complexity; and (6) High innovation.

Rare events, such as complex clinical cases or sporadic adverse events, are not an appropriate topic for a clinical audit, and should be analysed with more adequate methodologies (*i.e.,* Root Case Analysis)[5]. Once the topic has been selected, the purpose of the project must be defined, so that a proper audit methodology can be chosen and designed.

The aim of an audit project could include the implementation of new processes (for example laboratory protocols, surgical procedures, *etc.*) and/or the improvement of current strategies[6].

Moreover, before beginning a clinical audit, organisations should clearly declare the resources allocated to support the project management (data collection, hardware and software required) and for the training of the clinical staff, including education on clinical audit techniques, facilitation and data management[7,8].

Regarding the audit project team, it is advisable that it be customised for the specific audit project, with team members providing many of the skills needed. For example, if the topic of the audit is the management of vascular access in patients undergoing haemodialysis, it will be useful to include nephrologists, vascular surgeons

Table 1 Factors to consider in the decision on a topic for a clinical audit

For the choice of an appropriate theme for a clinical audit, assess that:
The problem to be audited has an important impact in terms of costs, resources, or risk
There is some strong scientific evidence available (guidelines, systematic reviews)
The improvements made on the subject in question can be easily evaluated and source of important clinical/organisational consequence.

and dialysis nurses in the audit team[9].

Step 2: Selection of indicators, criteria and standards and definition of intervention strategies

Once the preliminary issues of the audit have been defined, the next step is to set the standards, which the current clinical practice will be compared to. At this point, it is important to clarify some definitions: (1) Indicator: a variable that allows to describe complex phenomena and to measure changes in relation to defined criteria, in order to guide the decisions aiming at obtaining or maintaining the changes. It can be expressed as absolute number, percentage, rate, or average; (2) Criterion: it is a definable and measurable aspect of health care that describes its quality. The audit criteria are explicit statements that define an outcome to be measured. In a clinical audit, it is a declaration of what should happen on the basis of good practice, and it should be evidence-based[10]; and (3) Standard: it is the standard of care to be achieved for each specific criterion, usually expressed as a percentage. It represents the threshold of acceptability, that is, the value that defines the upper or lower limit, so that the quality of care is considered to be appropriate[11]. Some indicators are so important that the standards must be achieved in 100% of patients (*e.g.*, use of masks during the dressing of central venous catheters), but in general it is sufficient to meet the standard in a lower percentage (for example, in 80% of patients)[4].

The choice of criteria and standards is one of the most critical points in the design of a clinical audit and it requires the collaboration of all participants in the audit. Indeed, the quality of care provided (*i.e.*, the final result of the audit) will be evaluated just on the basis of a comparison with these parameters.

The sources where criteria and standards can be drawn from may be: international guidelines, scientific literature, expert consensus, data obtained by other health care facilities and personal case studies[12-14]. The stronger the evidence taken as a reference will be, the more the results of the comparison with daily clinical practice will be reliable. However, to design an effective clinical audit, it is important that the standard and criteria be shared with colleagues prior to the review of the collected data, since they should not be object of rearrangement in the course of verification, nor be changed retrospectively, in the light of the findings derived from the audit itself.

Finally, the audit team should also define the intervention strategies to be implemented in case of important discrepancies between standards and actual clinical practice. These strategies should be discussed, shared, clear and easy feasible according to a structured algorithm.

Step 3: Data collection

In clinical audit data can be collected prospectively or retrospectively[15]. Taking into consideration past clinical documentation, the latter method is certainly faster, but often the quality of the collected information is not optimal.

Perspective audits are more expensive in terms of time, but they allow a more accurate design, while offering a more realistic description of the current clinical practice. Before proceeding with data collection, it is necessary to carefully plan the variables to be recorded, and define the type of analysis to be conducted on the collected data. These points are important to prevent the collection of useless data or, conversely, the lack of essential information. A specific-designed form or a database should be arranged to collect patient records[16].

Moreover, it may be appropriate to carry out a sampling (preferably using randomized methods) if there is a very large number of patients to be examined, also in relation to the degree of confidence that one wants to achieve and the resources actually available (time, money, personnel)[17].

Collected data can be quantitative or qualitative, such as interviews, questionnaires or comments and data sources can be various, including medical records, results of biochemical and instrumental evaluations and/or other different archives[18,19]. The medical record is certainly the main source of information, but it is often incomplete. In this regard, highlighting the inadequacies of data management, already in the preliminary phase of data collection, the audit improves the existing information flow. Finally, it is worth pointing out that in every moment of data collection and analysis, patient privacy must be protected, making the information collected anonymous and explaining the reasons for the data collection, in case of direct involvement of patients themselves[20].

Step 4: Comparison of collected data with the standards and development of corrective actions

This is the central phase of clinical audit. In this phase, the team of professionals interested in the audit analyses the data and compares them with the pre-set standards. It is important to note that the critical nature of this moment lies in the fact that the professionals involved in the audit process can interpret the audit as an inspection of their clinical activity, thus becoming, unconsciously, an obstacle to an effective data analysis (Table 2)[21].

Table 2 Facilitating factors and barriers for effective clinical audit

Facilitating factors	Obstacles
Clarity of design and data collection	Not clear objectives and planning
Good planning	Lack of resources-heavy workload
Organisation support	Lack of clarity on the method
Dedicated staff	Lack of organizational support
Collective analysis of the results	Unwillingness to change

For this reason, the meeting where the results of the audit will be discussed must be carefully prepared, paying particular attention to all aspects of communication and social skills[22,23].

Moreover, these contents must be pre-emptively shared with those who have proposed the audit. From the comparison of actual data with the theoretical standards different results might emerge, and the standard could be reached or not. In the event that the standard is not met, it should be assessed whether or not there is the possibility of a real improvement. In fact, if the data are not in line with the standards but they are sufficiently close, one might decide that any further improvement is difficult to achieve, and therefore it would be useful to invest resources in the assessment of other problems. In the case there is a significant difference between information gleaned from the clinical documentation and standards, collegial discussion should highlight the barriers to the achievement of the standard[24]. Afterwards, audit methodology requires that the audit team elaborate intervention strategies and recommendations, according to the indications preliminarily set[25]. Such advices or recommendations should take into account organizational factors (in terms of economic resources, timing, dedicated staff) and the context in which the audit takes place. For this reason it is imperative that the developed recommendations be clear, explicit and shared[26]. The mere dissemination of educational materials, such as guidelines, has little effect if they are not accompanied by selected methods of implementation, such as training seminars or discussions among peers[27].

Instead, in case the results obtained from the audit can be considered satisfactory, it is equally indispensable to provide a form of monitoring. Finally, all the findings drawn from data analysis and the subsequent discussion, including strategies to implement change, should be reported in a detailed account to be distributed to all participants of the audit, as feedback and reminder of the work done.

Step 5: Check and maintenance of improvements

The audit cycle ends with the stage of verification and monitoring of implemented strategies[2,4].

Indeed, it is essential for a proper process of clinical audit to schedule periodic verifications of the effects of the changes introduced. It would be advisable to use a data collection and an organizational strategy similar to that used for the previous analysis, so that the results are comparable.

If it emerges that the objectives have not been achieved and the plan of improvements was not effective or sufficient, it could be necessary to make changes to planned strategies.

However, also in case of success, a monitoring plan should be equally scheduled in order to maintain the improvements made.

EFFICACY OF THE CLINICAL AUDIT

There is conflicting evidence on the effectiveness of clinical audit[28]. A systematic review of the Cochrane Study Group has considered 140 studies in which clinical audit and the corresponding feedback were tested alone or in comparison to other types of interventions (meetings, distribution of printed materials, *etc.*). In the studies included in this review, the results produced by the audit were widely variable, from a negative to a very positive effect. When the audit was effective, the effects generally ranged from small to moderate. The review concluded that the relative effectiveness of an audit is likely to be greater when baseline adherence to recommended practice is low and when feedback is carried out with greater intensity[29]. Therefore, at the moment, scientific evidence does not provide clear support about the real effectiveness of clinical audit. This finding could be a starting point to design studies and analyses to validate clinical audit in different operating contexts[30].

CLINICAL AUDIT IN NEPHROLOGY

Medical literature offers several studies on audits conducted in the field of clinical nephrology, especially in patients on haemodialysis (HD). The reported studies have evaluated different aspects of organizational management and clinical research, such as the problems associated with late referral, vascular access, the management of hypertension and anaemia[31-33]. A careful analysis of these studies shows that the research has been mainly focused on the comparison between data collected from several case studies and indications of the guidelines. Therefore, the majority of these studies lack in the processes of cyclicity and verification that, as aforesaid, are the distinctive and characteristic features of clinical audit. An example of a well-conducted audit has been reported in a paper of an Australian group that has performed an audit in order to assess the effect of a multi-disciplinary intervention on the choice of dialysis vascular access, aiming at reducing the use of central venous catheters[34]. The first data collection on 184 incident dialysis patients was useful to recognize the problems in limiting the use of arteriovenous fistula, such as communication difficulties with patients or organizational shortcomings. Then, basing on the difficulties identified, the audit team developed specific intervention strategies (*i.e.*, promotion of educational skills, facilitated access to the operating room, direct nurse involvement,

Table 3 Checklist for the planning and validation of a clinical audit

Item	Yes/ No
Promoting a clinical audit	The audit topic has been decided according to the needs of the working group.
	The objectives are clearly specified.
	Indicators, criteria and reference standards have been set according to literature, guidelines and/or the consensus among experts.
Design and planning	The audit has been organized in different stages and times, assigning specific responsibilities.
	Necessary resources have been allocated.
	The population/reference sample has been defined.
	Tools for data collection have been designed, preliminarily defining data management methods.
	The whole material has been proposed in advance to the participants.
Data collection	Those who participated in the preventive phase have been involved.
	The established phases have been met.
	Data have been correctly collected.
Data analysis	The results have been discussed with the participants to the audit and other interested parties.
Interventions	A structured strategy to implement changes has been defined.
	Written reports of the results have been made and sent to all the participants.
Checking the audit effectiveness	A check of the effectiveness of the changes introduced has been planned.
	The verification has been formally documented.

etc.), that resulted, 12 mo later, in a significant increase in the number of patients starting dialysis with an arteriovenous fistula (75% *vs* 56 % of control baseline, $P < 0.01$).

Many audit projects have been also focused on management of hypertension in HD patients and different aspects have been investigated, such as the role of sodium dialysate concentration and dialysate temperature in the determining blood pressure (BP) levels[35,36].

Interestingly, in a recent study we tested whether a clinical audit in se is effective in improving BP control in a population of patients on regular HD.

We studied 177 adult prevalent HD patients, recording data on factors affecting BP and anti-hypertensive drug regimen at months -1 (Pre), 0 (the date of the audit- Audit), and +1 and +6 after the audit.

Hypertensive patients were identified, cases were discussed and recommendations for improving BP management were recorded, and then returned to each physician as a reminder and a feedback of the audit process.

The interventions included the reduction of extracellular fluid volume in patients with fluid overload, use of interdialytic ambulatory blood pressure monitoring and bioimpedance, initiatives aimed to increase patient compliance and modulation of dialysis sodium content or temperature. Interestingly, the announcement of the audit by itself was associated with a decreased prevalence of hypertension (Pre 64.4% to Audit 58.7%) and a further decrease followed the audit (Post-1 51.1%, Post-6 47.6%, $P < 0.05$ *vs* Audit). Systolic BP in hypertensive patients also decreased (mean decrease was -8.5 and -14.1; $P = 0.007$ and $P < 0.001$ at Post-1 and Post-6), being also associated with a reduced number of drugs assumed, thus proving that clinical audit is an effective tool to improve BP control in HD patients[37].

Mineral metabolism disorders in Chronic Kidney Disease (CKD-MBD) are an example of a suitable topic for a clinical audit. Indeed, they are common in HD patients and are associated with a number of clinical symptoms and complications, including cardiovascular diseases[38].

However, although MBD in HD patients are the object of intense research activity, their prevention and treatment still remain unsatisfactory[39]. In this view, we performed two large multicentre audits aiming to enlighten the obstacles that hamper the successful control of MBD by a straightforward "patient-oriented" approach[40,41].

Overall, we collected information and discussed the cases of about 700 prevalent HD adult patients according to the audit methodology.

First of all, we confirmed the data regarding the difficulty to achieve therapeutic targets, showing that only 15%-20% of the evaluated patients presented Ca, P and PTH values simultaneously controlled[42].

Then, evaluating the factors related to unsatisfactory results, we found that low compliance with treatment was the major determinant of failure (43.5% of the cases).

However, we observed a discrepancy between the analysis of factors accounting for therapeutic failure and the interventions planned. In fact, while the low compliance was recognized as the main cause of therapeutic failure, most of the interventions were focused on pharmacological therapy. Consequently, six months after the audit we found that, against a significant increase in the amount of drugs prescribed, the control of MBD parameters did not improve.

Therefore, the results of the audit suggested that low compliance with treatments is a main but still neglected cause of failure in the achievement of MBD control in HD patients, while increase of drug administration, regardless the awareness to the compliance to the therapy, is insufficient to obtain an overall satisfactory rate of therapeutic success.

This finding is particularly important, since indicates that future therapeutic strategies, beyond the development of new drugs, should include the implementation of feasible educational programmes addressed to both

health personnel and patients. This kind of study shows the potentiality of a clinical audit that allows to effectively compare theoretical standards with daily clinical practice, providing suggestions to improve quality of care.

FUTURE APPLICATIONS

Audit methodology could be potentially extended to several other issues in the setting of clinical nephrology.

For example, it could be useful to evaluate the causes of treatment failure in patients undergoing peritoneal dialysis, such as to implement protocols to reduce the rate of central venous catheter-related infections. Moreover, clinical audit could be a feasible tool to solve organizational problems, such as the delays on the waiting list for kidney transplantation.

Finally, a clinical audit could be used to face more general topics, which may involve also renal patients, such as management of dyslipidaemia (for example, evaluating the appropriateness of statin prescription) and implementation of lifestyle change.

CONCLUSION

Quality control, and consequently the right allocation of resources, is becoming a central issue in the management of Health Care Systems. Several tools are deployed to provide a monitoring of the levels of care and improve its quality. Among them, clinical audit is one of the most popular and widespread. In the specific field of clinical nephrology, this method has proven its effectiveness in facing different problems, such as hypertension and mineral metabolism control. However, it still seems necessary to spread the understanding of clinical audit and promote its systematic application both nationally and locally, so that it can be part of the expertise of each health care provider, together with other quality improvement techniques. In Table 3 we present a checklist for the planning of a clinical audit.

ACKNOWLEDGMENTS

We thank Marina Nazzaro for the English editing.

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P- Reviewer: Nakhoul FM, Stavroulopoulos A, Showkat HI, Watanabe T **S- Editor**: Ji FF **L- Editor**: A **E- Editor**: Lu YJ

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DOI: 10.5527/wjn.v3.i4.256

World J Nephrol 2014 November 6; 3(4): 256-267
ISSN 2220-6124 (online)

MINIREVIEWS

From crystalluria to kidney stones, some physicochemical aspects of calcium nephrolithiasis

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Received: June 20, 2014 Revised: July 23, 2014
Accepted: September 6, 2014
Published online: November 6, 2014

Abstract

Nephrolithiasis seems to be the result of crystal formation, aggregation and retention in the kidney during crystalluria. These processes have to occur within the short urinary transit time through the kidney being in the order of few minutes. Recently much work was done on rather qualitative aspects of nephrolithiasis like genetics, metabolism and morphology. In this review we try to provide some quantitative information on urinary supersaturation with respect to stone minerals, especially Ca oxalate (CaOx), on the formation and aggregation of CaOx crystals and on crystal retention in the kidney. The paper is centered on idiopathic Ca nephrolithiasis being the most frequent stone disease with only partially known pathogenesis. New aspects of the role of urinary macromolecules in stone formation and of the mechanism of crystal aggregation are provided.

Key words: Calcium nephrolithiasis; Crystalluria; Crystal aggregation; Urinary macromolecules; Self aggregation

Core tip: The state of urinary saturation with respect to Ca salts is governed by pH, Ca and Ox concentration. Growth of calcium oxalate (CaOx) in urine is too slow that single crystals could acquire a size to be trapped in nephron. The aggregation (AGN) of CaOx in urine was lacking or severely delayed due to inhibition by urinary macromolecules (UM's). Albumin, after temporary adsorption on calcium phosphate, showed self aggregation and promoted AGN of CaOx. Self aggregated UM's probably overwhelm the electrostatic repulsion of crystals coated by negatively charged UM's. This mechanism may explain the effect of Randall's plaques.

Baumann JM, Affolter B. From crystalluria to kidney stones, some physicochemical aspects of calcium nephrolithiasis. *World J Nephrol* 2014; 3(4): 256-267 Available from: URL: http://www.wjgnet.com/2220-6124/full/v3/i4/256.htm DOI: http://dx.doi.org/10.5527/wjn.v3.i4.256

INTRODUCTION

Nephrolithiasis can be defined as the result of formation and retention of crystals within the kidneys[1] where during crystalluria stone formation mainly seems to occur by crystal aggregation (AGN)[2]. Urinary stones are large crystal aggregates being embedded in a proteinous matrix. During the last years much work was done with respect to genetic, metabolic and morphologic aspects of nephrolithiasis[3-6]. Crystallization of stone forming minerals often was studied in artificial solutions neglecting the important fact that in biological solutions like urine crystals are always coated by macromolecules which essentially influence results[7,8] and that crystallization being relevant for stone formation has to occur within urinary transit time through the kidney, being in the order of a few minutes[9,10]. In this review we try to give some quantitative information on crystal formation, growth, AGN and retention as they may occur in the kidney during stone formation. The paper is centered on idiopathic calcium oxalate (CaOx) nephrolithiasis being the most frequent stone disease[11]. To illustrate the different top-

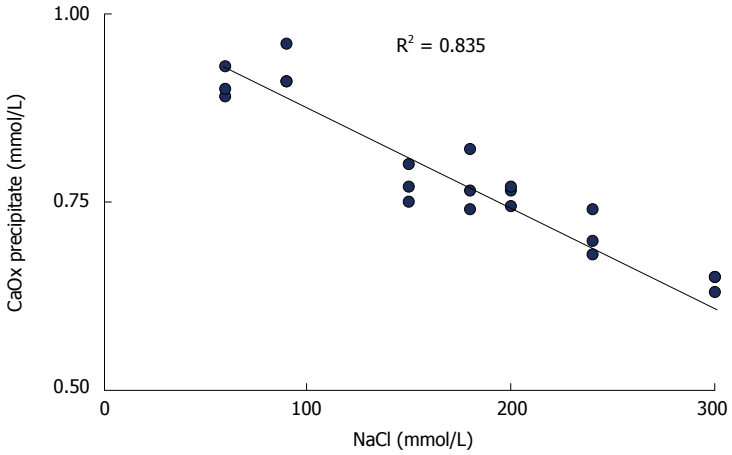

Figure 1 Influence of NaCl concentration on CaOx precipitation calculated from Ca decrease after the addition of 1.0 mmol/L sodium oxalate to aqueous solution of 1.5 mmol/L $CaCl_2$ buffered to pH 6.0 with 5 mmol/L sodium cacodylate.

ics, new figures were included which were drawn from own and only partially published experiments.

URINARY SUPERSATURATION WITH RESPECT TO STONE MINERALS, ESPECIALLY CAOX

The driving force for crystallization is urinary supersaturation which depends on the concentration of stone forming ions, their chelators, ionic strength and pH. Excessive excretion of Ca or Ox which almost exclusively can explain stone formation occurs in rare metabolic disorders like primary and secondary hyperoxaluria and some types of hypercalciuria[12]. In this review idiopathic Ca stone formation is addressed where not always and only relative mild forms of hypercalciuria or hyperoxaluria are found, which often are dependent from diet. After ingestion of a diet rich in Ox even in metabolic normal people an almost threefold increase of Ox excretion was observed[13]. To avoid dietary Ox excesses remains therefore essential in Ca stone metaphylaxis.

Urine is a complicated poly-ionic solution where multiple ions form various complexes between each other[14]. Some of these complexes like CaOx have an extremely poor solubility, precipitate already at a low concentration and -under special conditions being described below-form stones. In poly-ionic solutions the mobility and thus the activity of ions is reduced by the electrostatic forces exerted between the ions. Chemical reactions in solutions are therefore instead of ionic concentrations governed by ion activities (A, mol/L)[15]. A is calculated by the multiplication of the ion concentration (C) by an activity coefficient (f) as shown in equation (1). (f) can roughly be estimated by the ionic strength of the solution (I, mol/L).

(1) $A = f \bullet C$

In urine, ionic strength is mainly generated by sodium and chloride which are present in much higher concentrations than other compounds[16]. Increasing ionic strength decreases ion activities and thus supersaturation or the amount of substances which can be precipitated from supersaturated solutions. This is demonstrated by CaOx precipitation in solutions with constant Ca and Ox but increasing NaCl concentrations (Figure 1). Unfortunately, this effect cannot therapeutically be used because a high NaCl intake stimulates urinary Ca and reduces citrate excretion[17]. The high ionic strength in concentrated urine also does not protect from precipitation because the effect of decreasing ion activity is largely overwhelmed by the increase of supersaturation due to the increased ion concentrations[16]. A high diuresis remains therefore important for stone metaphylaxis.

Complex formation is schematically illustrated for CaOx by equation (2a). It is characterized by the reversible formation and dissociation of the soluble complex ($CaOx_S$) and by precipitation and dissolution processes between $CaOx_S$ and its crystalline precipitate ($CaOx_P$):

(2a) $Ca^{2+} + Ox^{2-} \leftrightarrow CaOx_S \leftrightarrow CaOx_P$

For each complex exists a dissociation constant (K_D, mol/L). It defines as shown in equation (2b) for CaOx the ratio between the mathematical product of free ion activities (A) in the solution and A of the solved complex:

(2b) $K_D = A_{Ca} \bullet A_{Ox}/A_{CaOxS}$

Since at a given temperature and with the precipitate in excess also the concentration of solved complexes is a constant (*e.g.*, 7.1 mg CaOx/L at 37 ℃). The status of complex forming ions in a solution can simply be expressed as activity product (AP).

In urine, multiple complexes are in competition to each other in reducing free ionic concentrations. Compounds which have a high tendency to form complexes with a high solubility are called chelators. Citrate is such a chelator which as shown in Figure 2 essentially reduces free Ca concentration (Ca^{2+}) and especially in patients with hypocitraturia has proofed to be efficient in stone metaphylaxis[18]. Contrary to other ions Ca^{2+} easily can be measured by Ca selective electrodes. Comparison of Ca^{2+} and total Ca in 20 urines showed a linear correlation with a chelation of 50%-70% of total urinary Ca being dependent from pH (Figure 3). A low pH or a high H^+ concentration respectively reduces by protonisation of phosphates and carboxyl groups the chemical

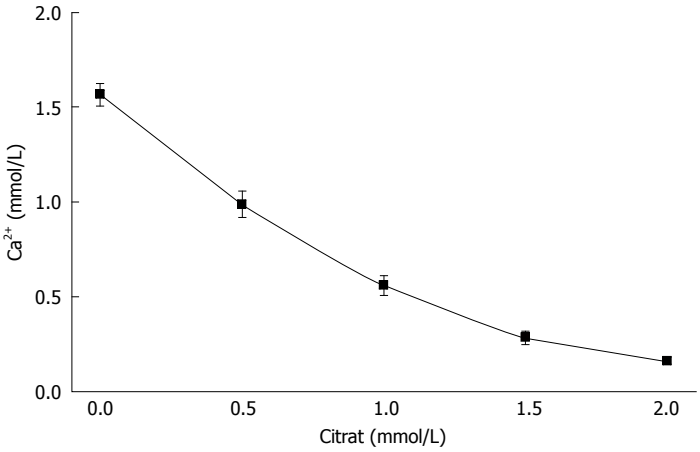

Figure 2 Influence of citrate concentration on free ionic Ca concentration (Ca^{2+}) in a solution like in Figure 1 but containing 100 mmol/L NaCl (mean ± SD, *n* = 5).

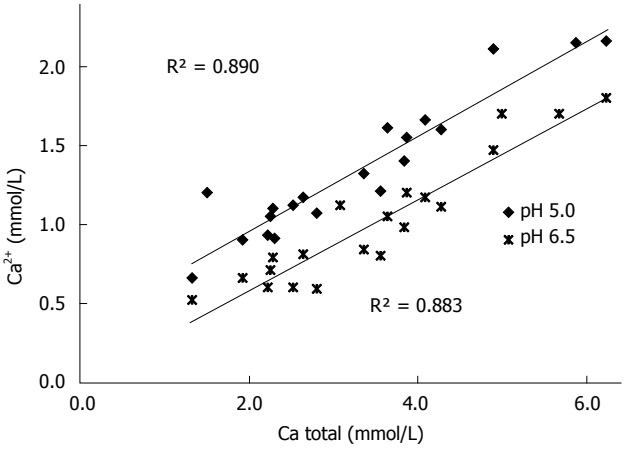

Figure 3 Correlation Ca^{2+} and total Ca concentration in 20 urines at pH 5.0 and 6.5.

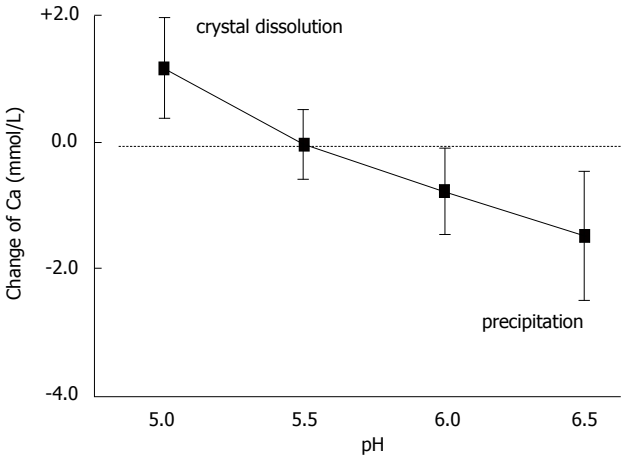

Figure 4 Influence of pH on solubility of hydroxyapatite demonstrated by the change of Ca concentration after equilibration of 20 urines with 10 mg/mL hydroxyapatite (mean ± SD).

valences of these compounds and thus their capacity of complex formation. This is demonstrated in Figure 3, where Ca chelation in urine was reduced and thus Ca^{2+} increased by lowering pH from 6.5 to 5.0. However, the most important effect of pH is observed with respect to phosphate. A high pH with its low H^+ concentration favors the formation of poorly soluble tertiary phosphates. This is demonstrated in experiments shown in Figure 4 where the change of Ca concentration was determined after equilibration of urine with hydroxyapatite (HAP, $Ca_{10}(PO_4)_6(OH)_2$). At an urinary pH above 5.5 the incubated HAP changed from dissolution to precipitation with a markedly decrease of urinary Ca. Struvite ($MgNH_4PO_4$) stones are exclusively found in alkaline urine where urease positive germs produce high concentrations of NH_4.

Due to all the various factors being involved, the state of urinary saturation with respect to stone minerals is difficult to determine. A generally accepted expression for this state is relative supersaturation (RS), the ratio of the products of ionic activities (AP) actually found in urine (e.g. $A_{Ca} \cdot A_{Ox}$) and the AP in the same urine being saturated with respect to the corresponding stone mineral (*e.g.,* CaOx)[15]. The latter AP is called solubility product (SP). An RS > 1.0 denotes supersaturation, an RS < 1.0 undersaturation. RS can be calculated by the sophisticated computer program Equil 93[14]. This program bases in its most extended version on the input of 15-23 chemical parameters and the calculation of about 100 complexes. Another approach is the calculation of an AP index from 5 parameters by equation (3) as demonstrated for CaOx[19]:

(3) $\text{AP index}_{CaOx} = 1.9 \cdot Ca^{0.84} \cdot Ox / Mg^{0.12} \cdot Cit^{0.22} \cdot \text{Urine-Vol.}^{1.03}$

The state of urinary saturation can also experimentally be determined by the calculation of a concentration product ratio (CPR) of stone forming ions before and after equilibration of urine with the corresponding stone forming mineral[20]. The comparison of CPR's and 6 chemical parameters measured in 76 urines of 19 idiopathic Ca stone patients showed only significant correlations between CaOx monohydrate saturation and Ox concentration ($P < 0.001$) and between brushite saturation and pH and Ca concentration ($P < 0.001$)[21]. Ca and Ox concentration and pH are thus the main parameters governing the state of urinary saturation with respect to stone forming Ca salts and which therapeutically can be influenced.

CRYSTAL FORMATION IN URINE

Stone minerals show as mentioned above at every state of saturation a bi-directional process of permanent precipitation and dissolution. With increasing supersaturation precipitation, which is also called crystal nucleation, prevails. However, low states of supersaturation do not have the energy to create stable particles and the precipitated crystal nuclei permanently dissolve[22]. To create stable particles a critical AP called formation product (FP) is mandatory which furnishes the energy being necessary to build up stable particle surfaces against surface tension. Supersaturation can thus be divided into two zones namely a labile zone above FP where crystal nucleation and growth occur and a metastable zone between FP and SP where already formed crystals grow without further nucleation. FP decreases with increasing incubation time, a fact that has to be taken in consideration in experiments simulating crystallization in the kidney with its short urinary transit times. Preexisting surfaces of solids where crystals can attach allow nucleation already in metastable supersaturated solutions. This special kind of nucleation is called heterogeneous nucleation.

Crystal nucleation and growth in urine are apart from supersaturation influenced by multiple urinary compounds called crystallization modulators[8]. These modulators comprise citrate, pyrophosphate, some glycosaminoglycans and a large group of proteins[23]. The most intensively studied and probably most important proteins are albumin, inter alpha inhibitor, nephrocalcin, osteopontin, prothrombin fragment 1 and Tamm Horsfall glycoprotein. Due to such modulators, often called inhibitors, crystallization processes in urine generally are decreased when compared to inhibitor free control solutions. Some substances can also act as nucleators. Despite of intensive research the role of all these compounds in stone formation could not definitively be clarified[24]. Studies in urine where all involved factors simultaneously are present are therefore of special interest.

Normal urine contains on average 4 mmol/L Ca and 0.4 mmol/L Ox and has a relative supersaturation (RS) of about 5[9], whereas for the spontaneous nucleation of CaOx an RS of 14 is mandatory[10]. In 60 urines of idiopathic stone patients and controls an Ox addition of 0.64 ± 0.11 mmol/L was necessary to induce CaOx crystallization without a difference between the two populations[7]. Such Ox concentrations are only achieved after excessive Ox ingestion[13]. Nevertheless, crystalluria is at least in some studies a frequent finding being generally more often found in urine of stone patients (9%-48%) than of healthy controls (2%-26%)[25]. Since urine often is only metastability supersaturated with respect to CaOx and storage and cooling have a minimal influence on CaOx formation[26], heterogenous nucleation seems to play an important role in crystalluria. Acid phospholipids accumulate Ca on cell membranes which thus become ideal nucleators for CaOx[23]. This heterogenous nucleation can occur by tubular cells in the kidney as well as by cellular debris in urine. Cellular material comprises about 50% of urinary deposits[27].

Nucleation and growth of CaOx can directly be followed in urine by measurement of the increase of optical density or the Ca decay after Ox addition[28]. Repeated measurement of the ionic Ca (Ca^{2+}) after different Ox additions to urine showed typical crystallization curves (Figure 5). being characterized by the half-life of Ca^{2+} decrease (h, min.) and by the ratio of Ca^{2+} decrease at time (t) and of total Ca^{2+} decrease at the end of crystallization (RD_t). RD_t can be calculated by equation (4a) from h, which is obtained by equation (4b) from Ox in urine after the Ox addition (Ox_i), a growth rate factor (g = 1.13 ± 0.36 mM^{-1} min^{-1}) and a factor for metastability (m = 0.60 ± 0.07 mmol/L).

(4a) $RD_t = t /(t + h)$

(4b) $h = 1 /g(Ox_i - m)$

Measurement of crystalluria by a coulter counter in freshly voided urine of stone patients and controls which was collected at 3 h intervals, showed after an oral Ox load single crystals with diameters on average of 6 μ and a maximum of 15 μ[10]. In the nephron at high urinary Ox concentrations (> 0.6 mmol/L) some nucleation can occur during a short time at the end of the descending limb of the loop of Henle but the main crystallization takes place at the end of collecting ducts[10]. The time for crystal nucleation and growth is therefore in the nephron about 1 min. For this time and a maximal Ox concentration of 0.9 mmol being assumed in idiopathic stone patients[9] a RD_t of 0.25 can be calculated. The size of the large single crystals obtained from bladder urine has therefore for the nephron to be reduced from 15 to 9.45 μ, which is far below the minimal internal diameter of 30 μ of renal collecting ducts[9]. The reduced crystal size (d) in the nephron was calculated basing on a reduction of an octahedron volume ($V = \sqrt{2}\, d^3/3$) of CaOx dihydrate crystals to 25%. This calculation shows that without AGN crystals seem to have only little chances to be mechanically trapped within renal tubules.

CRYSTAL AGGREGATION

Already in 1969 it was demonstrated that stone patients contrary to healthy controls especially after Ox ingestion have a tendency to excrete large crystal aggregates[29]. Such aggregates which can reach diameters up to 500 μ can obstruct collecting ducts and by further apposition of crystals can give raise to stone formation[12]. The growth of already existing stones can also be explained by aggregation (AGN)[2].

For AGN crystals have to collide. The natural driving forces for particle collision without shaking or stirring are diffusion by Brownian motion and sedimentation. The effect of these two forces recently was studied in the context of high physiological crystal concentrations, renal tubular and pelvic dimensions and urinary transit times in the kidney[30]. Crystals are even at the maximal

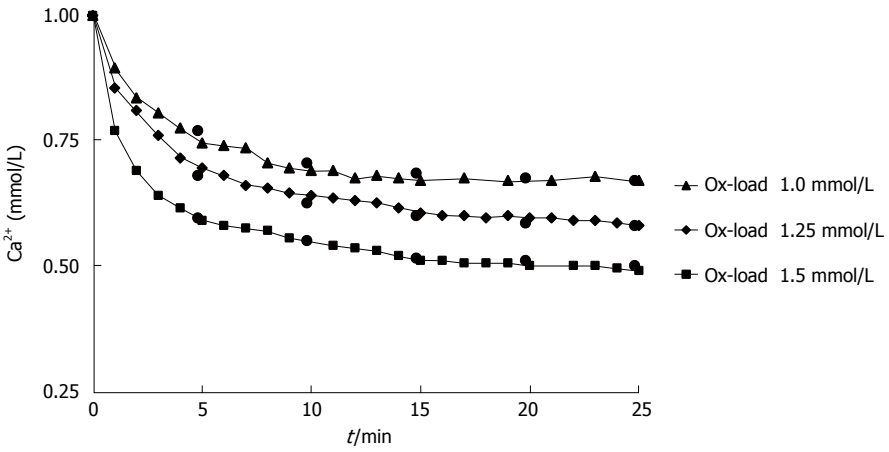

Figure 5 Decrease of Ca^{2+} in urine during observation time (*t*) after different Ox additions (1.0-1.5 mmol/L). Measured (triangle/diamond/square) and by RD_t (see text) calculated values (black circle).

concentration of 24000/cm^3 assumed in idiopathic Ca stone formers[9] on average in a distance of about 350 μ. Crystal motion by diffusion contrary to sedimentation is an undirected three- dimensional random process being only in the order of 10 μ/$min^{0.5}$. Crystal collision in the nephron by diffusion is therefore negligible and in the renal pelvis with a volume of 7 cm^3 may occur about 3 times per min. Collision of free floating single crystals by sedimentation is even more rare. It bases on differences in sedimentation rates due to differences in crystal sizes which in urine are too low for an efficient collision. However, sedimentation seems to be important for crystal accumulation on tubular walls where flow rate is slow due to fluid drag and on surfaces in the renal pelvic system where urinary transit time is prolonged. For collecting ducts being in a horizontal position a maximal accumulation of 1.3 crystals per min. was estimated. The accumulation of crystals on surfaces in the renal pelvic system by sedimentation (As, cm^{-2} $min.^{-1}$) can be calculated by equation (5) which contains the crystal concentration (C, cm^{-3}) and a sedimentation rate (vs, cm/min.).

(5) $As = C \bullet vs$

For maximal crystalluria of 24'000 crystals/cm^3 and a sedimentation rate of 0.026 cm/min. an accumulation of 624 crystals per cm^2 surface and min. can be calculated[30]. Crystal accumulation on kidney calcifications or stones seems to be an important mechanism in stone formation.

However, also with an important crystal accumulation on surfaces during crystalluria, for AGN crystals have to become attached to each other. In inorganic solutions this attachment generally is ascribed to an attraction by van der Waal forces being only effective at short distances of some 0.1 nm[31]. In biological fluids crystals are always surrounded by protein coats with a thickness of 10-30 nm[32]. These proteins have a negative electric charge of -15 to -30 mV which normally inhibits AGN by electrostatic repulsion of the identically charged particles[33]. From CaOx and CaP crystals being precipitated in urine six different proteins comprising albumin were isolated[34]. The composition of these proteins showed no difference whether they were precipitated from urine of stone patients or of controls. Inhibition of crystal AGN by urinary macromolecules (UM's) was demonstrated in various studies[23]. But a deficient urinary inhibitory activity in urine of stone patients could only be demonstrated in some studies[35-37] but not in all[38,39]. Examination of AGN often was performed in artificial solutions with the addition of an UM and of crystals which were previously produced in an inhibitor free medium.

However, the formation and AGN of CaOx can also be studied in urine by an oxalate titration with spectrophotometric follow of the crystallization process[7]. This is demonstrated in Figure 6A where after a critical Ox addition, which is a measure for metastability and was lower in a control solution (CS) than in urine, optical density (OD) steadily increased. The maximal OD which was reached at the end of titration and which mainly reflects particle concentration was lower in CS than in urine, where due to an inhibition of crystal growth more but smaller crystals were produced. After the end of Ox titration stirring was stopped and OD decrease reflecting particle sedimentation was followed during a further 30 min. In CS which in scanning microscopy performed at the end of Ox titration showed large crystal aggregates (Figure 6C), a rapid OD decrease immediately after the end of titration was observed, whereas in urine without AGN (Figure 6B) this rapid OD decrease was lacking or occurred with a delay of 15 and more minutes. Analysis of crystallization curves and scanning electron microscopy of crystal sediments revealed a good correlation between OD decrease and particle sizes[30]. Crystals produced by Ox titration showed in urine of 63% of healthy controls but only in urine of 33% of stone patients during 30 min observation time a complete inhibition of AGN ($P < 0.05$)[7]. In the remaining urine AGN occurred with a delay often being beyond urinary transit time through the kidney which is in the order of 15 min[40]. Most aggregates found in voided urine therefore seems to originate from crystal AGN in the urinary bladder. Since in ultrafiltrate of urine where urinary macromolecules (UM's) were retained on a 5 kD filter, AGN immediately occurred, inhibition of AGN could mainly be ascribed to the effect of UM's[41]. However, when UM's were isolated by a hemofilter procedure or

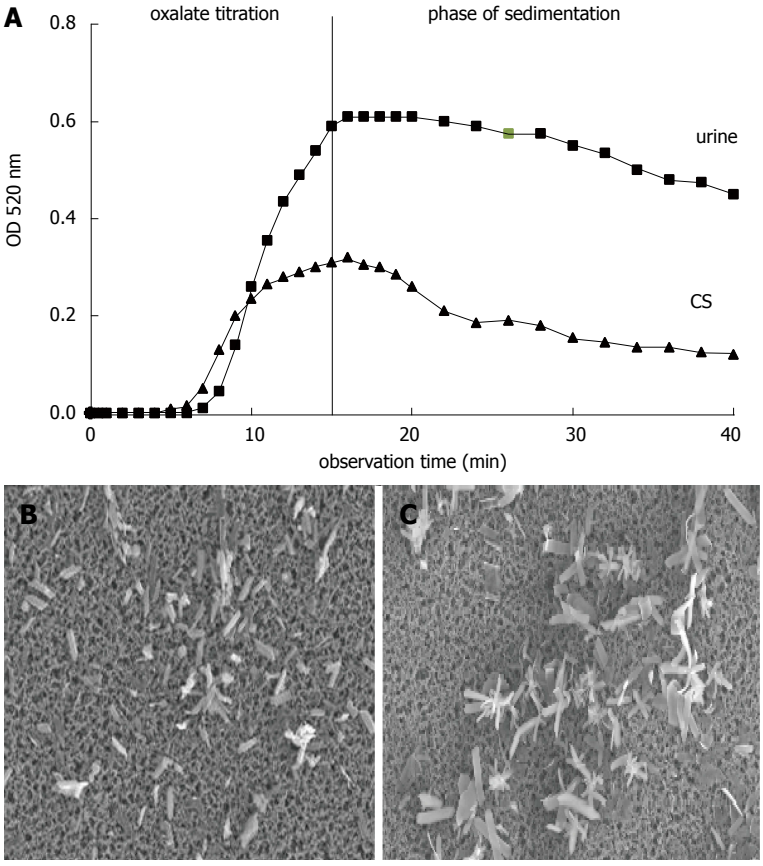

Figure 6 Ox titration. A: Spectrophotometric crystallization curve of Ox titration (0.1 mmol/min.) in urine and control solution (CS) both with pH 6.0, 100 mmol/L NaCl, initially 2 mmol/L Ca^{2+} and with total 1.5 mmol/L Ox addition by titration; B: Scanning microscopy of deposit on Millipore filter obtained at the end of Ox titration from urine and (C) from CS.

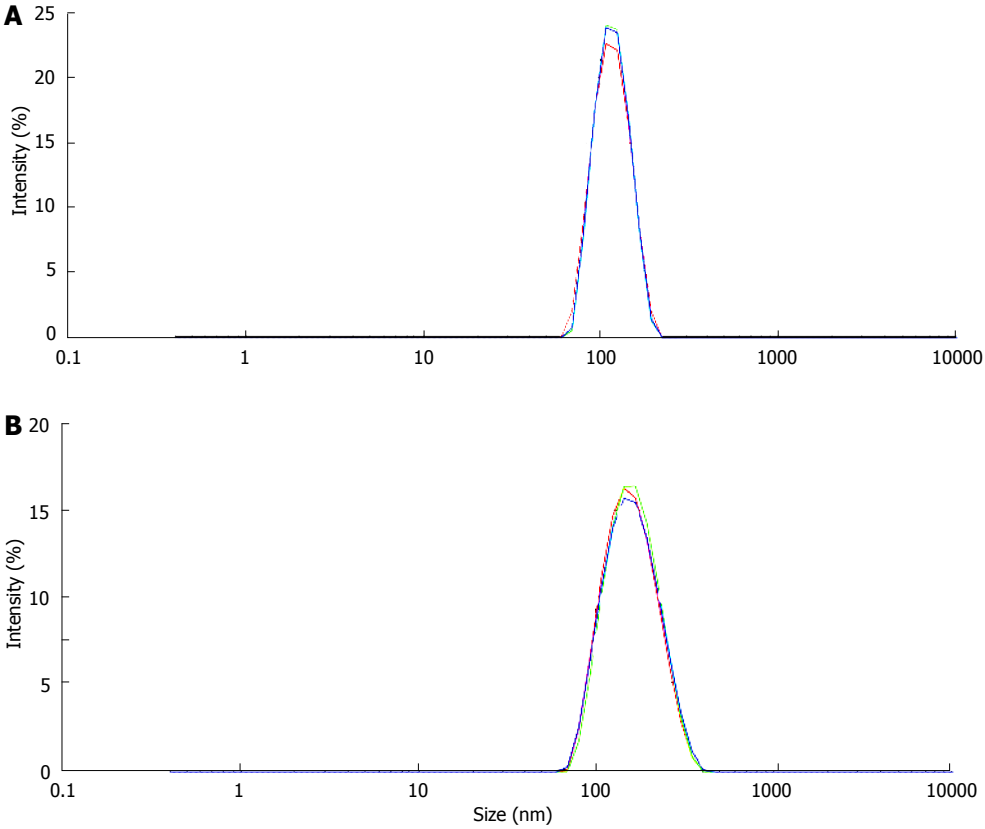

Figure 7 Particle size distribution in suspension of latex beads (size 100 nm, concentration 0.025%/mL) measured by a Zetasizer (A) in control solution, (B) in solution of urinary macromolecules obtained by CaP precipitation and dissolution of the precipitate.

by a CaP precipitation with dissolution of the sediment, AGN already occurred after a delay of about 7 min[33]. The same effect was observed after addition of hydroxyapatite crystals to urine[7]. A contact with surfaces even when temporary seems thus to destroy the inhibitory potential of UM's.

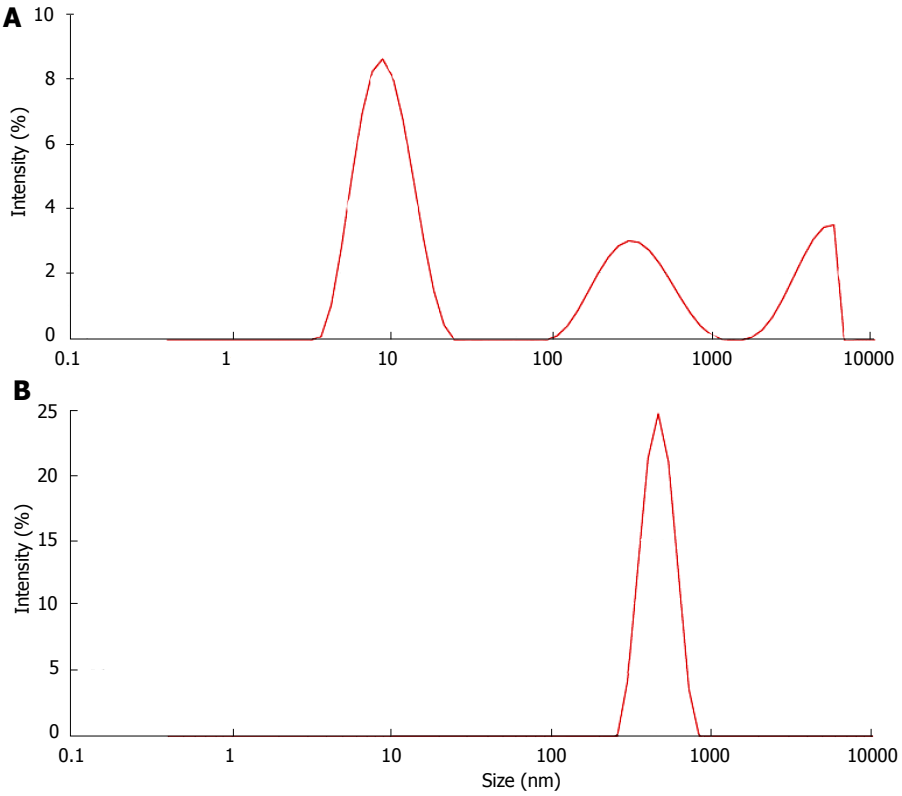

Figure 8 Particle size distribution (A) in albumin solution (AS, 20 μg/mL) and (B) in solution obtained from AS after CaP precipitation and dissolution of the precipitate (DPA).

UM's have a high and rather unspecific affinity to surfaces. As known from catheter incrustation they bind by hydrophobic forces even to latex. The adsorption of UM's on latex beads is demonstrated in Figure 7. Incubation in UM solution increased the maximal peak in particle size distribution of latex beads which was measured by a Zetasizer from 116 nm to 160 nm. Some UM's like albumin, osteopontin and Tamm Horsfall glycoprotein (THG) when concentrated are known to have a tendency to self AGN[23]. Self AGN turned THG from a potent inhibitor to a promoter of CaOx AGN[42]. Also polymers of albumin were found to be strong promoters of CaOx crystallization[43]. The tendency of albumin to self AGN is demonstrated in Figure 8 again by the measurement of particle size distribution with a Zetasizer. In an albumin solution apart of the main peak at 10 nm further peaks indicating albumin aggregates were found (Figure 8A). After CaP precipitation in the same solution and dissolution of the sediment where albumin temporary had been adsorbed to CaP crystals only one fraction of highly aggregated albumin with a peak at 480 nm was observed (Figure 8B). This highly aggregated albumin had, as shown in Figure 9, the same effect on CaOx crystallization curves as UM's isolated by hemofiltration or by CaP precipitation and as the addition of HAP to urine. Maximal OD after oxalate titration was diminished and already 7 min. after the end of titration a sharp OD decrease indicating CaOx AGN occurred. Temporary concentration of UM's by adsorption on surfaces seems thus to favor self AGN of UM's and to change their inhibitory potential with respect to the AGN of CaOx crystals.

The questions raises, how crystals aggregate despite of their electro-negative UM coats and electrostatic repulsion and how self AGN of UM's can favor crystal AGN. The answers to these questions are still speculative. Three different options for crystal attachment to each other are discussed: Incomplete isolation of crystals by protein coats , insufficient surface potential of coats and bridging between crystals by altered proteins. Scanning microscopy of crystal aggregates which were produced in protein solutions showed gaps in protein coats where crystals were in direct contact to each other[32]. But it could not be decided whether crystal coating had occurred before or after AGN. In other aggregates at points of crystal convergence large amorphous material was found suggesting crystal bridging by proteins.

The inhibition of crystallization processes by UM's is mainly attributed to anionic residues like carboxyglutamic acid[44,45], phosphate[46,47] and sialic acid[48,49] which have a high affinity to the Ca of crystals. Some of these anionic groups being responsible for the electro-negative charge of coated crystals were found to be reduced in UM's of stone patients. An insufficient crystal coating and electrostatic repulsion was therefore suggested to be responsible for AGN and stone formation. On the other hand it was shown that desialylation of Tamm Horsfall glycoprotein (THG) provoked self AGN of THG and AGN of CaOx crystals[49], effects which were also demonstrated in experiments performed with albumin (Figures 8 and 9). Desialylation reduces anionic domains and thus reinforces hydrophobic groups of THG being responsible for hydrophobic protein binding. This can favour not only self AGN of THG but also a bridging

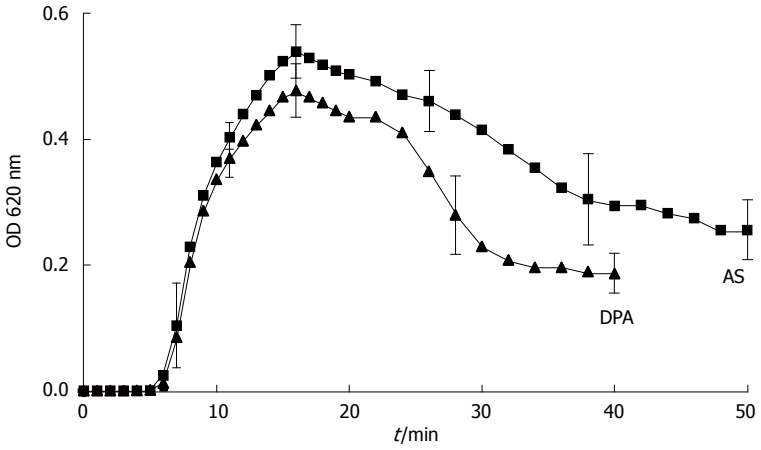

Figure 9 Spectrophotometric crystallization curve of Ox titration in AS and in DPA with pH 6.0, 100 mmol/L NaCl, initial 2.0 mmol/L Ca^{2+} and 1.5 m mmol/L Ox addition by titration. (Further details see Figure 8) (mean ± SD, *n* = 5).

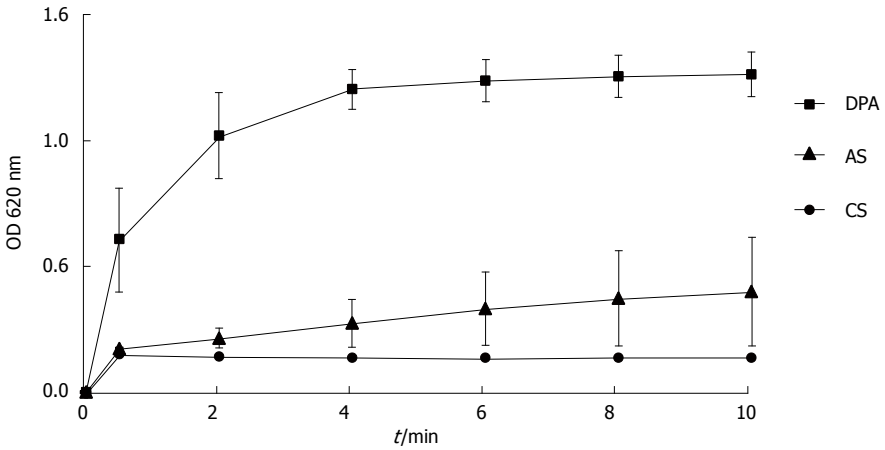

Figure 10 Increase of optical density of suspensions of latex beads reflecting increase of particle size by aggregation in control solution, in albumin solution and in aggreagted albumin. (Further details see Figure 8) (mean ± SD, *n* = 5). CS: Control solution.

function of THG between proteins of crystal coats. In electrolyte-containing solutions the electrostatic potential generated by electrically charged particles exponentially decreases with increasing distance from the particles[31]. Identically charged particles can therefore approach to each other to a critical distance of some nanometers where diffusion, sedimentation or mechanic forces like stirring or shaking are compensated by electrostatic repulsion. Large protein aggregates probably are able to bridge such zones of electrostatic repulsion.

Bridging and especially self AGN seem to be relative slow processes which could explain the delay of AGN observed in urine. In a spectrophotometer the velocity of albumin-induced AGN of latex beads can directly be followed by an OD increase which occurs in a linear correlation with the increase of latex aggregates[50]. Figure 10 shows that in the untreated albumin solution (AS) this increase was very slow whereas in a solution of aggregated albumin (DPA) latex-AGN was already complete within 4 minutes. Bridging of CaOx crystals with their irregular shape and large surface certainly takes more time than bridging of the small and spherical latex beads. In view of the delayed AGN of CaOx crystals in urine urinary transit time (UTT) through the kidney becomes a crucial factor for stone formation. In the nephron average UTT is about 3 min[10] and in the renal collecting system about 12 min[40]. These values were calculated for an urinary output of 1.5 L/24 h. Increasing diuresis which remains an important measure in stone metaphylaxis[51] essentially reduces UTT especially in the renal collecting system.

CRYSTAL AND STONE RETENTION IN THE KIDNEY

We showed that crystal growth at least in idiopathic stone patients generally is too slow for single crystals to be trapped in the upper urinary tract and that crystal AGN often is delayed beyond urinary transit time trough the kidney. Crystals are probably most often washed out by diuresis before AGN occurs. However, when CaOx crystals were produced in the presence of HAP crystals which were added to urine before Ox titration, AGN occurred as demonstrated in Figure 11 after about 7 min. Scanning microscopy of sediments showed that this AGN had occurred in narrow contact with the HAP crystals[52]. Tissue calcifications with HAP, after erosion to papillary surface seem therefore to be an ideal platform for a rapid attachment of urinary crystals. Randall developed already in 1937 the theory that Ca stones can start by CaOx growth initially fixed on papillary calcifications which are called Randall's plaques[53]. This mechanism allows crystal aggregates to grow to stones of a critical size where they can not anymore be washed out of the urinary tract by the urine flow. Systematic post-

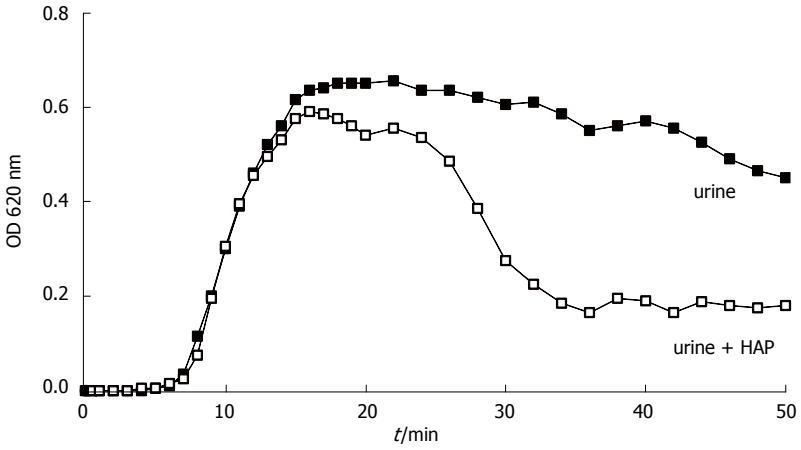

Figure 11 Spectrophotometric crystallization curve of Ox titration in urine without and with previous addition of 0.05 mg/L HAP.

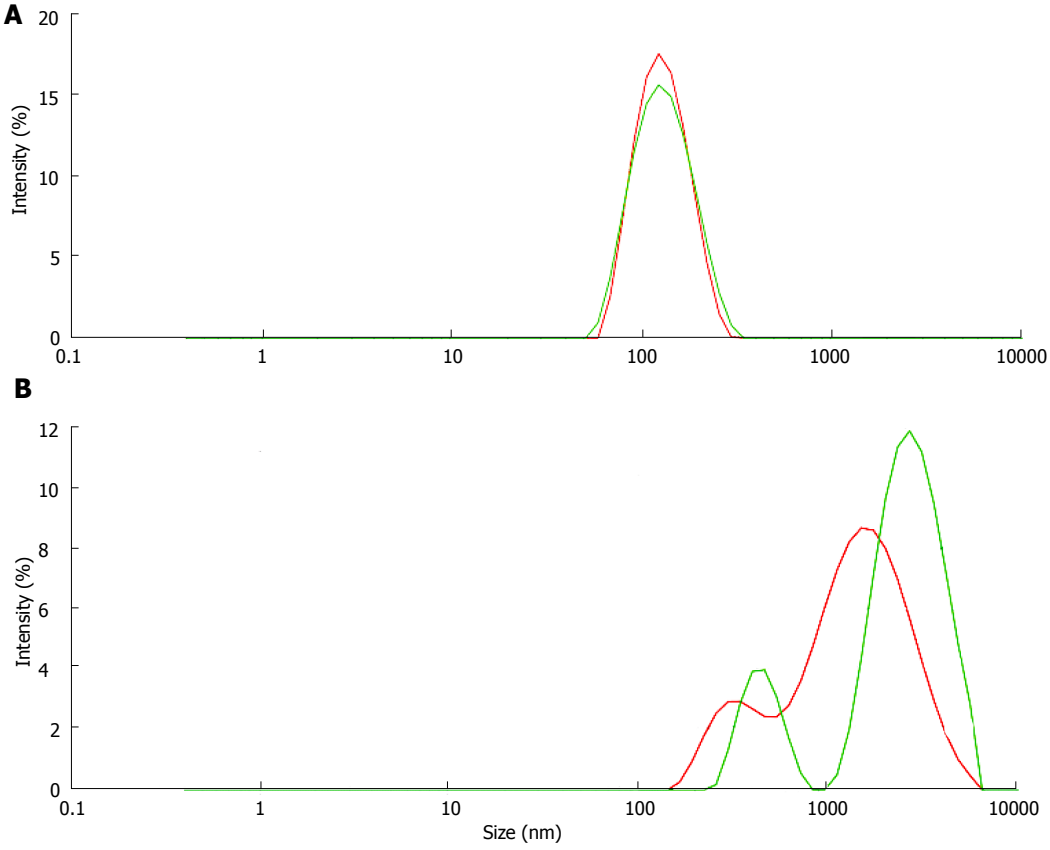

Figure 12 Particle size distribution of latex beads (A) at pH 6.0 and (B) at pH 5.0 in solution of urinary macromolecules obtained by CaP precipitation.

mortem examination of 100 kidneys revealed in 100% some calcifications but only in 7% stones[54]. Kidney calcifications are therefore not a singular cause of Ca stone formation. New endourologic methods allowing in vivo inspection of all renal papilla with the possibility of biopsies have brought new evidence for the role of Randall's plaques in idiopathic Ca stone formation[12,55]. CaOx stones found in calices often were attached to white plaques on the papilla containing amorphous hydroxyapatite within a protein matrix. Furthermore, most CaOx stones being endoscopically removed showed residual cores of apatite where they probably have been attached to the papilla.

Stone formation on Randall's plaques is complex and still poorly understood. Histological analysis with immunohistochemistry or infrared spectroscopy of Randall's plaques with an adherent stone showed that the plaques consisted of an osteopontin (OP) matrix with hydroxyapatite (HAP) deposits whereas the stone in addition to OP and HAP contained Tamm Horsfall glycoprotein (THG) and CaOx, the latter increasing with increasing distance from the plaque[12]. Stone formation thus seems to occur at the interface of HAP and CaOx crystals being embedded in proteins like OP and THG which both have a tendency to self AGN[23] and which in urine of some stone formers were found to have a deficiency in

acid groups[47,49]. A reduction of anionic groups reinforces as mentioned above hydrophobic activity of proteins and thus the tendency to self AGN and to the binding to hydrophobic domains of other proteins. AGN inhibition by anionic groups can indirectly be demonstrated studying the influence of pH on UM induced latex AGN (Figure 12). When latex beads with a diameter of about 100 nm were incubated at pH 6.0 in a UM solution obtained by the precipitation and dissolution of CaP in urine only a moderately increased particle size of 156 nm due to UM coating of the latex beads was observed (Figure 12A). The same procedure performed at pH 5.0 with a higher protonisation of anionic UM groups on the other hand produced a peak of particle intensity at 2400 nm showing a massive AGN (Figure 12B). Lowering pH from 6.0 to 5.0 reduced the surface potential of the latex beads being influenced by the anionic valence of the UM coats from -28 to -21 mV. An effect of pH on the activity of UM's and thus on AGN could be of interest from a therapeutic point of view and deserves further investigation.

CONCLUSION

It is astonishing that despite of the widespread occurrence of crystalluria and kidney calcifications not everybody produces stones. In stone patients without treatment average recurrence rate was only 1 stone within 8 years[56] and in 71% of patients stone residuals after percutaneous nephrolithotomy remained stable or even decreased during an observation time of 2-3 years[57]. The retention and growth of crystal aggregates in the kidney seems therefore only to occur under very special and rare conditions. Such conditions may be an extremely low diuresis with high concentrations of stone forming minerals and with a prolonged urinary transit time allowing crystal AGN already in the kidney and/or an extreme oxalate ingestion. Both conditions can produce an excessive crystalluria which can damage renal tubules and alter the production of UM's normally protecting from stone formation[23]. Which factors finally are decisive whether crystalluria provokes stone formation or crystalluria is a useful mechanism to eliminate heavy soluble substances with minimal water loss remains open to further research.

ACKNOWLEDGMENTS

We wish to thank Dr. Marion Nöel for their collaboration concerning Zetasizer measurements, performed in Bern University of Applied Sciences, Division of Architecture, Wood and Civil Engineering, Research and Development, Biel, Switzerland.

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P- Reviewer: Chang CC, Isaka Y, Shrestha BM **S- Editor**: Ji FF
L- Editor: A **E- Editor**: Lu YJ

World Journal of Nephrology

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Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.5527/wjn.v3.i4.268

World J Nephrol 2014 November 6; 3(4): 268-276
ISSN 2220-6124 (online)

MINIREVIEWS

T-cell ageing in end-stage renal disease patients: Assessment and clinical relevance

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Received: June 10, 2014 Revised: July 8, 2014
Accepted: August 27, 2014
Published online: November 6, 2014

Abstract

End-stage renal disease (ESRD) patients have a defective T-cell-mediated immune system which is related to excessive premature ageing of the T-cell compartment. This is likely to be caused by the uremia-associated pro-inflammatory milieu, created by loss of renal function. Therefore, ESRD patients are highly susceptible for infections, have an increased risk for virus-associated cancers, respond poorly to vaccination and have an increased risk for atherosclerotic diseases. Three ageing parameters can be used to assess an immunological T-cell age. First, thymic output can be determined by assessing the T-cell receptor excision circles-content together with CD31 expression within the naïve T cells. Second, the telomere length of T cells and third the T-cell differentiation status are also indicators of T-cell ageing. Analyses based on these parameters in ESRD patients revealed that the immunological T-cell age is increased by on average 20 years compared to the chronological age. After kidney transplantation (KTx) the aged T-cell phenotype persists although the pro-inflammatory milieu is diminished. This might be explained by epigenetic modifications at hematopoietic stem cells level. Assessment of an immunological T-cell age could be an important tool to identify KTx recipients who are at risk for allograft rejection or to prevent over-immunosuppression.

Key words: End-stage renal disease patients; Kidney transplantation; T-cell ageing; T-cell differentiation; Uremia

Core tip: The uremia-induced inflammatory environment in end-stage renal disease (ESRD) patients is associated with a prematurely aged T-cell compartment, resulting in defective T-cell-mediated immunity. ESRD patients are highly susceptible for infections, have an increased risk for virus-associated cancers, respond poorly to vaccination and have an increased risk for atherosclerotic diseases. Adequate renal replacement therapy in the form of kidney transplantation is able to diminish the uremic pro-inflammatory environment but unsuccessfully reverses the aged T-cell system. Assessment of T-cell ageing might be a tool to facilitate individualization of immunosuppressive regimes and prevent over-immunosuppression and its associated clinical complications in kidney transplant recipients.

Meijers RWJ, Betjes MGH, Baan CC, Litjens NHR. T-cell ageing in end-stage renal disease patients: Assessment and clinical relevance. *World J Nephrol* 2014; 3(4): 268-276 Available from: URL: http://www.wjgnet.com/2220-6124/full/v3/i4/268.htm DOI: http://dx.doi.org/10.5527/wjn.v3.i4.268

INTRODUCTION

Loss of renal function is strongly associated with a defective immune system which is known as uremia-associated immune deficiency[1-3]. Retention of uremic molecules and cytokines in end-stage renal disease (ESRD) patients are key mechanisms in generating oxidative stress and inflammation[2,4,5]. This creates a pro-

inflammatory environment in which both the innate (first line of defense, a-specific)[3,6-8] as well as the adaptive (specific) immune system are affected (Figure 1)[3,9,10].

T cells, members of the adaptive immune system, are the best-studied immune cells in ESRD patients and in the field of transplantation they are the main target of immunosuppressive medication[11]. The uremia-associated pro-inflammatory milieu causes T-cell defects associated with premature T-cell ageing when compared to healthy age-matched individuals (Figure 1)[12]. Analysis of the T-cell compartment in ESRD patients revealed that the immunological age of T cells is increased by 20 years compared to their chronological age (Figure 1)[12].

The dysfunctional immune system of ESRD patients has a substantial clinical impact on both the morbidity and mortality of ESRD patients. Patients are highly susceptible for infections[13,14], have an increased risk for virus-associated cancers[15], respond poorly to vaccination[16] and have an increased risk for atherosclerotic diseases[17,18].

In this review, the concept of uremia-associated age-related changes of T cells is highlighted focusing on the assessment of an immunological T-cell age, clinical implications and possible therapeutic options for ESRD patients.

CONCEPT OF T-CELL AGEING

With normal healthy ageing, the T-cell immune system ages as well[19]. Hematopoietic stem cells (HSCs), generated in the bone marrow, give rise to myeloid as well as lymphoid progenitor cells[20]. T cells are generated from the latter. With increasing age, HSCs are skewed towards myeloid-generating subsets at the expense of lymphoid-generating HSCs, resulting in a lower number of progenitor T cells. These progenitor T cells are further "educated" in the thymus in which naïve T cells will form specific receptors on their cell surface known as T-cell receptors (TCRs). With increasing age, the thymus involutes[21,22]. This process involves a decrease in tissue in combination with a loss of tissue organization with the net outcome that numbers of naïve T cells leaving the thymus, known as recent thymic emigrants (RTEs) are reduced. Involution of the thymus starts at birth and is accelerated during adolescence[23].

This explains the lymphopenic number in naive T cells with increasing age. Despite the fact that the naïve T-cell pool can also be maintained by homeostatic proliferation in which TCR triggering in combination with the cytokines Interleukin (IL)-7 and IL-15 expand T cells[24], the net effect is a diminished number of naïve T cells and the number of memory T cells in the peripheral blood of elderly individuals is preserved[25]. A relatively expanded number of naïve T cells by homeostatic proliferation results in a T-cell pool with a restricted TCR repertoire[24,26]. A diverse TCR repertoire is a necessary prerequisite for an adequate and effective T-cell response towards newly encountered antigens[27].

After encountering and activation by an antigen, a naïve T cell will proliferate and become a memory T cell. During physiological ageing the population of antigen-experienced memory T cells will increase and the majority of these cells will become highly differentiated. These cells are known to have an increase in pro-apoptotic markers[28] and loss in co-stimulatory molecule CD28[29,30]. CD28 plays an important role in the activation of T cells and a loss of CD28 can result in insufficient activation, shorter replicative lifespan and a higher toxicity[29]. Furthermore, highly differentiated cells are known to have a reduction in their telomere length[31].

A telomere is a region of repetitive nucleotides which is located at the end of each chromosome and prevents chromosomal instability. Loss of telomere length has been linked to an increased risk for tumor development and to T-cell ageing[32,33].

ASSESSING AN IMMUNOLOGICAL T-CELL AGE

A global assessment of the immunological age of the T-cell system can be performed by the analysis of three ageing parameters. During the formation of the T-cell receptor (TCR) in the thymus, DNA sequences in the TCR loci are deleted and circularized into episomal DNA molecules, so called single joint TCR excision circles (TREC), a process known as TCR rearrangement[34]. This TREC remains in the newly formed naïve T cells leaving the thymus. Upon replication of these cells in the periphery, the TREC is only transferred to one daughter cell resulting in a reduction of TRECs in the naïve daughter T cells. With an increasing age, the number of RTEs containing a TREC declines log linearity due to a lower thymic output of RTEs and an increase in proliferation of naïve T cells. The TREC content can be determined using a quantitative polymerase chain reaction (qPCR) method normalized to the single-copy albumin gene[34,35]. Next to the TREC content, these RTEs can be detected by measuring the expression of CD31 within the naïve T-cell pool[36,37]. In addition to the thymic output of T cells, the diversity of the TCR repertoire can be analyzed by sequencing in order to determine the loss of TCR specificities within the T-cell population and to assess the percentage of oligoclonal T cells[27,38]. Recently, a novel TREC assay in which the TCR diversity was combined with the TREC content to get quantitative insight into intra-thymic and post-thymic proliferative capacity of T cells and its alterations upon ageing[39].

As a second parameter for the assessment of an immunological T-cell age, the T-cell telomere length can be determined as a measurement for the proliferative history of a T-cell population[40]. A decline in telomere length is highly associated with an increased proliferative history. A commonly used method to assess a relative telomere length (RTL) is the fluorescent *in situ* hybridization (FISH) method[41,42]. During this procedure a labeled

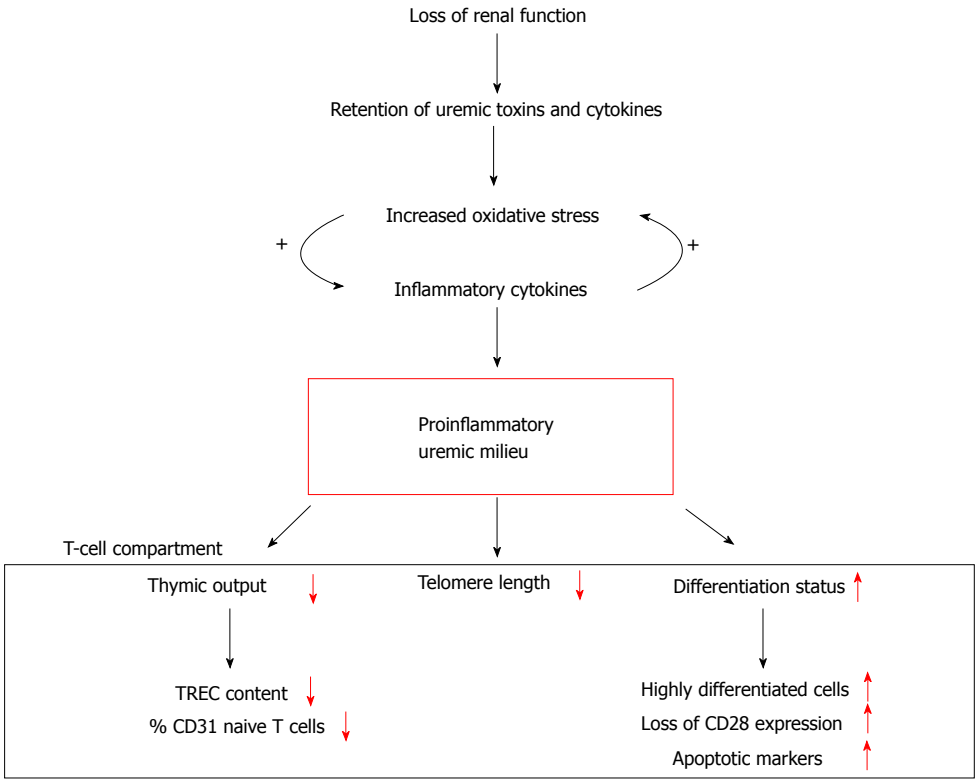

Figure 1 Schematic overview of the effects on the T-cell compartment caused by the uremia-induced pro-inflammatory milieu in end-stage renal disease patients. Loss in renal function creates a pro-inflammatory milieu by the retention of uremic toxins and cytokines which increases oxidative stress and the production of inflammatory cytokines. This pro-inflammatory uremic milieu is associated with premature T-cell ageing, which results in defective T-cell immunity. End-stage renal disease (ESRD) patients have a lower thymic output of naïve T cells which can be measured by the TCR excision circles (TREC) content and the percentage of CD31-expressing naïve T cells. Furthermore, ESRD patients have an expanded population of highly differentiated T cells with a loss in CD28 expression and an increase in apoptotic markers. Moreover, these expanded T cells have a high proliferative history causing a decline in telomere length which can be measured by the relative telomere length analysis.

peptide nucleic acid (PNA) probe binds to the telomere repeats which can be read-out by fluorescent microscopy or by fluorescence measurements using a flow cytometry (flow FISH). The RTL can be calculated by relating the intensity of the bound PNA probe to that of a T-cell lymphoblastic leukemia (1301 CCRF-CEM) cell-line, known for its long telomeres, as an internal control[41]. Inclusion of antibodies in this method makes it possible to analyze the telomere length in different T-cell populations (*i.e.,* $CD4^+$ and $CD8^+$ T cells)[2,41]. A limitation of this assay is the temperature (82 ℃) which is required for DNA annealing which makes the use of stable fluorochromes necessary[41,42]. Quantum dots (nanoparticles) were found to be highly fluorescent, bind to antibodies and have much better temperature stability. Quantum dots conjugated with antibodies directed to T-cell antigens were found to retain most of their fluorescence following the annealing step. The use of quantum dots can be a solution for the limitations in antibody use in the flow-FISH procedure and allows to assess a telomere length in different T-cell subsets within one assay[42].

In addition to the telomere length, the activity of the telomerase can be measured. Telomerase is responsible for maintaining telomere length and the cellular replicative potential and an impaired activity of telomerase results attrition of telomeres[19]. Measuring the activity of telomerase gives additional information on the telomere shortening. This assay is based on the capacity of a test sample to amplify a telomere template[43].

The differentiation status of the T-cell compartment can be used as a third parameter to assess an immunological age. The increase in highly differentiated memory cells with increasing age can be determined by analysis of the phenotype of circulating T-cells using multicolor flowcytometry. Based on the expression of the chemokine (C-C motif) receptor 7 (CCR7), enabling cells to migrate to secondary lymphoid organs, and CD45RO, an isoform of the leukocyte common antigen expressed on memory T cells, a distinction within the memory T-cell compartment can be made. The different memory T cell subsets include Central Memory (CM) ($CCR7^+$ and $CD45RO^+$), able to home to secondary lymph nodes and producing mainly IL-2 which is necessary for the proliferation of T cells, Effector Memory (EM) ($CCR7^-$ and $CD45RO^+$), able to migrate to peripheral tissues exerting direct effector functions and terminally differentiated effector memory $CD45RA^+$ (EMRA) ($CCR7^-$ and $CD45RO^-$), which exert cytotoxic activities and are highly susceptibility to apoptosis[44]. Moreover, these terminally differentiated cells often lose the expression CD28 which makes them less dependent on co-stimulation to become activated[45]. In addition, CD57 can be measured

as a marker for highly differentiated memory T cells[12,46]. CD95 (FAS) and CD279 (known as programmed death receptor-1 (PD-1)) are both commonly used as pro-apoptotic markers[12,28,47].

AGED T-CELL SYSTEM IN ESRD PATIENTS

Based on the analyses of the T-cell ageing parameters, *i.e.*, assessment of TREC- content, relative telomere length and differentiation status we showed that the immunological age of ESRD patients is advanced by 20 years compared to their calendar age[12]. As compared to an age-matched healthy control, ESRD patients had a lower thymic output of naïve T cells, a decline in the T-cell telomere length and an increase in the differentiation status towards the terminally differentiated memory phenotype with a large number of CD28-negative (or CD28null) T cells (Figure 1)[12]. Progressive loss of renal function was highly correlated with a lack of IL-7, a loss of naïve T cells and an increase in terminally differentiated $CD8^+$ T cells[48]. The effects of renal replacement therapy (RRT) on the T-cell ageing parameters seemed to be small and were limited to the $CD8^+$ T-cell compartment of young ESRD patients[2]. The type of RRT did not influence the ageing parameters since both hemodialysis (HD) and peritoneal dialysis (PD) patients showed signs of an aged T-cell compartment[2]. Moreover, the duration of dialysis did not seem to influence the ageing parameters[49]. Furthermore, the type of underlying kidney disease was not related to any parameter of immunological ageing[2] indicating that the loss of renal function is the dominant factor for a decreased thymic output of naïve T cells and increased differentiation/proliferation of memory T cells.

Cytomegalovirus (CMV) is known to affect the T-cell compartment which closely resembles ageing[46,50-52]. Infection with the virus results in chronic latency and the effects of CMV on the T-cell compartment are relevant, since approximately 70% of the ESRD patients is infected with CMV[50]. In these patients, CMV was associated with an increased number of highly differentiated $CD4^+$ and $CD8^+$ T cells and a relatively small decline in $CD8^+$ T-cell telomere length[46,50,53]. The effects were restricted to the memory T-cell compartment since the thymic output of T cells was not affected. Therefore we concluded that CMV only affects the differentiation status of circulating T cells[46,50,53].

CLINICAL IMPLICATIONS OF AN AGED T-CELL COMPARTMENT

The uremia-associated prematurely aged T-cell immune system has a substantial clinical impact leading to an increased morbidity and mortality. ESRD patients are highly susceptible for infections which might further contribute to the pro-inflammatory milieu. For instance periodontitis, which is common in patients with chronic kidney disease (CKD), often leads to inflammation[54].

T cells of ESRD patients have an impaired production of IL-2 and the inadequate T-cell proliferative capacity results insufficient T-cell responses[55-57]. This in combination with low numbers of T cells results into inadequate T-cell responses directed to viruses and a decreased tumor surveillance which significantly increases the risk for virus-associated tumors[15,58]. Next to IL-2, in hemodialysis (HD) patients it was found that activated T cells have impaired responses to tumor necrosis factor (TNF)-α, implying a state of tachyphylaxis[59].

Following vaccination against hepatitis B, the formation of antigen-specific $CD4^+$ EM T cells is severely impaired in ESRD patients[56]. The poor development of IL-2 producing $CD4^+$ EM T cells in patients with ESRD was strongly associated with a low generation of antibodies towards hepatitis B antigens[56]. The inability to maintain protective antibody titers after T-cell dependent vaccinations[60,61] or after a natural infection[62,63] might be caused by a loss of antigen-specific T cells as a result of their increased susceptibility for apoptosis[12,47].

Furthermore, the loss in TCR diversity of naïve T cells due to a lower number of RTEs but an increase in proliferated naïve T cells is linked to a decreased efficiency of vaccination but also to an increased susceptibility for infections and cancers[26,64].

$CD4^+$ T cells lacking CD28 expression, are found to be highly cytotoxic as they produce large amounts of interferon (IFN)-γ and TNF-α and release granzyme-B and perforin upon activation. In several studies[17,65] it is shown that these cytotoxic cells are present in unstable atherosclerotic plaques and are associated with an increased risk for recurrence of both acute coronary events and ischemic stroke resulting in a higher mortality rate[66]. As confirmed in ESRD patients, high numbers $CD4^+$CD28null T cells is strongly associated with a history of cardiovascular diseases[17,18,65,67].

$CD8^+$CD28null T cells contain a subpopulation of cells possessing immunosuppressive capacities[68,69] and has therefore been linked to a decreased vaccination responsiveness of healthy individuals[70]. These immunosuppressive capacities also suggest that these cells could be important in preventing allograft rejection after kidney transplantation (KTx). Indeed, we recently demonstrated that patients with an expanded population of highly differentiated (EMRA) $CD8^+$CD28null T cells had a lower risk for allograft rejection after KTx[71]. Another explanations might be that $CD8^+$CD28null T cells represents clonal expansions of particular antigen-specific $CD8^+$ T cells that compete for immunologic space which is associated with reduction of T-cell diversity[72]. This might affect the diversity of alloreactive T-cells as well. Next to these highly differentiated $CD8^+$ T cells in KTx recipients, a high proportion of highly differentiated $CD4^+$ T cells was also linked to a lower risk for allograft rejection[73].

PREMATURE T-CELL AGEING AND KIDNEY TRANSPLANTATION

After KTx, the levels of pro-inflammatory proteins and oxidative stress decrease rapidly to levels that are comparable to healthy individuals[74]. Despite this, the uremia-associated prematurely aged T-cell immune system existed after KTx. (Meijers et al, 2014 submitted)

Immunosuppressive treatment affected the number of highly differentiated cells directly post-KTx. However after tapering the immunosuppressive medication, these highly differentiated T-cell numbers were restored to pre-KTx values. Furthermore, the telomere length of the T-cell compartment did not change and thymic function was not improved the first year post-KTx (Meijers *et al* 2014 submitted). Even after T-cell depleting immunosuppressive therapy [*i.e.*, rabbit antithymocyte globulin (rATG)] T cells are repopulating by homeostatic proliferation instead of a higher thymic output of naïve T cells[75,76]. Therefore, the uremia-associated immunological ageing seems stably imprinted in the T-cell system and not reversible by KTx.

Normal ageing is associated with, epigenetic changes in HSCs resulting in a shift in the balance towards myeloid precursors at the expense of the lymphoid ones[77,78]. Healthy ageing results in genetic alterations affecting T cells at developmental stages leading to phenotypic as well as functional changes[79]. In ESRD patients, uremia is able to cause epigenetic changes[80]. Young *et al*[81] 2012 found that methylation of the KLOTHO gene is initiated by oxidative stress in ESRD patients. KLOTHO deficient mice created a syndrome that resembles human ageing[82]. Although KTx reverses the uremic proinflammatory environment[74] it is unable to induce changes at the epigenetic level. The persistence of the aged T-cell phenotype post-KTx has several clinical implications as it may increase the risk for infections, malignancies and cardiovascular diseases in KTx recipients. T-cell lymphopenia has been associated with a high risk for infections and malignancies post-KTx[83,84].

Due to ageing of the T-cell compartment, elderly patients are more vulnerable for drugs toxicity, infections and malignancies caused by over-immunosuppression. In these patients, the incidence of virus-associated cancers is even higher post-KTx as it is pre-KTx[58,85]. Over-immunosuppression might be prevented after mapping the T-cell immune system of the transplant recipient[73,86] as T cells are the main target of immunosuppressive medication[11]. A study of Ducloux *et al*[87] in 2010 showed that prolonged $CD4^+$ T-cell lymphopenia after severe T-cell depletion by rATG is associated with an increased risk for infections and mortality post-KTx. High TREC values implying for a "younger" T-cell compartment pre-KTx, is associated with a better reconstitution of T-cell numbers after rATG and lower risk for infections and cancer post-KTx[87].

THERAPEUTIC OPTIONS TO REVERSE T-CELL AGEING

As mentioned earlier, RRT did not reduce T-cell ageing since no major differences between patients on dialysis and predialysis patients with respect to the T-cell ageing parameters were observed[2]. Adequately targeting the presence of the pro-inflammatory environment in ESRD patients by KTx[74] did not successfully reverse the aged T-cell immune system.

Another method to reduce the level of oxidative stress and inflammation in ESRD patients is targeting the transcription factor Nuclear factor-erythroid-2-related factor 2 (Nrf2) which is an important regulator of genes encoding antioxidant and detoxifying molecules[88]. Treatment with bardoxolone methyl, which is an activator of Nrf2 may attenuate T-cell ageing in ESRD patients[88]. However, treatment is restricted due to the increased risk of cardiovascular diseases after treatment with bardoxolone[89].

Another therapeutic option that might be able to improve T-cell function in ESRD patient is treatment with IL-7, a key cytokine for homeostatic proliferation of naïve T cells, that is reduced in patients causing a depletion of naïve T-cell pool[48,90]. The first human studies, in which IL-7 was administered, are promising since an increased naïve T-cell pool with a broader TCR repertoire diversity was found[38,91]. At present, IL-7 administration has not been tested in patients with ESRD.

CONCLUSION

Progressive loss of renal function creates a pro-inflammatory milieu which is highly associated with a dysfunctional immune system. This is a logical explanation for the increased vulnerability for infections, poor vaccination responses, high risk for malignancies and high risk for atherosclerotic diseases. Analysis of the T-cell system showed that ESRD patients have a prematurely aged T-cell compartment resulting in an impaired function. ESRD patients have a lower thymic output of naïve T cells, T cells have shorter telomeres and the T-cell compartment is shifted towards more differentiated T cells.

Therapeutic options to minimize morbidity and decrease mortality by improving or even fully reversing the aged T-cell phenotype are warranted. Although improvement of renal function by adequate renal replacement therapy in the form of KTx, which drastically decreases the uremia-associated pro-inflammatory milieu, the prematurely aged T-cell phenotype appeared to be irreversible. Therefore the aged T-cell immune system remains an important determinant of the dysfunctional immune system post-KTx. More research is necessary to fully understand the uremia-associated premature T-cell ageing phenomenon, also at earlier developmental stages of T-cells, to be able to successfully intervene and increase the life-span of ESRD patients.

Until today, all KTx recipients receive the same standard immunosuppressive therapy to prevent allograft rejection. Recently it was shown that the effect of calcineurin-inhibitors and rapamycin on peripheral blood mononuclear cells (PBMCs) was different between young and elderly individuals[92]. Assessing an immunological T-cell age using T-cell ageing parameters as described in this review, may guide clinicians in decision-making with respect to transplanting an ESRD patient or not, adjusting immunosuppression following KTx to minimize its long-term-associated adverse events.

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World J Nephrol 2014 November 6; 3(4): 277-281
ISSN 2220-6124 (online)

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Nutcracker syndrome

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Received: June 6, 2014 Revised: July 16, 2014
Accepted: September 17, 2014
Published online: November 6, 2014

Abstract

The nutcracker phenomenon [left renal vein (LRV) entrapment syndrome] refers to compression of the LRV most commonly between abdominal aorta and superior mesenteric artery. Term of nutcracker syndrome (NCS) is used for patients with clinical symptoms associated with nutcracker anatomy. LRV entrapment divided into 2 types: anterior and posterior. Posterior and right-sided NCSs are rare conditions. The symptoms vary from asymptomatic hematuria to severe pelvic congestion. Symptoms include hematuria, orthostatic proteinuria, flank pain, abdominal pain, varicocele, dyspareunia, dysmenorrhea, fatigue and orthostatic intolerance. Existence of the clinical features constitutes a basis for the diagnosis. Several imaging methods such as Doppler ultrasonography, computed tomography angiography, magnetic resonance angiography and retrograde venography are used to diagnose NCS. The management of NCS depends upon the clinical presentation and the severity of the LRV hypertension. The treatment options are ranged from surveillance to nephrectomy. Treatment decision should be based on the severity of symptoms and their expected reversibility with regard to patient's age and the stage of the syndrome.

Key words: Nutcracker syndrome; Renal vein entrapment; Hematuria; Orthostatic proteinuria; Left renal vein hypertension

Core tip: The nutcracker phenomenon [left renal vein (LRV) entrapment syndrome] refers to compression of the LRV most commonly between abdominal aorta and superior mesenteric artery. Term of nutcracker syndrome (NCS) is used for patients with clinical symptoms associated with nutcracker anatomy. The symptoms vary from asymptomatic hematuria to severe pelvic congestion. The management of NCS depends upon the clinical presentation and the severity of the LRV hypertension.

Gulleroglu K, Gulleroglu B, Baskin E. Nutcracker syndrome. *World J Nephrol* 2014; 3(4): 277-281 Available from: URL: http://www.wjgnet.com/2220-6124/full/v3/i4/277.htm DOI: http://dx.doi.org/10.5527/wjn.v3.i4.277

INTRODUCTION

The nutcracker phenomenon [left renal vein (LRV) entrapment syndrome] refers to compression of the LRV most commonly between abdominal aorta and superior mesenteric artery. This phenomenon is characterized by impeded outflow from the LRV into the inferior vena cava (IVC) due to extrinsic compression.

The terms nutcracker phenomenon and nutcracker syndrome (NCS) are sometimes used as synonym in the literature. Nutcracker phenomenon descript anatomic findings suggestive of nutcracker are present without clinical symptoms. Term of NCS is used for patients with clinical symptoms associated with nutcracker anatomy.

Diagnosis of NCS could be difficult for some reasons. It was thought to be a rare condition. Also in the

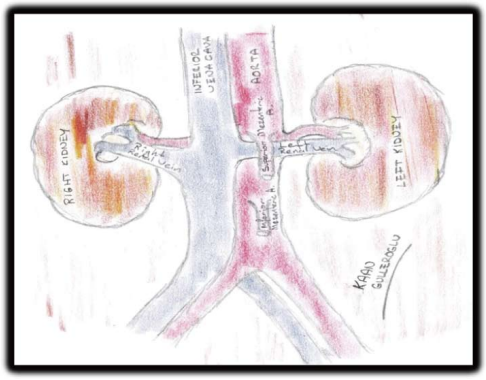

Figure 1 Anatomical configuration of nutcracker syndrome.

absence of clinical features it was necessitate a high suspicion. A noninvasive imaging must be followed by an invasive imaging for confirmation of the diagnosis.

ANATOMICAL CONFIGURATION AND PATHOPHYSIOLOGY

LRV entrapment divided into 2 types: anterior and posterior. Anterior NCS is the compression of a normally situated LRV by the abdominal aorta and the superior mesenteric artery (Figure 1). Posterior NCS is rare. It is presented with retroaortic LRV compressed usually between abdominal aorta and vertebral column. Other uncommon causes such as pancreatic neoplasm, paraaortic lymphadenopathy, retroperitoneal tumor, abdominal aortic aneurysm, overarching testicular artery, LRV duplication, and ectopic ventral right renal artery and strangulating fibrolymphatic tissue may play a role on the etiology of posterior NCS. Left renal ptosis, lordosis and decreased retroperitoneal and mesenteric fat tissue may cause to NCS[1-3].

Right-sided NCS is a more rare condition. Pregnancy is defining as a factor contributing to right-sided NCS by compression of large veins[1]. Left-sided IVC, hemiazygos continuation and persistent left superior vena cava combination is another rare cause of right NCS[4].

All of the anatomic mechanisms involved in renal vein compression are resulting with outflow obstruction leads to LRV hypertension with a measurable renocaval pressure gradient. The normal pressure gradient between the distal renal vein and IVC is < 1 mmHg. A renocaval pullback pressure gradient of ⩾ 2 mmHg is highly suggestive of a nutcracker phenomenon[5]. LRV hypertension is the underlying mechanism which may result in formation of varices and collaterals. Venous sinuses in the neighboring of renal calyces were taken in form by these varices and collaterals. Hematuria and proteinuria are the results of these venous sinuses[6].

DEMOGRAPHIC CHARACTERISTICS

Prevalence of NCP is unknown. NCP may be higher in female. Affected persons are ranging from children and adolescents to middle-aged and older people with seventh decade of life[7]. Most symptomatic patients are in their second and third decade of life and a second peak of NCS occurs in middle-aged women[8]. Coincidental cases in siblings have been reported, although NCP is not a hereditary phenomenon[9]. The rapid increase in body height and the maturation of the vertebral bodies during puberty is resulting with decrease in the angle between the superior mesenteric artery and aorta. A low body mass index has been shown to correlate positively with NCS[10].

CLINICAL FEATURES

Clinical features of patients with NCS are various. The symptoms vary from asymptomatic hematuria to severe pelvic congestion. Some patients have severe and persistent symptoms. Symptoms are aggravated by physical activity[7]. Symptoms include hematuria, orthostatic proteinuria, flank pain, abdominal pain, varicocele, dyspareunia, dysmenorrhea, fatigue and orthostatic intolerance[11-13]. The symptoms of autonomic dysfunction such as hypotension, syncope, and tachycardia could be seen but they are rare[14]. Henoch-Schönlein purpura, IgA, nephropathy, membranous nephropathy, and idiopathic hypercalciuria with nephrolithiasis associated with NCS have been reported[12,15].

NCS can differentiate clinically into 2 subtypes as follows: typical presentation (or renal presentation) and atypical presentation (or urologic presentation). Typical clinical presentation include hematuria (micro- to macrohematuria), orthostatic proteinuria with or without flank pain. Abdominal pain, varicocele, dyspareunia, dysmenorrhea, fatigue and orthostatic intolerance are the components of the atypical presentation (Table 1).

The most common symptom is hematuria. It is due to elevated LRV pressure resulting in the rupture of thin-walled septum between the varices and the collecting system in the renal fornix. Hematuria varies from micro- to macrohematuria. LRV is correspondent in this variation[14]. Isolated hematuria was reported 33.3% in children with NCS. Microhematuria is 4 times more common than macrohematuria[16].

Orthostatic proteinuria is another common symptom in NCS. The degree of proteinuria is variable. The incidence of orthostatic proteinuria is high during puberty. The mechanism of orthostatic proteinuria was not well understood yet. Changes of renal hemodynamic and the elevated levels of norepinephrine and angiotensin II were thought as the causes[17].

Pain is a result of the inflammatory cascade triggered by venous hypertension. Flank pain and abdominal pain are the consequences of that inflammatory process[1]. Left flank pain can be due also to urethral colic related to blood clots passing down to left ureter[7].

Varicocele affects 5.5%-9.5% of men and usually occurs on the left side. Development of varicocele is related with high LRV pressure and collateral circulation.

Table 1 Clinical features of the nutcracker syndrome

	Renal presentation	Urologic presentation
Hematuria	+	-
Orthostatic proteinuria	+	-
Flank pain	+	-
Abdominal pain	-	+
Varicocele	-	+
Dyspareunia	-	+
Dysmenorrhea	-	+
Fatigue	-	+
Orthostatic intolerance	-	+

Collateral veins could be demonstrated on pelvic and abdominal Doppler ultrasonography or venography[11].

DIAGNOSIS

Variations of normal anatomy must be considered before the diagnosis. Asymptomatic dilatation of LRV is frequently seen on ultrasonography or computed tomography, has been accepted as a finding of a normal variant[18]. NCS can exist without distended LRV. Normal flow also can exist in distended LRV[11]. Therefore, the first diagnostic need must be clinical examination. Existence of the clinical features constitutes a basis for the diagnosis. The presence of macroscopic or microscopic hematuria and proteinuria must evaluate. Urine analysis, urine phase contrast microscopy, urine culture and imaging of kidneys should be performed. Several imaging methods are used to diagnose NCS. Doppler ultrasonography, computed tomography angiography (CTA), magnetic resonance angiography (MRA) and retrograde venography are utilized.

Doppler ultrasonography can be used as the first diagnostic test in patients with suspected NCS. Length of the LRV is 6 to 10 cm and the average normal LRV diameter is 4 to 5 mm[7]. The normal pressure gradient between LRV and IVC is 1 mmHg or lower An elevated gradient > 3 mmHg between the LRV and the IVC can be used as a criteria of diagnosis for NCS[5]. Diameter of normal left gonadal vein is approximately 3 mm[19]. The normal superior mesenteric artery (SMA) originates behind the neck of the pancreas at the level of the first lumbar vertebra, and usually creates an acute angle at its origin from the aorta. Mean SMA angle is 51 ± 25° and mean SMA-aorta distance is 16 ± 6 mm in normal adults. Mean SMA angles in children are 45.8 ± 18.2° for boys and 45.3 ± 21.6° for girls. Mean SMA-aorta distances in children are 11.5 ± 5.3 mm for boys and 11.5 ± 4.5 mm for girls[20]. The standards of ultrasound diagnosis of NCS are descript by Zhang *et al*[21]: (1) the flow velocity of stenosis of the LRV in the supine position accelerates remarkably, and the acceleration, which is more than 100 cm/s, is more obvious after the patient has stood for 15 min; (2) the inner diameter ratio between ratio between the renal hilum and stenosis of the LRV in the supine position is > 3 and is > 5 after the patient has stood for 15 min[21]. Doppler ultrasonography has a sensitivity of 78% and a specificity of 100%[22]. However, in children the use of these criteria is limited because the smallest LRV sampling area and the largest Doppler angle than in adults[23].

CTA and MRA provide visualization of the anatomy. These tests can demonstrate the precise LRV compression point and/or prestenotic dilatation of the LRV together with perirenal and/or gonadal vein varices[24]. "Beak sign" is the abrupt narrowing of the LRV with a triangular shape at the aortomesenteric portion. It might be most useful finding among the various CT parameters, because it showed sensitivity 91.7% and specificity 88.9%[25]. MRA finding are similar to CT findings and MRA has the advantages of being less invasive with less amount of radiation than retrograde venography.

Retrograde venography is the gold standard for the diagnosis of NCS. It is not only confirming anatomic change, but also show a pressure gradient across the area of entrapment. Reflux of contrast into adrenal and gonadal veins from periureteral and perirenal venous collaterals, and pooling of contrast into the renal vein can be demonstrated[22]. Retrograde venography is the most informative method although it is an invasive test. It is not commonly performed in patients who have not severe symptoms.

Another invasive test such as cystoscopy may be helpful to identify hematuria from left ureteral origin. Notching from varicosities of the renal pelvis and ureters may be seen[26]. Cystoscopy is an indirect diagnostic method for NCS diagnosis.

TREATMENT

NCS is a type of spectral disease and varies in severity and symptoms, reflecting degrees of LRV compression, LRV hypertension and the compensatory stage related to the development of collaterals[11]. The management of NCS depends upon the clinical presentation and the severity of the LRV hypertension. The treatment options are ranged from surveillance to nephrectomy. Treatment decision should be based on the severity of symptoms and their expected reversibility with regard to patient's age and the stage of the syndrome[27]. Mild and tolerable symptoms can be followed conservatively. However, recurrent gross hematuria with anemia, severe flank pain, renal functional impairment, and inefficacy or aggravation of conservative treatment of the persistent orthostatic proteinuria after 24 mo of follow-up might require surgical treatment[18].

Spontaneous resolution by physical development during childhood is possible[18]. Conservative approach with observation during minimum 2 years without medication is the best option for patients younger than 18 years old. Seventy-five percent of patients with hematuria have complete resolution during this time[7]. Angiotensin inhibitors could be effective in patients with especially severe and prolonged orthostatic proteinuria[1].

Surgical procedures are used for treatment in patients with severe symptoms. Nephropexy, intravascular and

extravascular stent implantation, transposition of the LRV or SMA, gonadocaval bypass, renal autotransplantation and nephrectomy are surgical procedures.

Open surgical techniques for anterior NCS include LRV transposition, LRV transposition with patch venoplasty, patch venoplasty without LRV transposition, LRV transposition with saphenous vein cuff, gonadal vein transposition and saphenous vein bypass[28]. LRV transposition is the most frequent and most effective technique in which LRV is transposed distally to the IVC. The LRV is transected and re-anastomosed to the IVC in a more distal location and in a tension-free end-to-side fashion. LRV transposition with patch venoplasty is used in conditions as permanent distortion of the vein with prolonged compression of the LRV or overstretched LRV because of the prominent aorta. The great saphenous vein is used as a patch to augment the LRV-IVC confluence after transposition of the LRV. Patch venoplasty without LRV transposition technique is used when transposition is not favorable because of the short renal vein is short or it is not improve the external compression of the vein. In LRV transposition with saphenous vein cuff technique the saphenous vein is used to form a cuff extension to the LRV to create tension-free anastomosis. Decrease of pelvic congestion and decompression of LRV can be obtained by left gonadal vein transposition. Saphenous vein also can be used for the bypass of the decompressed segment of the LRV[28].

Anterior transposition of LRV is used for posterior variant of NCS. In this technique LRV is excised with a small rim of the caval wall, and transposed to IVC, in a proximal position, *via* anteaortic routing[29].

Surgical placement of an external stent to the LRV is another surgical approach to NCS [5]. Endovascular stenting is an alternative treatment option. It can be preferred to open surgery because of the long period of renal congestion, additional anastomoses and extensive dissection requirement of the open surgery. Thrombosis, stent migration, fracture and restenosis are the complications of the endovascular stenting but they are rare[30].

CONCLUSION

NCS is a type of spectral disease and varies in severity and symptoms. Variations of normal anatomy must be considered before the diagnosis. Existence of the clinical features constitutes a basis for the diagnosis. The management of NCS depends upon the clinical presentation and the severity of the LRV hypertension.

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P- Reviewer: Bianchetti MG, Greenwell TJ **S- Editor**: Song XX
L- Editor: A **E- Editor**: Lu YJ

World Journal of Nephrology

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Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.5527/wjn.v3.i4.282

World J Nephrol 2014 November 6; 3(4): 282-286
ISSN 2220-6124 (online)

MINIREVIEWS

Usefulness of hounsfield unit and density in the assessment and treatment of urinary stones

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Received: June 22, 2014 Revised: July 24, 2014
Accepted: September 6, 2014
Published online: November 6, 2014

Abstract

Computed tomography (CT) is widely used to examine stones in the urinary system. In addition to the size and location of the stone and the overall health of the kidney, CT can also assess the density of the stone in Hounsfield units (HU). The HU, or Hounsfield density, measured by CT, is related to the density of the tissue or stone. A number of studies have assessed the use of HU in urology. HUs have been used to predict the type and opacity of stones during diagnosis, and the efficacy has been assessed using methods including extracorporeal shock wave lithotripsy (ESWL), percutaneous nephrolithotomy (PCNL), ureterorenoscopic ureterolithotripsy (URSL), and medical expulsive treatment (MET). Previous studies have focused on the success rate of HU for predicting the type of stone and of ESWL treatment. Understanding the composition of the stone plays a key role in determining the most appropriate treatment modality. The most recent reports have suggested that the HU value and its variants facilitate prediction of stone composition. However, the inclusion of data regarding urine, such as pH and presence of crystals, increases the predictive accuracy. HUs, which now form part of the clinical guidelines, allow us to predict the success of ESWL; therefore, they should be taken into account when ESWL is considered as a treatment option. However, there are currently insufficient data available regarding the value of HU for assessing the efficacy of PCNL, URSL, and MET. Studies performed to date suggest that these values would make a significant contribution to the diagnosis and treatment of urinary system stones. However, more data are required to assess this further.

Key words: Hounsfield unit; Urinary stones

Core tip: Hounsfield units provide information not only for the diagnosis of urinary system tumors but also regarding a number of properties of urinary stones. Computed tomography is currently used most commonly to predict the type of stone and assess the potential efficacy of extracorporeal shock wave lithotripsy treatment. However, it might also assist urologists to decide which of percutaneous nephrolithotomy, ureterorenoscopic ureterolithotripsy, and medical expulsive treatment should be used to treat a patient.

Gücük A,Üyetürk U. Usefulness of hounsfield unit and density in the assessment and treatment of urinary stones. *World J Nephrol* 2014; 3(4): 282-286 Available from: URL: http://www.wjgnet.com/2220-6124/full/v3/i4/282.htm DOI: http://dx.doi.org/10.5527/wjn.v3.i4.282

INTRODUCTION

In recent years, the use of helical non-contrast computed tomography (CT) in patients with urinary system stones has increased. Hounsfield units (HU), a parameter generated from standard CT, are related to the density of the stone or structure of interest.

Sir Godfrey Newbold Hounsfield first introduced

Table 1 Some critical hounsfield unit values in recent literature

	Ref.	Year	Hounsfield units	Affected parameters
Prediction of stones	Motley *et al*[4]	2001	Density < 76 /mm	Non-calcium stone
	Patel *et al*[5]	2009	Mean, 879 ± 230	Calcium oxalate monohydrate stone
			Mean, 844 ± 346	Apatite stone
			Mean, 550 ± 74	Cystine stone
	Spettel *et al*[7]	2013	< 500	Uric acid stone
Prediction of Radio-opacity	Chua *et al*[9]	2012	> 498.5	Radio-opaque stone
	Huang *et al*[10]	2009	> 800 (ureteral stones)	Radio-opaque
			< 200	Radiolucent
Predicting the success of ESWL	Hameed *et al*[15]	2013	> 1350	Low ESWL success
	El-Assmy *et al*[16]	2011	> 1000	Low ESWL success
	El-Assmy *et al*[17]	2013	≤ 600 and stone length ≤ 12 mm (in children)	High ESWL success
	Ouzaid *et al*[18]	2012	> 970	Low ESWL success
	Foda *et al*[19]	2013	> 934	Low ESWL success
Use in PNL	Gücük *et al*[20]	2012	< 677.5	Low PNL success
	Gücük *et al*[21]	2013	< 677.5	Increases success with flexible nephroscope use
Use in URS	Kim *et al*[22]	2014	Any	No effect
Medical expulsive treatment	Erturan *et al*[24]	2013	Any	No effect

ESWL: Extracorporeal shock wave lithotripsy.

the principle to quantify the amount of X-rays that pass through or are absorbed by tissues, and developed the resulting radiodensity scale. CT images are made up of pixels, each of which has a gray scale value from 1 (black) to 256 (white). This value corresponds to the amount of X-rays that pass through the structure, and can be measured and expressed in Hounsfield units (HU). HU have since been used to evaluate and quantify tissues and fluids. When the radiodensity of water is defined as 0, fat has a negative HU, and blood and other tissues have a positive HU. Using this method it is possible to differentiate 256 shades of gray that are indistinguishable to the naked eye[1].

HU can also be used to assess the CT density of urinary system stones. In recent years, this has become an important diagnostic tool, not only for predicting the type of stone but also for determining the appropriate mode of treatment. The aim of this review is to assess the various areas in which HU is used to diagnose and treat urinary system stones (Table 1).

THE ROLE OF HU IN PREDICTING THE TYPE OF STONE

Understanding the composition of urinary system stones is critical for determining the optimal mode of treatment. Urine pH, the presence of crystals, urease-positive bacteria in urine, plain radiographs, and a history of urinary stones have long been used to predict the composition of stones; recently, HU also was used for this purpose[2]. Mostafavi *et al*[3] performed an *in vitro* study and reported that stone composition could be predicted with high accuracy using HU. Motley *et al*[4] attempted to determine stone composition using HU density, calculated by dividing HU by the greatest transverse diameter of the stone (in mm), and suggested that HU density was more effective than HU alone. However, the authors also reported that neither HU value nor density was sufficient for determining stone composition *in vivo*[4].

Patel *et al*[5] investigated whether HU values could be used for differentiating among subtypes of calcium stones, and reported they were particularly useful for diagnosing calcium oxalate monohydrate and dihydrate stones. In a similar study, the authors reported that calcium stones could be identified with high accuracy using HU values, but that there was an overlap between the HU values of cystine and uric acid stones, making it difficult to differentiate these types of stones[6].

Spettel *et al*[7] designed an *in vivo* study to predict uric acid stones using urine pH and HU, and argued that using the two parameters together were more effective for predicting uric acid stones than either one alone. Specifically, for a stone > 4 mm a HU ≤ 500 and pH ≤ 5.5 had a positive predictive value of 90% for uric acid composition[7]. To elucidate whether the composition of struvite stones could be predicted using HU values, Marchini *et al*[8] reported that the HU values of pure and mixed struvite stones overlapped, and concluded that struvite stone composition could not be accurately predicted by HU.

Recent studies suggested that HU and their variants are useful for predicting the composition of stones. However, they were insufficient for certain types of stone; the use of urinary parameters improved the accuracy in such cases.

THE ROLE OF HU IN PREDICTING RADIO-OPACITY

Knowing the radio-opacity of urinary system stones affords significant information to urologists for selecting the appropriate treatment and imaging modality to use during follow-up. Nevertheless, the relationship between the range/threshold of the HU values of stones measured using CT and radio-opacity is poorly understood.

Identifying radiolucent stones using CT has the advantage of preventing unnecessary radiographies during follow-up, preventing exposure to radiation, lowering anxiety, and reducing costs. Chua *et al*[9] also assessed the predictive potential of the radio-opacity of stones identified using plain radiographs and HU values. They examined 184 cases, and calculated that 498.5 HU was the appropriate cut-off value for determining if a stone > 4 mm was radio-opaque or radiolucent, with 89.3% sensitivity and 87.3% specificity[9]. Huang *et al*[10] performed a study that also included ureteral stones, and reported that stones of HU > 800 were visible on plain radiographic images, whereas those with a density < 200 HU were not. Taken together, data assessing the relationship between HU values and radio-opacity suggested that the follow-up of certain groups of patients could be performed adequately using plain radiographs rather than repeated CT examinations, reducing the time, cost, and exposure to ionizing radiation.

THE ROLE OF HU IN PREDICTING THE SUCCESS OF EXTRACORPOREAL SHOCK WAVE LITHOTRIPSY TREATMENT

Extracorporeal shock wave lithotripsy (ESWL) can successfully eliminate approximately 90% of renal stones in adults[11]. Successful ESWL depends on the type of lithotripter, and the localization, size, and hardness of the stone[12]. Many previous studies have investigated the relationship between CT parameters and successful ESWL. Data revealed that the energy of the shock wave needed for fragmentation was related to stone density, and that the higher the stone density, the stronger the shock wave energy needed to achieve fragmentation[13,14].

Hameed *et al*[15] reported that successful fragmentation using ESWL was decreased in stones with HU > 1350, which required application of more shock waves. El-Assmy *et al*[16] used the Hounsfield value of the stones to predict stone composition and density, and the fragmentation success using ESWL, and selected HU > 1000 as their cut off value. Another study of pediatric patients by the same group revealed that stones ⩽ 600 HU and ⩽ 12 mm in length were significant independent predictors of SWL success in children[17].

Ouzaid *et al*[18] performed a prospective study on 50 patients, and reported that a HU threshold of 970 was predictive of successful ESWL. Specifically, the stone-free rate was 96% and 38% with HU < 970 and > 970, respectively[18]. Foda *et al*[19] demonstrated that stone disintegration failed if the stone density was > 934 HU; therefore, they did not recommend ESWL in this group of patients.

Taken together, the available data suggest that the HU value, a parameter that is incorporated into clinical guidelines and enables prediction of successful ESWL, should be considered when making decisions regarding the use of ESWL.

THE ROLE OF HU IN PERCUTANEOUS NEPHROLITHOTOMY

Fluoroscopic imaging has been widely used during percutaneous nephrolithotomy (PCNL) operations to facilitate access to the collector system and renal anatomy, determine the placement of surgical tools, and identify and extract residual stones. The accurate assessment of post-operative residual stones significantly reduces morbidity. However, identifying residual stones using fluoroscopy depends largely on the size and opacity of the stone. In contrast, CT is an effective imaging tool for identifying all but indinavir stones. In addition, it allows the opacity of the stones to be quantified using HU. The HU value of stones affects the outcome of PCNL operations. Gücük *et al*[20] investigated the effects of certain parameters, including HU, on the outcome of 179 PCNL patients, and concluded that the HU value was an independent factor that affected the success of PCNL. Specifically, an HU value < 677.5 reduced the success of PCNL by 2.65-fold. The authors also reported a positive relationship between HU value and hemorrhage, and explained that this was associated with an increased frequency of endoscopic manipulation to extract residual stones. The identification of residual stones became easier with increasing HU value, and a higher HU value was also associated with increased renal trauma as a result of the higher energy required to breakdown stones[20]. The same group assessed the efficacy of routine flexible nephroscopic examination for identification and treatment of residual stones during PCNL operations, and reported that flexible nephroscopy was more effective in stones with low compared with high HU values. They suggested that this might be because flexible nephroscopy is used more commonly than fluoroscopy because stones with a low HU cannot be identified using fluoroscopy[21].

Although limited data are available regarding the association between HU and percutaneous nephrolithotomy, we conclude that consideration of HU values in patients scheduled for PCNL might assist selection of the appropriate treatment procedures and improve success rates.

THE ROLE OF HU IN URETEROSCOPIC LITHOTRIPSY

Ureteroscopic lithotripsy (URSL) is an important treatment modality for ureteral stones that is currently used for stones of all sizes present in any location within the ureter. The size and location of the stone are the prime factors that determine the success of URSL. However, it remains unclear whether HU is a determinant of URSL success. The only previous study of the relationship between HU value and URSL success was performed by Kim *et al*[22]. They examined the size, location, impaction, and HU value of stones using CT, as well as the effect of

these parameters on the success of URSL. Their results revealed that the HU value did not affect the success of treatment using URSL[22]. However, this study failed to assess several important parameters, such as the duration of the operation and of lithotripsy. As with ESWL, more energy might be needed and/or the procedure might be prolonged to fragment stones with a high HU using URSL. Therefore, further studies are required to elucidate whether higher energy and/or prolonged treatment are needed to successfully fragment stones with high HU values, and to identify any associated complications.

THE ROLE OF HU IN MEDICAL EXPULSIVE TREATMENT

Medical expulsive treatment (MET) is commonly used to facilitate the passage of ureteral stones in the absence of severe renal colic, infection, and obstruction. The spontaneous passage ratio can be as high as 98%, particularly in stones smaller than 5 mm. The most important factors that affect spontaneous passage are the size and location of the stone[23]. Erturhan *et al*[24] assessed the effect of HU value on the success of MET. This study, the only current report assessing this relationship, demonstrated that stones with a high HU would pass through the ureter slowly and with difficulty because of their compact structure. They compared two groups of stones with mean HU values of 625 and 507, and concluded that HU could not be used to predict the likelihood of success for MET[24]. However, that study included two groups in which the HU values were similar. As such, additional studies including stones with a wider range of HU values would make a significant contribution to current knowledge. Nevertheless, the available data suggest that HU values do not provide any additional benefit to MET.

CONCLUSION

Previous studies have revealed the benefit of HU values, parameters obtained from CT scans, on ESWL treatment and predicting the composition of urinary system stones. HU measurements now form part of the clinical guidelines because of the lower success rate of ESWL treatment of high HU stones[11]. Although HU is currently used most commonly during ESWL treatment and for prediction of stone composition, current data suggest that it could be used in other treatment modalities as our knowledge increases.

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P- Reviewer: Foda K, Olowu WA, Nowicki S
S- Editor: Song XX **L- Editor**: A **E- Editor**: Lu YJ

World Journal of Nephrology

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Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.5527/wjn.v3.i4.287

World J Nephrol 2014 November 6; 3(4): 287-294
ISSN 2220-6124 (online)

MINIREVIEWS

Renal biopsy practice: What is the gold standard?

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Received: July 2, 2014 Revised: July 22, 2014
Accepted: September 6, 2014
Published online: November 6, 2014

Abstract

Renal biopsy (RB) is useful for diagnosis and therapy guidance of renal diseases but incurs a risk of bleeding complications of variable severity, from transitory haematuria or asymptomatic hematoma to life-threatening hemorrhage. Several risk factors for complications after RB have been identified, including high blood pressure, age, decreased renal function, obesity, anemia, low platelet count and hemostasis disorders. These should be carefully assessed and, whenever possible, corrected before the procedure. The incidence of serious complications has become low with the use of automated biopsy devices and ultrasound guidance, which is currently the "gold standard" procedure for percutaneous RB. An outpatient biopsy may be considered in a carefully selected population with no risk factor for bleeding. However, controversies persist on the duration of observation after biopsy, especially for native kidney biopsy. Transjugular RB and laparoscopic RB represent reliable alternatives to conventional percutaneous biopsy in patients at high risk of bleeding, although some factors limit their use. This aim of this review is to summarize the issues of complications after RB, assessment of hemorrhagic risk factors, optimal biopsy procedure and strategies aimed to minimize the risk of bleeding.

Key words: Renal biopsy; Bleeding; Complications; Procedure

Core tip: Renal biopsy (RB) is useful for diagnosis, prognostic assessment and therapy guidance of various diseases affecting native kidneys or transplants. However, RB incurs a potential risk of bleeding complications of variable severity. This aim of this review is to summarize the issues of complications after RB, assessment of hemorrhagic risk factors, optimal biopsy procedure and strategies aimed to minimize the risk of bleeding.

Brachemi S, Bollée G. Renal biopsy practice: What is the gold standard? *World J Nephrol* 2014; 3(4): 287-294 Available from: URL: http://www.wjgnet.com/2220-6124/full/v3/i4/287.htm
DOI: http://dx.doi.org/10.5527/wjn.v3.i4.287

INTRODUCTION

Renal biopsy (RB) is often necessary for diagnosis, prognostic assessment and therapy guidance of various diseases affecting native and transplant kidneys. The final diagnosis differs from the main hypothesis in up to one third of cases[1]. Despite its necessity, RB incurs a potential risk of bleeding complications of variable severity, from transitory hematuria or asymptomatic hematoma to life-threatening hemorrhage[1-3]. Several studies identified risk factors for complications after RB[4-6]. However, controversies persist regarding the optimal assessment and management of bleeding risk. Two surveys, one conducted by the Society of Nephrology in France and another one in United Kingdom paediatric hospitals, highlighted significant variation in RB procedures[7,8]. Therefore, the gold standard for RB practice still re-

mains to be defined. We previously participated to the elaboration of consensual recommendations by the Society of Nephrology in France[8]. Optimizing procedures for RB may improve patient safety and may also provide some logistic benefits and save costs.

This review discusses the issue of complications after RB, optimal biopsy procedure, and strategies aimed to minimize the risk of bleeding. We only address biopsies for the investigation of medical kidney diseases, but not those performed for kidney tumors.

COMPLICATIONS AFTER PERCUTANEOUS RB

Several large prospective and retrospective studies provide an estimate of the frequency of complications after percutaneous RB[1-3,5,9-12]: (1) Death: < 0.1%; (2) Major bleeding requiring nephrectomy or surgical hemostasis: 0.1% to 0.5%; (3) Arteriovenous fistula requiring invasive intervention: 0.1% to 0.5%; (4) Blood transfusion requirement: 0.3% to 7.4%; (5) Uncomplicated hematoma: 10 to 90%; and (6) Transient macroscopic hematuria : 1% to 10%.

We recently published a series of 312 native kidney biopsies performed at our institution: 15% of patients developed a symptomatic hematoma, 5% macroscopic hematuria, 9% received a red blood cell transfusion and 1% required an angio-intervention[13].

The reported incidence of complications after RB varies in relation to numerous factors, including patient selection, definitions of complications, procedures, and monitoring protocols. Several studies were performed before the implement of ultrasound guidance and automated biopsy devices, which improved the safety and efficiency of RB procedures[4,9]. The rates of complications drawn from these reports may therefore not reflect the risk associated with RB performed nowadays.

Recent studies reported major bleeding and life-threatening complications in less than 0.1% of RB procedures[2,4]. Tøndel *et al*[12] recently published the largest report of RB complications: 9288 (715 children and 8573 adults) biopsies from the Norwegian kidney biopsy registry, the vast majority of which (99.7%) were guided by ultrasound. In this study, 0.9% of the patients needed blood transfusion, 0.2% required an invasive procedure (surgery or angiointervention), and 1.9% had a macroscopic hematuria[12].

The risk of bleeding complications appears lower for transplant than native kidney biopsie[14,15]. However, major complications can occur after transplant biopsy[16].

ASSESSMENT OF HEMORRHAGIC RISK FACTORS AND CONTRAINDICATIONS TO PERCUTANEOUS RB

An important step before RB is to search for factors increasing the risk of complications, particularly bleeding. Although there are no definitive ways to predict which patients will experience complications, several predisposing factors to bleeding have been identified, at times inconstantly.

High blood pressure, age, a decreased GFR, obesity, anemia, low platelet count and small center size (< 30 biopsies/year) are associated with an increased risk of bleeding[4-6,12,17-19]. Amyloidosis was reported to be associated with bleeding[4], although such association was not found in large study by Tøndel *el al*[12]. As discussed below, hemostasis disorders, anticoagulant or antiplatelet therapy, and certain anatomic conditions, may also contraindicate or complicate percutaneous RB.

A recent systematic review and meta-analysis of hemorrhagic complications after percutaneous native kidney biopsy using ultrasound guidance and automated spring-loaded biopsy device reviewed 34 publications and concluded that the predictors of erythrocyte transfusion were: the needle gauge (14 *vs* 16 or 18), sex (female), serum creatinine (⩾ 2 mg/dL), low hemoglobin prior biopsy (⩽ 12 g/dL) and acute kidney injury[18].

High blood pressure

Although high blood pressure is a well-recognized and modifiable risk factor of bleeding after RB[4,6,19], it is difficult to determine a cut-off level above which RB should not be performed. One study demonstrated a significant increase in the risk of bleeding when systolic blood pressure (SBP) was > 160 mmHg or diastolic blood pressure (DBP) was > 100 mmHg[6]. Some studies suggested that an upper limit value of 140/90 mmHg prior to an RB procedure would be appropriate to minimize this risk[4,6]. Interestingly, the risk of bleeding is increased in patients with a history of hypertension, irrespective of blood pressure at the time of biopsy[6]. It is possible that arteriolar hyalinosis associated with chronic hypertension limits the ability of vessels to contract following RB, regardless of the current blood pressure.

Hemostasis abnormalities

Screening for inherited or acquired hemostasis abnormalities relies on patient questioning, study of current and recent medications, and hemostatic tests. Even patients with mild bleeding disorders can bleed after surgery or invasive procedures[20]. In the general population, the most frequent mild bleeding disorders are Von Willebrand disease and platelet function disorders, each with an estimated frequency of up to 1%[21]. Thus, questioning patients about personal and familial bleeding history should not be neglected. However, our survey conducted in France highlighted that such information was not always assessed[8]. One issue may be that nephrologists are not familiar with this practice. The use of questionnaires prepared by hemostasis experts, such as the bleeding assessment tools[21] may be helpful to screen for inherited hemostasis abnormalities. However, these tools have not been validated in the setting of RB and cannot be used to predict bleeding after RB.

Careful examination of the list of current and recent medications, with a focus on anticoagulant and antiplatelet drugs, should be systematically performed before RB. The issue of RB in patients receiving anticoagulant or antiplatelet is discussed below.

It is universal practice to check blood cells count, prothrombin time and partial thromboplastin time before RB[8]. When a bleeding disorder is suspected based on a history of previous bleeding episodes, thrombopenia or abnormal hemostasis tests, thorough investigations should be carried out to determine whether percutaneous RB can be performed safely. It should be emphasized that hemostasis laboratory tests available do not reliably predict "uremic bleeding", which is the result of multifactorial alterations of hemostasis in a setting of chronic or acute renal failure[17]. Some nephrologists use bleeding time in an attempt to predict complications after RB, and some studies showed that a prolonged bleeding time was a risk factor for hemorrhagic complications[19]. However**,** the usefulness of this test is controversial. In the context of RB, several studies failed to demonstrate predictive value of the bleeding time for hemorrhagic complications[3,4,22,23]. It is now widely accepted that the bleeding time is not a good predictor of the risk of hemorrhage associated with surgical procedures and cannot reliably identify patients who have recently ingested antiplatelet agents; it is therefore no longer recommended as a routine preoperative test[24,25]. *Other laboratory* hemostasis tests have not been shown to improve prediction of bleeding after RB and are therefore not required.

RB in patient receiving anticoagulant or antiplatelet therapy

It is a standard of care to discontinue anti-platelet agents and non-steroidal inflammatory agents 5 to 7 d before an invasive procedure in order to reduce the risk of bleeding. However stopping an anti-platelet agent in a coronary patient can increase the risk of a thrombotic event[26], especially in patients with a high cardiovascular risk profile (extensive coronary disease, patients with recent stent placement: less than 6 wk after bare metal stent placement and less than 6 to 12 mo after drug eluting stent placement)[27,28]. In a cohort of 1358 consecutive patients admitted for a suspected acute coronary syndrome (ACS), 5% of those patients with a confirmed ACS had a history of coronary artery disease and had recently stopped their aspirin. The event happened after a mean of 11 d of aspirin cessation[29].

Some studies raised the possibility that withdrawal of antiplatelet therapy might not be mandatory before RB. In a retrospective study, the incidence of major hemorrhage after percutaneous RB was 1% (13/1270) in patients taking aspirin before RB, which was similar to the incidence of bleeding in patients not taking aspirin[30]. One important limitation of this study was that patients who stopped aspirin less than 10 d before RB, which is a common practice, were included in the "aspirin use" group. Additionally, the continuation of an anti-platelet agent was not identified as an increased risk factor of blood transfusion in a meta-analysis of 34 studies[18]. Mackinnon *et al*[31] reported 1120 RB from two different centers, in one, anti-platelets were stopped 5 d before the biopsy, whereas they were not discontinued in the other. There were no difference in the rate of major complications between the two centers but a significantly higher percentage of patients in the group still taking anti-platelet agents experienced a $\geqslant$ 1g/dL reduction in hemoglobin (23.5% *vs* 12.5%). The proportion of patients taking an anti-platelet agent was only specified for the elective biopsies (135 patients) where 75 had stopped the agents prior to biopsy whereas 60 patients were still taking an anti platelet agent (aspirin $n = 68$, clopidogrel $n = 7$) at the moment of the biopsy[31].

However, these studies about the safety of RB without cessation of aspirin have important limitations. In addition, the risk of bleeding associated with the continuation of other agents such as clopidogrel or newer agents like prasugrel or ticagrelor, is higher than the one with aspirin. It should be kept in mind that RB is a high bleeding risk procedure and, in our opinion, withdrawing anti-platelet agents before RB should be the standard of care in low-risk patients. It is therefore advisable to withhold these agents for 7 d before an elective kidney biopsy[32], and resume them 1 to 2 d after the biopsy. The management of patients at high risk of thrombotic events should be discussed with their cardiologist. The biopsy should be deferred if necessary or a transjugular biopsy, if available, should be considered.

Oral anticoagulant (anti-vitamin K) should be stopped 5 d before the biopsy and bridging with heparin should be considered in high and moderate risk patients. Oral anticoagulants should be resumed 12 to 24 h after the biopsy[28].

Although data are limited, platelet transfusion seems to be the best option in patients who are taking an anti-platelet agent and experience severe bleeding from a RB.

Solitary kidney and anatomic abnormalities

Renal ultrasound is usually performed in the assessment of kidney diseases and provides important information before RB about the size and morphology of kidneys. An anatomic or functional solitary native kidney is generally considered as a contraindication for RB, given the possibility that nephrectomy may be necessary in case of life-threatening bleeding. Complications requiring nephrectomy are however very rare and ultrasound-guided percutaneous RB with an automated biopsy device has been shown to be safe if contraindications, especially high blood pressure and abnormal haemostasis, are adressed. In three retrospective studies that included a total of 1955 ultrasound-guided percutaneous renal biopsies, only one case required nephrectomy[2-4]. Some authors advocated that otherwise uncomplicated adult patients with a solitary kidney might be considered for percutaneous biopsy[5]. Despite these reassuring data, un-

dertaking a solitary kidney biopsy remains an important decision that should be made only after carefully thinking about whether the RB result is likely to have important therapeutic implications.

Anatomic abnormalities of the kidney (congenital malformations, cysts, atrophy, hydronephrosis...) or blood vessels (arteriovenous fistula, aneurysm, microaneurysm...) can make RB difficult to perform. Such abnormalities have to be carefully characterized using appropriate imaging techniques in order to determine the risk and feasibility of the biopsy.

PREVENTION OF BLEEDING BEFORE RB

As it is for any invasive procedure, correction of coagulopathy is mandatory before RB. The platelet count threshold at which a RB can be safely conducted is not clear. Most platelet count thresholds for invasive procedure are based on weak observational evidence. For most major surgery, other than ocular and neurologic, platelet transfusion are considered if the platelet count is below 50000/microL[33]. It is not clear if this can be applied to RB. Many nephrologists consider RB contraindicated if platelet count is < 100000/microL, which seems more prudent. Of course, optimal methods for raising platelet count depend on the underlying condition.

In the setting of renal disease, the risk of bleeding can result from dysfunctional platelets resulting from uremia. Indeed, uremic bleeding is a well-known complication of renal failure. The exact underlying mechanisms remain largely unknown, but seem to be multifactorial. The pathophysiology of uremic bleeding and evidence based treatment recommendations were the subject of a review by Hedges *et al*[17]. Many factors contribute to platelet dysfunction including anemia, dysfunctional von Willebrand factor, platelet membrane abnormalities, uremic toxins inhibiting platelet aggregation, and increased prostacyclin and nitric oxide levels, which are strong anti-platelet aggregating factors[17]. Correction of anemia, deamino-8-D-arginine vasopressin (DDAVP), estrogens and cryoprecipitate have been shown to improve "uremic bleeding".

Desmopressin (DDAVP) is probably the most common agent used to treat or prevent bleeding in uremic patients. DDAVP improves hemostasis by releasing factor VIII from storage sites. DDAVP can reverse uremic platelet dysfunction rapidly (approximately within one hour of IV injection) for a short period of time (around 24 h)[17].

Several studies demonstrated that recombinant erythropoietin treatment prevents bleeding caused by uremic platelet dysfunction if the hematocrit is increased to more than 30%. Recombinant erythropoietin was shown to improve primary hemostasis in uremia through an increase of hematocrit but also through an effect on platelet function[17,34,35].

Several studies showed that intravenous conjugated estrogens can safely and effectively improve uremic platelet dysfunction and clinical bleeding. Intra-venous conjugated oestrogens improve bleeding time with a maximum effect at 5 to 7 d, lasting from 14 to 21 d[17].

Finally, cryoprecipitate is another therapeutic option in the setting of active uremic bleeding or in patients with high risk of bleeding[17,36]. Cryoprecipitate is prepared from plasma and contains fibrinogen, von Willebrand factor, factor Ⅷ and factor XⅢ. It has a rapid onset of action (around 1 h) and its effect lasts approximately 24 to 36 h.

The impact of dialysis on uremic bleeding is unsure. Studies are old, and the effect on platelet function and coagulation is inconstant.

In all, the evidence supporting recommendations for the prevention or treatment of uremic bleeding is limited, especially in the context of RB. Despite the absence of robust evidence, it may be prudent to avoid undertaking RB when the hematocrit is lower than 30%, and to consider DDAVP or oestrogens before RB when the glomerular filtration rate is lower than 30 mL/min per 1.73m^2, as suggested by some authors[37].

PROCEDURES FOR PERCUTANEOUS RB

Well-trained nephrologists can perform RB as well as radiologists[38,39]. Automated biopsy guns have superseded Tru-cut needles and are probably used in most centers[8]. Several studies suggested that 14-18G needles are appropriate for percutaneous RB[3,15,40]. The use of an automated biopsy gun in combination with real-time ultrasound guidance was reported to provide adequate samples in nearly 99% of cases, with severe hemorrhagic complications occurring in less than 0.1%. This method can be considered the gold standard[2,4]. The use of bedside ultrasound to assess the location and depth of the kidneys was reported as a reliable alternative to real-time guidance[39]. In some instances, especially in obese patients, it may be necessary to perform RB under guidance by CT-scan instead of ultrasound.

ALTERNATIVES TO PERCUTANEOUS RB

Transjugular RB has been reported to be a safe and reliable alternative to conventional percutaneous RB in patients with obesity[41] or those at risk for bleeding, including high-risk patients with coagulopathy and thrombocytopenia[42-44]. In these studies, transjugular RB provided diagnostic yield and safety similar to those of percutaneous approach. However, in most countries, the use of transjugular RB is limited to a few centers because of the necessity of skilled interventional radiologists.

Laparoscopic RB has also been reported as an alternative for patients in whom percutaneous approach was not feasible or was contraindicated, because of obesity, solitary kidney, anticoagulation or coagulopathy, or failed percutaneous biopsy[45,46]. However the number of patients included in these studies was limited and no study has compared the safety of percutaneous, transjugular

Table 1 Studies evaluating the safety of short observation time (< 24 h) after a percutaneous renal biopsy of native kidney

Study	Complications: minor/major	Timing of complications
Whittier *et al*[22] Retrospective 750 patients	6.6% minor complications 6.4% major complications (79% blood transfusion)	38 (42%) complications ⩽ 4 h post RB 61 (67%) complications ⩽ 8 h post RB 77 (85%) complications ⩽ 12 h post RB 81 (89%) complications ⩽ 24 h post RB
Lin *et al*[56] Retrospective 147 inpatients 183 outpatients	19.7% hematoma 6.4% macroscopic hematuria 0.9% pain No difference between in and out patients	2 outpatient admission (blood transfusion) all complications occurred within observation time of 6 h
Maya *et al*[57] Prospective *N* = 100	13% asymptomatic hematoma No major complications	All complications occured within 8 h of observation time 4% extended 24 h observation for decrease hematocrit
Margaryan *et al*[53] Retrospective, *N* = 146	Bleeding 2.8% Gross hematuria 1.4% Transfusion 0.69%, intervention 0	Hospital admission 5.6%, no late complications. Observation time 4-6 h
Jiang *et al*[52] Retrospective *N* = 475	6.9% minor complications 1.3% (6 patients) had major complications (transfusion or interventional radiology)	Median time for minor complications 2.5 h, 4/33 after 6 h 4/6 major complications occurred within 4 h, 1/6 at 12 h and 1/6 beyond 48 h
Carrington *et al*[50] Retrospective *N* = 192	3.6% (*n* = 7) immediate complications related to bleeding, 2/7 required blood transfusion and embolisation	All complications occurred within observation period of 8 h
McMahon *et al*[31] Prospective *N* = 105, low risk	11% required admission for complications (11/12 minor, 1 major complication)	9/12 during the observation time (5 h) 1 at 48 h (macroscopic hematuria), 2 at 5 d (AVF, hematoma)
Simard-Meilleur *et al*[13] Retrospective 164 inpatients 148 outpatients	15% symptomatic hematoma (pain, drop of more than 10 g/l Hb, gross hematuria, hypotension), 9% RBC transfusion, 1% angio-intervention	100% outpatient complications occurred during observation time (8 h)
Korbet *et al*[19] Prospective 1055 patients	Minor complications 8.1% Major complications 6.6%	57% of all complications occurred within 4 h, 72% within 8 h, 85% within 12 h and 89% within 24 h

RB: Renal biopsy.

and laparoscopic RB in patients at high risk for bleeding. In addition, when considering these procedures, one should carefully contemplate the risk of general anesthesia, perioperative risk and recovery time.

SURVEILLANCE AFTER RB

After RB, patients have to be monitored closely for the occurrence of complicatons such as gross hematuria, flank pain, hypotension and acute renal obstruction.

The standard practice after RB has traditionally been to observe the patient overnight, as suggested by early studies[47]. In our French survey, almost all nephrologists observed patients for at least 24 h after a native kidney biopsy[8]. However, controversies have emerged regarding the optimal duration of observation after RB and it has been proposed that patients be discharged after 6-8 h of observation[48,49]. Performing RB as an outpatient procedure offers several advantages but raises the concern of missing late complications. Whittier *et al*[22] reported a large series of 750 native kidney biopsies in adults. In this study, 13% patients developed biopsy-related complications; minor complications occurred in 6.6% and major complications (most requiring a blood transfusion) occurred in 6.4% patients. Around 30% of the patients had a biopsy performed using a manual biopsy device. The analysis of the timing of complications showed that 89% of complications were identified within 24 h after RB, and that an observation period less than 8 hours missed 33% of complications. On the contrary, several smaller studies suggested that outpatient observation time of 6 to 8 h is safe (Table 1)[13,19,49-57]. Most of outpatients in these studies were selected as low risk. Considering this, an outpatient biopsy may be an option in a carefully selected population with no risk factor.

Renal transplant biopsies are routinely performed as an outpatient procedure in some centers. In our survey in France, approximately 25% of nephrologists performed transplant biopsies with observation times limited to 4-8 h[8]. In a multicentric study by Furness *et al*[58] on 2127 protocol transplant biopsies, only 9 (0.42%) severe complications occurred, all presenting within four hours after biopsy. In another study, no severe complications were observed after 251 protocol transplant biopsies[59]. Therefore, an observation time of 4-8 h after a transplant biopsy appears to be a relatively safe practice, at least in patients without risk factors for bleeding.

Some protocols use a routine renal ultrasound or measurement of hemoglobin or hematocrit control before discharge, in addition to clinical monitoring. Systematic ultrasound reveals perirenal hematoma in 40%-90% of procedures[11,60]. Arteriovenous fistula may be detected in 10% of RB, but they usually disappear

spontaneously after a few months[61,62]. In biopsies that are otherwise uncomplicated with an asymptomatic course, hematomas are usually small (< 3 cm)[48,63]. These hematomas are almost always asymptomatic, and such a finding usually occurs without therapeutic consequence. In a study that evaluated the use of renal ultrasound one hour post-RB, the presence of a hematoma was poorly predictive of complications[63]. The absence of a hematoma was predictive of an uncomplicated course in after RB[63]. However, a period of observation is required after RB, even in the absence of hematoma right after the biopsy. Early routine repeat imaging is therefore of limited usefulness and is not necessary in patients otherwise asymptomatic.

The use of a hemoglobin or hematocrit measurement after a RB as a predictor of bleeding is controversial. Systematic hemoglobin monitoring was shown to be of little value in detecting complications after RB in one study[22], although in another study, a direct relationship was found between the change of hematocrit at 6 h and the hematocrit at 24 h following a RB, suggesting that the absence of fall at 6 h makes a significant fall of hematocrit at 24 h unlikely[64].

CONCLUSION

The RB is an indispensable tool to establish the diagnosis and management of kidney diseases. Although the overall incidence of serious complications is low, risk factors for bleeding must be carefully assessed and, whenever possible, corrected before the procedure. If contraindications, especially high blood pressure and hemostasis abnormalities, are respected, percutaneous RB with an automated biopsy device and ultrasound guidance is safe for the vast majority of patients. Some controversies remain regarding the optimal duration of observation and the possibility to perform RB as an outpatient procedure. To address these issues, further studies are warranted to improve our ability to predict and stratify the risk of bleeding.

ACKNOWLEDGMENTS

We are grateful to Dr. Stephan Troyanov for his assistance in improving the quality of writing.

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P- Reviewer: Mubarak M, Watanabe T **S- Editor**: Song XX
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World J Nephrol 2014 November 6; 3(4): 295-301
ISSN 2220-6124 (online)

MINIREVIEWS

Regulatory roles of nitric oxide and angiotensin II on renal tubular transport

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Received: June 28, 2014 Revised: September 5, 2014
Accepted: October 1, 2014
Published online: November 6, 2014

Abstract

Renal tubules regulate blood pressure and humoral homeostasis. Mediators that play a significant role in regulating the transport of solutes and water include angiotensin Ⅱ (AngⅡ) and nitric oxide (NO). AngⅡ can significantly raise blood pressure via effects on the heart, vasculature, and renal tubules. AngⅡ generally stimulates sodium reabsorption by triggering sodium and fluid retention in almost all segments of renal tubules. Stimulation of renal proximal tubule (PT) transport is thought to be essential for AngⅡ-mediated hypertension. However, AngⅡ has a biphasic effect on in vitro PT transport in mice, rats, and rabbits: stimulation at low concentrations and inhibition at high concentrations. On the other hand, NO is generally thought to inhibit renal tubular transport. In PTs, NO seems to be involved in the inhibitory effect of AngⅡ. A recent study reports a surprising finding: AngⅡ has a monophasic stimulatory effect on human PT transport. Detailed analysis of signalling mechanisms indicates that in contrast to other species, the human NO/guanosine 3′,5′-cyclic monophosphate/extracellular signal-regulated kinase pathway seems to mediate this effect of AngⅡ on PT transport. In this review we will discuss recent progress in understanding the effects of AngⅡ and NO on renal tubular transport.

Key words: Angiotensin Ⅱ; Nitric oxide; Proximal tubules; Thick ascending limb; Distal tubules; Na^+ transport

Core tip: Angiotensin Ⅱ (AngⅡ) and nitric oxide (NO) play important roles in the regulation of renal tubular transport. AngⅡ has a biphasic effect on renal proximal tubule (PTs) transport, and NO seems to inhibit the effect of AngⅡ. In human PTs, however, AngⅡ seems to have an NO-dependent monophasic stimulatory effect. We will discuss the recent findings in this field.

Horita S, Nakamura M, Shirai A, Yamazaki O, Satoh N, Suzuki M, Seki G. Regulatory roles of nitric oxide and angiotensin Ⅱ on renal tubular transport. *World J Nephrol* 2014; 3(4): 295-301 Available from: URL: http://www.wjgnet.com/2220-6124/full/v3/i4/295.htm DOI: http://dx.doi.org/10.5527/wjn.v3.i4.295

INTRODUCTION

Angiotensin Ⅱ (AngⅡ) is a strong pressor, acting on various organs and systems, including the kidney. It binds to angiotensin receptors, of which the main subtypes are angiotensin receptor type 1 (AT1R) and type 2 (AT2R)[1]. Although other classes of angiotensin and their receptors, such as AT7R[2], occur, the receptor with the dominant effect in the kidney seems to be AT1R. Recently, Coffman *et al*[3] demonstrated that renal AT1R is the essential target of AngⅡ-induced hypertension[3]. By showing the importance of renal AT1R in the emergence of hypertension, their study suggests that renal AT1R will be the target for therapy and the prevention

of hypertension.

Nitric oxide (NO) is a gaseous vasoactive substance produced by nitric oxide synthase (NOS). NO has been shown to play important roles in the regulation of renal tubular transport. However, its role seems to be pleiotropic and varies according to circumstances.

NOS has three isoforms, NOS1, NOS2 and NOS3, previously referred to as neuronal NOS (nNOS), inducible NOS (iNOS), and epithelial NOS (eNOS), respectively. Renal tubules have each of these NOS isoforms[4,5]; however, the details of their actions in the tubules are still unclear.

NO seems to inhibit NaCl reabsorption in the renal tubules and induces natriuresis. Inhibiting NOS decreased urine volume and NaCl excretion, without changing renal blood flow and the glomerular filtration rate[6-10]. Overall, NO is thought to inhibit the reabsorption of NaCl and fluid by tubules.

ANG Ⅱ AND TUBULES

Ang Ⅱ in proximal tubules

Ang Ⅱ has been widely known as a strong pressor and regulator of cardiovascular and renal function[11]. In the classical pathway, AT1R mediates the effects of Ang Ⅱ[1]. Proximal tubules (PTs) reabsorb approximately 60% to 70% of the sodium filtered in the glomeruli. Therefore, the regulation of sodium reabsorption in this segment is important for the maintenance of blood pressure and humoral homeostasis[12,13]. In the PTs, Ang Ⅱ is known to stimulate sodium and water transport. Although Ang Ⅱ affects transport processes in several nephron segments, as discussed below, its effect on PT transport may be its most important effect. In particular, the stimulatory effect of Ang Ⅱ in the PTs has significant importance for the emergence and progression of hypertension[14].

Ang Ⅱ acts mainly *via* type 1 and type 2 angiotensin receptors. The type 1 receptor has 1A and 1B subtypes and is thought to raise blood pressure[1]. AT2R is also thought to be located in the PTs[15-17]. Some investigators argue that AT2R may mediate the inhibitory effect of Ang Ⅱ[18]. However, most data, including our own obtained from AT1R knockout mice[13,19-21], indicate that AT1R is the dominant receptor mediating the biphasic effects of Ang Ⅱ in the PTs.

In PTs, the basolateral electrogenic sodium-bicarbonate cotransporter type 1 (NBCe1) and the apical sodium-proton exchanger type 3 (NHE3) mainly regulate sodium reabsorption[22]. In addition, sodium is reabsorbed and coupled with amino acids[23], glucose[24], phosphate[25], and other solutes from the apical side[14]. Sodium is also reabsorbed *via* Na^+-K^+-ATPase (NKA) from the basolateral side[26], which offers the driving forces for NBCe1 and NHE3.

Ang Ⅱ is known to have biphasic effect on the PTs of rats, mice and rabbits. Low concentrations (picomolar to nanomolar) of Ang Ⅱ stimulate PT transport, while high concentrations (nanomolar to micromolar) inhibit PT transport[27,28]. In PTs, Ang Ⅱ regulates major sodium transporters, such as NHE3, NBCe1, and NKA, in a biphasic manner[19,29-32]. The activation of protein kinase C and/or a decrease in cAMP concentration, followed by the activation of the extracellular signal-regulated kinase (ERK) pathway, may be responsible for the stimulatory effect of Ang Ⅱ[33-35]. On the other hand, the activation of the phospholipase A2/arachidonic acid/5,6-epoxyeicosatrienoic acid (EET) and/or the NO/guanosine 3',5'-cyclic monophosphate (cGMP) pathways[29,36,37] may be responsible for the inhibitory effect of Ang Ⅱ. The concentration of Ang Ⅱ is known to be much higher in kidney than plasma[38,39], suggesting that the inhibitory effect of Ang Ⅱ may also have some physiological significance in the regulation of renal tubular function and blood pressure.

Ang Ⅱ in the thick ascending limb

There are some reports that Ang Ⅱ stimulates net NaCl absorption in the thick ascending limb (TAL). Wang and colleagues showed that Ang Ⅱ stimulates basolateral Cl^- channels by activating the protein kinase C-dependent NADPH oxidase pathway, inducing net NaCl absorption[40]. Garvin *et al*[41] investigated the regulation of NKA activity in Ang Ⅱ-induced hypertension[41]. They showed that Ang Ⅱ-induced hypertension is accompanied by increased NKA activity in rat TAL, which may be at least partially due to Ang Ⅱ-stimulated superoxide production[42] *via* NADPH oxidase[43]. Moreover, Ang Ⅱ binding to AT1R was shown to inhibit ADH-stimulated transport in the rat TAL suspension cells[44]. Overall, Ang Ⅱ seems to stimulate Na^+ reabsorption in the TAL *via* AT1R.

Ang Ⅱ in the distal tubules

In the distal tubules, approximately 10% to 20% of the filtered Na^+ is reabsorbed. Na^+ enters the tubule cells *via* the sodium-chloride cotransporter (NCC) and exits from the basolateral side *via* NKA, while Cl^- exits *via* chloride channels (ClC-Kb)[14].

Recent studies indicate that With-No-Lysine Kinase (WNK), Oxidative stress-responsive kinase (OSR) 1, and STE20/SPS1-related proline alanine-rich kinase (SPAK) importantly regulate transport in distal tubules.

WNKs are atypical protein kinases, as their name "With No Lysine (K)" implies[45]. They are expressed in various organs and tissues, including renal distal tubules, and modulate several biological processes, such as solute transport, cell growth, and neurotransmission[46]. WNKs have subtypes, such as WNK1, WNK2, WNK3, WNK4 and kidney-specific (ks-) WNK1. The kidney expresses WNK1, WNK3, WNK4 and ks-WNK1, where they modulate the function of NCC in the distal tubules.

In distal tubules, Ang Ⅱ seems to activate NCC *via* phosphorylation. Hoorn *et al*[47] showed that Ang Ⅱ induces the phosphorylation of NCC, enhancing sodium retention in rat kidneys, independent of aldosterone[47]. On the other hand, Uchida and colleagues showed that,

although Ang Ⅱ increases NCC phosphorylation *via* the WNK-OSR1/SPAK pathway, the effect of aldosterone in this pathway is predominant[48]. Using WNK4 knockout mice, Gamba *et al*[49] showed that Ang Ⅱ stimulates NCC *via* a WNK4-SPAK dependent pathway and that WNK4 is involved in Ang Ⅱ-stimulated aldosterone secretion[49]. The detailed mechanisms by which Ang Ⅱ, WNK-SPAK/OSR1 and aldosterone regulate transport in the distal tubules transport remain to be clarified.

Ang Ⅱ in the connecting tubules and collecting tubules

In the last portion of the tubules, the connecting tubules (CNT) and the collecting tubules (CD), Na^+ is mainly reabsorbed *via* an epithelial Na^+ channel (ENaC) on the luminal side and NKA on the basolateral side. The amount of Na^+ reabsorbed from these segments represents only a small fraction of the total Na^+ absorption by the kidney, but its regulation contributes to the fine-tuning of sodium and fluid homeostasis.

ENaC is a heteromultimeric channel, with three homologous subunits (α, β, γ)[50,51]. Loss-of-function mutations of ENaC cause pseudohypoaldosteronism type I (PHA-I), while gain-of-function mutations cause Liddle's syndrome[52,53]. PHA-I features renal salt wasting associated with hyperkalaemia, while Liddle's syndrome shows arterial hypertension with hypokalaemia. Pharmacologically, amiloride directly and reversely blocks the ENaC.

In the CNT and CD segments, aldosterone has been thought to play a principal role in regulating basal and long-term ENaC activity[54]. Recently, however, Korbmacher *et al*[55] demonstrated that, in the distal convoluted tubules (DCT2) and CNT, ENaC function is largely independent of aldosterone[55]. They suggested that glucocorticoids and/or Ang Ⅱ may be responsible for the aldosterone-independent ENaC activity. Ang Ⅱ itself may directly stimulate amiloride-sensitive Na^+ reabsorption in CNT and CD, independent of aldosterone[56,57]. Indeed, several studies have reported that the Ang Ⅱ/AT1R pathway can regulate ENaC expression[58-61]. This effect of Ang Ⅱ is thought to be mediated *via* AT1R[62,63]. In obese Zucker rats, moreover, enhanced AT1R activity may result in the ENaC activation, suggesting a role for Ang Ⅱ in Na retention in diabetes and obesity[60].

THE EFFECT OF NO IN THE TUBULES

NO in the PTs

As described above, NO has been thought to inhibit net NaCl and fluid absorption through renal tubules. However, Wang and colleagues argued that NO has a biphasic effect on the PTs. Low concentrations of an NO donor, sodium nitroprusside (SNP; 10^{-6} mol), stimulated PT fluid (J_V) and bicarbonate absorption (J_{HCO3}) by 30%-50%, while high concentration of SNP (10^{-3} mol) inhibited Jv and J_{HCO3} by 50%-70%[64]. However, most other studies report that NO inhibits PT transport[65-67]. In particular, NO has been shown to decrease NHE3 and NKA activities[67,68]. Overall, NO is generally thought to inhibit NaCl, HCO_3^-, and volume reabsorption in the PTs.

NO in the TAL

In the TAL, approximately 30% of filtered Na^+ is reabsorbed[14]. The major Na^+ transporters here are the $Na^+/K^+/2Cl^-$ cotransporter (NKCC2) and NHE3 on the apical side as well as NKA on the basolateral side.

Garvin and colleagues found that NO donors inhibit Cl^- and HCO_3^- reabsorption[69,70]. They found that NO inhibits NKCC2 and NHE3 activity, but not NKA activity[71,72]. Using $NOS3^{-/-}$ mice, they also showed that NOS3 is responsible for NO production in the TAL[73,74]. HCO_3^- reabsorption in the TAL is accomplished by H^+ secretion *via* apical NHE3[75]. In the rat TAL, NO increases cGMP levels[76,77], and cGMP analogues inhibit J_{HCO3}[70] and J_{Cl}[78,79]. The inhibition of cGMP-dependent kinase (cGK) blocked the inhibitory effect of NO on J_{HCO3}, but not on J_{Cl}[70,80]. On the other hand, the inhibition of cGMP-stimulated phosphodiesterase (PDEII) blocked the inhibitory effect of NO on J_{Cl}[80]. Thus, the NO/cGK pathway seems to mediate the inhibitory effect on J_{HCO3}, while the NO/PDEII pathway seems to mediate the inhibitory effect on J_{Cl}[80].

NO in the CNT and CD

Recently, Wall and colleagues have showed that NO reduces Cl^- absorption through ENaC in mouse CD[81]. In the cultured *Xenopus laevis* distal nephron cell line 2F3, Bao and colleagues showed that the activity of ENaC was reduced by a cyclic GMP analogue or by an atrial natriuretic peptide[82]. Moreover, in cGKII knockout mice, ENaC inhibition induced a much greater increase in UNa^+V (2.6-fold) than in wild-type mice (1.9-fold), suggesting that ENaC activity is upregulated in the knockouts[83]. Integrating these results, NO and its signal transduction system appear to inhibit ENaC in CD and to induce natriuresis, therefore preventing sodium retention and hypertension.

The interaction between Ang Ⅱ and NO in PT

As previously described, the Ang Ⅱ effect on Na^+ reabsorption in the proximal tubule is biphasic in rodents and rabbits[27,28]. The inhibitory effect of Ang Ⅱ is mediated by the PLA2/arachidonic acid/EET and/or NOS/NO/cGMP pathways. In rat PTs, for example, the regulation of NKA by Ang Ⅱ seems to be dependent on the NO/cGMP pathway[35,36].

On the other hand, the effects of Ang Ⅱ on PT sodium transport in humans have not yet been clarified. To this end, we analysed the effects of Ang Ⅱ on human PTs isolated from the cortex of kidneys removed for renal carcinoma. Surprisingly, Ang Ⅱ, in contrast to other species, was found to induce a monophasic stimulation of human PT transport[84]. Specifically, Ang Ⅱ induced a dose-dependent stimulation of NBCe1, NHE3, and J_{HCO3} that was apparently mediated by both luminal and basolateral AT1Rs.

Table 1 Summary of angiotensin II effects on tubular transport

Nephron segment	Potential targets	Effects	Ref.
PT	NHE3, NBCe1	biphasic in rats, mice, rabbits	[19,27-32]
	J_{HCO3}	monophasic stimulation in humans	[84]
TAL	NKA, NKCC2, Cl channel	stimulation	[40-43]
	NADPH oxidase		
DCT	NCC	stimulation	[47,49]
	WNK4?		
CNT/CD	ENaC	stimulation	[55,59-61]

PT: Proximal tubule; TAL: Thick ascending limb; DCT: Distal convoluted tubules; CNT: Connecting tubules; CD: Collecting tubules; NHE3: Apical sodium-proton exchanger type 3; NBCe1: Basolateral electrogenic sodium-bicarbonate cotransporter type 1; NKA: Na^+-K^+-ATPase; NKCC2: $Na^+/K^+/2Cl^-$ cotransporter; NCC: Sodium-chloride cotransporter; ENaC: Epithelial Na+ channel.

Table 2 Summary of nitric oxide effects on tubular transport

Nephron segment	Potential targets	Effects	Ref.
PT	NHE3, NBCe1	inhibition in rats, mice, rabbits	[65-68,84]
	J_{HCO3}	biphasic in rats?	[64]
		monophasic stimulation in humans	[84]
TAL	NKA, NKCC2	inhibition	[69-72]
	J_{Cl}, J_{HCO3}		
CNT/CD	ENaC	inhibition	[81,83]

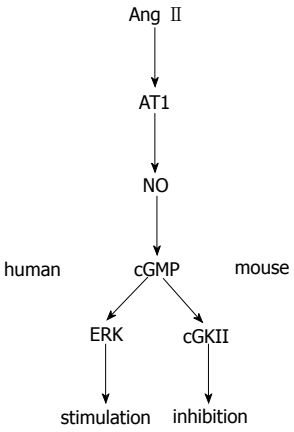

Figure 1 Species difference in angiotensin II/nitric oxide signalling in proximal tubules. In humans, NO/cGMP stimulates PT transport via ERK. In mouse, by contrast, NO/cGMP inhibits PT transport via cGKII. Ang II: Angiotensin II; NO: Nitric oxide; cGMP: Guanosine 3',5'-cyclic monophosphate; ERK: Extracellular signal-regulated kinase.

In contrast to other animals, both arachidonic acid and 5,6-EET failed to inhibit NBCe1 stimulation, which may partly account for the lack of an inhibitory effect of Ang Ⅱ in human PTs. Notably, however, we found that the contrasting responses to the NO/cGMP pathway could largely explain the different actions of Ang Ⅱ on PT transport in humans and other species. Thus, inhibition of the NOS/cGMP/cGKII pathway converted the inhibitory effect of 10^{-6} mol Ang Ⅱ on mouse PT transport into a stimulatory effect. SNP dose-dependently inhibited PT transport in wild-type but not in cGKII mice. By contrast, the inhibition of NOS/cGMP/ERK pathway completely suppressed the stimulatory effect of Ang Ⅱ on human PT transport. While the inhibition of cGKII did not affect the Ang Ⅱ effects, SNP dose-dependently stimulated transport in human PT. Western blotting with phosphor-specific antibodies revealed that Ang Ⅱ induced a dose-dependent cGKII activation in mouse but not in human kidney cortex samples. On the other hand, SNP induced a dose-dependent ERK activation in human but not in mouse samples. Collectively, these results indicate that while the NO/cGMP/cGKII pathway mediates the inhibitory effect of Ang Ⅱ in mouse PTs, the NO/cGMP/ERK pathway mediates the stimulatory effect in human PTs as shown in Figure 1.

We confirmed that human PTs do express cGKII. On the other hand, NO/cGMP failed to activate ERK in PTs from cGKII KO mice, indicating that the simple removal of cGKII from mouse PTs cannot reproduce the dose-dependent stimulatory effect of Ang Ⅱ in human PTs. Therefore, the reason why the NO/cGMP pathway, acting as the down-stream mediator of Ang Ⅱ, has contrasting effects on PT transport in humans and in other species is currently unknown. However, it is interesting to note that while the role of intrarenal NO in the adaptive natriuretic response to sodium loading has been well established in rodents, a similar role for NO has not been established in humans[85-90]. In any case, the human-specific stimulatory effect of the NO/cGMP pathway on PT transport may offer a novel therapeutic target for human hypertension.

CONCLUSION

The absorption of Na^+ in renal tubules is regulated by various factors, among which Ang Ⅱ and NO play

significant roles. In general, Ang Ⅱ stimulates sodium reabsorption and triggers fluid retention, leading to hypertension, while NO seems to induce natriuresis. However, our in vitro data suggest that NO may have distinct effects on PT transport in human and other species. Tables 1 and 2 summarize the effects of Ang Ⅱ and NO on renal tubular transport.

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P- Reviewer: Lymperopoulos A, Pedersen EB, Wu S
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DOI: 10.5527/wjn.v3.i4.302

World J Nephrol 2014 November 6; 3(4): 302-307
ISSN 2220-6124 (online)

MINIREVIEWS

Roles of the (pro)renin receptor in the kidney

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Received: June 25, 2014 Revised: September 14, 2014
Accepted: October 14, 2014
Published online: November 6, 2014

Abstract

Prorenin receptor (PRR) is a multi-functioning protein possessing at least four different roles: (1) working as a receptor for renin and prorenin producing angiotensin I from angiotensinogen thus enhancing the tissue renin-angiotensin system; (2) inducing intracellular signals when a ligand binds to PRR; (3) participating in the functions of vacuolar proton ATPase; and (4) constituting the Wnt signaling receptor complex. Here, the roles of PRR in kidney physiology and diabetic conditions as well as recent findings regarding a soluble form of PRR are discussed. We also propose the possible mechanism concerning diabetic nephropathy as "trade-off hypothesis" from a PRR point of view. In brief, under hyperglycemic conditions, injured podocytes degrade degenerated proteins and intracellular organelles which require V-ATPase and PRR for vesicle internal acidification. Sustained hyperglycemia overproduces PRR molecules, which are transported to the transmembrane and bind to increased serum prorenin in the diabetic condition. This enhances tissue renin-angiotensin system and PRR-mediated mitogen-activated protein kinase signals, resulting in increased injurious molecules such as transforming growth factor-β, cyclooxygenase2, interleukin-1β, and tumor necrosis factor-α ending in diabetic nephropathy progression. Although many findings led us to better PRR understanding, future works should elucidate which PRR functions, of the four discussed here, are dominant in each cell and kidney disease context.

Key words: Prorenin receptor; Atp6ap2; Soluble prorenin receptor; Kidney; Diabetic nephropathy; Podocyte

Core tip: Prorenin receptor (PRR) has shown its multi-functionality in at least four different aspects. In this review, the roles of PRR in kidney physiology and diabetic conditions as well as recent findings regarding a soluble form of PRR are discussed. Additionally, we propose the possible mechanism concerning diabetic nephropathy as "trade-off hypothesis" from a PRR point of view.

Oshima Y, Morimoto S, Ichihara A. Roles of the (pro)renin receptor in the kidney. *World J Nephrol* 2014; 3(4): 302-307 Available from: URL: http://www.wjgnet.com/2220-6124/full/v3/i4/302.htm DOI: http://dx.doi.org/10.5527/wjn.v3.i4.302

INTRODUCTION

Prorenin receptor (PRR), also known as ATP 6-associated protein 2 (Atp6ap2), was cloned in 2002 as a single transmembrane protein whose ligand is renin and its precursor prorenin[1]. Initially, the roles of PRR were thought to enhance the tissue renin-angiotensin system (RAS) by binding PRR to its ligand, while also inducing intracellular signal transductions such as mitogen-activated protein kinase (MAPK) pathways independent of the RAS. However, recent findings have revealed additional aspects of PRR, including it functioning as an accessory protein of vacuolar proton ATPase (V-ATPase) and constituting the Wnt receptor complex. This review discusses PRR in kidney physiology and diabetic nephropathy, and in addition to recent findings regarding the kidney

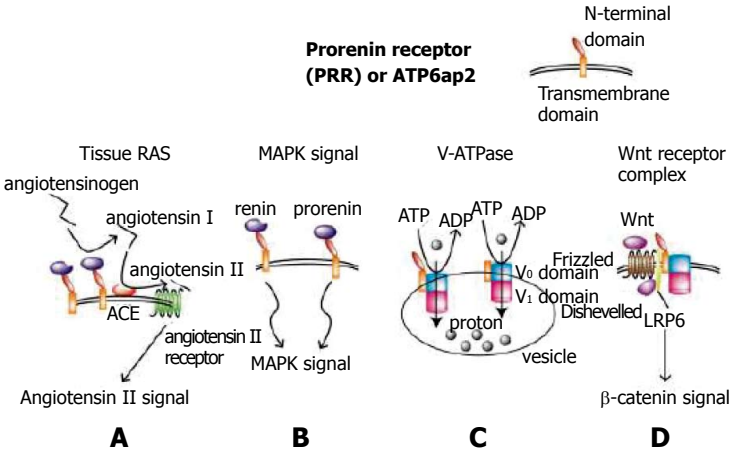

Figure 1 Four roles of prorenin receptor. A: When renin or prorenin binds to prorenin receptor (PRR), renin or prorenin enzymatic activity is enhanced through non-proteolytic conformational change, catalyzing angiotensinogen to angiotensin I. Produced angiotensin I is catalyzed by angiotensin-converting enzyme, yielding angiotensin II that induces angiotensin II receptor-mediated signal transduction, ending in enhanced tissue renin-angiotensin system (RAS)[45]; B: When PRR is bound to a ligand, renin, or prorenin, a mitogen-activated protein kinase (MAPK) signal is induced[1]; C: PRR, with or without the N-terminal domain, is required as a subunit of V-ATPase, which actively transports protons into vesicles such as endosomes, lysosomes, and autophagosomes using energy obtained by degrading ATP to ADP[9]. The V0 and V1 domains build up V-ATPase; D: PRR is required as an adaptor protein between V-ATPase and LRP6, which are members of the Wnt receptor complex[5,46].

and a soluble form of PRR.

PRORENIN RECEPTOR IN KIDNEY PHYSIOLOGY

After its discovery by cloning a single transmembrane protein PRR a dozen years ago, PRR has shown its multi-functionality in at least four different aspects (Figure 1). One of these is to enhance angiotensin I production from angiotensinogen by non-proteolytically increasing catalyzing activity of renin or prorenin when bound to PRR, resulting in enhanced RAS (Figure 1A). Another is to induce MAPK signal transduction pathway when PRR is bound to its ligand renin or prorenin[1] (Figure 1B). PRR is located on the X chromosome and is distributed widely in the kidney, heart, brain, liver, placenta and pancreas, although its' physiological role has not been elucidated until recently because of embryonic lethality of complete knockout of PRR in mice.

We previously generated floxed PRR mice and mated them with mice expressing Cre recombinase under the control of a podocyte-specific podocin promoter to create conditional PRR knockout mice in podocytes. Unexpectedly, these mice died of nephrotic syndrome and renal failure resulting from disturbed V-ATPase function. This provided evidence that, under physiological condition PRR is needed for maintaining vacuoles-such as endosomes, lysosomes, and autophagosomes- through normal V-ATPase function in mouse podocytes[2] (Figure 1C). Similar results in regard to podocytes[3] and cardiomyocytes[4] were obtained from another group and ours, respectively.

In *Xenopus*, PRR binds to V-ATPase and LRP6 to form a Wnt signaling receptor complex as an adaptor protein, showing that PRR is indispensable for normal Wnt signal transduction[5] (Figure 1D). The embryonic lethality of PRR full knockout in mice may be related to abrogated Wnt signals because Wnt signal is required for the formation of a primitive streak in early mouse embryogenesis[6]. PRR is also required for early embryogenesis in zebrafish[7]. PRR has been shown to be involved in nephrogenesis; mice with conditional PRR knockout in the ureteric bud developed renal hypodysplasia[8]. These mice were not embryonically lethal, presumably because the uretic bud is derived from the intermediate mesoderm, which develops long after the formation of the primitive streak.

Recently, the N-terminal of the PRR extracellular domain, which interacts with prorenin/renin, has been proven indispensable for V-ATPase biogenesis[9]. Although prorenin/renin does not influence overall V-ATPase activity[5,9], in vitro experiments using MCDK cells revealed prorenin and a handle region peptide that corresponds to the part of the prorenin responsible for binding to PRR and increases the initial linear phase of V-ATPase activity through binding to PRR[10]. Further investigations are expected to elucidate the roles of PRR in association with prorenin/renin, V-ATPase, and Wnt signals in physiological and developmental conditions in the kidney.

PRORENIN RECEPTOR IN DIABETIC NEPHROPATHY

In diabetes mellitus (DM), a sustained hyperglycemic state leads to diabetic nephropathy, causing end-stage renal disease. It has been reported that an elevated plasma prorenin concentration is associated with microalbuminuria[11] and diabetic nephropathy[12] in patients with DM. Tissue angiotensin Ⅱ levels are also increased in the kidneys of DM rats[13-15], suggesting activated tissue RAS. This is consistent with the multiple clinical trials showing that angiotensin-converting enzyme (ACE) inhibitors and angiotensin receptor blockers (ARB) have reno-protective effects against diabetic nephropathy[16-19], although these effects were limited. Adversely, plasma renin activity is suppressed in patients with diabetic nephropathy[20,21], reflecting a suppressed systemic RAS possibly resulting from activated tissue RAS in the kidneys. Recently, podocytes have been thought to play an important role in diabetic nephropathy and albuminuria[22,23]. Identifying PRR has led to a better understanding of diabetic nephropathy.

Albuminuria is caused by the breakdown of the

filtration barrier composed of endothelial cells, glomerular basement membranes, and podocytes. PRR has been heavily detected in mouse podocytes through immunoelectron microscopy[24]. Because transgenic rats overexpressing human PRR[25] developed slowly progressive proteinuria and enhanced MAPK signals mainly in the glomeruli podocytes[26], it is likely that podocytes are vulnerable to PRR-dependent signal transduction. The MAPK signals could be aggravated in the diabetic condition, in which both PRR and prorenin are upregulated, as discussed later in this article. This effect is believed to be independent of angiotensin Ⅱ because rat prorenin bound to human PRR did not show renin activity.

In rats with streptozotocin-induced diabetic nephropathy, PRR protein up-regulation were observed in their kidneys[27]. High glucose increased mRNA and protein PRR levels in cultured podocytes[28]. This experimental evidence shows that PRR is up-regulated in the podocytes of DM; thus, in DM, both the ligand prorenin and its receptor PRR increase.

Electron microscopic analysis in DM mice revealed that podocytes go through a hypertrophic state before the atrophic state. This hypertrophy reflects the occurrence of many vesicles (presumably lysosomes and autophagosomes) resulting from injured intracellular organelles[29]. Also, Golgi apparatus, rough endoplasmic reticulum, and free ribosome develop because of increased protein production demand resulting from cell injury[29] (Figure 2B). It is possible that these increased intracellular organelles might call for PRR up-regulation because PRR is required for V-ATPase activity, which acidifies intracellular vesicles (Figure 2C). V-ATPase is required for both maturation of synthesized proteins and degradation by lysosomes and autophagosomes that affects proteins and organelles.

As discussed previously, the serum prorenin level is increased in DM patients. Inhibition of prorenin binding to PRR by subcutaneous administration of a handle region peptide prevents diabetic nephropathy development, characterized by the inhibition of albuminuria and glomerulosclerosis in DM rats[15]. Moreover, nephropathy regression was observed in the peptide-treated handle region, but not inhibitor-treated ACE, in DM rats[30]. Handle region peptide treatment inhibited diabetic nephropathy in angiotensin Ⅱ type 1a receptor-deficient mice, suggesting the effectiveness of PRR signal transduction blockade in inhibiting diabetic nephropathy apart from angiotensin Ⅱ effects. This is consistent with the results of clinical trials showing a partial effect of ACE inhibitors or ARB in diabetic nephropathy in humans[16-19]. Other in vivo data showed that PRR increases transforming growth factor-β1[31], cyclooxygenase2[32], and cytokines such as interleukin-Iβ and tumor necrosis factor-α[33] in diabetic nephropathy. Our hypothesis here is that overproduced PRR counteracting cell injury could exhibit adverse effects through PRR-distinctive signal transductions, angiotensin Ⅱ-mediated effects, and other injurious molecules contributing to the progression of diabetic nephropathy. We term this the "trade-off hypothesis" (Figure 2E). According to our preliminary data using human PRR over-expression in transgenic rats, albuminuria was not seen in the early stages of diabetic nephropathy, whereas albuminuria was seen in wild-type rats. This experimentally supports our hypothesis.

V-ATPase is indispensable for the normal function of endosomes, lysosomes, and autophagosomes, whereas diabetic nephropathy is associated with endoplasmic reticulum stress[34] and autophagy[35]. Future experiments are needed to investigate the pathophysiological functions of PRR both in V-ATPase and signal aspects in diabetic nephropathy.

SOLUBLE PRORENIN RECEPTOR AND KIDNEY DISEASE

PRR is a single transmembrane protein with a proteinase cleavage site at the N-terminal domain near the transmembrane domain[36,37]. Cleavage of PRR by either furin[36] or ADAM19[38] results in the dissociation of the N-terminal fragment as soluble PRR (sPRR).

In healthy subjects, plasma sPRR did not exhibit circadian or posture variation or correlate with renin, prorenin or aldosterone levels[39], although serum sPRR negatively correlated with an estimated glomerular filtration rate independent of age, blood pressure, and glucose metabolism in essential hypertension and normotensive subjects[40]. sPRR correlated positively with urinary angiotensinogen[40], which is a biomarker of intrarenal RAS[41]. Moreover, in patients with chronic kidney disease, serum sPRR negatively correlated with estimated glomerular filtration rate and chronic kidney disease stage[42]. In human kidneys with end-stage renal disease, intrarenal PRR was immunostained mainly in tubules, suggesting a possible contribution of increased renal PRR expression to elevated sPRR in end-stage renal disease[43]. These findings suggest that sPRR might reflect the intrarenal RAS status.

However, in addition to the changes in sPRR levels being modest and not offering a set cut-off line for clinical use, these levels are affected by many other factors including, RAS inhibitor administration[39], lipid metabolites such as high-density lipoprotein cholesterol and triglycerides[40], age[40], and obstructive sleep apnea syndrome[44]. Moreover, the correlation between sPRR level and the activity of furin or ADAM19 has not yet been investigated under these conditions. Further investigations are expected to determine the relationships, if any, between kidney disease and sPRR, PRR, tissue RAS, V-ATPase and Wnt.

SUMMARY

In physiological and developmental conditions, it has been suggested from the experiments discussed previously that PRR primarily exhibits as V-ATPase or Wnt

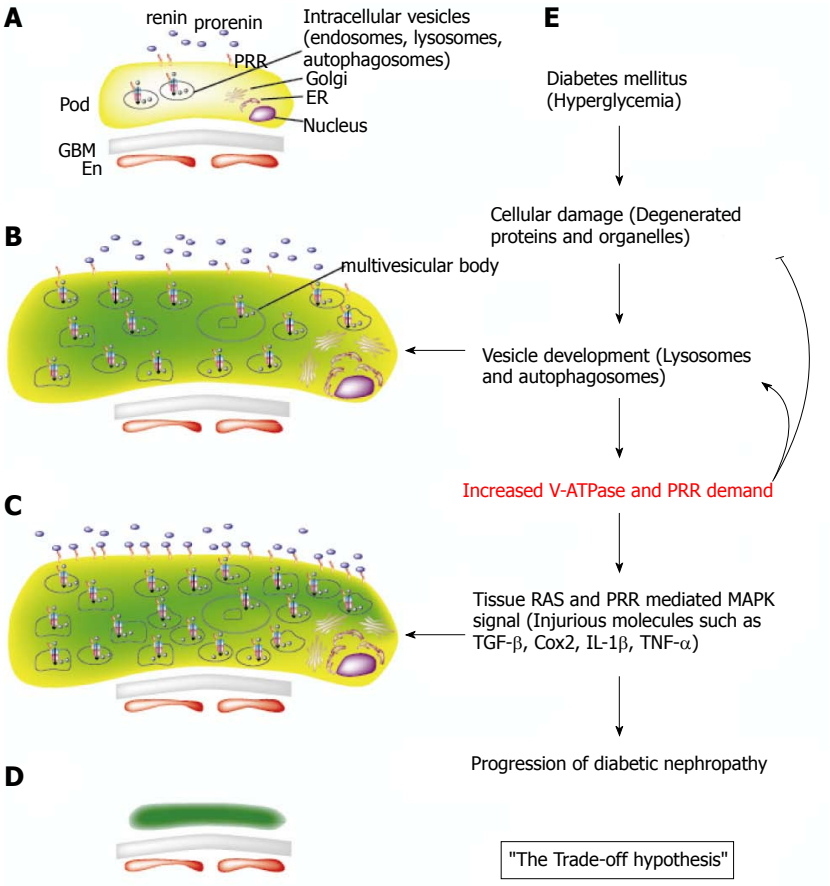

Figure 2 The trade-off hypothesis in diabetic nephropathy from the prorenin receptor perspective. A: Normal podocyte intracellular structure; B: Under hyperglycemic conditions, injured podocytes degrade degenerated proteins and intracellular organelles, forming vesicles such as lysosomes and autophagosomes, which require V-ATPase and prorenin receptor (PRR) for internal acidification; C: Sustained hyperglycemia overproduces PRR molecules, which are transported to the transmembrane and bind to increased serum prorenin in the diabetic condition. This enhances tissue renin-angiotensin system (RAS) and PRR-mediated mitogen-activated protein kinase (MAPK) signals; D: Atrophic or apoptotic podocytes after long-term hyperglycemic conditions. The changes in the glomerular basement membrane and endothelial cells are not shown; E: The schematic view of the trade-off hypothesis. In diabetes mellitus, podocyte injury occurs by producing degenerated proteins and organelles, which in turn are degraded in lysosomes and autophagosomes. This process requires V-ATPase and PRR production for internal acidification of the vesicles. PRR overproduction enhances tissue RAS and PRR-mediated MAPK signals, resulting in increased injurious molecules such as transforming growth factor-β, cyclooxygenase2, interleukin-1β, and tumor necrosis factor-α. Progression and deterioration of diabetic nephropathy occurs at the end. En: Endothelial cell; ER: Endoplasmic reticulum; GBM: Glomerular basement membrane; Pod: Podocyte.

signaling instead of RAS enhancement or PRR-distinct MAPK signal transduction. These former functions are related to fundamental cellular survival and embryo development. On the other hand, in DM, inhibition of prorenin binding to PRR has the beneficial effect of inhibiting diabetic nephropathy, which is not achieved in Ang II blockade. In DM, PRR primarily works as an RAS enhancement or MAPK signal on top of V-ATPase. Involvement of Wnt signals in diabetic nephropathy has not been investigated throughly.

The shift of PRR's function from a physiological condition to a DM condition could be attributed to altered PRR demand. As in the trade-off hypothesis, a hyperglycemic state leads to increased PRR demand caused by increased V-ATPase activity to recycle or degrade intracellular organelles and proteins. The overproduced PRR is transported to the cell membrane, which triggers RAS enhancement and PRR-dependent MAPK signals *via* prorenin binding. As a result, diabetic nephropathy progression occurs.

The significance of sPRR in kidney disease is not clearly defined because sPRR changes in kidney disease are too modest to set a cut-off line for clinical use. Yet, it is possible that sPRR reflects intrarenal RAS status.

CONCLUSION

Rigorous work has uncovered PRR physiology and pathophysiology in the kidneys. The multi-functioning protein PRR can shift its role from the physiological condition to the DM condition depending on underlying cellular conditions. V-ATPase is believed to help cells maintain a clean environment; however, in DM, it may have adverse effects in terms of overproduced PRR. This functional shift may occur because, unlike other molecules, PRR not only is important in fundamental cellular survival but also in disease progression. According to the trade-off hypothesis, over-expression of PRR may have beneficial effects in the very early stages of diabetic nephropathy, although PRR may have harmful effects in the late stages. Future works should elucidate which PRR functions, of the four discussed here, are dominant in each cell and kidney disease context.

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Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.5527/wjn.v3.i4.308

World J Nephrol 2014 November 6; 3(4): 308-316
ISSN 2220-6124 (online)

RETROSPECTIVE STUDY

Quality of life in end stage renal disease patients

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Received: May 28, 2014 Revised: July 24, 2014
Accepted: September 4, 2014
Published online: November 6, 2014

Abstract

AIM: To understand factors associated with quality of life (QOL), examine types of QOL instruments, and determine need for further improvements in QOL assessment.

METHODS: The method used databases (Pubmed, Google scholar) and a bibliographic search using key words QOL, end stage renal disease, Hemodialysis, Peritoneal dialysis, instruments to measure QOL, patients and qualitative/quantitative analysis published during 1990 to June 2014. Each article was assessed for sample size, demographics of participants, study design and type of QOL instruments used. We used WHO definition of QOL.

RESULTS: For this review, 109 articles were screened, out of which 65 articles were selected. Out of 65 articles, there were 19 reports/reviews and 12 questionnaire manuals. Of the 34 studies, 82% were quantitative while only 18% were qualitative. QOL instruments measured several phenomenon such as physical/psychological health, effects and burdens of kidney disease, social support etc. those are associated with QOL. Few studies looked at spiritual beliefs, cultural beliefs, personal concerns, as per the WHO definition. Telemedicine and Palliative care have now been successfully used however QOL instruments seldom addressed those in the articles reviewed. Also noticed was that longitudinal studies were rarely conducted. Existing QOL instruments only partially measure QOL. This may limit validity of predictive power of QOL.

CONCLUSION: Culture and disease specific QOL instruments that assess patients' objective and subjective experiences covering most aspects of QOL are urgently needed.

Key words: Quality of Life; Hemodialysis; Peritoneal dialysis; Patient; End stage renal disease; Quality of life instruments

Core tip: Quality of life (QOL) in end stage renal disease patients is an important outcome measure. This study tried to understand the dimensions of various QOL instruments and association of various risk factors with QOL. Since each instrument measures specific aspect of QOL, use of any one of these instruments allows studies to measure QOL only partially compromising on the validity of the predictive power of QOL. Furthermore, less attention has been given on conduct of qualitative and longitudinal studies. There is an urgent need to develop disease and culture specific instrument that covers most aspects of QOL.

Joshi VD. Quality of life in end stage renal disease patients. *World J Nephrol* 2014; 3(4): 308-316 Available from: URL: http://www.wjgnet.com/2220-6124/full/v3/i4/308.htm DOI: http://dx.doi.org/10.5527/wjn.v3.i4.308

INTRODUCTION

In medicine most assessments are conducted by laboratory tests or examinations from healthcare workers. Quality of Life (QOL), though equally important to assess the quality and outcomes of medical care, is not routinely measured. QOL instruments measure individ-

ual's own views of his wellbeing. The core components of QOL are physical, functional, psychological/emotional, and work/occupational[1]. This review will discuss QOL of adult end stage renal disease (ESRD) patients. For this review, we used the World Health Organization's (WHO) definition of QOL which is "individuals' perception of their position in life in the context of the culture and value systems in which they live and in relation to their goals, expectations, standards and concerns". It is a broad ranging concept affected by the person's complex physical health, psychological state, level of independence, social relationships, personal beliefs and their relationship to salient features of their environment[2]. QOL can be used to gauge health system performance, mortality indicators, and compare health of groups[3].

This review focused on adult ESRD patients since renal disease is a serious illness and treatment is challenging and prolonged. Globally, the estimated prevalence of chronic kidney disease (CKD) (the first four stages out of five) is 7.2% in adults over the age of 30 years[4]. CKD is a major determinant of poor health outcome of noncommunicable diseases affecting 5% to 8% of world's population[5]. Despite the substantial resources committed to the treatment of ESRD and significant improvements in the quality of dialysis therapy, patients continue to experience significant mortality and morbidity and a reduced quality of life[6]. With improved medication, medical treatment, medical care and health technology, patients may be living longer but are they living a better life? The effect of the treatment is not only measured in terms of survival, but also in terms of well-being. There is an ever expanding body of literature related to various factors that affect QOL, like genetic, environmental, psychosocial, stress, emotional, and co-morbidities. Findings have shown that lower scores on QOL were strongly associated with higher risk of death and hospitalization[7,8] than clinical parameters such as serum albumin levels[8] in cases of ESRD patients. It is also noticed that QOL in ESRD is most affected in the physical domains, and nutritional biomarkers are most closely associated with these domains compared to Kt/V (marker of dialysis adequacy), mineral metabolism indices, and inflammatory markers which are poor health related quality of life (HRQOL) correlates[9]. These findings demand more attention towards patients' essential QOL measures and indicators.

While assessing QOL, both subjective and objective information is necessary since they derive distinct types of information. Objective measures may be more suitable in detecting treatment effects, such as the number of days on dialysis. Subjective information (such as happiness, satisfaction, spiritual and religious beliefs) is also necessary to complete the QOL picture and enhance the interpretation of objective data. Both the illness and the treatment of ESRD influence subjective QOL factors.

Recently (2014), Boudreau JE has talked about the functional definition of concept of QOL by discussing three attributes: (1) the ability to engage in vigorous activities; (2) the ability to engage in social and occupational roles; and (3) the ability to perform activities of daily living (ADL)[10]. Reviews were conducted that included the type of measures, the instrument development process, study sample characteristics, particular quality of life domains, and reliability and validity testing. Some reviews provided an overview of the instruments used and judged the instruments in terms of their comprehensiveness, reliability, and validity[11]. Few studies sought to establish which domains of QOL are most affected by ESRD[9]. Review by Gentile[12] did provide a variety of generic and disease targeted health related QOL instruments for patients suffering from ESRD. Yet, reviews have rarely discussed whether existing QOL instruments have covered both objective and subjective patient experiences as per the WHO definition of QOL.

Based on this background, the aim of this review was to understand the factors associated with QOL of adult ESRD patients, examine the various dimensions that QOL instruments measure, and identify if there is a need to expand the measurements of QOL.

MATERIALS AND METHODS

The search strategy detailed in Figure 1 was used to identify published literature in the English language during the years 1990 to June 2014. The search was conducted during March - June 2014 using the search criteria (key words, year and language) as mentioned in Figure 1. The search was conducted with MEDLINE, PubMed and was further expanded with Google Scholar using the same search criteria mentioned above. Title and abstracts of the studies were checked with the key words to screen the articles. This process generated 109 studies including research papers, reviews, reports and manuals relevant to our scope of interest.

Inclusion and exclusion criteria (as mentioned in Figure 1) were applied to the selected abstracts for relevance. If the author was not satisfied with the content of the abstract, the full paper was accessed and the same inclusion/exclusion criteria were applied. A total of 62 research papers met the criteria. The bibliography of the research papers was then reviewed to identify additional literature published in English that met the inclusion criteria. Three more research studies were identified by this process. In total, 65 research papers, reports, reviews and quality of life questionnaire manuals were included in this review

These 65 research papers were then arranged into four principal categories as follows: (1) Reports, reviews, published series, discussion articles; (2) Quantitative studies; (3) Qualitative studies; and (4) Quality of life questionnaire manuals (Table 1).

RESULTS

For this review 109 articles were screened, out of which 65 articles were selected. Out of 65 articles, there were

Table 1 First Author, year of publication, study design and questionnaire used

Ref.	Study design	
Reviews, Published series, Reports, Discussion articles		
Donald[1], 2009	Published Series article	
POFS ABUSE[2], 1997	WHOQOL Instruments Report	
Romero *et al*[3], 2013	Discussion article	
EpiCast[4], 2014	Report	
Couser *et al*[5], 2012	Policy Forum	
Obrador *et al*[6], 2014	Review	
Schatell *et al*[7], 2012	Report	
Berman *et al*[9], 2008	Systematic Review	
Edgell *et al*[11], 1996	Review	
Gentile *et al*[12], 2003	Review	
Kimmel *et al*[21], 2006	Review	
Johansen[30], 2007	Report	
Kutner[31], 2010	Rehabilitation Report	
Valderrábano *et al*[46], 2001	In-depth Review	
Fleck *et al*[53], 2007	Discussion	
Carver *et al*[57], 1995	Review	
Blinkhorn[61], 2012	Review	
O'Connor *et al*[64], 2012	Review	
Catania *et al*[65], 2013	Report	
Quantitative	Study design	Questionnaire used
Mapes *et al*[8]	Longitudinal	KDQOLSF-36
Kao *et al*[13], 2009	Cross sectional	SF-36
Abraham *et al*[14], 2008	Case control, follow up	WHOQOL-BREF
Kimmel *et al*[15], 2008	Prospective	Satisfaction with Life Scale (SLS), McGill QOL, Single item
Patel *et al*[16], 2002	Prospective	McGill QOL, Beck Depression
Griva *et al*[17], 2009	Cross sectional	SF-36
Elder *et al*[19], 2008	Cross sectional, case mix	KDQOLSF-36
Sanner *et al*[20], 2002	Cross sectional	SF-36, Nottingham Health Profile
Tondra[22], 2014	Conceptual Framework, CS	Quality of Life Index Dialysis,
Mingardi *et al*[23], 1999	Prospective	SF-36
Seica *et al*[24], 2009	Cross sectional	SF-36, KDQOLSF-36
Bakewell *et al*[25], 2002	Longitudinal /intervention	KDQOLSF-36
Theofilou[26], 2012	Cross sectional/ Observational	WHOQOL-BREF, GHQ-28
Kim *et al*[28], 2013	Cross sectional	KDQOLSF-36
White *et al*[29], 2002	Retrospective cohort	SF-36
Painter *et al*[32], 2000	Experimental/Intervention	SF-36
Ouzouni *et al*[33], 2009	RCT	SF-36, Quality of Life Index
Agakhani *et al*[34], 2012	Case control/comparative	SF-36
Hegazy *et al*[35], 2013	Intervention/Pre-post	Karnofsky performance scale
Abraham *et al*[36], 2009	Prospective, intervention	Karnofsky performance scale
Moattari *et al*[37], 2012	RCT	SF-12
Brennan *et al*[38], 2007	Intervention, report	SF-36
Cukor *et al*[39], 2013	RCT	KDQOLSF-36, Beck Depression Inventory
Lii *et al*[40], 2007	Intervention/Experimental	SF-36
Sathvik *et al*[52], 2008	Cross sectional	WHOQOL-BREF
Pagels *et al*[50], 2012	Cross sectional	SF-36
WHOQOL-SRPB[54], 2005	Cross cultural/sectional study	WHOQOL-SRPB
Yong *et al*[63], 2009	Prospective cross sectional	SF-36, Charlson Comorbidity Index
Qualitative		
Baudeau *et al*[10], 2014	Concept analysis	
Fennegan-John *et al*[18], 2013	Interviews, FGD	
Arabi[41], 2006	Interview	
Rygh *et al*[59], 2012	Interviews with patients	
Stroetmann *et al*[60], 2000	Observational	
Jablonski[62], 2007	Observational	
QOL instruments		
Choices for Healthy Outcomes In Caring for End Stage Renal Disease[27]		
Sickness Impact profile[42]		
SF-36[43]		
SF-12[44]		
Nottingham Health Profile[45]		
EQ-5D[47]		
McGill Quality of Life Questionnaire[48]		
GHQ-28[49]		
WHO-BREF[51]		
Dialysis Symptom Index[55]		

KDQOL-SF36[56]	
CKD Questionnaire[58]	

QOL: Quality of life; CKD: Chronic kidney disease.

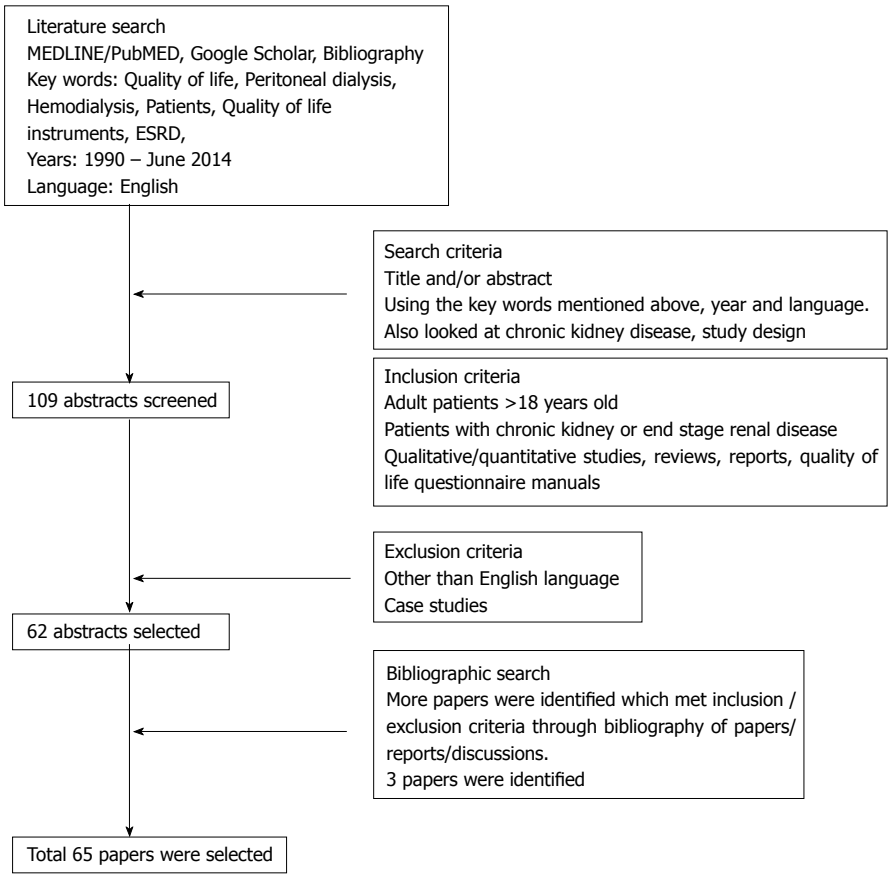

Figure 1 Literature search strategy. Flow chart below shows how the studies were selected for this article.

19 reports/reviews and 12 questionnaire manuals. Of these 34 studies, 82% were quantitative while only 18% were qualitative. Most quantitative studies were cross sectional. Only two studies used longitudinal design.

Association of various factors with QOL and outcome

The treatment for ESRD patients imposes heavy restrictions that affect QOL. QOL usually includes both objective and subjective evaluations of both the positive and negative aspects of life. Researchers have reported demographic, clinical, social, psychological, and treatment related associations with QOL[1].

It has been proven that the patient's perception is more important than the clinical assessment in determining QOL[15]. Figure 2 illustrates several factors having linear, inverse or no association with QOL. Studies have commented that QOL can be enhanced by intervention techniques as mentioned in Figure 2. The same figure further shows that lower scores on all three summary scores of QOL (physical component summary, mental component summary and kidney disease component summary) were strongly associated with death and hospitalization as revealed by Mapes in DOPPS study[8] (predictive power of QOL).

Qualitative research

Qualitative research produces rich information that is not possible to get by quantitative research. Qualitative research conducted on ESRD patients has reported some of the themes (subjective measures) for QOL. These themes were physiological impact, impact of treatment, impact on daily life, psychological impact, impact on relationships, social impact and coping responses[20]. Another study came up with three themes and sub themes as: (1) "life restricted" with sub-themes "being tied down", "feeling left out", and "doing without"; (2) "staying alive" with sub-themes "love from others", "accept illness as part of life", and "trust in God"; and (3) "feeling good" with sub-themes "personal satisfaction" and "being happy"[41].

QOL instruments

Some QOL instruments provide a standard assessment of health. These instruments include questionnaires designed to be applicable for general population such as the Sickness Impact Profile (SIP)[42], the SF-36[43], SF-12[44], the Nottingham Health Profile[45,46] (used for primary care), the European Quality of Life Instrument - EQ-5D[47], the McGill QOL (MQOL)[48] scale

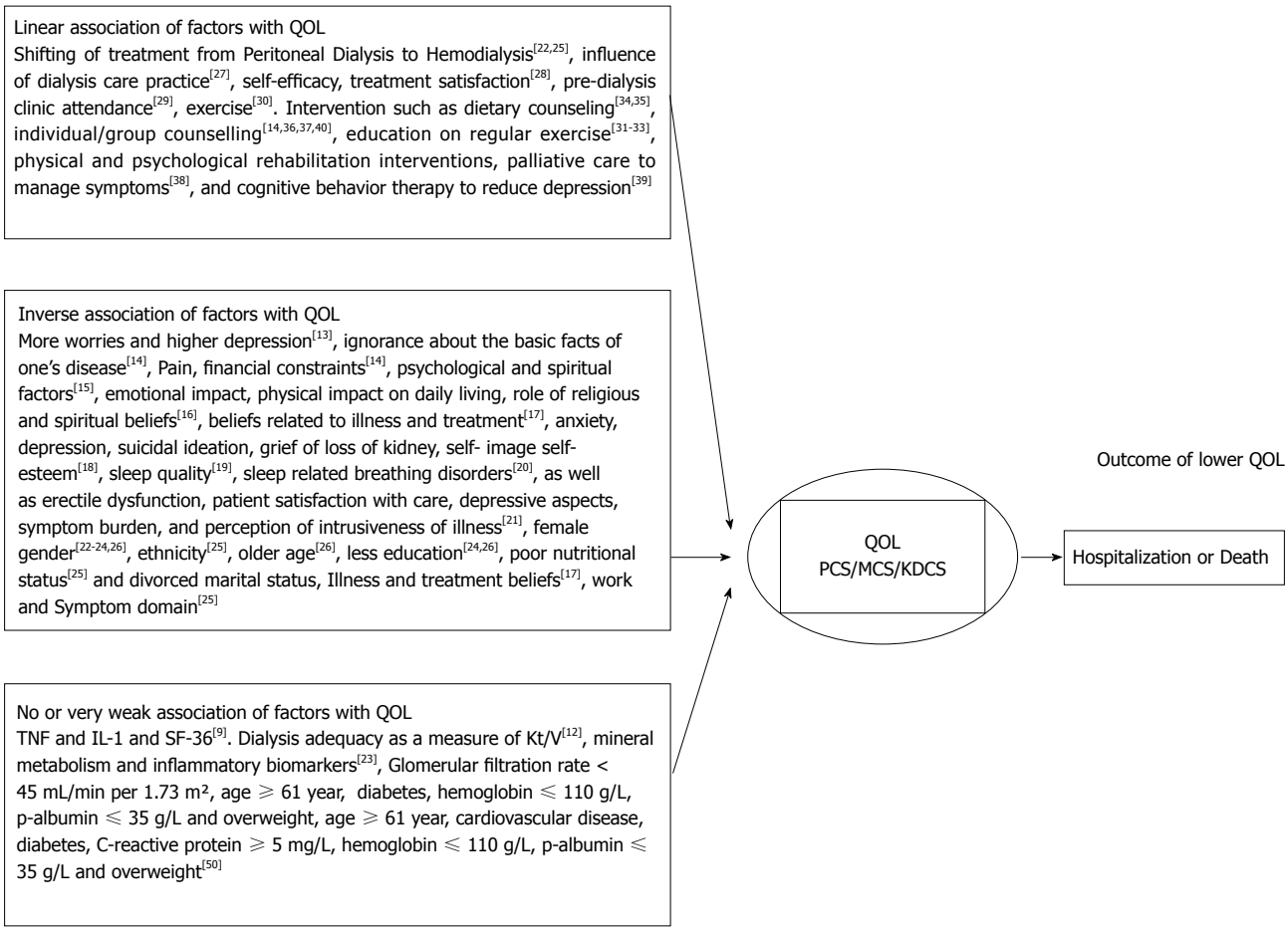

Figure 2 Factors associated with quality of life and predictive power of quality of life. Quality of life (QOL) is assessed based on several factors that show linear / inverse/no relationship with QOL. Based on these relationships QOL predicts Hospitalization or death. QOL: Quality of life; PCS: Physical component summary; MCS: Mental component summary; KDCS: Kidney disease component summary.

and GHQ- 28[49]. Instruments designed by WHO such as WHOQOL[50] WHOQOL-BREF[51,52] are used by researchers. WHOQOL-SRPB[53,54] is also used to assess spiritual, religious and personal beliefs (SRPB) within quality of life. In the CHOICE study, the research team is conducting several research projects for the development of patient-centered instruments for assessment of health-related quality of life[27].

There are three disease-targeted questionnaires developed for ESRD patients undergoing dialysis. Dialysis Symptom Index (DSI)[55], the Kidney Disease Quality of Life instrument Short Form- KDQOL-SF36[56]. The Choices for Healthy Outcomes in Caring for End-Stage Renal Disease ([ESRD] CHOICE)[27]. Additionally, few researchers use The Kidney Disease Questionnaire - KDQ[58], Renal Quality of Life Health Profile (RQLP), and Quality of Life Index-D. Each QOL tool covers a number of domains (measurements of different characteristics) and they measure quantitative outcomes[13]. Culture specific validation has been reported for these instruments in many countries. Every instrument is scored on different domains. There is no one instrument that measures all the domains or most of the patients' perceptions towards their disease or life. The most common instruments used were SF-36 and KDQOL. Data collected by administering these questionnaires was analyzed using quantitative methods. Most studies use descriptive cross sectional design[57]. In this review, out of 65 articles, 48% had used SF-36, 20% had used KDQOL, 16% had used WHOQOL and the remaining 16% had used GHQ-12, GHQ-28, McGill, DSI, QOLI (Table 1).

DISCUSSION

Researchers have reported a linear or inverse relationship between factors that improve or lower QOL. Researchers have defined attributes[10] or used frameworks[22] or models[3] that encompasses certain aspects of QOL, such as demographic data, information on diet, treatments and their impact, anthropometric biomarkers[50], and data related to mental health such as depression or anxiety. Most of the existing QOL instruments derived mainly quantitative information. Since QOL is subjective, more qualitative evidence needs to be gathered, assessed and understood. Although it is expensive and time consuming, incorporating qualitative methods will generate rich information.

Considering the WHO definition of QOL and its multidimensional aspects, the instruments and models reviewed only partly assess QOL. Some of the domains were omitted, such as patients' thinking, learning, memory concentration, self-esteem, patient's perception about his body image, patient's feelings about his health and the surrounding environment, patient's age, patient's dependence on medication or treatments, financial burden of treatment, and spiritual/religious beliefs[57]. While studies that have used WHO QOL have covered some of the above-mentioned characteristics, they have not specifically covered these in relation to kidney disease. Although Paul Kimmel has commented that there is a need for proper measurements for judging QOL for chronic kidney disease patients[21], not much attention has been given. There remains a need for an instrument that will capture the greatest number of QOL characteristics to get a broader understanding.

The results also reveal the need to conduct more longitudinal studies where researchers are able to detect changes in the characteristics of the population at a group level. Few longitudinal studies were conducted to report the usefulness of these instruments to find improvement in QOL over time. With longitudinal studies it would be possible to detect Minimal Clinically Important Difference (MCID) *i.e.*, a smallest change in treatment outcome that a patient himself would identify as important.

Culture plays a vital role in shaping individual QOL. An individual's values affects perception of QOL and this can differ between cultures as shown in DOPP study[8].

Furthermore, the current instruments were developed some time ago. [KDQOL-SF36 (1995), KDQOL-36, SF-36 (2002), SF-12, EQ-5D (2004)] Since then (1995), medical technologies (e-health) and medical services have improved. Although telemedicine[59], electronic/digital processes in health, healthcare practice using the Internet, video conferencing with patients, and electronic medical records have been implemented, these services are not evaluated for QOL. For example, there is little published research on telehealth in renal units[61]. Patients generally prefer to stay at home and telecare can extend homecare to peritoneal dialysis patients[60], but use of telehealth is under researched[61]. QOL instruments may be incorporated into telehealth assisted technologies for wider understanding and application.

For those who are not able to receive dialysis treatment, non dialytic management of ESRD seems to be a viable option. Patients managed conservatively had reported high symptom burden underscoring the need for concurrent palliative care[64]. Hence, physicians are now considering palliative care services that specialize in symptom management for ESRD patients[62,63]. This is especially important in frail, illiterate, elderly multimorbid patients with limited physical activity, where prognosis may not be altered by dialytic therapy. In such scenarios, palliative care will help improve quality of life. Though Catania G. has come up with a frame work to assess QOL with palliative care intervention[65], he has explained the complexity involved in measuring palliative care as an intervention. The existing QOL instruments have rarely looked at palliative care aspects for improvement of patient well-being. Inclusion of newer technologies and therapies measured over time may also help to establish the minimally important differences that would constitute a real change in scores as well as clinically meaningful differences.

Studies have shown that QOL has improved with hemodialysis treatment as compared to peritoneal dialysis[22,25]. Another study has shown that QOL is better for patients treated at home[60]. In most cases peritoneal dialysis treatment is given at home. These two results may look contradictory but they are reported by two different studies. Is it the type of treatment or the place of treatment that affects QOL? It will be interesting to know what will be the result when both aspects are looked at by the same study. When several other factors are studied and included in the model, with the help of statistical analysis it will be possible to identify which factor affects QOL the most. Most instruments do not cover health literacy, which also has an impact on QOL. In the case of illiterate patients, sufficient data may not be available, so pictorial forms of the instruments may help.

Since patient-reported baseline QOL levels provide additional predictive information[7], it is important to consider a patient's evaluation of their own QOL along with other aspects. A possible limitation of the study is that we were only able to review a portion of the research studies.

In summary, QOL is multidimensional where many indicators are intertwined and that affect person's overall QOL. Indicators based solely on certain characteristics of the patients pose serious restrictions to the measure of QOL. Ultimately, this may limit the predictive power of QOL. In examining QOL of ESRD patients, much work remains. The challenge for the next decade will be to continue to design a QOL instrument that takes both disease specific and culture specific subjective and objective factors into account so that it would be possible to get the complete assessment of QOL of ESRD patients.

ACKNOWLEDGEMENTS

Author would like to acknowledge the contribution of Ms. Abigail Levin Tatake for editing this article for English grammar and clarity.

COMMENTS

Background

Quality of life (QOL) is considered as an important outcome measure. Researchers have claimed that it is even better than clinical parameters. Understanding QOL of end stage renal disease patients is necessary because renal disease is a serious illness and treatment is challenging and prolonged. Though there are various instruments to measure QOL, it is necessary to understand the dimensions used for assessment by these instruments and if there is a

need to improve the existing QOL instruments.

Research frontiers

There are several instruments to measure QOL. Each instrument measures certain dimensions of human characteristics. Most of the instruments record objective information and measure QOL quantitatively. Most of the studies use cross sectional design that gives only snap shot information. An instrument designed by WHO measure subjective information but do not assess information related to kidney disease. These instruments rarely record the modern technologies such as telemedicine, e-health, conservative care *etc.*

Innovations and breakthroughs

There is an urgent need to develop QOL instrument that will try to look at the majority of (objective and subjective) characteristics of patients as well as the effect of new technologies like e-health and therapies like palliative care. QOL instruments, those are currently in use, have been developed some time ago. [KDQOL-SF36 (1995), KDQOL-36, SF-36 (2002), SF-12, EQ-5D (2004)] Since then (1995), medical technologies (e-health) and medical services have improved.

Applications

The newly designed QOL instrument that takes both diseases specific and culture specific, objective and subjective factors into account will help physicians to plan targeted intervention strategies based on strongest and weakest factors that affect QOL. With availability of complete QOL assessment, it will be possible to predict disease outcome effectively.

Terminology

Studies have reported that QOL can be used as an outcome measure in terms of hospitalization and mortality. The strength of this prediction would depend on how rigorously and comprehensively QOL was assessed. This is indicated as validity of the predictive power of QOL.

Peer review

This is an interesting topic.

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DOI: 10.5527/wjn.v3.i4.317

World J Nephrol 2014 November 6; 3(4): 317-323
ISSN 2220-6124 (online)

OBSERVATIONAL STUDY

Residual urinary output in high body mass index individuals on chronic hemodialysis: A disregarded life vest?

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Received: April 18, 2014 Revised: September 19, 2014
Accepted: September 23, 2014
Published online: November 6, 2014

Abstract

AIM: To assess residual diuresis and diverse variables according to body mass index (BMI).

METHODS: Cross-sectional study (n = 57), with 3 groups. Group A: BMI < 25, n = 22; Group B: BMI 25-30, n = 15; Group C: BMI > 30, n = 20. Diuresis, hematocrit, albumin, C-reactive protein, Malnutrition inflammatory score, Pro-BNP, Troponin T, leptin and insulin levels are expressed as median and ranges (r).

RESULTS: Albumin (g/dL): GA *vs* GC, 3.70 (r2.20-4.90) *vs* 3.85 (r3.40-4.90), P = 0.02. Diuresis (mL/d): GA 690 (r0-1780); GB 660 (r60-1800); GC 840 (r40-2840). Diuresis GA *vs* GC, P = 0.01. Leptin (ng/mL): GA *vs* GC, 3.81 (r0.78-69.60) *vs* GC, 32.80 (r0.78-124.50), P < 0.001. Insulin (μU/mL): GA *vs* GB, 7 (r2-44) *vs* 11.50 (r4-38), P = 0.02; GA *vs* GC, 7 (r2-44) *vs* 19.5 (r5-155), P = 0.0001. Troponin T and Pro-BNP levels were not different. Significant correlations: GC, Insulin-UF: ρ = 0.53; P = 0.03; TroponinT-diuresis: ρ = -0.48, P < 0.05; Pro-BNP-diuresis: ρ = -0.39, P < 0.01; Troponin T-ProBNP: ρ = 0.77, P < 0.0001; albumin-Troponin T: ρ = -0.66, P < 0.0001; albumin-ProBNP: ρ = -0.44, P < 0.05.

CONCLUSION: High BMI associated positively with higher diuresis and albuminemia, and negatively with TropT and Pro-BNP. High BMI-associated better survival may be explained by better urinary output, lowering cardiovascular stress.

Key words: Hemodialysis; Residual diuresis; Body mass index; Troponin T; Pro-BNP; Insulin

Core tip: Cardiovascular disease is the major cause of death in hemodialysis, while residual diuresis and increased body mass index (BMI) are associated with better survival. We found that an elevated BMI > 30 associated positively with higher diuresis, insulin levels and albuminemia. This higher urinary output dialysis individuals with BMI > 30%, may reflect water retention, in part due to hyperinsulinemia, hyperleptinemia and secondary higher ultrafiltration rates. The ability to excrete water correlates negatively and significantly with Troponin T and Pro-BNP levels, reflecting lower myocardial and vascular overload. High BMI-associated better survival may be explained by better diuresis, and lower cardiovascular stress.

Trimarchi H, Raña MS, Karl A, Andrews J, Dicugno M, Pomeranz V, Young P, Forrester M, Alonso M, Lombi F, Muryan A. Residual urinary output in high body mass index individuals on chronic hemodialysis: A disregarded life vest? *World J Nephrol* 2014; 3(4): 317-323 Available from: URL: http://www.wjgnet.com/2220-6124/full/v3/i4/317.htm DOI: http://dx.doi.org/10.5527/wjn.v3.i4.317

INTRODUCTION

The International Task Force has established that worldwide 1.5 billion adults are overweight or obese, with nearly 500 million being obese[1]. Only in the United States, a third of the adult population is overweight and another third is obese[2]. In the general population, obesity is associated with higher rates of hypertension, diabetes, metabolic syndrome, cardiovascular disease, and death[3,4]. Morbidly obese adults present a 6-fold higher risk of diabetes compared with their lean peers[5]. Moreover, approximately 70% of hypertension can be attributed to excess weight[6,7]. Because a large proportion of chronic kidney disease is attributed to both diabetes and hypertension, conditions associated with high body mass index (BMI), it seems logical to suppose that obesity should be associated with bad prognosis in hemodialysis (HD) individuals. However, in HD patients obesity is independently associated with reduced all-cause mortality[8-11]. In this regard, there is a negative correlation between BMI and death, generally referred to as the obesity paradox[12]. In hemodialysis individuals, obesity seems to act as a protective factor[13,14] and in general, obese subjects display a better nutritional status, regardless of portraying a more severe cardiac condition[15]. While diabetes and obesity are two usually associated conditions, in HD diabetics tend to present increased morbidity and mortality rates but obesity has been reported to display better survival. Many variables have been ascribed as potential factors that could explain this apparent paradox about obesity in HD: A better nutritional status, higher albumin levels, and a lower inflammatory milieu as assessed by C-reactive protein (CRP), among other factors[8-11].

In end stage kidney disease, residual renal function or remnant diuresis is considered an important variable associated with better survival[16-18]. Besides a better volume management, residual diuresis has been associated with better preserved renal functions, such as calcium, phosphorus and vitamin D homeostasis, erythropoietin levels, and removal of middle molecules[19-24]. In this regard, residual renal function has been shown to present a greater influence on dietary protein intake and nutritional status[25-27]. However, with respect to urinary output, in obese hemodialyzed people the reported results are scant or controversial. In this regard, some studies have found an inverse association between obesity and diuresis, while this association has also been reported to be inconclusive[28,29].

In addition, insulin and particularly hyperinsulinemia itself due to peripheral tissue resistance and deeply involved in the pathogenesis of metabolic syndrome and obesity, has been reported to be elevated in high BMI individuals on HD[15,30]. Insulin causes myocardial hypertrophy and water and salt retention and is associated with diabetes and hypertension, conditions that contribute to high morbidity and mortality rates, particularly in end stage kidney disease[31]. Finally, leptin and insulin not only present similar metabolic and hemodynamic actions, but also display the same patterns of distribution with respect to BMI in HD[30,32].

We investigated cardiac and metabolic biomarkers in HD subjects with respect to urinary output, and propose another potential protective cardiovascular mechanism high BMI individuals display in HD. Finally, a consideration is addressed with respect to the importance of defining elevated body weight in hemodialysis, as obesity may not always be the case when other factors intervene, as fluid overload or muscle wasting.

MATERIALS AND METHODS

Design

Cross-sectional, prospective, observational study undertaken in 57 chronic clinically stable HD individuals.

Patients

The Teaching and Research Committee of the Hospital Británico de Buenos Aires approved this observational study. Each patient signed the respective informed consent. Fifty-seven patients with more than 3 mo of HD were enrolled. Exclusion criteria: Patients younger than 18 years old, or with an active cancer, acute infections, hepatopathy, non-treated hypothyroidism, anuria or BMI > 40 kg/m^2. Anuria was defined as a urinary output < 140 mL/d and proteinuria was considered positive when the daily excretion was > 0.15 g/d. One included patient was HIV positive and another one was HbsAg positive. Failed transplant patients were excluded. The population was divided into three groups as to BMI tertiles as described above. Group A, BMI < 25 (n = 22); Group B, BMI 25-30 (n = 15) and Group C, BMI > 30 (n = 20). Median age (range): Group A: 65 (36-83) years; Group B: 71 (26-88) years; Group C: 63 (33-79) years, P = 0.61. Moreover, the three groups were not different with regard to gender, time on chronic HD, estimated glomerular filtration rate at the beginning of HD, etiology of kidney disease, hypertension, diabetes mellitus, CRP levels, nutritional status evaluated by the malnutrition inflammatory score (MIS), daily diuresis, ultrafiltration rates and no difference in mean estimated GFR in the three groups when dialysis was initiated (Table 1). The rates of decline of diuresis were 13%, 17% and 6 %, respectively. Determinations: Mean automatic intradialytic ultrafiltration rates, mean average blood pressure per session, Troponin T (TropT), Pro-BNP, albumin, insulin levels and HOMA. Serum concentrations of albumin and CRP were measured by routine procedures. TropT levels were determined by electrochemoluminiscence, Cobas e411, Roche Diagnostics, Indianapolis, IN, United States, (normal value: < 1 ng/mL); Pro-BNP levels were measured by chemiluminescence, VITROS 5600®, Johnson and Johnson, New Jersey, United States: (reference values: < 125 pg/mL for subjects < 75 years and < 450 pg/mL for those > 75 years). Insulinemia was measured by electrochemoluminiscence, Cobas e411, Roche Diagnostics®, Indianapolis, Indiana United States, (normal value: 2-15

Table 1 Patient characteristics *n* (%)

Variable	Group A	Group B	Group C
N	22	15	20
Male gender	10 (45)	9 (60)	14 (70)
Diabetics	4 (18)	3 (20)	10 (50)
Hypertensives	15 (68)	12 (80)	17 (85)
Median age (yr)	65	71	63
Range	36-83	26-88	33-79
Median time on HD (mo)	12	26	15.5
Range	4-101	9-92	4-55
BMI (Mean ± SD) (kg/m2)	21.3 ± 2.4[b]	27.6 ± 1.4[b]	33.9 ± 4.2[b]
Median MIS	5.5	4	3
Range	1-21	2-8	0-13
Causes of ESRD			
Glomerulonephritis	8	7	11
Diabetes	2	2	3
Nephroangiosclerosis	7	3	4
Obstructive uropathy	1	0	1
Interstitial nephritis	1	1	1
Polycystic kidney disease	3	2	0
Median C-Reactive protein (mg/dL)	1.2	1.1	1.1
Range	0.50-12	0.20-8	0-4.5
Median urinary output (mL/d)	690	660	840
Range	140-1780	160-1800	140-2840
Median initial urinary output (mL/d)	790	800	890
Range	170-1860	180-1970	940-2900
Median albumin (g/dL)	3.70[a]	3.8	3.85[a]
Range	2.2-4.9	3.2-4.4	3.4-4.9

[b]$P = 0.001$, [a]$P = 0.02$. MIS: Malnutrition Inflammatory Score; ESRD: End-stage renal disease; HD: Hemodialysis.

μUI/mL). HOMA was calculated as follows: (Insulin x glycemia)/405. Leptin levels were determined by ELISA, Millipore®, Missouri United States.

Blood samples were obtained in fasting condition prior to the dialysis session. Depending on the dialysis schedule, 24-h urine samples were collected on Sundays or Mondays. All the determinations were performed at the Hospital Británico.

Hemodialysis aspects

High-flux biocompatible membranes were employed in the hemodialysis sessions (Polyflux 21 R®, Gambro, Sweden and Sureflux 190®, Nipro, Japan). Dialysis was performed using bicarbonate bath with a mean blood flow: 450 ± 50 mL/min, and a dialysate flow: 500 mL/min; mean time of the sessions lasted 4.0 ± 0.5 h. The ultrafiltration rate recorded was the one obtained by the automatic dialysis machines (Surdial 190, Nipro® Japan or Diamax, Nipro® Japan) in coincidence with the session when the blood samples were obtained.

Medications

The majority of the patients were receiving aspirin, beta-blockers, angiotensin converting enzyme inhibitors, angiotensin II receptor blockers, and other commonly used drugs in HD: Subcutaneous erythropoietin, iv iron, calcium salts, potassium chelators, folic acid, vitamins, iv L-carnitine, statins, proton-pump inhibitors and benzodiazepines.

Statistical analysis

The results are expressed as the median (range), unless explained otherwise. Fisher exact test or Student test were used to determine categoric variables; for continuous variables, Mann-Whitney test was employed; for intervariable correlations Spearman Rank and ρ coefficient were calculated. *P* values $\leqslant 0.05$ were accepted as statistically significant. To compare the different variables with respect to BMI, χ^2 coefficients were calculated and the Kruskal-Wallis test was used.

RESULTS

Groups were not different as to age, gender, time on HD, hematocrit, ultrafiltration rates (UF), inflammation status evaluated by CRP levels and MIS (Table 1). Median glomerular filtration rates (mL/min) were: GA: 10 (r: 7-15), GB: 9 mL/min (r: 8-17), GC: 10 (r: 8-14), mL/min. Although the nutritional status, assessed by MIS, was not different among groups, albumin levels were statistically different between subjects with low BMI compared to those with BMI > 30: GA *vs* GC, 3.70 g/dL (r 2.20-4.90) *vs* 3.85 g/dL (r 3.40-4.90), $P = 0.02$ (Table 1). Urinary outputs measured in ml/day were also different between both groups: GA 690 (r 0-1780) *vs* GC 840 (40-2840), $P = 0.01$ (Table 1). Leptin levels increased significantly from GA to GC and correlated significantly with insulin (Tables 2 and 3). Insulin levels increased positively and significantly with BMI determi-

Table 2 Blood measurements and ultrafiltration rates in all groups

Group	TropT (ng/mL)	UF rates (L)	Pro-BNP (pg/mL)	Insulin (μU/mL)	HOMA	Leptin (ng/mL)
GA	40	2	4970	7.00[a,b]	1.30[c,b]	3.81[b,d]
	(9-1081)	(0.8-4)	(216-234000)	(2-44)	(0.3-22.4)	(0.8-69.6)
GB	48	2.5	2180	11.50[a]	2.50[c]	18.60[d]
	(5-179)	(0.8-4)	(226-102000)	(4-38)	(1.10-9.30)	(4.7-47.40)
GC	41	3	2040	19.50[b]	3.75[b]	32.80[b]
	(4-186)	(0.5-4.0)	(139-166000)	(5.0-155.0)	(1.0-59.3)	(0.78-124.80)

[a]$P = 0.02$; [b]$P = 0.0001$; [c]$P = 0.03$; [d]$P = 0.01$. UF: Ultrafiltration; Pro-BNP: Pro-brain natriuretic peptide.

Table 3 Different correlations in Groups A and C

	GA BMI < 25	GC BMI > 30
VARIABLE	ρ; *P*	ρ; *P*
Insulin-ultrafiltration rate	0.21; 0.44	0.53; 0.03
TroponinT-diuresis	-0.46; 0.07	-0.48; < 0.05
Pro-BNP-diuresis	-0.43; 0.09	-0.39; < 0.01
TroponinT-proBNP	0.44; 0.09	0.77; < 0.0001
Albumin-TropT	-0.04; 0.87	-0.66; < 0.0001
Albumin-proBNP	-0.1; 0.72	-0.44; < 0.05
Leptin-insulin	0.34; 0.26	0.52; < 0.03

Pro-BNP: Pro-brain natriuretic peptide.

nations (μU/mL): GA *vs* GB, 7 (r 2-44) *vs* 11.50 (4-38), $P = 0.02$; GA *vs* GC, 7 (r 2-44) *vs* 19.5 (r 5-155), $P = 0.0001$ (Table 2). With respect to cardiac and hemodynamic biomarkers, TropT and Pro-BNP levels were not different amongst groups (Table 2). However, the following significant correlations were observed, all in high BMI patients: In GC, TropT-diuresis: $\rho = -0.48$, $P < 0.05$; Pro-BNP-diuresis: $\rho = -0.39$, $P < 0.01$; TropT-ProBNP: $\rho = 0.77$, $P < 0.0001$; insulin-UF rate: $\rho = 0.53$, $P = 0.03$; albumin-TropT: $\rho = -0.66$, $P < 0.0001$; albumin-ProBNP: $\rho = -0.44$, $P < 0.05$ (Table 3).

DISCUSSION

In the present study, we observed that subjects with high BMI displayed higher diuresis, albumin, leptin and insulin levels. In this group, higher urinary outputs correlated significantly with lower TropT and Pro-BNP levels. Albumin inversely and significantly correlated with TropT and Pro-BNP. As expected, insulin levels raised accordingly with BMI, but correlated significantly with UF rates only in individuals with high BMI. Besides, all groups were not different according to the time on HD, and initial urinary outputs were similar in the whole cohort (Table 1). Noteworthy, the rates of decline in diuresis were lower in patients with high BMI (6%) in comparison with those from either Groups A (13%) or B (17%).

In the literature, many manuscripts refer to obesity as a variable associated with a better survival in HD subjects[8-11,33]. Many causes have been attributed to explain this phenomenon. It is possible that one variable associated with good prognosis could be remnant dieresis. In the present work, this association occurred independently of the time on HD (Table 1). However, many studies have reported that obese subjects on HD present with low renal residual function[28,29]. In addition, in our study patients with BMI > 30 presented significantly higher albumin levels that correlated with better residual kidney function. This clinical picture of a better oncotic pressure coupled with a preserved diuretic function could lead to a lower vascular stress. Consequently, a smoother hemodynamic scenario would originate. According to our findings, higher albumin levels could not be ascribed to a better nutritional status, as MIS was not different among groups, or to a better inflammatory milieu, as CRP levels were similar in all subjects (Table 1). Noteworthy, residual renal function has been associated with higher albumin levels[25-27]. However, notwithstanding the cause, these higher albumin levels in GC could be exerting a more efficient intravascular oncotic pressure, removing more interstitial water. As a consequence, vessels could be better replenished, being more volume delivered to the kidneys. The result would be a higher urinary output. This hemodynamic situation is also illustrated by the fact that albumin is negatively correlated with TropT and Pro-BNP, two cardiovascular biomarkers that are increased in overfilling states and myocardial stretching[34-39] (Table 3). Although we reported significantly low ProBNP levels in high BMI individuals, we could not demonstrate this phenomenon in the present work[15] (Table 2). It is possible that this smoother hemodynamic setting could explain in part one of the causes of a higher survival rate in high BMI subjects. Finally, it could also contribute to the absence of hypertension in GC, which in the general population is associated with elevated BMI[40,41]. Interestingly, Trop T can increase not only due to vascular causes as myocardial infarction, vascular shear stress, endothelial damage, cardiomyocyte hypertrophy, but also due to muscular wasting in obese subjects on HD[35,36,42,43]. In this regard, TropT is a non-specific marker of cardiovascular origin. Taken together, we propose that in obese subjects on HD, the higher urinary output that patients with high BMI present is correlated with lower TropT and Pro-BNP levels. A better residual renal function could also contribute to lower TropT levels, as this molecule is cleared by the kidneys[35,36,42].

Moreover, an interesting additional factor that could play a role in preserved remnant diuresis in obesity is in-

sulin. As it occurred in one of our previous publications, in subjects with BMI > 30, insulin is again associated with higher UF rates in high BMI patients, albeit UF rates were not different amongst groups[30]. As expected, insulin increased in parallel with BMI, but did not correlate significantly with any other variable except fluid removal and only in high BMI individuals (Table 3). This phenomenon could be related to the ability of insulin to retain salt and water[44]. In turn, this fluid retention would be the trigger for a pressure-diuresis phenomenon and a maintained urinary output, probably potentiated by higher albumin levels. This increase in insulin could also reflect an insulin-resistant state in high BMI patients, which is inherent to obese individuals[44,45]. In addition, in our study high BMI was associated with lower Pro-BNP levels, which is in agreement with other publications that report the association between hyperinsulinemia and low Pro-BNP patients in obesity[15,45-47].

In our study, leptin is significantly high in GC, where its correlation with insulin is positive and significant. Recently, insulin has been reported to upregulate leptin gene expression. With respect to leptin sodium and water handling, the results are controversial. While some studies have shown leptin presents natriuretic effects, many others have reported its association with water and salt retention, sympathetic nervous system activation and hypertension, which could add to insulin hemodynamic effects[32,48]. With respect to the cardiovascular system, leptin (as insulin) is involved in the pathogenesis of myocyte hypertrophy[32,48].

Finally, the obesity paradox in hemodialysis has always been related to an elevated weight and assumed to be due to fat. However, BMI correlates with body fatness or density[49], but in these studies and in our present manuscript, the increase in body weight has not been discriminated in tissue compartments. An elevated BMI could be due to an increase in fat, water, bone density and/or muscle mass[50]. Therefore, increased body weight, particularly in end-stage kidney disease patients, is not a synonymous of obesity. Moreover, assuming overweight dialysis patients as obese, may be a misleading statement. We assume our GC subjects as obese due to high leptin and insulin levels and an elevated HOMA index, a characteristic profile encountered in obesity (Table 2).

Our manuscript contains several pitfalls. It is a cross-sectional study including a limited number of patients. Our findings must be interpreted with caution, as it joins previous studies with respect to the evaluation of body tissue and fluid composition and distribution. We call the attention of future authors to make the appropriate distinction when overweight patients are studied in the dialysis setting. Obesity is not a synonymous of high BMI in renal failure. Water retention and muscle wasting are to be addressed. Finally, these variables can operate simultaneously in these individuals. In this regard, bioimpedancy studies are mandatory. It is possible that whether this issue is taken into account, the obesity paradox in hemodialysis may not be such. In this regard, residual renal function would be more related to fluid overload and a pressure-diuresis forced situation.

In conclusion, our study shows that high BMI HD patients display higher diuresis rates, albumin and insulin levels. This higher urinary output dialysis individuals with BMI > 30 present, may reflect water retention, in part due to hyperinsulinemia, hyperleptinemia and secondary higher UF rates. The ability to excrete water correlates negatively and significantly with TropT and Pro-BNP levels, which would reflect a lower myocardial and vascular stress and a better hemodynamic status. Whether these events are associated with a better survival rate in HD should be appropriately assessed.

COMMENTS

Background

Cardiovascular disease is the most important cause of mortality in dialysis, while residual diuresis and increased body mass index (BMI) are associated with better survival. The authors studied residual diuresis and diverse variables according to BMI.

Research frontiers

To be able to discern between BMI and fluid retention in dialysis patients. Residual diuresis may be an important in outcome in these subjects, and high BMI subjects may display higher dieresis rates, lowering cardiovascular stress.

Innovations and breakthroughs

High BMI hemodialysis patients display higher diuresis rates, albumin and insulin levels. This higher urinary output dialysis individuals with BMI > 30 present, may reflect water retention, in part due to hyperinsulinemia, hyperleptinemia and secondary higher ultrafiltration rates rates. The ability to excrete water correlates negatively and significantly with TropT and Pro-BNP levels, which would reflect a lower myocardial and vascular stress and a better hemodynamic status.

Applications

In the every-day assessment of dialysis subjects, this paper suggests that obesity may not always be the reflection of a fat tissue, but the fluid overload must be taken into account. This water retention may explain the residual renal function this group may display, in relation with the pressure-diuresis phenomenon.

Terminology

The obesity paradox in dialysis states that this cohort of patients do better than other groups with lower BMIs. This is in contradiction with what occurs in the general population. The authors state that an elevated BMI may not always be a mere reflection of a higher fat tissue mass, but to an accumulation of water. The residual renal function displayed by these subjects may be due to a pressure-diuresis phenomenon.

Peer review

The present study aimed to investigate the associations between several cardiac and metabolic biomarkers as well as residual diuresis with BMI in chronic dialysis patients. The study is interesting and it could be published provided that the discussion should be re-written taking into account that some differences between groups.

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