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Of sight, and insight into melatonin's role in breast cancer?

José Maria Soares Junior[1], Tugrul Kesicioglu[2], Demet Sengul[3*], Ilker Sengul[2,4]

A *Deucalione*, melatonin, is synthesized by the pine cone-shaped gland of the cerebrum, named as the conarium or epiphysis cerebri. Since the 17th-century philosopher René Descartes hypothesized the brain's pineal gland in order to represent the location of the *homo sapiens* soul, paleontologists described it as an ancestral "third eye," and modern psychology declares perception beyond physical visual function, which remains poorly understood to date[1]. The pineal gland is not only a crucial organ for melatonin but also vital for many activities; for example, we postulated that pinealectomy leads to many morphological alterations, including interstitial cell morphology, of rat ovaries that are associated with functional changes in steroidogenesis and attenuation in progesterone receptor expression[2].

This hormone is involved in the regulation of sleep, circadian rhythms, breast milk, and gut-brain signaling. In addition to its role in sleep-wake cycles, melatonin possesses antioxidant, anti-inflammatory, and antitumor properties[3]. Breast cancer is the most common cancer among women worldwide, accounting for over 2 million new cases each year. The development of breast cancer is a complex process that involves multiple genetic and environmental factors[4,5]. Hormones, such as estrogen and progesterone, are known to play a crucial role in the development of this malignity[6]. Melatonin has been revealed to inhibit the growth of breast cancer cells both in vitro and in vivo. *A posteriori*, melatonin has been found to attenuate the proliferation of breast cancer cells and induce apoptosis, or programmed cell death, in cancer cells. Of note, this effect is thought to be due to melatonin's ability to reduce oxidative stress and inflammation, two factors that are known to contribute to the development of cancer. Melatonin, *per se*, is a hormone with different oncostatin actions, which are particularly effective in breast carcinoma[7,8]. It displays antioxidant properties via the scavenging of free radicals, protecting cells from carcinogen-mediated deoxyribonucleic acid modifications of oxidative damage, which leads to preventing the initiation of malignant transformation[9,10]. Of note, melatonin is also reported to exhibit antiproliferative effects on Michigan Cancer Foundation-7 (MCF-7) human breast cancer cells by inducing a delay in the cell cycle G1-S transition. As such, a subsequent accumulation of the cells in the G0/G1 phase[10] emerges, which gives rise to arresting the cell cycle in the G1 phase and attenuating the invasion and migration of breast cancer cells. Moreover, this hormone possesses oncostatic activity using antiangiogenic actions in breast cancer cells[10-13]. In addition, Veiga and colleagues[14] reported a systematic review and meta-analysis, including a sum of 570 articles, and 9 manuscripts in which the authors analyzed women with breast cancer and control cases, of which 10 and 90% were in the reproductive period and after menopause, respectively. They emphasized that the lowest level of melatonin had been found in approximately 55% of studies with breast cancer in post-menopause and postulated that low levels of melatonin might be a risk factor for this malignity.

Recently, melatonin's role in the tumor microenvironment has been notified. To this end, laying out the paracrine interactions between malignant epithelial and proximal endothelial cells via downregulation of vascular endothelial growth factor expression in human breast cancer cells, which leads to significant attenuation in angiogenesis, has been emphasized[15]. Besides its direct effects, melatonin modulates the activity of estrogen receptors, which play a key role in breast cancer development. In addition, melatonin attenuates the expression of estrogen receptors in breast cancer cells, which may reduce the availability of estrogen for these cells in order to utilize in promoting their growth. The antitumor properties of melatonin have also been observed in animal models of breast cancer, such as inhibiting the growth of breast tumors and reducing the incidence of mammo-malignity, which are due to melatonin's ability to enhance the immune system response to cancer cells[16]. Despite the promising results of these studies, the clinical implications of melatonin in the prevention and treatment

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of breast carcinoma are not yet clear. While melatonin is safe and well tolerated in humans, more research is required to determine the optimal dose and timing of melatonin supplementation for breast cancer prevention and treatment modalities. Principally, although diagnosis[17,18] remains crucial for malignant phenomena, the debate is still ongoing on diagnostic tools as well as therapeutic agents in order to pay dividends for providers. In conclusion, melatonin appears to play a significant role in the regulation of breast cancer growth. Its ability to inhibit the proliferation of breast cancer cells, modulate estrogen receptor activity, and enhance immune system function makes it a promising candidate for preventing and managing breast cancer. As a matter of fact, this issue merits further investigation.

AUTHORS' CONTRIBUTIONS

JMSJ: Conceptualization, Data curation, Methodology, Project administration, Validation, Visualization, Writing – review & editing. **TK:** Investigation, Project administration, Validation, Visualization, Writing – original draft. **DS:** Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Resources, Software, Validation, Visualization, Supervision, Writing – original draft, Writing – review & editing. **IS:** Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Resources, Software, Validation, Visualization, Writing – original draft, Writing – review & editing.

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Brazilian pulmonology guidelines on Delphi panel for post-coronavirus disease 2019

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The Guidelines Project, an initiative of the Brazilian Medical Association, aims to combine information from the medical field to standardize how to conduct, and to assist in the reasoning and decision-making of doctors. The information provided by this project must be critically evaluated by the physician responsible for the conduct that will be adopted, depending on the conditions and the clinical condition of each patient.
Guideline submission: April 2023
Guideline acceptance: May 2023.
Societies: Brazilian Medical Association and Brazilian Society of Pulmonology and Phthisiology.

BACKGROUND

The coronavirus disease 2019 (COVID-19) pandemic has had a direct impact on health care systems worldwide. By February 1, 2023, more than 753 million people had been infected with the virus, and more than 6.8 million deaths had occurred[1]. These death rates are related to the spread of the virus and are currently more common in places where vaccination rates are low. The worldwide incidence still fluctuates, with over 5 million cases per week. Failure to control viral transmission facilitates the occurrence of new mutations and immune escape, which may determine the persistence of the disease for a much longer period than expected[1].

An additional problem is the high prevalence of patients with persistent signs and symptoms after acute COVID-19 infection[2-4]. This condition involves several organs with different severities related to the pathophysiological mechanisms of viral infection[5,6]. Cellular penetration through linkage with angiotensin-converting enzyme (ACE)-2 receptors, which are present in different cell types, can cause damage and lead to the perpetuation of inflammatory processes[7,8]. Additionally, it has been hypothesized that an autoimmune process with an exaggerated innate immune response and activation and persistence of cytokine release may be involved in the pathophysiology of this long-term syndrome. The cross-reactivity of specific antibodies against SARS-CoV-2 with host proteins, resulting in autoimmunity, has been reported. In fact, patients with severe COVID-19 may present elevated serum levels of inflammatory markers, such as interleukins 1, 6, and 1-beta, granulocyte colony-stimulating factor, and alpha tumor necrosis factor. Thus, respiratory, cardiocirculatory, gastrointestinal, hepatic, renal, and other systems can be affected directly and perpetually, even when the acute infection is resolved[7-10].

Other theories have been proposed, which may explain the sequelae of organs during acute infections. Some findings show that patients with COVID-19 with persistent symptoms may stock the virus in various potential tissue reservoirs throughout the body, which may not be identified by nasopharyngeal swabs[11]. Another theory suggests that delayed viral clearance is secondary to immune exhaustion, which may lead to chronic inflammation and inadequate tissue repair. Mitochondrial dysfunction, impaired immunometabolism, and changes in the microbiome may also occur and may be involved in the persistence of symptoms[9].

In this context, the incomparable and long-term impact of signs and symptoms may lead to devastating repercussions, with a reduction in the quality of life, professional performance, and exercise capacity. Evidence from the literature still shows great difficulty and variability in defining this clinical condition, as it is not possible to clearly define whether the long-term manifestations are caused directly or indirectly by the

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virus. In addition, the pathophysiological mechanisms related to these conditions are not yet fully defined[12,13].

The time after acute infection and terms used to define these long-term conditions are also still variable. The National Institute for Health and Care Excellence (NICE) defines long-term COVID-19 as a symptom that continues or develops after COVID-19 infection, which cannot be explained by an alternative diagnosis[12]. This term includes ongoing symptomatic COVID-19 4–12 weeks post-infection and post-COVID-19 syndrome beyond 12 weeks post-infection. On the contrary, the National Institutes of Health (NIH) and the CDC define long COVID-19 as a condition of sequelae that extend beyond 4 weeks after the initial infection[13]. Thus, there is still a need for a more accurate and more standardized definition for all specialists to uniformly use in clinical practice and research. Therefore, we need to understand the best denomination for these conditions to be used, i.e., post-COVID-19, long COVID-19, post-COVID-19 syndrome, or chronic COVID-19[14].

The present study aimed to identify, through the opinion and knowledge of national pulmonologist specialists, the most commonly used nomenclature, symptomatic manifestations, and prevalence of the main symptoms in patients with post-COVID-19 involvement. In this way, national educational actions can be programmed so that health professionals and systems can receive information that can be applied to each local reality.

METHODS

This study was a Delphi consensus-seeking interactive survey that evaluated the impressions of Brazilian pulmonologist experts. The Delphi method is a structured communication technique that was originally developed as a systematic, interactive, and forecasting method that relies on a panel of experts[15]. The participants of this survey on post-COVID-19 conditions included only active members of the Brazilian Thoracic Society. We aimed to represent clinicians with expertise in evaluating patients with post-COVID-19 conditions and their prevalence. There were no specific exclusion criteria for participants. The survey began with a statement explaining the objective of the project, and the answers to the survey implied consent to participate. The participants were allowed to withdraw at any time.

The participants were obtained from the internal database of the Brazilian Thoracic Society after the internal approval of the project. They were invited via an online recruitment letter requiring participation and engagement, along with an explanation of the study objectives, instructions, and outputs. This invitation was extended two times over an interval of 2 months.

The survey was performed using RedCap and contained listed options regarding terms (long COVID-19 and chronic COVID-19) and time (3 or 10 weeks) to consider in the definition, which were kept as broad and comprehensive as possible (Supplementary File). We previously used a list of terms identified in the literature to obtain information and develop the survey (search strategy: long-COVID OR long-haul COVID OR post-acute COVID syndrome OR persistent COVID-19 OR post-acute COVID-19 syndrome OR long-hauler COVID OR long COVID OR post-acute sequelae of SARS-CoV-2 infection OR long-haul COVID OR chronic COVID syndrome).

The domains contained information on the time of experience related to pulmonology assistance, acute COVID-19 and post-COVID-19 management experience, recognition of signs and symptoms related to post-COVID-19, and their prevalence. The survey responses were anonymous and tabulated according to their frequency.

All of the questions were evaluated on a nine-point Likert scale, from 1 (least important/non-agreement) to 9 (most important/high agreement), and the participants were asked to choose the level of importance for each variable in the definition. We considered a Likert scale score of ≥7 or ≤3 as a significant concordant consensus. The participants had the opportunity to add comments to an open question that evaluated their best interpretation of post-COVID-19 infection.

The sample size estimation considered the responses of at least 30 participants from the same panel[15]. Statistical analysis provided a summary of the group's view on each item, with calculated median, mean, and percentage scores for each statement to provide an indication of the level of agreement among the respondents. The description and frequency of qualitative answers were used to demonstrate the experts' best definitions of post-COVID-19 infection terms.

RESULTS

On December 15, 2022, we closed the survey capture, which was initially evaluated by 90 participants. Six participants declined participation, 15 gave consent without answering, and 69 responded to the survey. Among 69 respondents, 47.7% were females, the mean age was 46.5±11.9 years, only 1 participant affirmed being an infectious disease specialist, and 54.5% had between 6 and 29 years of pulmonology practice experience while 25.8% had <6 years of experience. Only 13.0% of the participants did not assist patients with acute COVID-19 in the last 6 months, and only three (4.3%) did not assist patients with post-COVID-19 infection. In total, 58 (84%) participants agreed with the recognition of a pathological condition of post-COVID-19 infection.

Additionally, 24 (41%) participants gave scores ≥7 on whether they agreed they were uncomfortable in treating patients with post-COVID-19, and 20 (34%) participants (Likert ≤3) were comfortable attending to such patients.

The agreement scores for the time to define long COVID-19 were higher with 10 weeks at 7.0 (1.8–9.0) compared to 3 weeks at 6.0 (1.0–9.0). The agreement score for chronic COVID-19 time definitions was moderately low in both times used, with 3 and 10 weeks at 4.0 (1.0–9.0) and 6.5 (1.0–9.0), respectively. We observed that an equal number of participants agreed or did not agree regarding the difference between the definition of long COVID-19 (29 participants) and chronic COVID-19 (29 participants).

Regarding the qualitative definition for post-COVID-19 conditions, 34 (49%) participants suggested the term "long COVID-19," followed by 18 (26%) with "post-COVID-19 syndrome," 3 (4%) with "chronic COVID-19," and 2 (2%) with "sequelae post-COVID-19." According to the duration to define post-COVID-19 conditions, 24 (34%) participants considered after 3 months and 20 (28%) considered after 4 weeks. One participant (1%) considered 15 days; four (5%) considered 3 weeks; two (2%) considered 2 months; two (2%) considered 6 months; and one (1%) considered several months.

The agreement of possible risk factors for the post-COVID-19 condition is presented in Table 1. We observed that smoking, age >60 years, obesity, previous diagnosis of lung disease, cardiovascular disease, kidney disease, diabetes mellitus, use of mechanical ventilation, and high-flow oxygen presented an agreement with a median Likert score of ≥7 points.

The agreement on the recognition of each sign or symptom related to the post-COVID-19 condition is shown in Table 2. The agreement with a median Likert score of ≥7 points was fatigue, cough, anosmia, anxiety/depression, loss of concentration, dysgeusia, headache, loss of memory, loss of peripheral muscle function, loss of strength, myalgia, autonomic dysfunction, and telogen effluvium. The concordant non-signs

Table 1. Agreement of possible risk factors of the post-coronavirus disease 2019 condition according to Likert score (concordant agreement considered median of ≥7 or ≤3).

	Median (interquartile interval) n=53
Allergy	4 (1.5–8)
Age >60 years	7 (6–9)
Cardiovascular disease	7 (5–8)
Diabetes mellitus	7(5–8)
High flow oxygen	8 (6–9)
Hypertension	6 (4–8)
Inflammatory bowel disease	5 (2–7)
Kidney disease	7 (5–9)
Liver disease	6 (4–7)
Mechanical ventilation	8 (7–9)
Obesity	8 (6–9)
Peripheric vascular disease	5 (3–7)
Respiratory disease	7 (6–9)
Rheumatologic disease	6 (4–8)
Smoking	7 (5–8)
Unvaccinated/incomplete vaccine – COVID-19	8 (7–9)

Table 2. Agreement of signs and symptoms related to the post-coronavirus disease 2019 condition according to Likert score (concordant agreement considered median of ≥7 or ≤3).

	Median (interquartile interval) n=57
Ageusia/Dysgeusia	7 (5–9)
Anorexia	4 (2–7)
Anosmia	8 (6–9)
Anxiety/Depression	8 (7–9)
Arthralgia	6 (4–7)
Auditive loss	4 (2–5)
Autonomic dysfunction	7 (5–7)
Chills	2 (1–5)
Communication abnormality	6 (4–8)
Cough	8 (7–9)
Diarrhea	3 (1.5–5)
Dizziness	4 (2–6.8)
Fatigue	9 (8–9)
Fever	2 (1–4)
Functional loss	7 (5–8.5)
Headache	7 (5–8.5)
Memory loss	8 (6–9)
Mental concentration abnormality	8 (7–9)
Myalgia	7 (5–8)
Rhinitis	3 (5.5–7.8)
Sicca syndrome	3.5 (1–5)
Sinusitis	5 (2–7)
Strength loss	7 (5–9)
Skin lesions	4 (2–5)
Telogen effluvium	7 (3–9)
Tremor	4 (2–6)
Thoracic pain	5 (3.5–7)
Visual abnormality	4 (2–6)
Voice abnormality	5 (3–7)

or non-symptoms presented in the post-COVID-19 condition with a median Likert score of ≤3 points were chills, fever, and diarrhea.

The agreement on the prevalence of each sign or symptom is shown in Table 3. The agreement with a high concordant Likert score of ≥7 points was fatigue, cough, dysgeusia, anxiety/depression, loss of concentration, and loss of memory. The agreement with a high concordance of infrequent signs or symptoms (Likert score of ≤3 points) was chills, fever, skin lesions, tremors, visual loss, auditive loss, dizziness, Sicca syndrome, and diarrhea.

Table 3. Agreement of sign and symptom prevalence related to the post-coronavirus disease 2019 condition according to Likert score (concordant agreement considered median of ≥7 or ≤3).

	Median (interquartile interval) n=53
Ageusia/Dysgeusia	7 (5–8)
Anorexia	4 (1.5–5.5)
Anosmia	6 (5–8)
Anxiety/Depression	8 (7–9)
Arthralgia	5 (3–6.5)
Auditive loss	2 (1–4)
Autonomic dysfunction	6 (5–8)
Chills	2 (1–4)
Communication abnormality	4 (2–6.5)
Cough	8 (6–9)
Diarrhea	2(1–4)
Dizziness	3 (2–5)
Fatigue	9 (8–9)
Fever	2 (1–3)
Functional loss	6 (4–8)
Headache	6 (5–7)
Memory loss	8 (6–8)
Mental concentration abnormality	8 (6–8.5)
Myalgia	6 (4–8)
Rhinitis	4 (2–7)
Sicca syndrome	2 (1–4)
Sinusitis	4 (2–6.5)
Strength loss	6 (4–8)
Skin lesions	2 (1.5–5)
Telogen effluvium	6 (3–8.5)
Tremor	3 (1–5)
Thoracic pain	4 (2–6)
Visual abnormality	3 (1–4.5)
Voice abnormality	4 (1–5)

DISCUSSION

A large proportion of patients continue to experience health-related consequences after acute COVID-19 infection, regardless of disease severity, and this number continues to increase, with a heavy impact on health care systems and work and educational activities[12,16]. Even non-hospitalized patients with mild COVID-19 during the acute phase may develop post-COVID-19 syndrome[13,16]. Available studies on the long-term outcomes of COVID-19 infections are heterogeneous. Different names have been used to define the occurrence of long-term symptoms secondary to COVID-19, such as long COVID-19, post-acute COVID-19 syndrome, chronic COVID-19, long haul COVID-19, and post-COVID-19 condition, which reinforces the lack of a unified definition[3,13].

The main findings of this Delphi study about the post-COVID-19 condition, which were based on the impression and knowledge of Brazilian pulmonologists, were as follows: (1) the vast majority of the participants attended to patients with acute and post-COVID-19 infection; (2) the majority recognized the existence of the post-COVID-19 condition; (3) several respondents were uncomfortable attending to patients with post-COVID-19 condition; (4) there was no profuse agreement regarding the time point in the follow-up that defines chronic and long COVID-19; (5) there was no consensus in the terms used to define the occurrence of long-term symptoms secondary to COVID-19; (6) risk factors for the post-COVID-19 condition were smoking, age >60 years, obesity, previous diagnosis of lung disease, cardiovascular disease, kidney disease, diabetes mellitus, the use of mechanical ventilation, and high-flow oxygen; and (7) clinical manifestations of the post-COVID-19 condition were fatigue, cough, anosmia, anxiety/depression, loss of concentration, dysgeusia, headache, loss of memory, loss of function, loss of strength, myalgia, autonomic dysfunction, and telogen effluvium.

There is still no consensus on the definition and time points of post-COVID-19 conditions[12]. The CDC defined post-COVID-19 conditions as a wide range of new, returning, or ongoing health problems experienced by patients with COVID-19 4 weeks after infection[13]. The NICE guidelines and an international Delphi consensus panel defined post-COVID-19 condition for adults as the presence of symptoms for at least 3 months from the onset of probable or confirmed SARS-CoV-2 infection that cannot be explained by an alternative diagnosis[12,14]. Onset of new symptoms may be identified

after initial recovery from an acute COVID-19 episode or persist from the initial illness and may fluctuate or relapse over time[12,14]. The heterogeneous definitions and time points in the literature regarding post-COVID-19 conditions are similar to the results of our Brazilian Delphi study, since there was no consensus among our participants about a standardized term and time points to define this situation.

In this post-COVID-19 scenario, patients may present with several symptoms involving multiple organs or even autoimmune conditions, with various durations[8,13,17]. In a study from Germany, the main manifestations of post-COVID-19 syndrome 4 and 7 months after the acute infection were anosmia (12%), ageusia (11%), fatigue (10%), and dyspnea (9%), and anosmia (15%), fatigue (15%), and dyspnea (14%), respectively[16]. In a systematic review and meta-analysis, Alkodaymi et al. demonstrated that the most common symptoms after acute respiratory syndrome due to COVID-19 were fatigue (32%), dyspnea (25%), and sleep disorder (24%) from 3 to <6 months; effort intolerance (45%), fatigue (36%), and sleep disorders from 6 to <9 months; fatigue (37%) and dyspnea (21%) from 9 to <12 months; and fatigue (45%) ≥12 months[4]. Additionally, according to the NICE guidelines, fatigue (47%), sleep disturbances (36%), and anxiety or depression (23%) were the most common symptoms ≥12 weeks after COVID-19 diagnosis[12]. Our study demonstrated some similarities between the clinical manifestations of post-COVID-19 condition in previous studies, since our participants reported that fatigue, anosmia, anxiety/depression, and dysgeusia were common in this context.

The estimates of the prevalence of post-COVID-19 conditions vary widely among studies[17,18]. In a systematic review and meta-analysis by Chen et al., the prevalence of the post-COVID-19 condition at least 28 days after acute infection was 43% (54% in hospitalized and 34% in non-hospitalized patients)[3]. This condition may develop in children and adolescents of different ages and in patients with different severities during the acute phase[3,13,16,17]. Risk factors for long COVID-19 include female sex; the presence of comorbidities such as diabetes, asthma, connective tissue disorders; obesity; smoking; lower income; more symptoms during the acute phase; higher severity during the acute infection, especially those who were hospitalized or needed ICU care; and people who did not get a COVID-19 vaccine[3,12,16,17]. Several risk factors for the post-COVID-19 condition demonstrated in previous studies, such as smoking, obesity, the presence of comorbidities, and higher severity during acute infection, were also reported by the participants of our study.

Additionally, the mechanism of persistent COVID-19 infection, especially in immunosuppressed individuals is still unknown. Persistent infection can lead to the development of a range of disease severities with persistent symptoms for a long time, and this condition may be more relevant in defining the chronic COVID-19 condition[11]. However, it is a challenge to propose continuous virus identification and quantification in these scenarios to determine the relationship between persistent symptoms and viral infections, and we may still confuse different post-COVID-19 infections in the same way.

This Delphi study had some limitations that need to be addressed. First, the study population was restricted to pulmonologists from Brazil. However, they are representative of the entire country, as they are registered with the Brazilian Thoracic Society. Although the number of responses reached was sufficient for the consistency of the results, the response rate was low despite the questionnaire being sent to all the members of the society. Moreover, the questionnaire was developed only in Portuguese, which limited its applicability to other languages.

RECOMMENDATIONS

- Long COVID-19 is the term to be used for post-COVID-19 condition after 10 weeks.
- Chronic COVID-19 is the term to be used for persistent COVID-19 infection after 10 weeks.
- Smoking, age >60 years, obesity, previous diagnosis of lung disease, cardiovascular disease, kidney disease, diabetes mellitus, the use of mechanical ventilation, and high flow oxygen are considered risk factors to develop long COVID-19.
- Fatigue, cough, anosmia, anxiety/depression, loss of concentration, dysgeusia, headache, loss of memory, function, and strength, myalgia, autonomic dysfunction, and telogen effluvium are considered the main long-COVID-19 symptoms.

CONCLUSIONS

This Delphi study demonstrated the need of continuous medical educational program offered by different institutions to amplify the knowledge of long COVID-19, including clinical manifestations and risk factors. It is urgent to standardize the terms and time points in the follow-up that define post-COVID-19 conditions in order to properly determine its prevalence, best method of prevention and management, and impact on the health care systems. This concept needs to be continuously reviewed and updated based on the availability of more information.

AUTHORS' CONTRIBUTIONS

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Radiofrequency us and overdiagnosis of atherosclerosis in individuals with psoriatric arthritis

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Dear Editor,

Atherosclerosis is an inflammatory vascular disease, caused by the deposition of cells, lipids, and tissue debris in the vascular intimal layer. Among the risk factors for this disease are sedentary lifestyle, poor diet, systemic arterial hypertension, and smoking[1].

Psoriasis is a common chronic inflammatory disease of the skin, with characteristic features of erythematous plaques demarcated by whitish scales. It is not only restricted to skin involvement but can also affect joints and other organs. Thus, psoriasis is not a specifically dermatological pathology but a systemic one, as demonstrated in studies that indicate a higher prevalence of diabetes and cardiovascular disease correlated with the severity of psoriasis[2].

Thus, when reading the article by Ozisler C, Kaplanoglu H, Sandikci SC, Ozisler Z, entitled "Evaluation of subclinical atherosclerosis by ultrasound radiofrequency data technology in patients with psoriatic arthritis,"[3] one can see the importance of highlighting elements relevant to the management of psoriatic arthritis, atherosclerosis. Thus, the magnitude of the structural differences in the arteries between the group with psoriasis and the control is notorious. Hence, when comparing the variables in Table 2 of the aforementioned article, through the calculation of Cohen's d, it is possible to show that in all of them there are effects considered to have a moderate to very large real impact.

In view of this, it is possible to conclude that these probabilistic and structural measurement differences may have diagnostic or prognostic utility. However, they are still just differences. Furthermore, the identification of structural alterations still in the preclinical phase can reduce the incidence of cases with clinical manifestations and, therefore, false positives and overdiagnoses[4]. Therefore, the finding of greater thickening and stiffness in the structure of the carotid artery in individuals with psoriasis, as well as this identified subclinical state, need to be clarified as really pathological in view of their possible impacts on the outcomes of clinical manifestation and death.

Thus, it is worth mentioning that the indiscriminate use of diagnostic/prognostic tests without estimated effectiveness on the prescription and therapeutic results causes psychological, financial, and physical loss to individuals undergoing unnecessary treatments, in addition to excessive costs to the health system. We therefore recommend that future studies should focus on the follow-up of individuals with psoriasis, assessing the difference in outcomes under and without treatment, and in the population, in which atherosclerosis and psoriasis do not coexist.

AUTHORS' CONTRIBUTIONS

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Comments on "Assessment of pain and quality of life in patients undergoing cardiac surgery: a cohort study"

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First, Viana et al.[1] evaluated postoperative pain and quality of life in patients undergoing median sternotomy (via comparisons in a cohort study). However, while comparing outcomes, it is important to present the clinical relevance of the differences found because the p-value shows only a statistical observation related to an alpha error probability[2,3]. Classical statistical significance is still the predominant way to analyze cohort studies, but clinical significance analysis has been slowly incorporated into the analysis of health-related studies. Statistical significance does not assure that the results are clinically relevant. The dichotomy that emerged from hypothesis testing[4], namely, the decision to accept or reject the null hypothesis based on the predetermined levels of probability[5] does not provide any insights into whether the results of the study are important for patients, clinicians, or decision-makers, limiting the value of the tests in the world of evidence-based practice[4,6,7]. It can be solved by adding the effect size to the significant values ($p \leq 0.05$)[8] or the minimal clinically important difference[9] of the instruments: Visual Analog Scale (VAS)[10], Brief Pain Inventory (BPI)[11], and World Health Organization Quality of Life Questionnaire (WHOQOL)[12]. These adjustments facilitate probabilistic reasoning in the clinical applicability of scientific evidence.

Second, the authors used convenience sampling and suggested further studies with larger samples. A convenience sample is one that is drawn from a source that is easily accessible to study. This sample, nonetheless, may not be representative of the population at large; e.g., a convenience sample of students can be drawn from a nearby medical college, but these students may not be representative of all students, such as students in other professional and nonprofessional colleges[13]. According to Andrade[14], the sample size for a study needs to be estimated at the time the study is proposed; too large a sample is unnecessary and unethical, and too small a sample is unscientific and also unethical. The necessary sample size can be calculated using software, based on certain assumptions[15-17]. As such, contributing to the authors and helping later studies with sampling, we designed a sample size a priori using G*Power 3.1.9.7.[18]. Regarding the difference between two dependent means (matched pairs), we used the following parameters: effect size=0.5, α=0.05, β=0.90, non-centrality parameter δ=3.3166248, critical t=2.0166922, and df=43 (n=44). Regarding the difference between two independent means (two groups), we used the following parameters for prior sample calculations: effect size=0.5, α=0.05, β=0.90, allocation ratio N2/N1=1, non-centrality parameter δ=2.9580399, critical t=1.6559704, and df=138 (n=140), with 70 patients per group.

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Reply to the letter: Comment on "Relationship between the number of comorbidities, quality of life, and cardiac autonomic modulation in patients with coronary disease: a cross-sectional study"

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Dear Editor,

We read with great appreciation the comment by Wang et al.[1] for our original article entitled "Relationship between the number of comorbidities, quality of life, and cardiac autonomic modulation in patients with coronary disease: a cross-sectional study[2]." We would like to thank the authors for their interest in our article and their time to express their concerns.

Indeed, as very well pointed out by the authors, heart rate is affected by many factors, and, consequently, heart rate variability (HRV)[3] that was used in the study to evaluate cardiac autonomic modulation[4]. However, HRV is a widely used instrument, and when it follows patterns of the capture of RR intervals, analyses, and interpretations[4], it provides important information regarding cardiac autonomic modulation.

Wang et al.[1] noted that heart rate is affected by thyroid, pituitary, kidney, and adrenal diseases[3] and that these factors should be considered when using HRV. We agree with this statement, so we had concerned to assess the presence of these comorbidities in our sample using the "Self-Administered Comorbidity Questionnaire[5]." We would like to highlight that only one participant had kidney disease, as shown in Table 2. Furthermore, among those who reported other comorbidities (n=5), none had any of the aforementioned conditions. Therefore, we can state that these diseases probably did not significantly impact our results.

Regarding the baseline data of the sample, Wang et al.[1] suggested the presentation of factors such as occupation, culture, income, and permanent residence, as they could impact the quality of life levels. We understand the importance of this information, and we have collected data about the current occupation and permanent residence of the participants. Of the participants included in the study, 85% were retired, and only one of them had permanent residence in another city. As the sample seems relatively homogeneous and we judged that it would not probably affect the interpretation of our findings, we have not included these data in the article; however, we have appreciated the opportunity to share this information here. We also took the opportunity to perform correlation analyses controlling for occupation and permanent residence. In this new analysis, a significant negative correlation between the number of comorbidities and the pain domain was also found (r=-0.444; p=0.03). Therefore, we conclude that these factors did not alter our main finding that the number of comorbidities is inversely related to the pain domain of the Medical Outcome Study 36-Item Short Form Health Survey (SF-36)[6].

Finally, Wang et al.[1] suggested that we should improve the baseline data and conduct random grouping so that the observation group (the experimental group) and the control group were balanced. However, we would like to emphasize that in our study only one group composed of individuals diagnosed with coronary artery disease, patients from a cardiac rehabilitation program, was included, and we performed correlation analyses between the number of comorbidities, HRV indexes, and quality of life, as assessed by the SF-36[6].

We are thankful for the letter. This discussion extends perspectives for future investigations.

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Lung and physical function in post COVID-19

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Dear Editor,

We would like to share our ideas on the article entitled "Lung and physical function in post COVID-19 and clinical and functional associations: a cross-sectional study in Brazil[1]". Nascimento et al.[1] investigated the relationship between lesion-level characteristics assessed by chest computed tomography, probable sarcopenia, and the percentage of diffusing capacity of the lung for carbon monoxide with clinical and functional variables in COVID-19 survivors[1]. Muscle impairment and lung dysfunction are widespread in COVID-19 survivors, according to Nascimento et al.[1] and hospitalization was related to the worst muscle force and carbon monoxide diffusing capacity of the lung[1]. According to Nascimento et al.[1] the findings underscore the need for long-term follow-up of such patients as well as rehabilitation programs[1].

The long-term consequences of COVID-19 have a concern that must be addressed immediately. There are significant issues that need to be resolved. Despite the likelihood that the patient had undiscovered co-morbid conditions, COVID-19 supported the patient's first apparent clinical diagnosis. The patient might also get a second COVID-19[2]. It is important to discuss about prior vaccines as well. To draw a conclusion on how the disease influences health issues, there must be enough data and adequate knowledge. In many instances, the patient's symptoms are most likely not the primary cause of extended COVID-19, also known as prolonged COVID.

AUTHORS' CONTRIBUTIONS

RM: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **VW:** Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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ORIGINAL ARTICLE

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Cardiac anomalies in pediatric patients with pectus excavatum

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SUMMARY

OBJECTIVE: Pectus excavatum is the most prevalently encountered deformity of the thoracic wall. It can be accompanied by congenital anomalies.

METHODS: The cardiac findings of 36 children who were diagnosed at the Thoracic surgery outpatient clinic of our university between 10 February 2021 and 1 October 2021 and 57 healthy children in a similar age group were analyzed.

RESULTS: We determined that the pectus excavatum patients in our study had a higher risk of having mitral insufficiency, mitral valve prolapse, tricuspid valve prolapse, cardiac malposition, and congenital heart disease.

CONCLUSION: Our study showed that the prevalence of cardiac pathologies was higher in pediatric pectus excavatum patients than in healthy children in the control group. Thus, we recommend clinicians to refer pediatric pectus excavatum patients to pediatric cardiology outpatient clinics for the early diagnosis of potential cardiac pathologies.

KEYWORDS: Pectus excavatum. Mitral valve prolapse. Tricuspid valve prolapse.

INTRODUCTION

Pectus excavatum (PE) is the most prevalently encountered deformity of the sternum and constitutes 90% of all congenital thoracic wall deformities[1]. In this deformity that is seen in one in every 300–400 live births, the female-male ratio is 1:4, where the prevalence of the condition is higher in males[2]. As the person ages, especially during the rapid development in adolescence, the deformity becomes more pronounced.

PE starts in childhood, and the deformity increases progressively. These patients, who have exercise intolerance at the beginning, start to have reduced cardiopulmonary function levels as the deformity increases[3]. The complaints of patients include fatigue, malaise, lower exercise capacity, palpitations, and chest pain. Through aging, these complaints increase.

Congenital cardiac diseases are observed in 2% of pediatric PE patients[4]. Patients with Marfan, prune belly, and Turner syndromes have an increased probability of having PE[5,6].

Varying degrees of systolic ejection murmurs can be heard in the physical examinations of individuals with PE deformity. This situation is more noticeable, especially following exercise. The closure of the distance between the pulmonary artery and the posterior sternal cortex or contact between these structures is shown as the cause of these murmurs.

In this study, we aimed to examine the electrocardiography (ECG) and echocardiography (ECHO) findings of pediatric patients with PE deformity comparatively with a control group without a thoracic deformity and discuss the results with the literature.

METHODS

This study was carried out in compliance with the Declaration of Helsinki. Before the inclusion of the patients, ethical approval was obtained from the Non-Interventional Clinical Studies Ethics Committee of our university on February 8, 2021 (meeting no: 2021/5 and decision no: 2). Additionally, the families of the patients provided informed consent.

Study design

The cardiac findings of 36 children diagnosed with PE at the Thoracic Surgery outpatient clinic of our university between February 10, 2021 and October 1, 2021 and 57 healthy control group patients in the same age group were analyzed. Routine posteroanterior and lateral chest radiographs of all patients were taken. Each patient was screened for cardiac pathologies by a pediatric cardiology specialist with ECG and ECHO.

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The demographic characteristics, complaints at the time of presentation, and ECG and ECHO results of all patients were recorded.

ECG: The patients were examined by 12-lead ECG. ECG examinations were made using a Gehealthcare Mac 2000 device. The electrocardiographs were examined in detail in terms of rhythm, heart rate, P-wave, ST-segment, QRS interval and amplitude, QTc interval, and T-wave values.

ECHO: All patients were examined by a pediatric cardiology specialist. All examinations were made with a Vivid 7 Pro ECHO device (GE Healthcare Vingmed Ultrasound AS) using the appropriate cardiac sector probe based on the patient's age and weight and in the left lateral decubitus position. The patients were examined with conventional ECHO methods based on the pediatric ECHO guidelines of the American Society of Echocardiography[7]. Patients who were followed up for congenital heart diseases (CHDs) and were diagnosed before the start of the study were excluded.

Statistical analyses

The statistical package for the Social Sciences for Windows 22 software was used for the statistical analyses. Descriptive variables are presented as frequency (n)–percentage (%) and mean±standard deviation values. The normal distribution of the variables was tested using the Kolmogorov-Smirnov test. The normally distributed parameters were analyzed by one-way analysis of variance (ANOVA) or Student's t-test. Kruskal-Wallis test or Mann-Whitney U test was used for the numerical variables that did not show normal distribution. Student's t-test, Mann-Whitney U test, and χ^2 test were used to find statistical significance. The risk factors were evaluated with univariate and multivariate logistic regression models. The variables found to be significant in the univariate analyses were included in the logistic regression analysis. $p<0.05$ was considered statistically significant.

RESULTS

Among the children who were included in the study, 24 were females, and 69 were males. The mean age of the patients was 11.10±4.04 (4–17.9) years. There was no statistically significant difference between the PE patients (PE group) and the healthy group (control group) in terms of sex or age (p=0.265 and p=0.506, respectively) (Tables 1 and 2).

In the comparison of the cardiological findings of the PE and control groups, no significant difference was found between the aortic valve pathology rates of the groups (p=0.740). The cardiac pathology, cardiac malposition, mitral valve prolapse (MVP), mitral insufficiency (MI), tricuspid valve prolapse (TVP), and CHD rates of the PE group were significantly higher than those of the control group (p<0.001, p<0.001, p<0.001, p<0.001, p<0.001, and p=0.027, respectively) (Table 1).

In the comparison of the ECHO findings, no significant difference was found between the PE and control groups in terms of their age, right ventricle end-diastole Z-score (RVED ZS), left ventricle end-diastole internal diameter Z-score (LVIDd ZS), end-diastolic left ventricle posterior wall thickness Z-score (LVPWd ZS), left ventricle end-systole internal diameter Z-score (LVIDs ZS), ejection fraction (EF), shortening fraction (SF), aortic sinus Z-score (Aort ZS), main pulmonary artery diameter Z-score (MPA ZS), Mitral annulus Z-score (Mannulus ZS), or pulmonary circulation values (p=0.506, p=0.093, p=0.146, p=0.195, p=0.238, p=0.656, p=0.900, p=0.736, p=0.921,

Table 1. Echocardiographic findings in patients with pectus excavatum and healthy control.

	Healthy control (57) n (%)	Patients with pectus excavatum (36) n (%)	p-value
Sex			
Female	17 (29.8)	7 (19.4)	0.265
Male	40 (70.2)	29 (80.6)	
Cardiac pathology	3 (5.3)	23 (63.9)	**<0.001**
Cardiac malposition	0 (0)	8 (22.2)	**<0.001**
MVP	4 (7)	20 (55.6)	**<0.001**
Mitral insufficiency	0 (0)	9 (25)	**<0.001**
TVP	1 (1.7)	9 (25)	**<0.001**
CHD	0 (0)	3 (3.2)	0.027
Aortic valve pathology	1 (1.8)	1 (2.8)	0.740

Statistics: Crosstabs, chi-squared tests. MVP: mitral valve prolapse; TVP: tricuspid valve prolapse; CHD: congenital heart disease. Statistically significant values are indicated in bold.

Table 2. Echocardiographic morphometric measurements in patients with pectus excavatum and healthy control.

	Healthy control (57) x±SD	Patients with pectus excavatum (36) x±SD	p-value
Age (years)	10.87±3.89	11.46±4.31	0.506
RVDD ZS	0.953±0.395	0.811±0.376	0.093
IVSd ZS	0.423±0.492	0.190±0.586	**0.042**
LVIDd ZS	-0.391±0.683	-0.622±0.823	0.146
LVPWd ZS	0.349±0.471	0.180±0.675	0.195
LVIDS ZS	-0.744±0.704	-0.912±0.602	0.238
EF	72.378±3.701	72.722±3.746	0.656
SF	41.386±3.211	41.472±3.193	0.900
Aort ZS	-0.555±0.785	-0.613±0.794	0.736
MPA ZS	-0.226±0.710	-0.242±0.807	0.921
Mannulus ZS	-0.447±0.461	-0.637±0.581	0.089
Tannulus ZS	-0.458±0.511	-0.722±0.518	**0.020**
LA ZS	0.700±0.585	0.109±0.833	**<0.001**
TAPSE ZS	-0.322±0.829	-0.809±0.784	**0.007**
Ascending Aorta ZS	-0.371±0.768	0.140±0.796	**0.003**
Pulmonary flow	1.045±0.059	1.044±0.090	0.941

Statistics: Student's t-test. RVDD ZS: end-diastole right ventricle diameter Z-score; IVSd ZS: diastolic interventricular septum diameter Z-score; LVIDd ZS: end-diastole left ventricle diameter Z-score; LVIDs ZS: end-systole left ventricle diameter Z-score; EF: ejection fraction; SF: shortening fraction; MPA ZS: main pulmonary artery diameter Z-score; LA ZS: left atrium diameter Z-score; TAPSE ZS: tricuspid annular plane systolic excursion Z-score. Statistically significant values are indicated in bold.

p=0.089, and p=0.941, respectively). The diastolic interventricular septum thickness Z-score (IVSd ZS), tricuspid annulus Z-score (Tannulus ZS), left atrium diameter Z-score (LAD ZS), and tricuspid annular plane systolic excursion Z-score (TAPSE ZS) values of the PE group were significantly lower than those of the control group (p=0.042, p=0.020, p<0.001, and p=0.007, respectively). The ascending aortic sinus Z-score (aorta ZS) of the PE group was significantly higher than that of the control group (p=0.003) (Table 2).

According to the results of the risk analysis that we carried out with the logistic regression analysis method for MVP and TVP development in the PE patients, MVP development increased 16.56-fold, and TVP development increased 18.66-fold in the PE patients (p<0.001 and p<0.001, respectively) (Table 3).

DISCUSSION

PE constitutes 90% of congenital thoracic cage deformities, and it is seen frequently in men[8]. In our study, similar to the literature, 80% of the PE deformity cases consisted of male children. In this deformity, while the first and second costal cartilages and the manubrium are usually in their normal positions, the lower costal cartilages adhering to the sternum and the body of the sternum are concave. Although the affected cartilages are curbed inward, the costae at the lateral of the costochondral junction remain unaffected. In approximately half of all cases, the sternum forms a curvature, mostly to the right on the frontal[9].

It can lead to reduced cardiopulmonary functions and physical capacity by causing a lower thoracic volume and compression in the heart. While its symptoms are rarely seen in early childhood, an increase can be observed in symptoms as the person gets older[9].

As the thoracic wall is flexible in young patients, the heart moves to the left, and this situation allows the compression on the heart to decrease to some extent. However, throughout the aging process, the flexibility of the thoracic wall decreasesand it becomes harder, the leftward deviation of the heart decreases, and compression on the heart and symptoms increase[10]. In the early adolescence period, complaints of early exhaustion are seen after started doing exercise. Moreover, exercise dyspnea, reduced stamina, chest pain, palpitations, exercise-triggered wheezing, and frequent upper respiratory tract infections can be seen[11]. While most of our patients did not have any complaints, the most common complaint in patients with symptoms was shortness of breath. Similar to the cases in the literature, in

Table 3. Regression values of mitral valve prolapse and tricuspid valve prolapse.

	OR	95%CI	p-value
MVP	16.56	4,937–55,563	<0.001
TVP	18.66	2,249–154,956	<0.001

Statistics: Logistic regression analysis. MVP: mitral valve prolapse; TVP: tricuspid valve prolapse.

our clinical study, it was found that the number of complaints increased with increasing age.

Cardiovascular functions can become more complicated with the displacement of the heart and rotation of the large blood vessels as the compression created by the sternum increases[12]. In our study, 8 (22%) patients had malposition on standard ECHO examination. This deformity, which is characterized by the depression of the sternum, may result in a significant decrease in the posterior-anterior diameter of the thoracic cage. This decrease may lead to a reduction in the stroke volume during systole as a result of the insufficient expansion of the heart during diastole[12]. This can lead to a failure in meeting the increased metabolic need, especially during exercise, and result in a lower LAD ZS value, as found in our study.

CHDs that can accompany PE are a vascular ring, pulmonary stenosis, atrial septal defect (ASD) primum, idiopathic hypertrophic subaortic stenosis, ASD secundum, total anomalous pulmonary venous return, transposition of the great arteries, tetralogy of Fallot, complete atrioventricular canal, tricuspid atresia, dextrocardia, truncus arteriosus, Ebstein anomaly, ventricular septal defects, aortic regurgitation, and patent ductus arteriosus. In our study, we found CHD in 3 (3.2%) patients: small secundum ASD that did not require intervention in one patient, and secundum ASD that required transcatheter closure in one patient and patent ductus arteriosus (PDA) in another patient.

As this deformity is in a concave form, it reduces venous return and myocardial perfusion by exerting compression on both the lungs and the ventricle. The compression of the right atrium and the right ventricle with an increased sternal depression index value in severe PE cases can be easily detected in echocardiographic tests[13]. TAPSE ZS is a value that shows the systolic function of the right ventricle[14]. In our study, the PE group had significantly lower tricuspid annulus diameter, tricuspid valve diameter, and TAPSE ZS values, which could be secondary to the increased sternal depression mentioned above. Furthermore, in the ECHO examination of 8 (22%) patients, malposition secondary to this sternal compression was detected.

In agreement with the information in the literature, in our study, the PE group had significantly higher rates of cardiac pathology, cardiac malposition, MVP, MI, TVP, and CHD ($p<0.001$, $p<0.001$, $p<0.001$, $p<0.001$, $p<0.001$, and $p=0.027$, respectively) (Table 1). Besides, when we conducted a risk analysis regarding MVP and TVP development in the PE patients using logistic regression analysis, we determined that the PE patients had a 16.56-fold increased risk of developing MVP and an 18.66-fold increased risk of developing TVP ($p<0.001$ and $p<0.001$, respectively) (Table 3).

In advanced-stage PE patients, as a result of the reduced volume based on the different positions of the heart, it is possible to observe tachycardia, right axis deviation in ECG, ST-segment depression (68% of patients), functional systolic murmurs originating from the compression of the left ventricular outflow tract (18% of patients), MVP (7–20% of patients), and conduction defects (branch blocks) (16% of patients)[15]. In our study, none of the patients showed significant ECG findings. The ECG findings that have been reported in the literature may be developing most probably due to the chronic effects of long-lasting sternal compression at older ages.

The fact that our hospital is a tertiary health center may have led to the inclusion of patients with severe thoracic deformities in the sample. Despite these limitations, the fact that this study is one of the very few studies in the literature that made a cardiac assessment of pediatric PE patients makes this study valuable.

Consequently, our study demonstrated that the prevalence of cardiac pathologies in pediatric PE patients was higher than the healthy control group. Accordingly, we recommend that pediatric PE patients are referred to pediatric cardiology outpatient clinics for the early diagnosis of cardiac pathologies.

AUTHORS' CONTRIBUTIONS

AA: Conceptualization, Data curation, Formal Analysis, Writing – original draft, Writing – review & editing. **UUG:** Data curation, Formal Analysis, Writing – original draft. **ŞG:** Conceptualization, Writing – review & editing.

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ORIGINAL ARTICLE

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Is there a difference between aortic and brachial vein blood lipoprotein and total cholesterol levels?

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SUMMARY

OBJECTIVE: Atherosclerosis is a disease of the arteries that is not practically observed in veins. There are a lot of proposed mechanisms underlying this phenomenon. We aimed to compare the lipoprotein and total cholesterol levels in aortic and venous blood samples.

METHODS: A total of 125 patients ≥18 years of age were included in the study. After overnight fasting, we drew blood from the proximal ascending aorta and brachial vein. Serum lipid profiles were compared between these samples.

RESULTS: Out of 125 patients, 45 (36%) were females, and 80 (64%) were males. The mean age of the patients was 62 years (24–85 years). Notably, 39 (31%) patients were using statin treatment. Coronary angiography showed that 103 (82%) patients had coronary artery disease. Mean arterial total cholesterol (low-density lipoprotein), high-density lipoprotein, and triglyceride levels were significantly lower than mean venous total cholesterol, low-density lipoprotein, high-density lipoprotein, and triglyceride levels (187.3±45.3 mg/dL vs. 204.5±52.6 mg/dL, p<0.001; 116.7±41.5 mg/dL vs. 128±45 mg/dL, p<0.001; 40.8±12.9 mg/dL vs. 45.3±13.3 mg/dL, p<0.001; and 142.8±81.5 vs. 161.5±100.3 mg/dL, p<0.001, respectively).

CONCLUSION: Aortic lipoprotein and total cholesterol levels are significantly lower than venous lipoprotein and total cholesterol levels in patients presenting to the hospital for coronary angiography.

KEYWORDS: Lipoproteins. Atherosclerosis. Coronary angiography. Cholesterol.

INTRODUCTION

Atherosclerosis is a disease of the arteries and is practically not observed in venous structures except for saphenous veins, which carry arterial blood when used for coronary artery bypass grafting. There is no unequivocal explanation for this phenomenon. However, some proposed mechanisms are as follows: the differences between the hemodynamic loads observed in arteries and veins, the structural differences between these vessels, the differences in the lipid composition of arteries and veins, the receptor differences in the walls of the vessels, and the shear stress differences in arteries and veins.

The hemodynamic load hypothesis is the most accepted one. A study on this issue observed that when arteries were interposed to veins, they did not develop atherosclerosis and underwent atrophic remodeling in cholesterol-fed rabbits[1]. Also, in patients with coronary bypass grafting, increased hemodynamic load on the grafted vein was one of the most important mechanisms underlying vein graft atherosclerosis, besides many other factors[2].

The literature is poor regarding the comparison of lipoproteins between the arteries and veins. Suppose there is a meaningful difference between the lipoprotein levels in these vessels. In that case, this can explain the basis of the difference in atherosclerotic processes in veins and arteries. So, we planned an investigation of patients undergoing coronary angiography and tried to compare the levels of lipoproteins in aortic and brachial venous blood. We also compared the frequency of lipid metabolism disorders in aortic and venous blood samples.

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METHODS

All procedures performed in this study were in accordance with the ethical standards of the institutional and national research committees and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study was approved by the Ethics Committee of Near East University, Cyprus.

A total of 125 patients hospitalized for coronary angiography procedures who were ≥18 years of age were included in the study. The indication for coronary angiography was acute coronary syndrome in 95 (76%) patients. There were no exclusion criteria. We drew blood from all patients after overnight fasting from the brachial vein and proximal ascending aorta during the coronary angiography procedure just before contrast material ingestion. Venous blood sampling was mostly done before aortic blood sampling, except for one patient. The time interval between blood samplings was less than 24 h in 80 (64%) patients, and it was more than 24 h in 45 (36%) patients. The blood samples were sent to a central laboratory, and the serum specimens were centrifuged, aliquoted, and stored at -80°C until the analysis time. At the end of the study, the lipid profile tests were analyzed on the Architect c8000 clinical chemistry system (Abbott Laboratories, Abbott Park, IL, USA). The low-density lipoprotein (LDL) concentration of all serum samples was measured by the direct method. We compared the lipid profile test results between the arterial and venous samples.

Statistical analysis

Statistical Package for the Social Sciences (SPSS), version 17.0, was used for the study's statistical evaluation. For the comparison of venous and arterial blood test results, paired samples t-test was used. The subgroup analyses (for sex, statin usage, and coronary artery disease status) of the venous and arterial blood sample results were again performed with paired samples t-test. The relationship between blood sample type and lipid metabolism disorders (hyperlipidemia, hypertriglyceridemia, and hipohigh-density lipoproteinemia) was analyzed with the Pearson chi-square test. A p-value <0.05 was considered significant.

RESULTS

Out of 125 patients, 45 (36%) were females, and 80 (64%) of them were males. The mean age of the patients was 62 years (24–85 years). Notably, 71 (57%) patients had a history of hypertension, whereas 36 (29%) were diabetic, and 39 (31%) patients were using statin treatment before enrollment in the study. As a result of the coronary angiography procedure, 103 (82%) patients were found to have coronary artery disease. Also, 30 (24%) patients had elective coronary angiography, whereas 95 (76%) patients had coronary angiography for acute coronary syndrome.

Mean arterial total cholesterol levels were significantly lower than mean venous total cholesterol levels (187.3±45.3 mg/dL vs. 204.5±52.6 mg/dL, p<0.001). The mean arterial LDL levels were also significantly lower than the mean venous LDL levels (116.7±41.5 mg/dL vs. 128±45 mg/dL, p<0.001). Mean arterial high-density lipoprotein (HDL) levels were also significantly lower than mean venous HDL levels (40.8±12.9 mg/dL vs. 45.3±13.3 mg/dL, p<0.001). Finally, mean arterial triglyceride levels were also found to be significantly lower than mean venous triglyceride levels (142.8±81.5 vs. 161.5±100.3 mg/dL, p<0.001) (Table 1). When the data was put into a subgroup analysis for the sex status of the patients, this subgroup analysis showed that the results did not change according to sex (Table 2). Another subgroup analysis was done for the statin usage status of the patients. This showed that statin users and non-users had reduced total cholesterol, LDL, and HDL levels in aortic blood compared with venous blood. Aortic triglyceride levels were significantly reduced compared to venous triglyceride levels in the statin non-user group, but in statin-users, aortic and venous triglyceride levels were not significantly different (Table 3). Moreover, we analyzed the data for coronary artery disease status according to coronary angiography, and this subgroup analysis showed that the results did not change according to the coronary artery disease status of the patients. Aortic lipoprotein and total cholesterol levels were lower than venous blood lipoprotein and total cholesterol levels, both in patients with coronary artery disease and in patients free of coronary artery disease.

When hyperlipidemia was defined as a serum total cholesterol level ≥200 mg/dL, according to aortic blood sample test results, 47 (38%) patients had hyperlipidemia. According to venous blood sample results, 64 (51%) patients had hyperlipidemia. Hypertriglyceridemia (defined as a serum triglyceride

Table 1. Lipoprotein and total cholesterol levels in aortic and venous blood.

Lipoprotein type	Blood type		p-value*
	Aortic	Venous	
Total cholesterol (mg/dL)	187.3±45.3	204.5±52.6	**<0.001**
LDL (mg/dL)	116.7±41.5	128.0±45.0	**<0.001**
HDL (mg/dL)	40.8±12.9	45.3±13.3	**<0.001**
Triglyceride (mg/dL)	142.8±81.5	161.5±100.3	**<0.001**

*Aortic lipoprotein and total cholesterol levels were significantly lower than venous lipoprotein and total cholesterol levels. HDL: high-density lipoprotein; LDL: low-density lipoprotein. Bold indicates statistically significant p-values.

level ≥150 mg/dL) was observed in 41 (33%) patients based on arterial blood sample results and in 58 (46%) patients based on venous sample results. Low HDL levels were defined as values <40 mg/dL for men and <50 mg/dL for women. Low HDL levels were detected in 76 (61%) patients according to arterial blood sample results and in 56 (45%) patients according to venous blood sample results. According to the Pearson chi-square test, all of the differences in lipid metabolism disorders (hyperlipidemia, hypertriglyceridemia, and hypohigh-density lipoproteinemia) were related to the blood sample type (p-values: 0.030, 0.028, and 0.011, respectively).

DISCUSSION

The present study's main finding is that all lipoproteins are found in lower amounts in aortic blood when compared with peripheral venous blood. The results do not change when stratified by sex or the coronary artery disease status of the patients. However, when we stratified the patient group for statin usage status, we observed that, in statin users, venous and aortic triglyceride levels were similar. In contrast, total cholesterol, HDL, and LDL levels were significantly lower in aortic blood than in venous blood. In statin non-users, total cholesterol levels and lipoproteins were lower in aortic samples than in venous samples.

Very few studies in the literature compare the lipoproteins of arteries and veins. In one of these studies, LDL was higher in aortic blood versus femoral venous blood; all other lipoproteins were similar[3]. This study also showed that when incubated with mouse peritoneal macrophages, arterial LDL, and very-low-density lipoprotein (VLDL) increased cholesterol accumulation and enhanced cholesterol esterification within these macrophages, whereas venous lipoproteins had less effect. They concluded that this difference in the function of venous and arterial lipoproteins might explain the atherosclerosis seen in arteries. Another historical study found that arterial HDL and triglyceride levels were lower when compared with venous blood. Also, arterial platelet activity was higher than venous platelet activity, which might serve as a mechanism for the atherosclerotic process in the arteries[4]. Another study compared the uptake and degradation of labeled LDL between arteries and veins. It showed that the uptake of LDL was similar between these vessels, but degradation was two times higher in the arteries compared to the veins[5]. They argued that this difference might be due

Table 2. Lipoprotein and total cholesterol levels in aortic and venous blood stratified for sex.

Sex	Blood type	Lipoprotein level (mg/dL)	p-value*
Female	Aortic total cholesterol	196.1	**<0.001**
	Venous total cholesterol	214.2	
	Aortic LDL	120.1	**<0.001**
	Venous LDL	135	
	Aortic HDL	46	**<0.001**
	Venous HDL	51.6	
	Aortic triglyceride	130.9	**0.016**
	Venous triglyceride	143.2	
Male	Aortic total cholesterol	182.3	**<0.001**
	Venous total cholesterol	199	
	Aortic LDL	114.7	**<0.001**
	Venous LDL	124.1	
	Aortic HDL	37.9	**<0.001**
	Venous HDL	41.8	
	Aortic triglyceride	149.5	**0.002**
	Venous triglyceride	171.8	

*Aortic lipoprotein and total cholesterol levels are lower than venous blood lipoprotein and total cholesterol levels both in female and male patients. HDL: high-density lipoprotein; LDL: low-density lipoprotein. Bold indicates statistically significant p-values.

Table 3. Lipoprotein and total cholesterol levels in aortic and venous blood stratified for statin usage.

Statin usage	Blood type	Lipoprotein level (mg/dL)	p-value*
Yes	Aortic total cholesterol	165.6	**<0.001**
	Venous total cholesterol	177.2	
	Aortic LDL	93.8	**0.002**
	Venous LDL	103.2	
	Aortic HDL	42.5	**<0.001**
	Venous HDL	46.7	
	Aortic triglyceride	133.1	0.204
	Venous triglyceride	140.1	
No	Aortic total cholesterol	198.2	**<0.001**
	Venous total cholesterol	218.3	
	Aortic LDL	128.5	**<0.001**
	Venous LDL	140.4	
	Aortic HDL	39.8	**<0.001**
	Venous HDL	44.4	
	Aortic triglyceride	149.4	**<0.001**
	Venous triglyceride	174.3	

*Aortic lipoprotein and total cholesterol levels are lower than venous blood lipoprotein and total cholesterol levels both in statin users and non-users, except for triglyceride levels in statin users. In statin users, aortic and venous triglyceride levels are not significantly different. HDL: high-density lipoprotein; LDL: low-density lipoprotein. Bold indicates statistically significant p-values.

to increased plasmalemmal vesicles, which work in the endocytosis of LDL through the vessel wall in the endothelium of large arteries compared to large veins[6].

These studies were ancient and also enrolled very few patients. The results were inconclusive. So, we tried to compare the lipoprotein levels in aortic and venous blood samples in a broader patient population presenting to the hospital for coronary angiography. The levels of arterial lipoproteins and total cholesterol levels were significantly lower than their venous counterparts. Mainly, the patients were not using statins at the time of enrollment in the study. However, in 31% of the patients who were using statins, total cholesterol, LDL, and HDL levels were reduced in the arterial blood. In contrast, triglyceride levels were not significantly different between arterial and venous samples.

The results are new and may be similar to the historical study in 1989[4]. There is no proven mechanism that may explain the difference in lipoprotein levels between arteries and veins. The most plausible mechanism underlying this phenomenon may be the differences in receptors in the aortic and venous walls. We know that LDL enters the arterial wall through endocytosis (to the arterial endothelium) and transcytosis (directly to the arterial wall beneath the endothelium). Some receptors and ligands are found in the arterial endothelium that work for this process, like the LDL receptor, scavenger receptor B1 (SR-B1), CD36, activin-like kinase 1 (ALK1), and so on, which transfer LDL from the lumen to the arterial wall through endocytosis and transcytosis[7-11]. The SR-B1 receptor was found much more abundantly in atherosclerotic regions of arteries relative to normal arterial regions[7]. These receptors may be found much less frequently in the venous wall. Nevertheless, this is just a hypothesis that needs to be proved. A historical study in rats has shown that the endothelium of large arteries contains about twice as many plasmalemmal vesicles as that of large veins[12]. More lipoproteins entering the vessel wall may explain the lower levels in the arterial lumen. There are also studies comparing the structures of arterial and venous grafts to understand the difference in atherosclerosis development in these conduits. One of these studies showed that arterial grafts undergo less lipid synthesis, slower lipid uptake, and more lipid lipolysis[13]. This is not a direct comparison between normal arteries and veins because venous grafts are prone to atherosclerosis due to increased hemodynamic load after the grafting procedure, and they carry arterial blood. However, it may give an opinion about the differences in lipid metabolism in arteries and veins, which need further studies to be elucidated. If the receptor hypothesis underlying low levels of aortic lipoproteins is true, more lipoproteins enter the aortic wall, which supports the atherosclerotic process seen in arteries.

As a result of the lower total cholesterol, LDL, HDL, and triglyceride levels in aortic blood, the frequency of lipid metabolism disorders was also significantly different in aortic blood and venous blood. Of course, the abnormal values used to define lipid metabolism disorders were traditionally derived from venous blood samples. So, this may not mean that these levels are also abnormal in aortic blood because we do not know the average lipoprotein values in aortic blood. This brings up the question: which one is more predictive for future cardiovascular events, venous or arterial lipoprotein levels? We do not know the answer because this has never been tested before. However, when we think of atherosclerosis as an arterial process, one can guess that arterial lipoprotein levels may matter more than their venous counterparts. We hope that this question can be answered in the future.

On the contrary, taking venous blood samples from patients or healthy people to check their cholesterol levels is much easier and more feasible than taking arterial blood samples. Moreover, using drugs for patients with lipid metabolism disorders will probably also lower arterial lipoprotein levels, as was the case in our study. However, arterial blood sampling may be used primarily for high-risk patients if arterial lipoprotein levels foresee future cardiovascular events better than venous lipoprotein levels. We think these are the future implications of the present study.

Limitations of the study

The main limitation of the present study is the time interval between aortic and venous blood sampling. Venous blood sampling was mostly done before aortic blood sampling due to angiography laboratory conditions. Another limitation may be the place where the venous blood was taken. The brachial vein is a peripheral vein, whereas the aorta is a central arterial site. So, we compared blood samples between central blood and a peripheric blood sample site. Moreover, the patient population may be seen as a small sample for this kind of study. Finally, statin usage may have affected the results, but the results were mostly similar between statin users and non-users.

CONCLUSION

Aortic lipoprotein and total cholesterol levels are significantly lower than venous lipoprotein and total cholesterol levels in a patient population presenting to the hospital for coronary angiography.

AUTHORS' CONTRIBUTIONS

UY: Conceptualization, Data curation, Investigation, Methodology, Resources, Writing – original draft. **LC:** Data curation, Project administration, Supervision, Validation, Writing – review & editing. **BY:** Data curation, Formal Analysis, Resources, Validation. **SU:** Conceptualization, Data curation, Formal Analysis, Resources. **EC:** Conceptualization, Data curation, Investigation, Methodology, Project administration, Resources. **OHE:** Data curation, Formal Analysis, Methodology, Resources. **OA:** Conceptualization, Formal Analysis, Supervision, Project administration, Writing – review & editing. **HD:** Conceptualization, Project administration, Supervision, Writing – review & editing.

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ORIGINAL ARTICLE

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Transcultural adaptation of a scale for exclusive breastfeeding to be used in Brazil

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SUMMARY

OBJECTIVE: The aim of this study was to perform a cross-cultural adaptation of the Breastfeeding Self-Efficacy Scale in Exclusive Breastfeeding for use in a Brazilian-Portuguese context.

METHODS: The cross-cultural adaptation process involved the translation from original English into Brazilian Portuguese by two qualified and independent translators. Both translations were synthesized into a single version that was back-translated into English. An expert committee was created to assess linguistic equivalences, formulating a pre-final version that was tested on ten nursing women attending a maternity hospital. To assess its psychometric properties, a cross-sectional study was carried out. The population consisted of 99 nursing women from a reference maternity hospital in southern Brazil. The scale's stability and internal consistency were measured through Cronbach's alpha. The Pearson's correlation coefficient and the intraclass correlation coefficient between two applications were assessed to ascertain the Breastfeeding Self-Efficacy Scale in Exclusive Breastfeeding-Br scale's reliability. The construct validity was evaluated through exploratory factorial analysis.

RESULTS: The Breastfeeding Self-Efficacy Scale in Exclusive Breastfeeding-Br showed a general Cronbach's alpha of 0.849. The test-retest analysis showed a Pearson's correlation coefficient of 0.483 and intraclass correlation coefficient of 0.645. The exploratory factorial analysis showed two domains among the nine items of the Breastfeeding Self-Efficacy Scale in Exclusive Breastfeeding-Br: the functional domain, including six items, and the cognitive domain, including three items, explaining 59.77% of the variance.

CONCLUSION: The Breastfeeding Self-Efficacy Scale in Exclusive Breastfeeding-Br was considered adequate for the cultural context and reliable and valid for Brazilian nursing women.

KEYWORDS: Exclusive breastfeeding. Surveys and questionnaires. Validation study. Psychometrics.

INTRODUCTION

Exclusive breastfeeding for at least 6 months has a profound relationship with the prevention of disease and influences babies' cognitive development[1]. Despite the importance of the exclusive breastfeeding process identified in scientific studies, it is the lactating mother's knowledge that significantly influences whether this practice is adopted. Thus, the relationship between breastfeeding knowledge and adherence to exclusive breastfeeding is direct and expressive[2]. The better the maternal understanding of breastfeeding, the greater the chance of success in exclusive breastfeeding[2]. National and international organizations have taken measures to improve breastfeeding practices worldwide and have been analyzing breastfeeding programs[1]. Incentives for breastfeeding practices are necessary, making it important to measure them based on indicators, such as breastfeeding self-efficacy.

A systematic review with meta-analysis showed that breastfeeding self-efficacy is a modifiable factor that health professionals can target to improve breastfeeding rates in mothers of full-term infants[3]. Moreover, higher breastfeeding self-efficacy is associated with a lower risk of expressed human milk feeding and a longer duration of any and exclusive breastfeeding[4].

Thus, researchers emphasize the importance of studies that validate and develop instruments that can increase knowledge about the causal factors underlying breastfeeding decisions and how the breastfeeding process can be better supported[5].

The first known instrument to assess breastfeeding self-efficacy, known as the Breastfeeding Self-Efficacy Scale (BSES) was published by Dennis and Faux in 1999[6]. The BSES includes 33 items that seek to measure mother's expectations regarding self-efficacy; in other words, her confidence in her ability to breastfeed her new child. In 2003, Dennis proposed and

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validated another version of the self-efficacy scale, the so-called Self-Efficacy Scale Short Form (BSES-SF), which includes 14 items[7]. This version was validated for several different languages and populations[8-10], including Brazilian women[11].

More recently, Boateng et al.[12] adapted the BSES-SF scale to measure exclusive breastfeeding self-efficacy among women in Uganda. The result was an instrument called the Breastfeeding Self-Efficacy Scale-Exclusive Breastfeeding (BSES-EBF), composed of nine items that measure the cognitive and functional dimensions of exclusive breastfeeding in women in Uganda[12]. Authors pointed out that the BSES-EBF was valid and reliable for measuring exclusive breastfeeding self-efficacy in northern Uganda and was considered ready for adaptation and validation for clinical and programmatic use elsewhere[12].

The Brazilian literature lacks a valid and reliable instrument to measure the self-efficacy scale for exclusive breastfeeding. As self-efficacy is an important determinant of breastfeeding behaviors[12], and the existing measures do not specifically assess the self-efficacy of exclusive breastfeeding but rather the self-efficacy of any breastfeeding, it is important to have an exclusive scale for this purpose in Brazil. Considering the need to identify possible mothers at risk of non-adherence to the exclusive breastfeeding process or premature cessation of this practice, this study aims to propose a Brazilian version of the BSES-EBF scale and assess its psychometric properties.

METHODS

The guidelines of the COSMIN Study Design Checklist for Patient-Reported Outcome Measurement Instruments[13] were followed in this study. The author of the original scale authorized using the BSES-EBF to perform the transcultural adaptation to Brazilian Portuguese and validate its psychometric properties.

The cross-cultural adaptation and psychometric analysis to validate the BSES-EBF scale were carried out from June 2020 to March 2021, involving nursing mothers admitted to the childbirth of a maternity hospital in southern Brazil, after the local research ethical committee approved it.

The process started with the direct translation of BSES-EBF in its original English version into the Portuguese language spoken in Brazil. The translation was carried out by two independent translators, one native to Brazil and the other native to a country in which English is spoken as a native language, producing two different translations. Then, the researchers compared these two versions and synthesized them into a single version that was translated back into English by a third independent translator who was a native English speaker and had a master's degree in the Portuguese language. This process made it possible to correct minor misunderstandings or unclear wordings in the initial translations.

Next, a committee of experts comprised two obstetricians, a nurse specialized in obstetrics, an epidemiologist, and a medical student who evaluated all the processes and linguistic equivalences, discussing synonyms, reformulating questions to facilitate the understanding of questions by people with different levels of education, and finally formulating a pre-final version. This was tested on 10 nursing women attending the selected maternity hospital who had agreed to participate in the research and had signed the informed consent form. The researchers evaluated possible interpretation or understanding difficulties, any constraints caused by the questions, or eventual inadequacies in the given answers. Without the need for adjustments, the Brazilian version was proposed (BSES-EBF-Br).

An epidemiological study of cross-sectional design was carried out to analyze the psychometric properties of BSES-EBF-Br. The scale was applied on two different occasions with an interval of 2 weeks, in accordance with the test-retest method. The second approach was performed by phone, after consent was obtained.

The sample size was calculated using the proportion of 10 interviewees per question[14], plus 10% for losses, totaling 99 women. The inclusion criteria were women whose birth event took place between 24 and 48 h before the interview, who were breastfeeding, who were aged 18 years or over, who knew how to read and write in Portuguese, and who agreed to participate in the study by signing the informed consent form. The sample selection was performed consecutively according to the date of the birth event. Patients with psychiatric disorders that prevented their participation in the data collection were excluded.

The resulting data were entered into and analyzed by the Statistical Package for Social Science for Windows (SPSS), version 18.0 (IBM©, Chicago, Illinois, USA). The Pearson's correlation coefficient and the intraclass correlation coefficient (ICC) between the two applications were assessed to ascertain the scale's reliability. A Bland-Altman graph was created to determine the distribution of responses for the two applications. The scale's internal consistency was tested using Cronbach's alpha, calculated with the results of the first moment of response for general analysis by domains and by items.

The instrument's apparent validity was defined and assessed by the experts involved in the study. For the construct validity of the proposed scale, exploratory factor analysis (EFA) was used after observing its suitability with the linear correlation matrix, Kaiser-Meyer-Olkin test (KMO), and Bartlett's sphericity test. The Kaiser criterion for eigenvalues more significant or close to one and the Scree plot were used to define the number of factors

extracted. The main components' extraction was performed by rotation of Varimax to determine the BSES-EBF-Br items and to minimize the number of variables with high loads in each factor.

RESULTS

The transcultural adaptation process led to a Brazilian-Portuguese version of the BSES-EBF scale. For the instrument's psychometric analysis, 99 nursing women were interviewed, and of these, 42 undertook a second interview.

The participants' mean age was 26.8±6.1 years, with a minimum age of 14 years and a maximum age of 45 years. In the socio-demographic evaluation, 31.3% had completed more than 11 years of schooling, and 84.8% lived with their partner. During the interviewees' obstetric information evaluation, 1–6 previous pregnancies were observed, with 35.4% of women having delivered their first child and 31.3% having delivered their second child. Of the postpartum women interviewed, 45.5% had never breastfed, and only 33.4% had breastfed their children for more than 6 months previously (Table 1).

Table 1. Descriptive statistics of socio-demographic, obstetric, and breastfeeding characteristics of Brazilian study participants.

Characteristics	n	%
Household income/month (US$)		
Up to 250	17	17.2
Between 251 and 500	44	44.4
More than 500	38	38.4
Living with partner		
Yes	84	84.8
No	15	15.2
Educational level		
Up to 8 years of study	25	25.3
Between 8 and 11 years of study	31	31.3
More than 11 years of study	42	42.4
Previous pregnancies		
0	39	24.7
1	37	23.4
2	14	8.2
≥3	12	6.3
Previous breastfeeding time		
Never breastfed	45	45.5
1–4 months	13	13.1
4–6 months	7	7.1
More than 6 months	34	34.3

Brazil, 2021 (n=99).

The test-retest analysis demonstrated moderate stability, with a Pearson's correlation coefficient of 0.483 (p<0.001) and an intraclass correlation coefficient (ICC) of 0.645 (95%CI 0.335–0.810; p<0.001). The uniform distribution of the responses to the two applications of the instrument corroborated its reliability.

The BSES-EBF-Br presented a general Cronbach's alpha of 0.849. If each item was removed from the scale, the Cronbach's alpha of the instrument identified values close to or above 0.810, showing good internal consistency in maintaining all items of the proposed scale (Table 2).

To determine the adequacy of the EFA against the data, the linear correlation matrix between the items was calculated and showed Pearson's correlation indices between 0.300 and 0.800 in most cases. The KMO test value was 0.751, and a Bartlett's sphericity test with statistical significance (p<0.001) demonstrated the suitability of the data set for use in the EFA.

By extracting the main components and through visual confirmation using the Scree plot, two domains were obtained among the nine items of the BSES-EBF-Br: the functional domain, consisting of six items reflecting the participants' confidence and ability to breastfeed. The other domain was cognitive, comprising three items reflecting the participants' beliefs about the importance of maintaining exclusive breastfeeding. The Cronbach's alpha results for each domain were 0.803 and 0.597, respectively. Only items 4, 6, 7, and 9 scored in a single domain, and the others were included in the domain in which they presented the highest factor load (Table 3).

The initial analysis of the eigenvalues of the two domains after rotation explained 59.77% of the variance, and the commonality values varied between 0.31 and 0.79. The items with lower commonalities were as follows: "*I can always determine that my baby is getting enough milk*" (0.31), "*I can always deal with the fact that breastfeeding can be time-consuming*" (0.41), and "*I can continue exclusively breastfeeding for as long as I want*" (0.51). Thus, as most of the items presented high factor loads, the two extracted domains could explain the expected variance of the indicators.

DISCUSSION

The scale under study is a modification of the original BSES-SF to target exclusive breastfeeding. According to the authors[12], the following two dimensions of the BSES-EBF emerged: cognitive and functional. The authors concluded that it was a valid and reliable scale ready for adaptation and validation for clinical and programmatic use elsewhere. The present study is the first to be developed to propose a Brazilian version of the BSES-EBF

Table 2. Reliability analysis from the Cronbach's alpha of the Breastfeeding Self-Efficacy Scale in Exclusive Breastfeeding-Br.

Items BSES-EBF-Br	Cronbach's alpha if an item is deleted
1. I can always give my baby only breast milk without using animal milk, formula, or other liquids or foods as a supplement / *Eu sempre consigo dar ao meu bebê apenas leite materno, sem usar leite de origem animal, fórmula ou outros líquidos e alimentos como suplemento.*	0.829
2. I can continue exclusively breastfeeding for as long as I want / *Eu posso continuar amamentando exclusivamente durante o tempo que eu quiser.*	0.829
3. I can always exclusively breastfeed without my baby receiving even a drop of water or any other liquid / *Eu sempre consigo amamentar exclusivamente o meu bebê sem que ele receba nem uma gota de água ou qualquer outro líquido.*	0.828
4. I can always stop someone from trying to feed my baby liquids or foods other than breast milk, including purchased baby foods (e.g., infant formula, milk, porridge, juice, tea [whatever is commonly given]), before 6 months of age / *Eu sempre consigo impedir qualquer um que tente alimentar meu bebê com líquidos ou outros alimentos além do leite materno, incluindo alimentos infantis como fórmula infantil, leite, mingau, suco e chá, antes dos seis meses.*	0.845
5. I can always determine that my baby is getting enough milk / *Eu sempre consigo perceber se meu bebê está mamando o suficiente.*	0.846
6. I can always be satisfied with my breastfeeding experience / *Eu sempre sinto satisfação com minha experiência em amamentar.*	0.833
7. I can always deal with the fact that breastfeeding can be time consuming / *Eu sempre consigo lidar com o fato de que a amamentação pode ser demorada.*	0.844
8. I can always continue to breastfeed my baby for every feeding / *Eu consigo amamentar meu bebê quando necessário.*	0.822
9. I can always manage to keep up with my baby's breastfeeding demands / *Eu sempre consigo atender as necessidades de amamentação do meu bebê.*	0.819

Brazil, 2021 (n=99).

Table 3. Analysis of each item's factorial components on the Breastfeeding Self-Efficacy Scale in Exclusive Breastfeeding-Br obtained by the Varimax rotation method.

Items on the BSES-EB-Br	Factors	
	1	2
Functional		
2. I can continue exclusively breastfeeding for as long as I want / *Eu posso continuar amamentando exclusivamente durante o tempo que eu quiser.*	0.539	
5. I can always determine that my baby is getting enough milk / *Eu sempre consigo perceber se meu bebê está mamando o suficiente.*	0.497	,256
6. I can always be satisfied with my breastfeeding experience / *Eu sempre sinto satisfação com minha experiência em amamentar.*	0.771	
7. I can always deal with the fact that breastfeeding can be time consuming / *Eu sempre consigo lidar com o fato de que a amamentação pode ser demorada.*	0.632	
8. I can always continue to breastfeed my baby for every feeding / *Eu consigo amamentar meu bebê quando necessário.*	0.815	
9. I can always manage to keep up with my baby's breastfeeding demands / *Eu sempre consigo atender as necessidades de amamentação do meu bebê.*	0.863	
Cognitive		
1. I can always give my baby only breast milk without using animal milk, formula, or other liquids or foods as a supplement / *Eu sempre consigo dar ao meu bebê apenas leite materno, sem usar leite de origem animal, fórmula ou outros líquidos e alimentos como suplemento.*		0.810
3. I can always exclusively breastfeed without my baby receiving even a drop of water or any other liquid / *Eu sempre consigo amamentar exclusivamente o meu bebê sem que ele receba nem uma gota de água ou qualquer outro líquido.*		0.722
4. I can always stop someone from trying to feed my baby liquids or foods other than breast milk, including purchased baby foods (e.g., infant formula, milk, porridge, juice, tea [whatever is commonly given]), before 6 months of age / *Eu sempre consigo impedir qualquer um que tente alimentar meu bebê com líquidos ou outros alimentos além do leite materno, incluindo alimentos infantis como fórmula infantil, leite, mingau, suco e chá, antes dos seis meses.*		0.814

Brazil, 2021 (n=99).

scale for assessing the self-efficacy of exclusive breastfeeding in Brazilian nursing mothers.

The uniform distribution of the two applications of the instrument corroborated its reliability. The scale's stability in the Brazilian instrument was superior to that observed in the original scale (correlation coefficient=0.54), probably due to the long interval between the original study interviews[10]. Other transcultural adaptations of BSES-EBF were not found in the literature for comparing these indicators.

The internal consistency confirmed by the Cronbach's alpha of the full BSES-EBF-Br scale was considered excellent, demonstrating good internal consistency in maintaining all items of the proposed instrument in the same way as the original scale[10]. No tests were applied to assess external validity.

When testing suitability using the KMO test and the Barlett test, the results found for the BSES-EBF-Br were comparable to the original scale. They showed that the sample size was adequate to perform the EFA. These data were identical to those found for the original scale[10].

When submitting the data to the EFA, the total variance explained on the BSES-EBF-Br scale resulted in two domains, including the one determined by Boateng et al.[10] However, the distribution of the items in each domain was different, so that, in the original scale, the domain "Functional," reflecting the competence and ability of the participants to breastfeed, consisted of five items, while the domain "Cognitive," reflecting the belief of participants in exclusive breastfeeding, consisted of four items[10]. This difference may be attributable to the time elapsed between birth and the scale application, which was different in the two studies.

As most of the items presented high factor loads, the initial analysis of the two domains' eigenvalues, after rotation, was able to explain the expected variance of the indicators in the same way as observed by Boateng et al. for the original scale[10].

Some limitations require caution when interpreting the results of this study. The absence of a control group, which would have allowed further analyses and the calculation of an essential cutoff point to determine which score should be considered to indicate a higher risk of early breastfeeding interruption in the Brazilian population. Another limitation was the non-inclusion of a comparative questionnaire to help with the external validation of the studied instrument.

As the research was carried out at a single public health institution in Santa Catarina, the scale may behave differently when applied to other socioeconomic strata or in different Brazilian regions.

It is believed that the variability in the psychometric parameters observed in the versions of the scale under study is the result of the socio-cultural characteristics peculiar to each country, which reinforces the need for rigorous scientific transcultural adaptation. Thus, the few psychometric differences found in the original version do not indicate flaws in the process of transcultural adaptation.

The proposed scale is understandable and appropriate to the Brazilian cultural context and can be reliable and valid for Brazilian nursing women. We believe that it could help identify nursing women with low confidence in exclusive breastfeeding and allow for stimulus measures to be strengthened.

CONCLUSION

The Brazilian version of the BSES-EBF can be considered adequate for the cultural context and reliable and valid for Brazilian nursing women.

AUTHORS' CONTRIBUTIONS

LKV: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **LSG:** Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. **GKR:** Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. **RDN:** Conceptualization, Formal Analysis, Investigation, Methodology, Project administration, Visualization, Writing – original draft, Writing – review & editing. **JT:** Conceptualization, Formal Analysis, Investigation, Methodology, Project administration, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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ORIGINAL ARTICLE

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Factors associated with low skeletal muscle index among patients with Crohn's disease

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SUMMARY

OBJECTIVE: Disease-related skeletal muscle loss is highly prevalent among patients with Crohn's disease. Low skeletal muscle mass lead to disability and interventions to prevent skeletal mass loss as an effective strategy to prevent disability. The aim of this article was to identify the factor associated with skeletal muscle loss of Crohn's disease and seek for management target for the prevention of sarcopenia-related disability.

METHODS: Patients with Crohn's disease were divided into low and normal skeletal muscle mass groups based on L3 skeletal muscle index using abdominal CT scans. The clinical and laboratory parameters and colonoscopy were compared between the two groups. Univariate and multivariate regression logistic models were built to identify the prognostic markers of Crohn's disease-associated muscle loss.

RESULTS: A total of 191 Crohn's disease patients were enrolled in this study, of whom 116 (60.73%) were detected to have low L3 skeletal muscle index, including 71 (68.26%) males. The multivariate logistic regression analysis showed that age (OR: 1.031, 95%CI: 1.006–1.057), female gender (OR: 2.939, 95%CI: 1.386–6.233), disease duration (OR: 0.988, 95%CI: 0.980–0.996), endoscopic disease activity (simple endoscopic score for Crohn's disease) (OR: 0.923, 95%CI: 0.855–0.996), serum albumin (OR: 1.079, 95%CI: 1.009–1.154), and serum creatinine (OR: 1.037, 95%CI: 1.011–1.063) were associated with L3 skeletal muscle index among Crohn's disease patients.

CONCLUSION: The gender, age, and duration of disease were uncontrollable factors associated with muscle loss of Crohn's disease. The treatment target of mucosal healing and improved nutritional status may be beneficial for maintaining muscle mass among Crohn's disease patients.

KEYWORDS: Crohn's disease. Sarcopenia. Body composition. Endoscopy.

INTRODUCTION

Crohn's disease (CD) is an inflammatory bowel disease (IBD) characterized by chronic, relapsing, systemic inflammation of the gastrointestinal tract, complex gastrointestinal symptoms, extraintestinal manifestations, and comorbidities[1]. The bone and skeletal muscle are vulnerable to be affected by the disease. Low muscle mass has been proven to be associated with adverse outcomes including the severity of CD, the presence of surgery related to CD, increase in the intestinal surgery-associated complications, and death[2,3]. CT is considered the gold standard technique for the detection of muscle quality and accurate assessment of body composition in patients with CD[4]. When considering only the low muscle mass based on CT or MRI, 31–61.4% of CD patients were complicated with sarcopenia[5]. The data showed that the prevalence, incidence, years of life lived with disability (YLDs), and disability-adjusted life years (DALYs) of IBD had increased in China over the past three decades. Focus on muscle loss prevention may be an important policy to manage CD-related disability[6].

Multiple factors are involved in the muscle dysfunction and sarcopenia of IBD, such as poor nutrition, physical inactivity, hormonal changes, prolonged corticosteroid therapy, high degree of lipid peroxidation or oxidative stress, and muscle protein synthesis pathways[7]. The aim of this article was to identify the controllable factors associated with skeletal muscle in the context of CD. We establish the relationship between the clinical and laboratory parameters and the skeletal muscle mass in the active stage of disease in order to seek for management target for the prevention of sarcopenia-related disability.

METHODS

Patient selection

This is a single-center retrospective study of CD data collected from the Affiliated Hospital of Yangzhou University, a Chinese tertiary teaching hospital, between January 2013 and August 2020. Hospitalized patients were consecutively enrolled in the

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study with a confirmed diagnosis of CD. The inclusion criteria were as follows[1]: age ≥14 years and[2] all patients who underwent abdominal CT scan within 2 weeks before or after admission and colonoscopy during hospitalization. The exclusion criteria were as follows[1]: re-admission after recruitment and[2] clinical data or laboratory information unavailable.

Assessment of muscle mass on CT

The L3SMA measurement was done on CT images using Picture Archiving and Communication Systems (PACS, IMPAX6.3.1.4095, AGFA HealthCare NV, Belgium). The L3 skeletal muscle index (L3 SMI) is denoted by L3SMA (cm^2)/height^2 (m^2). The diagnostic criteria of low L3SMI are below 42 cm^2/m^2 for men and below 38 cm^2/m^2 for women[8].

Study design and data collection

All patients were divided into two groups, i.e., low L3SMI group and normal L3SMI based on the cutoff value of L3SMI. Clinical parameters included disease duration, the Montreal classification of CD[9], main symptoms of hospital admissions, simple Cohn's disease activity index (simple CDAI), simple endoscopic score for CD (SES-CD)[10], and treatments during hospitalization. The nutritional assessments used nutritional risk screening (NRS2002) and prognostic nutritional index (PNI). Laboratory parameters included blood cell count, neutrophil-lymphocyte ratio (NLR), platelet-lymphocyte ratio (PLR), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), D-dimer, and serum levels of calcium, albumin, prealbumin, cholinesterase, creatinine, urea, and retinol-binding protein.

Ethical approval

This study was performed in accordance with the Declaration of Helsinki (2000) of the World Medical Association. The Human Research Ethics Committee of the Affiliated Hospital of Yangzhou University approved this retrospective trial.

Statistical analysis

Statistical analyses were performed using SPSS 23.0 (IBM, Armonk, NY, USA). All demographic, clinical, and laboratory characteristics were compared between the two groups. Normal distribution test of quantitative variables used the Shapiro-Wilk test. Normally distributed variables were described as mean± standard deviation, using an independent-samples t-test. Non-normally distributed variables were described as the median and interquartile range (IQR) using the Mann-Whitney U test. The qualitative variables were described as numbers (percentages) using the chi-square test. Univariate and multivariate regression logistic models were built to identify the prognostic markers of CD-associated muscle loss. The Pearson correlation analysis was conducted between clinical and laboratory parameters. Two-tailed $p<0.05$ was considered to be statistically significant.

RESULTS

Demographic characteristics between groups

A total of 191 CD patients were enrolled in this study, including 104 males (54.45%). The average age of patients was 40 years. A total of 116 (60.73%) patients were detected to have low L3SMI, including 71 (68.26%) males.

Clinical characteristics between groups

The duration of disease was longer in the low skeletal muscle group, but there was no statistical significance in the univariate analysis. Patients with low L3SMI had higher disease activity disease severity scores and endoscopic lesions severity scores. The simple CDAI was 5.00 (IQR: 4.00, 6.00) vs. 4.00 (IQR: 3.00, 5.00), and the SES-CD was 6.00 (IQR: 3.00, 9.75) vs. 3.00 (IQR: 0.00,6.00) (all p-values<0.01) (Table 1).

Laboratory parameters between groups

Hemoglobin and serum levels of calcium, albumin, prealbumin, retinol-binding protein, cholinesterase, creatinine, urea, and creatinine/cystatin C were significantly lower in the low L3SMI group than those in the normal L3SMI group. PLR as a systemic inflammatory marker was higher in the low L3SMI group (Table 2).

Factors associated with Crohn's disease-associated low skeletal muscle mass

Age, gender, disease duration, endoscopic activity, and serum levels of albumin, urea, and creatinine were associated with L3SMI based on univariate analysis. After multivariate regression, female gender, younger age, longer disease duration, SES-CD, and lower levels of serum albumin and serum creatinine were more likely to be diagnosed with low L3SMI (Table 3).

DISCUSSION

In this study, muscle loss diagnosed by the low L3SMI was highly prevalent among CD patients. A total of 60.73% patients had decreased skeletal muscle mass, including 71 (68.26%) males. This was in line with the previous research of Zhang et al.[2] using the same diagnostic criteria among adult Chinese patients with CD. Skeletal muscle loss showed sex-specific variations. Male patients were more prone to muscle loss in the context of CD.

Table 1. Clinical characteristics of Crohn's disease patients between the low and normal L3 skeletal muscle index groups.

Clinical parameters	All patients (n=191)	Low L3SMI (n=116)	Normal L3SMI (n=75)	p-value
Age (years)	40.00 (28.00, 50.00)	38.50 (27.00, 53.00)	40 (31.00, 53.00)	0.070
< 18	12 (6.28%)	10 (8.62%)	2 (2.70%)	
18–59	154 (80.63%)	96 (82.76%)	58 (73.33%)	
>60	25 (13.09%)	10 (8.62%)	15 (20.00%)	
Gender (n, %)				
Male	104 (54.45%)	71 (61.20%)	33 (44.00%)	0.026*
Female	87 (45.55%)	45 (38.80%)	42 (56.00%)	
Disease duration (months)	36.00 (10.00, 84.00)	36.00 (6.00, 84.00)	24.00 (6.00, 72.00)	0.078
Age at diagnosis of CD (n, %)				
A1	12 (6.28%)	10 (8.62%)	2 (2.70%)	0.125
A2	105 (54.97%)	66 (56.90%)	39 (52.00%)	
A3	74 (38.74%)	40 (34.48%)	34 (45.30%)	
Behavior of CD (n, %)				
B1	103 (53.93%)	57 (49.14%)	46 (61.33%)	0.100
B2	57 (29.84%)	36 (31.03%)	21 (28.00%)	
B3	19 (9.95%)	12 (10.34%)	7 (9.33%)	
B2+B3	12 (6.28%)	11 (9.48%)	1 (1.33%)	
Cause of hospital admission (n)				
Abdominal pain (yes/no)	163/28	101/15	62/13	0.410
Diarrhea (≥times/day) (yes/no)	32/159	21/95	11/64	0.560
Fever (yes/no)	105/86	61/55	44/31	0.458
Bloody stool (yes/no)	24/167	13/103	11/64	0.508
Disease activity (simple CDAI)	4.92 (3.00, 6.00)	5.00 (4.00, 6.00)	4.00 (3.00, 5.00)	0.003*
Endoscopic disease activity (SES-CD)	5.92 (0.00, 8.00)	6.00 (3.00, 9.75)	3.00 (0.00, 6.00)	0.001*
BMI (kg/m^2)	21.00±3.06	20.89±2.91	21.18±3.30	0.521
NRS2002	1.88 (1.00, 3.00)	2.00 (1.00, 3.00)	2.00 (0.00, 3.00)	0.303
PNI	45.56±6.31	44.64±6.83	46.99±9.47	0.051
Treatments during hospitalization, (n)				
Aminosalicylic acid (yes/no)	28/163	13/103	15/60	0.099
Thiopurinen (yes/no)	51/140	29/87	22/53	0.509
Corticosteroids (yes/no)	25/166	18/98	7/68	0.274
Anti-TNF (yes/no)	39/152	27/89	12/63	0.272
Enteral nutrition (yes/no)	80/111	54/62	26/49	0.012*

*Two-tailed p<0.05 was considered to be statistically significant. CDAI: Cohn's disease activity index; SES-CD: simple endoscopic ore for Crohn's disease; PNI: prognostic nutritional index.

Previous research had suggested that muscle loss was higher in males than females[11]. Sarcopenia was considered to be an age-related disease[2]. However, for CD patients with disease-related muscle loss, there was no statistical difference in age between the low and normal skeletal muscle groups. The high prevalence of skeletal muscle loss among adolescent CD patients was partially associated with the growth impairment caused by chronic intestinal inflammation and chronic caloric insufficiency. Younger IBD patients were prone to have active inflammation, with profound malnutrition and immunosuppression[12].

Table 2. Laboratory parameter of Crohn's disease between low and normal L3 skeletal muscle index groups.

Laboratory parameters	All patients (n=191)	Low L3SMI (n=116)	Normal L3SMI (n=75)	p-value
White blood cells (10^9/L)	5.96±2.64	5.89±2.68	6.07±2.59	0.647
Neutrophils (10^9/L)	4.02±2.33	4.04±2.40	3.99±2.23	0.882
Lymphocytes (10^9/L)	1.34±0.63	1.28±0.65	1.44±0.60	0.094
Eosinophils (10^9/L)	0.12±0.13	0.12±0.14	0.12±0.12	0.981
Hemoglobin (g/L)	123.58±22.46	117.27±17.80	133.34±25.38	<0.001*
Platelets (10^9/L)	233.00 (180.50, 297.50)	240.00 (178.75, 332.75)	229.00 (188.00, 261.00)	0.193
NLR	3.84±4.10	4.10±4.64	3.44±3.44	0.285
PLR	181.60 (125.50, 269.45)	206.10 (137.89, 354.62)	146.02 (111.76, 217.88)	0.006*
ESR (mm/h)	14.00 (5.50, 29.00)	16.50 (8.75, 40.25)	12.00 (5.00, 27.00)	0.064
CRP (mg/L)	3.60 (0.62, 20.78)	6.57 (0.54, 52.63)	6.04 (0.81,17.71)	0.466
D-Dimer (mg/L)	0.26 (0.12, 0.42)	0.26 (0.14, 0.39)	0.25 (0.10, 0.49)	0.415
Serum calcium (mmol/L)	2.32±0.21	2.29±0.24	2.36±0.17	0.017*
Serum albumin (g/L)	39.30±5.70	38.26±5.34	40.97±5.92	0.001*
Serum prealbumin (g/L)	222.80±68.66	204.23±65.65	252.99±62.90	<0.001*
Serum retinol-binding protein (g/L)	36.72±16.21	33.66±14.95	41.60±17.05	0.002*
Serum cholinesterase (μ/L)	7192.06±1929.63	6746.36±1761.80	7897.74±1984.42	<0.001*
Serum creatinine (μmol/L)	62.32±19.44	57.61±17.31	69.77±20.38	<0.001*
Serum urea (mmol/L)	4.43±2.01	3.98±1.89	5.14±2.00	<0.001*
Serum cystatin C (mg/L)	0.79±0.29	0.76±0.21	0.82±0.36	0.192
Serum creatinine/cystatin C	83.08±29.98	78.88±32.08	89.78±25.10	0.020*

NLR: neutrophil–lymphocyte ratio; PLR: platelet–lymphocyte ratio; ESR: erythrocyte sedimentation rate. *Two-tailed p<0.05 was considered to be statistically significant.

Table 3. Univariate and multivariate logistic analysis of predictor on Crohn's disease-associated muscle loss.

Variable	HR	95%CI	p-value	OR	95%CI	p-value
	Univariate analysis			Multivariate analysis		
Gender (female)	4.204	1.555–11.366	0.005*	2.939	1.386–6.233	0.005*
Age (years)	0.965	0.935–0.996	0.027*	1.031	1.006–1.057	0.016*
Disease duration (months)	1.011	1.001–1.021	0.028*	0.988	0.980–0.996	0.003*
Disease activity (simple CDAI)	1.083	0.868–1.351	0.480			
Endoscopic activity (SES-CD)	1.086	0.989–1.192	0.044*	0.923	0.855–0.996	0.040*
PNI	0.962	0.924–1.001	0.055	1.082	0.966–1.212	0.174
Lymphocyte (10^9/L)	0.936	0.399–2.196	0.880			
Hemoglobin (g/L)	0.976	0.944–1.009	0.157			
PLR	1.003	0.999–1.008	0.128			
ESR (mm/h)	0.992	0.975–1.010	0.382			
Serum albumin (g/L)	1.128	0.991–1.285	0.069	1.079	1.009–1.154	0.027*
Serum prealbumin (g/L)	0.991	0.979–1.002	0.116			
Serum cholinesterase (μ/L)	1.000	0.999–1.000	0.101			
Serum calcium (mmol/L)	0.252	0.033–1.954	0.187			
Serum urea (mmol/L)	0.723	0.527–0.993	0.045*	1.255	0.998–1.579	0.052
Serum creatinine (μmol/L)	0.936	0.892–0.981	0.006*	1.037	1.011–1.063	0.005*
Serum retinol-binding protein (g/L)	1.018	0.977–1.061	0.384			
Creatinine/cystatin C	1.000	0.983–1.018	0.990			

The variables with p<0.100 were calculated by logistic regression. *Two-tailed p<0.05 was considered to be statistically significant. PLR: platelet–lymphocyte ratio; ESR: erythrocyte sedimentation rate; PNI: prognostic nutritional index.

The CD-associated skeletal muscle loss was largely related to the duration of disease and the severity of disease activity, especially endoscopic disease activity. In our study, disease duration and SES-CD were independently associated with low L3SMI. The chronic, relapsing, persistent systemic inflammation increased disease severity with complications, longstanding active disease, and disease affecting small bowel absorption leading to CD-associated muscle loss[12]. Endoscopic disease activity may contribute to the development of malnutrition and sarcopenia by the mechanisms of malabsorption, enteric nutrient loss, and reduced energy intake due to disease manifestations[13]. Endoscopic disease activity was associated with high-level inflammation markers (such as NLR, PLR, and CRP) and poor nutrition markers (such as serum albumin, prealbumin, hemoglobin, and PNI). Thus, inflammation and nutrition play important roles in the occurrence and development of sarcopenia. This means that the primary treatment target of endoscopic healing in CD may be a benefit for the disease-related skeletal muscle loss.

Malnutrition was a highly prevalent complication in patients with IBD driven to bad outcomes. It was considered to be a principal mechanism involved in the genesis of sarcopenia[14]. During the malnutrition screening of NRS2002 and PNI, there was no significant difference between the two groups, partially because current malnutrition screening tools do not incorporate IBD-specific characteristics such as physician global assessment, steroid therapy, and endoscopic disease activity. These tools were considered to be less adequate for screening malnutritional CD patients[13]. The common nutritional status markers including albumin, prealbumin, retinol-binding protein, and cholinesterase were significantly reduced in low muscle mass patients. Notably, serum albumin independently predicts the low muscle mass of CD patients.

We established the relationship between the clinical and laboratory factors and CD-related skeletal muscle loss in order to provide evidence for the effective prevention of CD-related skeletal muscle loss. Meanwhile, the limitations were obvious. First, this is a single-center retrospective study. Patients in the study cannot be complete homogeneous. Second, the disease manifestations and complications were complex, and the skeletal muscle mass of patients was changeable during the acute and remission stages of the disease. We enrolled the first admission of patients with an acute attack of the disease during the study and were unable to discern the skeletal muscle dynamics in the disease.

In conclusion, the factors that affected CD-related muscle loss were complex and multifaceted. The gender, age, and duration of the disease were uncontrollable factors. The treatment target of mucosal healing and improved nutritional status may be beneficial for maintaining muscle mass. We initially discussed the skeletal muscle metabolic markers based on laboratory parameters. Impaired skeletal muscle synthesis rather than muscle catabolism is associated with skeletal muscle mass among CD patients, but further research is needed.

AUTHORS' CONTRIBUTIONS

JZ: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Writing–original draft, Writing –review & editing. **BJ:** Data curation, Formal Analysis, Methodology, Writing – review & editing. **SQ:** Data curation, Formal Analysis, Investigation, Project administration, Writing – review & editing. **LL:** Data curation, Formal Analysis, Supervision, Project administration, Writing – review & editing. These authors contributed equally to this article.

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ORIGINAL ARITCLE

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Vascular endothelial growth factor gene insertion/deletion polymorphism is associated with Vitamin D level in Turkish patients with coronavirus disease 2019

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SUMMARY

OBJECTIVE: Coronavirus disease 2019 emerges as a disease caused by severe acute respiratory syndrome coronavirus 2. It is a systemic disease associated with vascular inflammation and endothelial damage. In this study, we aimed to investigate whether vascular endothelial growth factor gene insertion/deletion polymorphism is associated with coronavirus disease 2019 in the Turkish population.

METHODS: The study included 179 participants (79 patients with coronavirus disease 2019 and 100 controls). DNA isolation was made from peripheral blood, and then the polymerase chain reaction analysis was performed.

RESULTS: When we analyze vascular endothelial growth factor gene insertion/deletion polymorphism in the study group, we found that the DD genotype and D allele were found to be statistically significantly different when compared to coronavirus disease 2019 patients with high vitamin D value (p=0.005 for DD genotype and p=0.006 for D allele) in the control group. In this high-level control group, when we analyze II+ID genotype versus DD, a statistically significant difference was also detected (p=0.007).

CONCLUSION: As a result of the study, we found that DD genotype and D allele were associated with vitamin D level in Turkish patients with coronavirus disease 2019.

KEYWORDS: Vascular endothelial growth factors. Vitamin D. Coronavirus disease 2019. Polymerase chain reaction.

INTRODUCTION

Pathogens of the coronavirus family can infect both humans and animals. A novel coronavirus (nCoV) was recently identified, leading to severe pneumonia cases in the Chinese city of Wuhan at the end of 2019. With its rapid spread, it became a global threatening pandemic after causing an epidemic throughout China. The World Health Organization (WHO) designated the viral disease as COVID-19 (i.e., coronavirus disease 2019) in February 2020. The virus that causes COVID-19 infection has been renamed severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) to replace the previous name 2019-nCoV. The purpose of this review was to outline the three-phase clinicogenomic course of COVID-19 immune syndrome as identified in a recent bioinformatics study[1].

Considering the pathogenesis of COVID-19, the infection starts with the binding of the virus to the angiotensin converting enzyme 2 (ACE 2) receptors expressed in various tissues, thus triggering an excessive immune response. Cytokine storm resulting from the overproduction of proinflammatory cytokines has been associated with severe progression of COVID-19 infection and organ damage. The pathophysiological mechanism of COVID-19 infection has not been fully elucidated, but the effects of genetic variations in genes in inflammation-related pathways on the course and severity of the infection are being investigated. In older adults and people with comorbidities, COVID-19 infection is more severe. COVID-19 infection is characterized by various symptoms such as fever, cough, shortness of breath, weakness, muscle aches, taste and smell disorders, diarrhea, and headache. The results of studies on genetic variations show differences between populations[2]. The vascular endothelial growth factor (VEGF) family and its receptors are key regulators of angiogenesis and barrier function. The VEGF family consists of VEGF-A, PlGF, VEGF-B, VEGF-C, VEGF-D, and VEGF-E. The researchers detected elevated plasma levels of VEGF-A in the serum of COVID-19 patients, which were found to be correlated with disease severity[3,4]. VEGF-B levels

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did not appear to be altered in COVID-19, while VEGF-D level, which promotes angiogenesis and lymphangiogenesis, was lower in COVID-19 compared to healthy control[5]. Kong et al. identified high VEGF-D as the most important indicator of disease severity in a small cohort of COVID-19 patients[6]. Further studies are needed to clarify these contrast findings and the potential mechanistic role of VEGF-D in COVID-19. Based on these findings, we aimed to investigate the relationship between *VEGF* gene insertion/deletion polymorphism and COVID-19 in the Turkish population.

METHODS

Study sample

This study included 179 participants (79 patients with COVID-19 and 100 controls). The study was conducted with 79 patients who were diagnosed with COVID-19 and 100 healthy controls in Samsun Education and Research Hospital between 2021 and 2022. The University of Samsun Health Science Clinical Ethical Committee approved the study (the ethical number is GOKA 2021/10/18). All the participants were informed about the study and signed the informed consent form. Patients' information was obtained and white blood cells, neutrophils, lymphocytes, platelets, C-reactive protein (CRP) (mg/dL), D-dimer (ng/mL), vitamin D (ng/mL), monocytes, prothrombin time, activated partial thromboplastin time, international normalized ratio, ferritin, and procalcitonin (PCT) values were recorded.

DNA isolation and polymerase chain reaction methods

DNA isolation was performed from 179 samples belonging to the patient and control groups. It was carried out from 2 mL peripheral blood sample taken into a tube with EDTA. After DNA isolation, a polymerase chain reaction (PCR) was performed using primers. The promoter region of the vascular endothelial growth factor gene was amplified by PCR. For the reaction, 50 ng of DNA, 1 µM from each primer, 10 µM dNTP from each dNTP, 1.5 mM $MgCl_2$, 0.2 units of Taq polymerase, and 10´ PCR buffer were placed into 25 µL total mixture. The PCR conditions were as follows: the initial denaturation was applied for 4 min at 94°, 35 cycles, and the denaturation was applied for 45 s at 95° and 45 s at 62°. Forward primers such as 5'-GCTGAGGATGGGGCTGACTAGGTA-3' and reverse primers such as 5'-GTTT CTGACCTGGCTATTTCCAGG-3' were used. Elongation was performed at 72° for 45 s, and the final extension was performed at 72° for 7 min. We visualized the amplification products on a 2.5% agarose gel. The amplified PCR product size of VEGF was the 228 bp fragments showing the D allele and the 221 bp fragments showing the I allele. To check the results, 20% of the randomly selected samples were reworked and a 100% match was found.

STRING analysis

STRING database annotates the functional interactions between the proteins in a cell. In this study, VEGF protein interactions were evaluated.

Statistical analysis

The SPSS 20 program was used in the statistical analysis of our study. Chi-square analysis was used to calculate genotype distributions and allele frequencies. The OpenEpi program was also used for genotype distributions and grouped genotype and allele comparisons. In the statistical analysis results, the p-value >0.05 was accepted as statistically significant.

RESULTS

The mean age of the control group was 56.44±19.08 years, and the mean age of the patients was 55.84±13.82 years. In the patient group, the rate of women was 55.7%, while the rate of men was 44.3%. In the control group, the rate of women was 32% and the rate of men was 17%. Table 1 represents the clinical characteristics of COVID-19 patients. When the control group with normal vitamin D value was compared with the

Table 1. Clinical characteristics of coronavirus disease 2019 patients.

Clinical findings of patients	Mean±SD
White blood cells	9.48 (7.30)
Neutrophils	6.67 (6.25)
Lymphocytes	1.19 (0.73)
Platelets	244.40 (101.68)
C-Reactive protein (mg/dL)	81.03 (89.81)
D-dimer (ng/mL)	2.65 (5.71)
Vitamine D (ng/mL)	12.96 (6.26)
Monocytes	0.51 (0.35)
Prothrombin time	12.52 (1.54)
Activated partial thromboplastin time	24.15 (7.64)
International normalized ratio	1.10 (0.14)
Ferritin	533.50 (570.36)
Procalcitonin (PCT)	0.55 (2.01)
Services	29 (36.7)
Intensive care units	50 (63.3)

patients (p=0.232), there was no statistical difference between genotype distributions and allele frequencies, but a statistically significant difference was found when compared with the control group with high vitamin D value (Table 2). The results of our study showed that the DD genotype and D allele were found to be statistically significantly different when compared to COVID-19 patients with high vitamin D value (p=0.005 for DD genotype and p=0.006 for D allele) in the control group. In this high-level control group, when we analyze II+ID genotype versus DD, a statistically significant difference was also detected (p=0.007) (Table 3).

STRING analysis

By analyzing the VEGF protein with the STRING database, we found the predicted functional partners of the protein as follows: vascular endothelial growth factor receptor 3 (FLT4), vascular endothelial growth factor receptor 2 (KDR), neuropilin 1, the membrane-bound isoform 1 (NRP1), vascular endothelial growth factor receptor 1 (FLT1), neuropilin 2 (NRP2), hypoxia-inducible factor 1-alpha (HIF1A), high-affinity nerve growth factor receptor (NTRK1), cadherin 5 (CDH5), fibronectin 1 (FN1), and fibroblast growth factor 2 (FGF2). The interaction network of these proteins is shown in Figure 1.

DISCUSSION

COVID-19, caused by SARS-CoV-2, has become a persistent health emergency since its outbreak in late 2019[6]. Progression of COVID-19 often involves excessive pro-inflammatory cytokines and mediators[7]. VEGF, a key factor involved in vascular permeability and inflammation[8], was found to be skyrocketed in the blood of COVID-19 patients and related to disease severity[9].

Vitamin D regulates the immune modulatory mechanisms by decreasing the proinflammatory environment *in vivo* and

Table 2. Genotype distribution and allele frequencies of coronavirus disease 2019 patients and control with high vitamin D level.

Vascular endothelial growth factor insertion/deletion	COVID-19 group n=79 (%)	Control group n=100 (%)	χ^2	OR (95%CI)	p-value
Genotypes					
II	9 (11.4)	2 (4.1)			**0.005**
ID	53 (67.1)	45 (91.8)			
DD	17 (21.5)	2 (4.1)			
II+ID:DD	62:17	47:2	7.275	0.157	**0.007**
DD+ID:II	70:9	47:2	2.058	0.333	0.151
Alleles					
I	71	49	0.6227	0.4925–1.352	0.4301
D	87	49			

Bold values indicate that p<0.05 is statistically significant.

Table 3. The distribution of the genotypes and alleles in coronavirus disease 2019 and control groups.

Vascular endothelial growth factor insertion/deletion	COVID-19 group n=79 (%)	Control group n=100 (%)	χ^2	OR (95%CI)	p-value
Genotypes					
II	9 (11.4)	17 (17)	2.921		0.232
ID	53 (67.1)	71 (71)			
DD	17 (21.5)	12 (12)			
II+ID:DD	63:17	88:12	2.814	0.5073	0.093
DD+ID:II	71:9	83:12	0.078	1.14	0.779
Alleles					
I	71	105	2.001	0.7409	0.157
D	87	95			

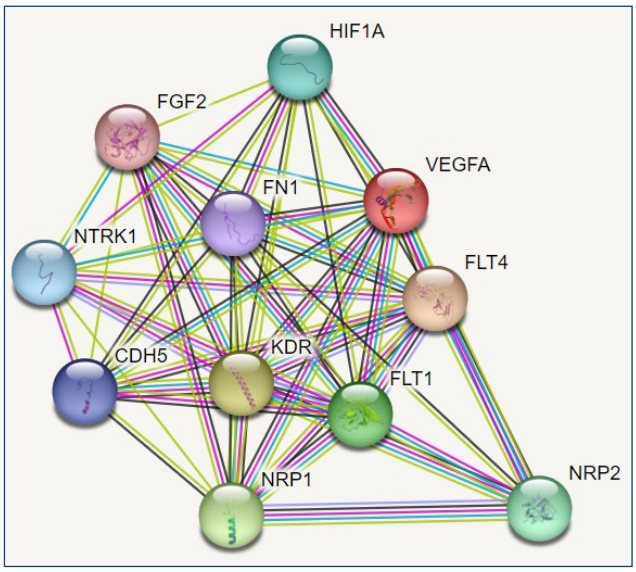

Figure 1. Interactions of vascular endothelial growth factor-A protein, according to STRING database predictions.

increasing the secretion of anti-inflammatory cytokines. It has been reported that 25(OH)D deficiency, a physiologically quantifiable form of vitamin D, is strongly associated with unfavorable clinical outcomes. Finally, no adverse effects of using high doses of vitamin D in COVID-19 and other circumstances have been reported[10].

Overall, melatonin is an intriguing compound, not unlike vitamin D, which is pleiotropic in activity and responsive to light-dark cycles. From a scientific perspective, melatonin acts as a powerful antioxidant that can cross the blood-brain barrier, inhibit inflammation, and interact with the gut microbiome. From a clinical point of view, melatonin imbalance may indicate "darkness deficiency" in much the same way that vitamin D may infer whether or not someone has a "light deficiency"[11].

Soluble levels of a circulating form of the VEGF-A receptor are markedly increased in COVID-19 patients and correlated with disease severity[2-4]. Under normal conditions, this form is electrostatically bound to proteoglycans and thus sequestered[12]. Its elevated level could therefore theoretically be a result of damage. Its overexpression has been well shown to promote endothelial dysfunction, particularly during preeclampsia[13]. Dupont et al. reported that plasma levels (n=46) at admission to the ICU are associated with the need for mechanical ventilation, the need for vasopressor support, the development of severe acute kidney injury, and death[14]. However, unlike preeclampsia, its high levels in COVID-19 are clearly not accompanied by a decrease in PlGF[15,16]. This finding is apparently very consistent, as the rate remains low in pregnant women. It allows a good distinction between COVID-19 pneumonia and true preeclampsia and preeclampsia-like symptoms due to COVID-19[17]. VEGF plays a primary role in maintaining the growth, development, and maintenance of a healthy circulatory system, thereby ensuring normal angiogenesis[18]. They bind with VEGFR and activate the endothelial cell. Alveolar immune regulation is important and is maintained by the integrity of the endothelial barrier in lung tissue, which is crucial in patients affected by COVID-19[19]. Serum levels of VEGF have been found to be elevated in people affected by SARS-CoV-2. Based on these findings, we aimed to investigate whether VEGF insertion/deletion gene polymorphism was associated with COVID-19 and we found that the DD genotype and D allele was associated with vitamin D level in Turkish COVID-19 patients. In COVID-19, neutrophils, monocytes, and macrophages become hyperactivation, and as a result, it shows that it can lead to dysregulation in the inflammatory response and cytokine storm (Fernandes 2022)[20-22]. These changes have also been associated with an increase in various interleukins and VEGFs in COVID-19 patients. A large-scale study reports that vitamin D mechanisms also play an important role in these processes[23].

CONCLUSION

In accordance with our results, it is previously reported that it has been hypothesized that sufficient vitamin D levels could prevent cytokine storm while promoting an adequate adaptive immune response in patients with COVID-19. In the literature, there are no studies about investigated relation between *VEGF* gene insertion/deletion polymorphism and COVID-19. Our results would provide important contributions to the literature. Genetic polymorphisms are very important in the field of medicine and will allow for the discovery of new treatment strategies and drugs as their mechanisms are understood.

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AUTHORS' CONTRIBUTIONS

SY: Conceptualization, Project administration, Resources, Validation, Writing – original draft. **ST**: Conceptualization, Formal Analysis, Resources, Software, Visualization, Writing – review & editing. **RA**: Data curation, Formal Analysis, Resources. **OS**: Data curation, Formal Analysis, Resources.

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ORIGINAL ARTICLE

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Effect of adenomyosis on prognosis of patients with endometrial cancer

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SUMMARY

OBJECTIVE: Our goal was to contrast the prognoses of patients with endometrial cancer who had adenomyosis against those that did not.

METHODS: All patients who had received surgical staging for hysterectomy-based endometrial cancer had their medical data retrospectively examined. The analysis covered 397 patients, who were split into two groups depending on the presence of adenomyosis. Comparisons were made between patients covering type of surgery, histopathology, endometrial cancer stage, lymphovascular space invasion, presence of biochemical or histochemical markers, adjuvant therapy, presence of adenomyosis in the myometrial wall, and outcomes in terms of overall survival and disease-free survival.

RESULTS: There is no statistically significant difference in the 5-year disease-free survival or overall survival rates between endometrial cancer patients with and without adenomyosis. This is based on comparisons of tumor stage, tumor diameter, histological type and grade of tumor, myometrial invasion, lymphovascular space invasion, and biochemical markers that affect the course of the disease. The median follow-up times were 61 months for the adenomyosis-positive group and 56 months for the group without adenomyosis.

CONCLUSION: Coexisting adenomyosis in endometrial cancer has no bearing on survival rates and is not a prognostic factor.

KEYWORDS: Adenomyosis. Endometrium cancer. Gynecology. Prognosis. Survival.

INTRODUCTION

Endometrial cancer (EC) is the sixth most detected cancer and the 14th most prevalent cause of cancer death in women globally[1], affecting 2.8% of women at some point in their lifetime[2]. Patients typically present with uterine-confined pathology and have high survival rates. The key predictors of cancer outcome are histological character, tumor grade and size, age, degree of myometrial invasion, lymph node involvement, and disease stage[3].

One of the most prevalent pathological signs in hysterectomy tissues is ectopic endometriosis (known as adenomyosis), which spreads from the endometrium into the uterus, the myometrium, and the endometrial glands. With a varying prevalence of 12–66%[4], it is one of the most common ancillary histopathological results of EC, especially of the endometrioid histotype.

Numerous studies have investigated whether adenomyosis is present in EC patients. Those that investigated the importance of adenomyosis in endometrial adenocarcinoma suggested that it had negative impacts on EC[5,6]. However, in other studies, adenomyosis with EC has been linked to early-stage malignancy and extended surveillance[7]. Furthermore, subsequent investigations have demonstrated that adenomyosis does not negatively affect the prognosis and questionnaires of EC patients[8,9]. It is therefore still unclear whether EC and adenomyosis are related.

Long-term evaluation of the prognosis and surveillance of patients with EC has focused on the occurrence of adenomyosis in this population. As a result, our goal was to assess how adenomyosis affected the prognosis of women with EC.

METHODS

All women (n=425) operated on for EC between January 2016 and December 2021 had their medical records retrospectively evaluated. We established the inclusion and exclusion criteria to qualify patients for additional investigation. To be included, patients required a preoperative assessment, a thorough medical history record, surgical therapy entailing at least a hysterectomy, and a record of postoperative pathology results. Patients were excluded if they had received preoperative chemotherapy

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or endocrine treatment, had primary tumors in other parts of the body (such as the breast or colon), or if significant data were lacking. The National Ethics Committee gave the Bakırköy Dr. Sadi Konuk Training and Research Hospital in Istanbul permission to conduct this study (No. 2020-01-07, Bakırköy Dr. Sadi Konuk Training and Research Hospital).

The pathological reports from the chosen patients (n=397) were thoroughly examined for EC. We excluded 28 individuals, 12 of whom had previously undergone chemotherapy and surgery for breast cancer, and 16 of whom had already undergone chemotherapy and surgery for colon cancer.

All patients had had hysterectomy procedures, either with or without pelvic/paraaortic lymphadenectomy and pelvic wash cytological analysis. They all had been closely monitored, received treatment, had radiotherapy or chemotherapy combined with radiotherapy according to their stages, and received follow-ups every 6 months if deemed necessary following the multidisciplinary team conference. Once the clinical presentations and prognoses of the two concurrent disorders were understood, the clinicopathological traits and oncological outcomes were assessed. The data variables considered included age, menopausal status, tumor grade, stage, preoperative cancer antigen CA-125 level, and adenomyosis status. Tumor stage and histological grade were established in accordance with the National Comprehensive Cancer Network guidelines[10]. Our primary conclusion is that the prognostic factors used are poor. These include tumor stage, histological type and grade, lymphovascular space invasion, myometrial invasion, age and tumor diameter, biochemical and histochemical markers, and overall survival (OAS) and disease-free survival (DFS) rates.

High (II–III) and low (I) histological grades were distinguished. From hospital records, patient information was also gathered, including age, body mass index (BMI), gravity, nulliparity, medical comorbidities, operation type, adjuvant treatment (chemotherapy and/or radiotherapy), follow-up, and relapse. Patients were divided into two groups based on the presence or absence of adenomyotic tissue. Women in group A had adenomyosis in addition to EC, and those in group B had EC only. The data related to the two groups were statistically compared.

The SPSS statistics software for Windows version 21.0 (IBM Corp., Armonk, NY) was utilized for the statistical analysis. The W2 test was used to compare categorical data, the Kaplan–Meier tests were used to compute OAS and DFS, and the log-rank test was used to compare the results. For OAS, Cox proportional hazards regression models were run with single and multiple covariates and a 95% confidence interval (CI). Statistics were found to be significant at $p<0.05$.

RESULTS

The demographic data, surgical procedures, and outcomes are evaluated in Table 1. The comparisons in Table 1 include age (p:0.342), BMI (p:0.257), gravity (p:0.947), nulliparity (p:0.448), menopausal status (p:0.757), surgical method (laparoscopy vs. laparotomy) (p:0.279), degree of pelvic-only lymphadenectomy (p:0.070), and pelvic and paraaortic lymphadenectomy (p:0.808). The analysis of the demographic information and the surgical approach showed no statistically significant differences between the groups. The clinical and pathological characteristics of the study, preoperative and postoperative Ca-125 values, and other biochemical and histochemical data (p:0.562–p:0.455) are shown in Table 2. These include tumor grade (p:0.309), perineural involvement (p:0.782), uterine lower segment involvement (p:0.368), depth of myometrial invasion (p:0.565), lymphovascular space invasion (LVSI) (p:0.302), tumor size (cm) median (range) (p:0.595), and cervical involvement (p:0.068). These markers are poor prognostic indicators for EC. Our analyses identified no difference between these predictive indicators when examining both groups. In the same table, estrogen receptor (ER) positivity and progesterone receptor (PR) positivity, as well as p16 and p53 positivity were also evaluated as biochemical and histochemical markers and are included as positivity of the ER (p:0.382), positivity of the PR (p:0.242), the presence of p16 (p:0.437), and the presence of p53 (p:0.699). When comparing the two groups,

Table 1. Patient charecteristic demographic variables and surgical variables.

Variable	Adenomyosis		p
	Yes (n=99)	No (n=297)	
Age at surgery, median (range)	60.59	60.50	0.342
Gravity	3.65	3.64	0.947
Nulliparity	4	14	0.448
BMI (kg/cm^2)†	36.42	36.83	0.257
Menapausal status			0.757
Premenopausal (n:%)	10 (10)	33 (11.1)	
Postmenopausal (n: %)	90 (90)	264 (88.9)	
Surgical approach, (n: %)			0.279
Laparoscopy	81 (81.8)	240 (80.8)	
Laparotomy	17 (18.2)	57 (19.2)	
Lymphadenectomy, (n: %)			
Pelvic alone	12 (12.1)	33 (11.1)	0.070
Pelvic and para-aortic	7 (7.4)	30 (10.5)	0.808

p<0.05 accepted as statistically significant. †Body mass index.

Table 2. Tumor characteristics, biochemical and histochemical markers.

Variable	Adenomyosis		p
Tumor grade, n (%)	Yes (n=99)	No (n=297)	0.309
1	27 (27.6)	62 (21.8)	
2	55 (56.1)	157 (53.3)	
3	16 (16.3)	65 (22.9)	
Perineural ınvolvement	21 (21.2)	57 (19.3)	0.782
Uterine lower segment involvement	14 (14.3)	50 (16.9)	0.368
Tumor size (cm) Median (range)	3.31	3.19	0.595
Deep (≥50%) myometrial invasion, n (%)	22 (23.6)	71 (23.7)	0.565
Lymphovascular space involvement, n (%)	89 (89)	252 (84.8)	0.302
Cervical involvement, n (%)	2 (2.02)	12 (4.02)	0.394
Adnexial involvement, n (%)	4 (4.04)	14 (4.6)	0.459
Positive peritoneal cytology, n (%)	4 (4.04)	10 (3.3)	0.496
Preoperative Ca-125 (U/mL)	82	238	0.562
Postoperative Ca-125 (U/mL)	74	221	0.455
ER (+) n (%)†	49 (49)	126 (42.4)	0.382
PR (+) n (%)‡	34 (34)	131 (44)	0.242
P53 existence n (%)	26 (26)	95 (34)	0.699
P16 existence n (%)	12 (12)	46 (15.5)	0.437

p<0.05 accepted as statistically significant.†Estrogen receptor. ‡Progesteron receptor.

no statistically significant differences exist. The group showing PR positive with adenomyosis is higher, but no statistically significant differences were found (p:0.242).

The 5-year DFS and OAS rates between the two groups did not differ in a way that was statistically significant. Median time to recurrence was longer in the adenomyosis-negative group than in the adenomyosis-positive group (61 months vs. 56 months) (p:0.278). For patients with and without adenomyosis, the 5-year OAS was 97 vs. 91.4% (HR 1.51; 95%CI 0.52–4.20; p=0.230) and the 5-year DFS was 94 vs. 92% (HR 1.57; 95%CI 0.51–5.20; p=0.440), respectively. Kaplan-Meier plots are shown in Figure 1. When patients with and without adenomyosis were compared, it was shown that the OAS after 5 years was higher in the adenomyosis-free people. However, no statistically significant changes were found.

DISCUSSION

There has long been interest in the clinical relevance of the coexistence of adenomyosis and EC. Numerous studies have been documented on the relationship between EC and adenomyosis. Our research sought to ascertain whether adenomyosis had a favorable or unfavorable prognostic impact on EC.

Poor prognostic factors for EC include tumor grade, perineural involvement, uterine lower segment involvement, tumor size, depth of myometrial involvement, LVSI, tumor size (cm), cervical involvement, positive peritoneal cytology, and stage of the disease. When these poor prognostic indicators were examined, some studies found that groups with adenomyosis had a worse prognosis than those without adenomyosis[11]. However, in our investigation, no statistically significant difference was discovered.

In one study, OAS in EC with adenomyosis was evaluated as relatively higher than OAS reports in EC alone[12]. This favorable prognostic outcome could be explained by the mechanical role of adenomyosis in preventing cancer invasion through the hypertrophic and hyperplastic myometrial stroma that surrounds it[11]. When cases of EC that developed from adenomyosis were examined in a meta-analysis, it was discovered that the effects of poor prognostic indicators were amplified by deep myometrial tumor involvement, high-grade, complicated stage, and the presence of positive node metastases[13]. In our research, we demonstrated that the prognosis of the disease is unaffected by the existence of adenomyosis in EC.

Although the exact cause of malignant transformation in adenomyosis is still unknown, several writers have suggested

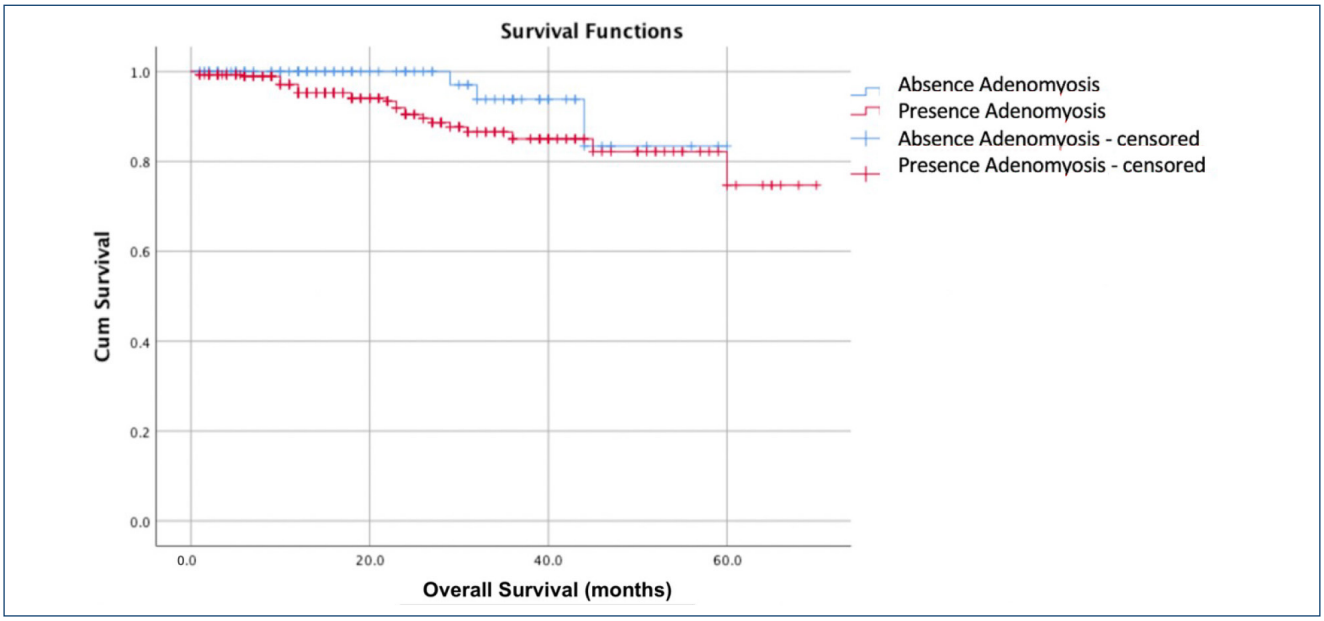

Figure 1. Overall survival curve for the groups.

that genetic and epigenetic factors may be involved. Due to the absence of an anatomic set in the basal part of endometrial tissue, cancer first develops within the myometrial part and smoothly spreads to the myometrial stromal layer[14]. Cancer that has directly invaded the myometrial stromal tissue extends rapidly to the lymphatic and circulatory systems. However, many molecular elements of the malignant development of adenomyosis remain unknown. The relationship between adenomyosis and the disruption of heterozygosity in the DNA mismatch repair gene has only been briefly described in research[15].

Our results suggest that the superior EC prognosis of patients with adenomyosis given to individuals without adenomyosis is not explained by clinical characteristics. After the establishment of the Proactive Molecular Risk Classifier for Endometrial Cancer (ProMisE) and The Cancer Genome Atlas (TCGA) Research Network discoveries, EC may now be divided into four molecular prognostic categories: mismatch repair defective, POLE-mutated, p53-mutated, and p53 wild-type. Groups with POLE mutations and p53 wild-type patients have improved prognoses[16]. Immunohistochemical and hematological markers are important indicators in determining the prognosis of EC[17,18]. Additionally, we assessed the positivity of p53 and p16 in groups with and without adenomyosis. When we compared the two groups, we were unable to detect any statistically significant difference.

A few clinical characteristics have been recognized as EC prognostic indicators[17]. Particularly, it has been found that parous EC women have a much better prognosis than nulliparous women[13]. Age has also been demonstrated to be associated with the prognosis for EC; according to a German population-based investigation, 5-year relative survival fell from 90.0% in the age group of 15–49 years to 74.8% in the age group of over 70 years[19]. When these factors were compared between the two groups in our study, there was no statistically significant difference between them, in contrast to the demographic literature data mentioned above.

Additionally, in EC patients, a greater ER/PR expression status was linked to a better DFS[20]. In our study, we assessed the positivity of ER and PR in both groups. Patients with PR-positive adenomyosis experienced OAS more frequently. However, no statistically significant change was found.

Due to the limited scope of the current investigation, only pathological examinations of women who had been treated with surgery for EC were carried out. The group with adenomyosis was chosen within these findings and contrasted with the group that could not be tracked in a blind manner. This study compared the impact of adenomyosis on EC and observed the p16 and p53 status.

Our research has some drawbacks. First, there is a deficiency of pathological evaluation to differentiate between ECs with adenomyosis and ECs developing from adenomyosis foci. These two disorders are histopathologically and clinically diverse, with different diagnostic criteria and biological characteristics. Histologically speaking, EC emerging in adenomyosis (EC-AIA) is identified by the presence of adenocarcinoma in the epithelium of the adenomyosis foci but not in the typically located

endometrium. In summary, the key difference between these two entities is whether EC is present in the eutopic endometrium[13]. ECs-AIA are strongly related to weak DFS, according to a recent comprehensive analysis comparing cancer results between ECs coexisting with adenomyosis and ECs-AIA. This finding was made after checking for grade, stage of the disease, and histotype[13]. Nevertheless, the rate of EC formation from adenomyosis, as demonstrated in this study, is less than 1%. This meta-analysis only includes case reports for EC, which arises from the backdrop of adenomyosis and exhibits poor prognostic features. Second, our study did not allow us to assess POLE mutations. We think that one of the crucial conditions for upcoming research on EC is the examination of the POLE mutation.

Our research concluded that adenomyosis is not significantly linked to the development of cancer. These results lead us to recommend that the presence of adenomyosis cannot be considered or further studied as a prognostic factor in EC.

AUTHORS' CONTRIBUTIONS

EŞ: Investigation, Methodology, Software, Writing – Original draft. **SY:** Methodology, Writing – Original draft, Writing-review &editing. **SK:** Data curation, Methodology, Visualization. **SG:** Conceptualization, Formal Analysis. **ÖAY:** Conceptualization, Formal Analysis, Software. **İAÖ:** Formal Analysis, Resources, Supervision. **LY:** Investigation, Supervision.

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ORIGINAL ARTICLE

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The clinical significance of lymphovascular space invasion in patients with low-risk endometrial cancer

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SUMMARY

OBJECTIVE: The aim of this study was to assess the effect of lymphovascular space invasion on recurrence and disease-free survival in patients with low-risk endometrial cancer.

METHODS: The study included patients with stage 1A, grade 1–2 endometrioid endometrial cancer who underwent a total hysterectomy and bilateral salpingo-oophorectomy with pelvic lymphadenectomy. Independent prognostic predictors of endometrial cancer recurrence were assessed using the Cox regression model. Binary logistic regression analysis was used to identify the predictors of distant recurrence. Kaplan-Meier analysis was used to describe survival curves, and the log-rank test was used to compare the differences in survival curves.

RESULTS: A total of 189 patients met the inclusion criteria, of whom 24 (12.7%) had lymphovascular space invasion. The median follow-up time was 60 (3–137) months. Distant recurrence was present in 11 of 22 patients who developed recurrence. Kaplan-Meier survival analysis showed that the 5-year disease-free survival rates of patients with lymphovascular space invasion(+) and lymphovascular space invasion(-) were 62.5 and 91.9%, respectively, which were significantly lower (p<0.001). In multivariate Cox regression analysis, the presence of lymphovascular space invasion (p<0.001) and age ≥60 years (p=0.017) remained as prognostic factors for reduced disease-free survival. In binary logistic regression analysis, only lymphovascular space invasion (adjusted OR=13, 95%CI=1.456–116.092, p=0.022) was a prognostic factor for distant recurrence.

CONCLUSION: lymphovascular space invasion is a prognostic risk factor for recurrence and distant metastasis and also a predictor of poorer disease-free survival outcomes in low-risk endometrial cancer.

KEYWORDS: Disease-free survival. Endometrial cancer. Recurrence.

INTRODUCTION

Low-risk endometrial cancer (EC) is defined as tumors confined to the uterine corpus with grade 1 or 2 endometrioid histology or invading less than 50% of the myometrium[1]. The 5-year survival rate for patients with low-risk EC is above 90%; however, 5–10% of these patients develop distant or locoregional recurrences afterward[2].

Lymphovascular space invasion (LVSI) is defined as the presence of adenocarcinoma of any extent within lymphatic vessels and/or small capillaries, outside the invasive tumor[3]. Although LVSI is considered a prognostic factor for EC by the European Society of Gynaecological Oncology (ESGO), the European Society for Radiotherapy and Oncology (ESTRO), and the European Society of Pathology (ESP), it has not yet been included in the International Federation of Gynecology and Obstetrics (FIGO) staging system[4,5]. Numerous studies have confirmed that the presence of LVSI is an independent predictor of distant or locoregional recurrence and decreased disease-free survival (DFS) in EC patients with FIGO stage I–III[6-15]. The prognostic significance of LVSI in low-risk EC is still unclear.

Therefore, we aimed to evaluate the impact of LVSI on recurrence and DFS in patients with low-risk EC.

METHODS

The study was approved by the Ethics Committee of Tepecik Education and Research Hospital (Ref: 2022/10-20) and conducted in accordance with the principles of the Declaration of Helsinki. The medical reports of patients who underwent

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surgery between January 2010 and December 2021 were used to collect pathological, surgical, and demographic data.

The inclusion criteria were as follows: (1) patients with an endometrioid histological subtype of EC, less than 50% myometrial invasion, and grade 1–2; (2) patients who had undergone pelvic lymph node dissection; (3) patients with no intraoperative evidence of extrauterine spread; and (4) patients with complete medical files containing information about age, comorbidities (hypertension or diabetes), detailed surgical procedures, pathological results (i.e., tumor size, LVSI, histological type, and grade), and postoperative adjuvant treatment.

The exclusion criteria were as follows: (1) patients with a non-endometrioid or mixed histological subtype of EC, or grade 3; (2) patients with FIGO stage higher than IA; (3) patients with concurrent malignancies; (4) patients with a history of radiotherapy or chemotherapy before surgery; and (5) patients with incomplete medical records and follow-ups.

All surgeries were performed by gynecological oncologists. The patients underwent a total hysterectomy and bilateral salpingo-oophorectomy surgeries, with a minimum requirement of pelvic lymphadenectomy. Pelvic lymphadenectomy was defined as the removal of lymphatic tissue from the external, internal, common iliac, and obturator regions. Expert gynecological pathologists evaluated all surgical specimens.

The multidisciplinary tumor board made decisions regarding adjuvant treatments based on ESGO, ESTRO, and the National Comprehensive Cancer Network (NCCN) guidelines. Patients were followed up every 3 months for the first 2 years, biannually for up to 5 years, and annually thereafter. At each follow-up visit, pelvic examination and ultrasonography of the pelvis and abdomen were performed. If there was suspicion of recurrence, a biopsy was performed if possible. If not, a computed tomography, magnetic resonance imaging, bone scintigraphy, or positron emission tomography scan was performed. Radiologists or gynecological pathologists with experience confirmed the recurrence on the multidisciplinary tumor board. Recurrences at the vaginal cuff, vagina, bladder, rectum, and pelvic lymph nodes were classified as locoregional, while all other recurrences (lymph nodes outside the pelvis, peritoneal carcinomatosis, liver, bone, lung, and abdomen) were classified as distant. If there were multiple recurrence sites, the recurrence was classified as distant. DFS was defined as the time from the date of surgical staging to the date of recurrence.

The study data were analyzed using version 20.0 of SPSS (statistical software package) by IBM Corp. in Armonk, NY, USA. The data were presented using number, percentage, mean, standard deviation, median, minimum, and maximum values. The t-test was used as a parametric test, while the Mann-Whitney U test was used as a non-parametric test based on the results of the normal distribution conformity test. The analysis of categorical data was conducted using the χ^2 test. The assessment for predictors of distant recurrence was performed using binary logistic regression analysis. Variables with $p<0.05$ in univariate regression were included in a multivariable model. The multivariate Cox regression model included independent prognostic predictors of EC recurrence with a p-value<0.05 in the univariate Cox regression model. To describe the survival curves, Kaplan-Meier analysis was used, and the differences between survival curves were compared using the log-rank test. A p-value <0.05 was considered statistically significant.

RESULTS

A total of 189 eligible patients were identified, and the characteristics of low-risk EC patients by LVSI status are shown in Table 1.

Tumor recurrence was reported in various locations, including the vaginal cuff (isolated) in nine patients, the pelvic and inguinal lymph nodes in one patient, the vaginal cuff and pelvic lymph nodes in two patients, the vaginal cuff and rectosigmoid colon in one patient, the lung and bone in one patient, the inguinal lymph nodes (isolated) in one patient, the small intestines in one patient, the liver and brain in one patient, and the peritoneum (peritonitis carcinomatosa) in five patients (not shown in table).

The patients were followed up for a median of 60 months (range, 3–137 months). Analysis using the Kaplan-Meier method showed that the 5-year DFS rates were significantly lower in patients with LVSI(+) (62.5%) compared to those with LVSI(-) (91.9%) ($p<0.001$) (Figure 1).

Furthermore, the results of univariate Cox regression analysis demonstrated that the presence of LVSI ($p=0.001$) and age ≥60 years ($p=0.04$) were associated with a reduced DFS. After conducting multivariate Cox regression analysis, the presence of LVSI ($p<0.001$) and age ≥60 years ($p=0.017$) were still found to be independent prognostic factors for a reduced DFS (Table 2).

Out of 22 patients who developed recurrence, 11 had a distant recurrence. Among those with distant recurrence, five had no LVSI while six had LVSI, and seven received adjuvant brachytherapy while four did not. Additionally, five out of six patients with LVSI and distant metastases received adjuvant brachytherapy. Binary logistic regression analysis was performed with age (<60 vs. ≥60 years), tumor size (<2 cm vs. ≥2 cm), grade (1 vs. 2), LVSI (no vs. yes), and adjuvant brachytherapy (no vs. yes) to evaluate the predictors of distant recurrence.

Table 1. Characteristics of low-risk endometrial cancer patients according to lymphovascular space invasion status.

Characteristics	Total n=189	LVSI (+)	LVSI (–)	p-value
		n=24 (12.7%)	n=165 (87.3%)	
Age (years, mean±SD)	57.3 (±9.09)	56.63 (±8.32)	57.39 (±9.21)	0.7
Age (years)				
<60	113 (59.8%)	16 (66.7%)	97 (58.8%)	0.462
≥60	76 (40.2%)	8 (33.3%)	68 (41.2%)	
Tumor size (cm)				
<2 cm	33 (17.5%)	2 (8.3%)	31 (18.8%)	0.262*
≥2 cm	156 (82.5%)	22 (91.7%)	134 (81.2%)	
Grade				
1	59 (31.2%)	2 (8.3%)	57 (34.5%)	0.01
2	130 (68.8%)	22 (91.7%)	108 (65.5%)	
Adjuvant brachytherapy				
No	125 (66.1%)	0 (0%)	125 (75.8%)	<0.001
Yes	64 (33.9%)	24 (100%)	40 (24.2%)	
Recurrence				
No	167 (88.4%)	16 (66.7%)	151 (91.5%)	0.002*
Yes	22 (11.6%)	8 (33.3%)	14 (8.5%)	

LVSI: lymphovascular space invasion; SD: standard deviation. *Fisher's exact test.

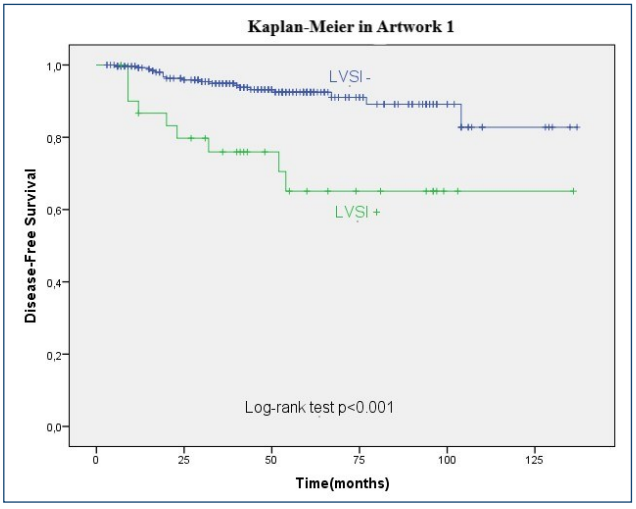

Figure 1. Kaplan-Meier curve.

Univariate analysis showed that LVSI (unadjusted OR=10.667, 95%CI=2.957–38.477, p£0.001) and adjuvant brachytherapy (unadjusted OR=3.715, 95%CI=1.045–13.204, p=0.043) were associated with distant recurrence. In multivariate analysis, only LVSI (adjusted OR=13, 95%CI=1.456–116.092, p=0.022) remained a prognostic factor for distant recurrence (data not shown in table).

DISCUSSION

This study demonstrated that LVSI is a significant predictor of decreased DFS and an independent risk factor for the development of distant recurrence in patients with low-risk EC. This result is consistent with previous studies by Tortorella et al.[16] and Ørtoft et al.[10] which also reported LVSI as a prognostic risk factor for recurrence, distant metastasis, and reduced DFS[10,16]. Although the Tortorella's study utilized a three-tiered scoring system for LVSI, distinguishing between absent, focal, and substantial involvement, it is worth noting that 45.7% (16/35) of patients with focal LVSI and 27.3% (6/22) of patients with substantial LVSI did not undergo lymph node evaluation[16]. According to the study by Ørtoft et al.[10] it was found that only a small proportion (9.4%) of patients in the low-risk group (defined as grades 1 and 2 with <50% myometrial invasion) underwent lymph node resection. However, there were no available data on the percentage of patients who tested positive for LVSI and underwent lymph node resection in this group[10]. Jorge et al.[17] demonstrated that the presence of LVSI elevated the risk of lymph node metastasis from 0.7 to 11.4% in stage 1A grade 1 tumors and from 1.3 to 13.2% in stage 1A grade 2 tumors[17]. According to the Tortorella's study, the lack of lymph node evaluation in a considerable proportion of LVSI-positive patients (38.8% or 22/57) and, according to the Ørtoft's study,

Table 2. The univariate and multivariate Cox regression analysis for disease-free survival in women with low-risk endometrial cancer.

Variables	Univariate analysis		Multivariate analysis	
	Hazard ratio (95%CI)	p-value	Hazard ratio (95%CI)	p-value
Age (years)				
<60	1			
≥60	2.435 (1.044–5.682)	0.040	2.849 (1.210–6.711)	0.017
Tumor size (cm)				
<2 cm	1			
≥2 cm	3.090 (0.687–13.905)	0.142	–	–
Grade				
1	1			
2	1.004 (0.417–2.421)	0.992	–	
LVSI				
No	1			
Yes	4.219 (1.767–10.073)	0.001	4.887 (2.024–11.798)	<0.001
Adjuvant brachytherapy				
No	1			
Yes	1.074(0.456–2.529)	0.869		-

LVSI: lymphovascular space invasion; CI: confidence interval.

the absence of information on the proportion of LVSI-positive patients who underwent lymph node resection may have led to confusion in assessing the prognostic significance of LVSI, as patients with occult lymph node metastasis may have been included. In contrast, our study performed lymph node dissection in all patients, eliminating the possibility of occult lymph node metastasis and ensuring greater clarity in the assessment of the prognostic value of LVSI.

The study conducted by Iida et al.[18] involved 98 patients with stage 1A, grade 1–2 EC, where all patients underwent lymph node dissection. Their results indicated that LVSI is neither a prognostic factor for recurrence nor a predictor of poor DFS[18]. According to the study by Iida et al.[18] the small number of patients who experienced recurrence (only three individuals) and had LVSI (only nine patients) may have limited the statistical power to detect a significant effect of LVSI on DFS rates and could have influenced its ability to act as a prognostic factor for recurrence.

According to the Nwachukwu et al.'s[19] study, only patients with stage 1A, grade 1 EC were evaluated, and LVSI was not found to be a predictive factor for recurrence in EC[19]. However, the exclusion of patients with grade 2 EC, despite being in the low-risk group, may have limited the evaluation of the effect of LVSI on recurrence in that study. In our study, we specifically aimed to assess the impact of LVSI on recurrence in patients with low-risk EC.

Similar to our study, Cusano et al.[7] showed lower DFS rates in patients with LVSI, but they were unable to demonstrate a significant association between LVSI and recurrence[7]. However, the inclusion of patients with stage 1B and grade 3 tumors in the multivariate analysis may have caused LVSI to lose its significance as a prognostic factor.

One of the most comprehensive studies on LVSI in EC was conducted by Ayhan et al.[9] Their study found that patients with LVSI had worse DFS, but the presence of LVSI was not an independent prognostic factor for recurrence[9]. It is worth noting that in this study, 86.3% of all patients and 88.7% of patients with LVSI underwent lymph node dissection, which reduces the likelihood of missing patients with occult lymph node metastases, although not completely. However, according to the Ayhan et al.'s[9] study, the evaluation of LVSI status with hematoxylin-and-eosin stained slides may have caused an underestimation of the true overall rate of tumors with LVSI, which could have led to the loss of its significance as an independent risk factor for recurrence.

In our study, we used D2-40 staining in addition to hematoxylin-and-eosin staining when there was a technical problem in the evaluation of lymphatic vessels. This superior technique

may have contributed to the higher LVSI positivity rates in our study compared to other studies evaluating low-risk EC. It is important to note that our study aimed to assess the effect of the presence of LVSI on recurrence in patients with low-risk EC, similar to the study by Nwachukwu et al.[19] However, unlike their study, which only evaluated stage 1A, grade 1 EC patients, we included all low-risk EC patients regardless of grade and stage[8,9,11,16].

Our study has some limitations. First, this is a retrospective, single-center study. The second limitation is the absence of a three-tiered scoring system used for the evaluation of LVSI status, which is considered an important factor for recurrence. Additionally, the small number of patients with recurrence in low-risk EC, which generally has a good prognosis, was the third limitation of our study investigating the factors affecting recurrence.

Despite these limitations, our study stands out as one of the most important studies in the literature on the low-risk EC group, as all patients underwent lymph node dissection, and the operations were performed by qualified gynecological oncologists with standardized surgical procedures. Furthermore, the surgical specimens were evaluated by expert gynecological pathologists, which enhances the reliability and accuracy of our results.

CONCLUSIONS

The presence of LVSI in low-risk EC is a significant prognostic risk factor for recurrence and distant metastasis, as well as a predictor of decreased DFS. Therefore, when LVSI is detected, adjuvant treatment options should be re-evaluated, considering the risk of distant metastasis. This is particularly important until the data on the definition of molecular classification become clear.

ABBREVIATIONS

EC: endometrial cancer; LVSI: lymphovascular space invasion; ESGO: European society of gynaecological oncology; ESTRO: European society for radiotherapy and oncology; ESP: European society of pathology; DFS: disease-free survival; FIGO: international federation of gynecology and obstetrics; NCCN: national comprehensive cancer network.

ETHICS APPROVAL

This study was approved by the Tepecik Education and Research Hospital ethics committee (Ref:2022/10-20) and conducted in accordance with the Declaration of Helsinki.

AUTHORS' CONTRIBUTIONS

İÇ: Conceptualization, Data curation, Writing – original draft. **CA:** Conceptualization, Data curation, Formal Analysis. **EB:** Data curation, Investigation, Methodology. **VG:** Formal Analysis, Writing – review & editing. **MS:** Formal Analysis, Methodology, Validation. **MÖ:** Software, Supervision, Validation. **SE:** Supervision, Validation, Writing – original draft, Writing – review & editing. **KG:** Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **ZEÇ:** Visualization, Writing – original draft. **MG:** Writing – original draft, Writing – review & editing.

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ORIGINAL ARTICLE

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Vitamin D levels in patients with seborrheic dermatitis

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SUMMARY

OBJECTIVE: Seborrheic dermatitis is a common papulosquamous skin disease with unknown pathogenesis. The aim of our study was to determine the serum level of 25-hydroxy vitamin D in patients with seborrheic dermatitis SD.

METHODS: A total of 53 patients and 60 healthy controls were included in the study. Serum vitamin D, calcium, phosphorus, and parathormone levels were measured in the patient and control groups, and a comparison was made between the two groups regarding these parameters.

RESULTS: Severe vitamin D deficiency was more frequent among patients with seborrheic dermatitisSD compared to controls (52.8 vs. 25.8%, p=0.003). In patients with severe vitamin D deficiency, seborrheic dermatitis SD was detected more frequently at an early age (p=0048) and in women (p=0.015). No correlation was found between the seborrheic dermatitis skin involvement site and vitamin D level.

CONCLUSION: The fact that vitamin D levels decreased in patients with seborrheic dermatitis SD and patients with severe vitamin D deficiency develop seborrheic dermatitis SD earlier suggests that the low levels of vitamin D are related to seborrheic dermatitis.

KEYWORDS: Seborrheic dermatitis. Vitamin D. Calcium. Phosphorus. Parathormone.

INTRODUCTION

Seborrheic dermatitis (SD) is encountered with a frequency of 3–5% (2.35–11.3) in the society. It is a chronic inflammatory disease that is more common, especially in men[1]. Scalp, eyebrows, nasolabial region, ear, sternal region, and flexor regions are frequently involved. It is frequently observed in infants in the first years of life, in pubertal age when sebaceous gland activity increases, and in advanced ages[2,3]. The most accused ones in its etiopathogenesis are microbial factors such as seborrhea, malassezia, and demodex, androgenic hormones, immunological disorders, drugs, hereditary factors, and stress[1,4-6]. Skin diseases, such as psoriasis, atopic dermatitis, acne, and rosacea, are also frequently associated with SD[4]. The etiopathogenesis is not fully known. It is considered an abnormal focal inflammatory immune response to Malassezia species and their metabolites[1-3]. Moreover, it has been reported that the lesions exacerbate in winter and undergo remission in summer with the curative effect of UV rays[6].

A total of 95% of active vitamin D (vit D) is formed in the skin, especially after exposure to UVB (290–320 nm) rays. A very small amount of it is taken with the diet[7]. Vit D is known as the basic hormone that plays a key role in calcium (Ca)-phosphate (P) metabolism and shows its effects on the intestines, kidneys, and musculoskeletal system[8]. Apart from Ca and bone metabolism, vit D plays a role in cell proliferation, differentiation, regulation of hormone secretion, and immunological functions[9,10-12].

Vit D, which is found in cells such as keratinocytes, mast cells, melanocytes, fibroblasts, the immune system, and many tissues, shows its effect through its nuclear receptors (VDR)[8,13,14]. VDR is mostly expressed in the skin[14,15].

The effect of vit D has been shown in many systemic diseases, including skin diseases[10,15-18]. It is used in the treatment of various skin diseases such as psoriasis, vitiligo, and morphea alopecia areata, in which vit D deficiency is detected[12-14]. Vit D supplements or natural sunlight were shown to be beneficial in SD[19].

Studies have been conducted to investigate vit D levels in patients with the assumption that vit D deficiency may play a role in the development of the disease due to its effects on the inflammatory process and immune response in SD etiopathogenesis[20-23]. There are few studies on this subject in our country[24,25]. For this reason, we wanted to investigate vit D levels in patients with SD.

METHODS

This study was planned as a single-center case-control study. A total of 53 volunteer patients over the age of 18 years who were diagnosed with SD in our outpatient clinic and 60 healthy

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controls were included in the study. Patients' age, gender, age of disease onset, disease duration, lesion localization, smoking status and alcohol use, subjective stress history, and presence of additional skin and systemic diseases were collected and recorded. Pregnant women, those with systemic disease and neoplastic disease, those who have received systemic and topical drug therapy in the last 3 months, and those who have used drugs containing vit D and Ca were not included in the study. Routine biochemistry tests were performed, and vit D, Ca, P, and parathormone (PTH) levels were measured in patients' sera. Vit D and PTH values were measured using Simens Atellica immunoassay autoanalyzer, and routine biochemistry and Ca and P levels were measured using Simens Atellica IM/CIH biochemistry autoanalyzer. The study was carried out between November and May.

The control groups were selected from healthy people who came for general control purposes. Before the study, permission was obtained from the local ethics committee (ethics committee no. 26/0172022; date and number E1-22-2347). The study was conducted according to good clinical practices and Helsinki Declaration. Consent was obtained from the patient and control groups. The informed consent form was obtained from the patients.

In the patient group, the diagnosis of SD was made based on the clinical findings. The lesion localization was differentiated.

Serum vit D levels were compared between the patient and control groups.

Vit D levels were evaluated according to 2 units (ng/mL and nmol/L). Vit D levels <20 ng/mL were identified as severe deficiency, 20–30 ng/mL as mild-moderate deficiency (moderate), and 150> ng/mL as toxicity risk. Similarly, vit D levels <50 nmol/L were identified as severe deficiency, 50–75 nmol/L as mild-moderate deficiency (moderate), and >375 nmol/L as toxicity risk.

Ca, P, and PTH levels were compared between the patient and control groups (the normal Ca values were accepted to be 8.7–10.4 mg/dL, the normal P-values were accepted to be 2.4–5.1 mg/dL, and the normal PTH values were accepted to be 18.4–80.1 ng/L). The Ca, P, and PTH levels were compared between patients with and without severe vit D deficiency.

Duration of the disease: It was divided into three groups, namely, 1–5 years, 6–10 years, and more than 10 years.

A comparison was made between those with or without severe vit D deficiency in terms of age, gender, age of onset, duration, area of involvement, subjective stress history, smoking status, alcohol use, and presence of additional systemic and skin diseases. The relationship between disease localization and duration of the disease and vit D levels was evaluated. Involvement areas were grouped as scalp, forehead, face, nasolabial, chin, eyebrows, ear, and mid-chest.

Statistical method

All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) 21.0 for Windows (SPSS, Inc., Chicago, USA) package program. While descriptive values were expressed as numbers (n) and percentage (%) for categorical values, they were expressed as the mean (standard deviation, SD) if normally distributed and as the median (interquartile range, IQR) if not normally distributed. Pearson chi-square and Fisher's exact tests were used to compare categorical variables. Whether the continuous variables fit the normal distribution or not was evaluated with Kolmogorov-Smirnov and Shapiro-Wilk tests, and comparisons were made using Student's t-test for numerical variables that fit normal distribution and Mann-Whitney U test for numerical variables that did not fit normal distribution. The statistical significance level was accepted as $p<0.05$ in all comparisons.

While calculating the sample size in our study, it was predicted that the frequency of severe vit D deficiency would be 53% in patients with SD and 26% in the control group. Thus, the total (patient+control) number of participants required for an effect size of 0.28 was calculated as 108 (strength 80%, type 1 error 5%, and df=1). Sample size calculation was done using G-power.

RESULTS

The median age of the patients was 28 years (IQR: 25–37.5). The median age of the controls was 32 years (IQR: 26–47.3). The patient and control groups were similar in terms of age (p=0.14). In the patient group, 58.5% were males and 41.5% were females (male/female ratio 1:4). In the control

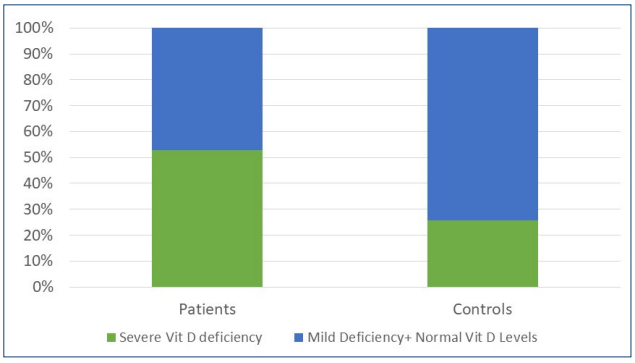

Figure 1. Presence of severe vitamin Dvit D deficiency in the patient and control groups.

group, 48.3% were males and 51.7% were females. The patient and control groups were similar in terms of gender (p=0.28).

The duration of the disease was 1–5 years in 77.4% of the patients, 6–10 years in 20.8% of the patients, and more than 10 years in 1.9% of the patients. The median age of onset of the disease was 24 years (IQR: 21–32).

Figure 1 shows the distribution of vit D deficiencies in the patient and control groups. The presence of severe vit D deficiency was significantly more frequent in the patient group than in the control group (p=0.003) (Figure 1). There was no relationship between vit D levels and disease duration or disease localization.

Patients with severe vit D deficiency were younger than patients with mild vit D deficiency and normal vit D levels (p=0.048). Additionally, female gender was more frequent in patients with severe vit D deficiency (57.1% in the severe vit D deficiency group vs. 24% in the mild deficiency+normal vit D level group) (p=0.015). In the control group, no statistically significant association was observed between vit D levels and age or sex (p-values=0.94 and 0.25, respectively). All characteristics of patients with and without severe vit D deficiency, disease localization, and rates are summarized in Table 1.

When calcium, phosphorus, and PTH levels in the patient and control groups were compared, phosphorus was found to be significantly lower in the patient group (p=0.013) (Table 2).

Patients with and without severe vit D deficiency were similar in terms of serum levels of Ca, P, and PTH (Table 3).

DISCUSSION

SD is a common inflammatory disease that can last for years with relapses and remissions. As the disease is chronic and has no definite treatment, it negatively affects the quality of life of patients. Various studies have been conducted on the etiology of the disease, and many theories have been proposed. However, no definite conclusion has been reached[2,4].

In various studies, increased CD16+expression in NK cells, activation of complement systems, and increased inflammatory interleukins in the skin of patients with SD were observed[25,26]. In addition to this, various studies have shown that Malassezia-type yeasts, which play a role in the etiopathogenesis of SD, increase the release of inflammatory cytokines such as IL-6, IL-8, and TNF-α from keratinocytes[3,27,28]. This suggests that there is increased subclinical inflammation in SD[27].

Several studies have shown the effect of vit D on many inflammatory and autoimmune skin diseases such as psoriasis, atopic dermatitis, polymorphous light eruption, systemic lupus erythematosus, vitiligo, and alopecia areata[15-17]. In these studies, vit D levels were generally found to be low[29-31]. In these diseases in which vit D deficiency is detected, it is recommended to keep the vit D level normal[16,25]. In the etiopathogenesis of SD, vit D deficiency may have a role in the development of the disease due to its effects on the inflammatory process and immune response[11]. Moreover, the fact that SD shows spontaneous improvement in summer and benefit from vit D preparations used in the treatment brought up the relationship between vit D and disease, and SD and vit D levels were investigated[20-25]. In these studies, the questions "Is remission achieved in SD by eliminating vit D deficiency?" and "What role does vit D play in skin diseases?" were tried to be answered.

The data in studies on the use of topical vit D analogs in the treatment of SD are still contradictory. While beneficial results are obtained in some studies, others are not found to be effective enough[32-37].

In 2013, Dimitriova et al. conducted the first study on vit D levels in SD. They found that optimal vit D levels could not be determined in any of the patients, and vit D was found to be deficient or insufficient in all patients. Moreover, no significant difference was found between male and female genders. In 2017, the same authors examined remission in their study by giving vit D supplements to patients with SD and found that their attacks decreased[19,20].

On the contrary, Rahimi et al. found that vit D levels were significantly lower in the study group, which included 118 patients and 171 controls, compared to the control group. This difference was found to be more pronounced in patients with facial involvement compared to scalp involvement. The authors found that the vit D level was lower in patients with severe SD in the scalp than in the scalp involvement and reported that vit D was inversely proportional to the severity of the disease[21]. Sobhan et al. found that the vit D level of the patients with SD was significantly lower than the controls, but no correlation was found with the severity of SD in this study[22]. In a retrospective study conducted in Turkey, vit D levels were found to be lower in patients with SD compared to references[24]. Inan et al. investigated vit D levels in patients with telogen effluvium and SD and found low vit D levels in the presence of SD and both acute and chronic telogen effluvium, but they could not detect a significant relationship between vit D levels only in patients with SD[25].

Scalp involvement is normally around 50–70% in SD[1,2,6]. In a study by Byung et al., they reported that sebaceous gland activity increased in 20 and 30 years of life as well as in

Table 1. All characteristics of patients with and without severe vitamin D deficiency, disease localization, and rates.

	Patients with severe vit D deficiency	Patients without severe vit D deficiency	p-value
Age, average (standard deviation)	28.9 (9.7)	35.2 (13.1)	0.048
Sex, n (%)			
Female	16 (57.1)	6 (24)	0.015
Male	12 (42.9)	19 (76)	
Disease duration, years, median (IQR)	3 (1–5)	3 (1.7–8.5)	0.34
Age of onset, median (IQR)	23 (18.9–28.4)	26 (22–34.5)	0.14
Number of sites involved, median (IQR)	1.5 (1–3.8)	2 (1–2.5)	0.85
Scalp involvement, n (%)			
Yes	26 (92.9)	22 (88)	0.55
No	2 (7.1)	3(12)	
Facial involvement, n (%)			
Yes	8 (28.6)	6 (24)	0.71
No	20 (71.4)	19 (76)	
Nasolabial involvement, n (%)			
Yes	3 (10.7)	1 (4)	0.36
No	25 (89.3)	24 (96)	
Eyebrow involvement, n (%)			
Yes	6 (21.4)	5 (20)	0.89
No	22 (78.6)	20 (80%)	
Ear involvement, n (%)			
Yes	6 (21.4)	5 (20)	0.89
No	22 (78.6)	20 (80%)	
Chin involvement, n (%)			
Yes	2 (7.1)	2 (8)	0.91
No	26 (92.9)	23 (92)	
Chest involvement, n (%)			
Yes	6 (21.4)	4 (16)	0.61
No	22 (78.6)	21 (84)	
Smoking status, n (%)			
Yes	7 (25)	3 (12)	0.23
No	21 (75)	22 (88)	
Alcohol use, n (%)			
Yes	3 (10.7)	2 (8)	0.73
No	25 (89.3)	23 (92)	
Additional systemic disease, n (%)			
Yes	10 (35.7)	7 (28)	0.55
No	18 (64.3)	18 (72)	
Additional skin disease, n (%)			
Yes	1 (3.6)	5(20)	0.089
none	27 (96.4)	20 (80%)	
Subjective stress, n (%)			
Yes	17 (60.7)	14 (56)	0.73
No	11 (39.3)	11 (44)	

Table 2. Comparison of calcium, phosphorus, and parathormone levels in the patient and control groups.

	Patient group	Control group	p-value
Calcium (mg/dL), average (standard deviation) (n=8.7–10.4)	9.61 (0.76)	9.69 (0.61)	0.52
Phosphorus (mg/dL), average (standard deviation) (n=2.4–5.1)	3.47 (0.52)	3.75 (0.54)	0.013
Parathormone (ng/L), median (IQR) (n=18.4–80.1)	35.7 (28–55.3)	40.5 (32.5–54.5)	0.63

Table 3. Comparison of calcium, phosphorus, or parathormone levels in patients with and without severe vitamin D deficiency.

	Those with severe vit D deficiency	Those without severe vit D deficiency	p-value
Calcium (mg/dL), average (standard deviation)	9.61 (0.79)	9.8 (0.59)	0.75
Phosphorus (mg/dL), average (standard deviation	3.63 (0.57)	3.66 (0.54)	0.72
Parathormone (ng/L), median (IQR)	39.9 (30.8–53)	36.7 (28.5–56.8)	0.87

adolescents. They stated that this may be due to the intense number and activity of sebaceous glands in the scalp, thus facilitating the development of SD[38]. In our study, scalp involvement was also higher.

Similar to other studies, Gray et al. reported that half (52%) of the patients with SD had vit D deficiency. These authors also stated that the lesions are mostly located on the scalp, and like some researchers, they reported that the severity of the disease in the scalp is associated with higher and lower serum vit D levels and that it is seen at younger ages such as the third decade[23]. In this study, we found vit D levels in patients with SD to be severely low at a rate of 52.8%, similar to previous studies. It was also lower in young female patients.

The known function of vit D is to regulate Ca and P metabolism. It achieves this effect by increasing the absorption of both Ca and P in the intestine; vit D decreases PTH secretion and increases bone resorption and Ca and magnesium resorption from the kidneys[39]. While there is a negative correlation between PTH phosphate levels, vit D has a stimulating effect on both Ca and P homeostasis. When the vit D level decreases, Ca absorption decreases, PTH increases, and urinary excretion of P increases. PTH increases due to hypocalcemia and hypophosphatemia, but there may be cases where it does not[40]. However, the reason for this is not known exactly. In their studies, Aydın et al. found PTH elevation only in 6.3% of the patients with low vit D[39].

In this study, besides the vit D level, Ca, P, and PTH levels were also examined in the patient and control groups. In the analysis, PTH increase was not detected in any of the patients with low vit D levels. There was no difference between Ca levels, but P levels were found to be significantly lower in the patient group. No pathology was found in any of the patients to decrease blood P. Therefore, it was found that low P could be due to vit D deficiency or incidental. It was concluded that P may have another unknown effect on the pathogenesis of SD, and further research is needed on this subject. Furthermore, there was no difference between Ca, P, and PTH values in comparison of those with and without severe vit D levels.

The limitations of the study can be summarized as the small number of participants, not specifying the severity scale of SD and skin type, not performing questioning of the lifestyle and clothing style, sun exposure history, and diet questioning, which could be potential sources of bias and uncertainty.

CONCLUSION

Recently, vit D has been extensively studied by researchers not only for its role in Ca metabolism but also for its immunoregulatory, antiproliferative, and differentiation-controlling properties. The obtained data show that vit D is promising to researchers. In this study, vit D levels were found to be low in patients with SD. The fact that vit D levels decreased in patients with SD and patients with severe vit D deficiency develop SD earlier suggests that the low levels of vit D are related to SD.

AUTHORS' CONTRIBUTIONS

AA: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **FK**: Conceptualization, Methodology, Writing – review & editing. **SS**: Data curation, Writing – review & editing. . **YH**: Conceptualization, Methodology, Writing – review & editing.

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ORIGINAL ARTICLE

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Predictors of mortality in patients with geriatric trauma in the emergency service

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SUMMARY

OBJECTIVE: In our study, it was aimed to compare the power of trauma scores (Glasgow Coma Score, Revised Trauma Score, Abbreviated Injury Scale, Injury Severity Score, and Trauma Score-Injury Severity Score) in order to predict mortality in patients with geriatric trauma and to determine the predictive values of these scores in mortality.

METHODS: Demographic data, clinical features, etiological causes, laboratory results, and trauma scores of the patients were statistically analyzed. SPSS 20 for Windows was used for this evaluation.

RESULTS: It was determined that as the Glasgow Coma Score value of the patients increased, the Abbreviated Injury Scale and Injury Severity Score scores decreased and the Trauma Score-Injury Severity Score score increased. Abbreviated Injury Scale and Injury Severity Score values increased and Revised Trauma Score and Trauma Score-Injury Severity Score values decreased as the lactate levels of the patients increased. It was determined that the Abbreviated Injury Scale and Injury Severity Score scores of the patients hospitalized in the intensive care unit were significantly higher, while their Trauma Score-Injury Severity Score scores were lower.

CONCLUSION: Glasgow Coma Score, Revised Trauma Score, Trauma Score-Injury Severity Score, Abbreviated Injury Scale, and Injury Severity Score scores and blood lactate levels are important parameters that can be used in the emergency department for the early detection of high-risk patients in geriatric trauma and the evaluation of the prognosis of geriatric trauma patients.

KEYWORDS: Geriatrics. Trauma. Injury. Mortality.

INTRODUCTION

The elderly population is gradually increasing due to the increase in living standards in our country, as in all developed and developing countries[1,2]. Patients over 65 years of age constitute 28% of trauma-related deaths. For patients over the age of 65 years, traffic accidents and falls are the most common causes of trauma. High morbidity and mortality are observed in geriatric patients. Many scoring methods are used in order to understand the severity and consequences of trauma that are inconsistent with the clinical picture in the early period and to reduce the deaths due to this inconsistency[1-4].

The following five trauma scores were used in our study: Glasgow Coma Score (GCS), Revised Trauma Score (RTS), Injury Severity Score (ISS), Abbreviated Injury Scale (AIS), and Trauma Score-Injury Severity Score (TRISS).

The GCS is used to evaluate the state of consciousness. GCS scores ranging from 3 (fatal) to 15 (minor) indicate the patient's level of consciousness, and scores of 8 and below indicate that the patient is in a coma[3]. RTS is the combination of respiratory rate, systolic blood pressure, and GCS. The AIS is a glossary in which trauma is scored from 1 (minor) to 6 (fatal). ISS is calculated as the sum of the squares of the AISs of the three most severely injured regions of these organs (head, neck, face, thorax, abdomen, extremities, and others). The score ranges from 1 (minor) to 75 (fatal). ISS indicates 16 or more major traumas. The trauma and injury severity score (TRISS) is a combined scoring system that evaluates the probability of survival of a trauma patient based on RTS, ISS, AIS, and the patient's age (1–4 years).

In our study, it was aimed to compare the power of trauma scores (GCS, RTS, AIS, ISS, and TRISS) in order to predict mortality in patients with geriatric trauma and to determine the effectiveness of the hospitalization decision/prognosis and the epidemiological and clinical characteristics.

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METHODS

Patients aged 65 years and older and 295 volunteer patients who presented to the Emergency Department of Kahramanmaraş Sütçü İmam University (KSU) Faculty of Medicine due to acute trauma or complications in 2018–2019 were evaluated in this descriptive study.

Clinical features, etiological causes, laboratory results (hemogram and biochemical parameters), and trauma scores of the patients were statistically analyzed. After the emergency follow-up of the patients, sociodemographic information; the occurrence and mechanism of trauma; the place where the trauma occurred; trauma sites in the body and other accompanying injuries prognostic factors, such as hospitalization status (service, intensive care) and length of stay; and referral to another center were determined from the hospital automation program and examined.

SPSS 20 for Windows was used for this evaluation. Categorical variables were expressed using numbers and percentages. The Kolmogorov-Smirnov analysis was performed to evaluate the normal distribution of quantitative data based on measurement. Pearson chi-square test was used for the statistical analysis of categorical data, and Mann-Whitney U test, Kruskal-Wallis test, and post hoc Tamhane's T2 test were used for the statistical analysis of quantitative data. A value of $p<0.05$ was considered statistically significant.

Ethics committee approval was obtained from the KSÜ Clinical Research Ethics Committee with resolution number 12 in session 2019/07 on April 17, 2019. The study is consistent with the principles of the Declaration of Helsinki.

RESULTS

Age, gender, social security, comorbid diseases, trauma site, type of trauma, and prognosis information of the 295 patients included in our study were evaluated. A need for intensive care in 34 patients (%11.5) was detected. The prognosis of 295 patients was evaluated: recovery/discharge in 181 patients (61.4%), sequelae in 107 patients (36.3%), and exitus in 7 patients (2.4%). Age (76.53±8.44 years), length of stay in the emergency department (3.26±3.58 h), length of stay in intensive care (6±6.81 day), heart rate (80.53±12.86/min), respiration rate (17.12±3.16), and systolic blood pressure (139.58±25.98 mmHg) were detected in patients included in our study. No significant correlation was found between the gender, educational status, trauma sites, trauma type, presence of comorbidities, site of the accident, fever degrees, hemoglobin and hematocrit values, and prognosis of the patients included in our study. Sequelae and mortality rates were found to be higher in patients with a median age of over 76 years. Sequelae and mortality rates were found to be higher in high-energy traumas. Sequelae and mortality rates were found to be higher in patients with surgical fractures. The death rate was found to be higher in patients with a high heart rate. Sequelae and mortality rates were found to be higher in patients with a higher respiratory rate. The prognosis of the only patient with a low respiratory rate resulted in death.

GCS values, lactate values, and trauma scores (AIS, ISS, RTS, and TRISS) of the patients were detected: AIS (2.44±1.74), ISS (5.89±7.59), RTS (11.93±0.55), TRISS (96.25±9.18), GCS (14.90±0.78), and lactate (1.69±1.29).

No significant correlation was found between intensive care hospitalization and GCS, admission lactate level, RTS score values, and the length of stay in the emergency department (Table 1). While the AIS and ISS scores of the patients hospitalized in the intensive care unit were found to be significantly higher, the TRISS scores were significantly lower.

The evaluation of trauma scores predicting prognosis is shown in Table 2.

The comparison of trauma scores is shown in Table 3.

DISCUSSION

In the study conducted by Yousefzadeh-Chabok et al., falls and motor vehicle accidents are the most common causes of trauma in the elderly population[5]. In the study conducted by Ümit İ. Güneytepe et al., the first cause of trauma in the elderly population was motor vehicle accidents (62%), followed by falls (31%). In the same study, fall-related injuries occupied the first place among those aged 75 years and over. In addition to changes in bone mass, the inability to absorb fall and fall energy adequately due to muscle strength and coordination problems plays a role in the formation of fractures due to falls in the elderly. Furthermore, there may be balance problems due to metabolic endocrine disorders such as syncope, seizures, and sodium imbalances that pave the way for falls in the geriatric population. In the literature, it was demonstrated that the areas injured after trauma were mostly the head region and extremities in the elderly population[1]. In the patients included in our study, it was determined that 70.8% of traumas occurred due to falls in the home environment, followed by traffic accidents. In our study, the head and neck region was the most common injury site, and the lower extremities were the second most common injury site, which supports the literature.

It is possible to detect high-risk patients in geriatric traumas in the early period and to prevent mortality by better stabilizing these patients with appropriate treatment[6]. Various studies

Table 1. Evaluation of the prognosis of intensive care patients according to trauma scores.

	Intensive care hospitalization		p-value Z
	Yes Median (min–max) Mean ± Std. Dev	No Median (min–max) Mean ± Std. Dev	
Age (years)	82.5 (65–89) 80.11±7.79	75 (65–109) 76.07±8.43	0.004 -2.914
GCS	15 (11–15) 14.82±0.75	15 (3–15) 14.91±0.79	0.221 -1.223
Lactate	1.35 (0.3–4.1) 1.72±1.03	1.4 (0.1–13.9) 1.69±1.32	0.831 -0.214
AIS score	3 (1–12) 3.52±2.07	2 (0–13) 2.30±1.64	0.000 -4.322
ISS score	9 (1–54) 10.32±9.33	4 (0–75) 5.32±7.15	0.000 -4.964
RTS score	12 (11–12) 11.94±0.23	12 (3–12) 11.93±0.58	0.402 -0.839
TRISS score	96.75 (41.03–98.31) 94.87±9.68	97.84 (1.85–98.44) 96.43±9.11	0.000 -4.728
Length of stay in the Emergency Department	3.5 (1–12) 3.64±2.42	3 (0–48) 3.21±3.71	0.126 -1.529

Mann-Whitney U test was used.

Table 2. Evaluation of trauma scores predicting prognosis.

	Prognosis			p-value χ^2
	Recovery median (min–max) Mean±Std. Dev	Sequelae median (min–max) Mean±Std. Dev	Exitus median (min–max) Mean±Std. Dev	
Age (years)	75 (65–109) 75.64±8.41	78 (65–98) 77.82±8.05	77 (66–104) 80.14±12.53	0.053 6.097
GCS	15 (12–15)[a] 14.97±0.24	15 (11–15)[a] 14.94±0.43	15 (3–15)[b] 12.71±4.42	0.000 44.078
Lactate	1.3 (0.1–6.5)[a] 1.56±0.94	1.5 (0.3–4.2)[a] 1.67±0.92	3.4 (1.2–13.9)[b] 5.44±4.88	0.003 11.709
AIS Score	1 (0–7)[a] 1.82±1.15	3 (1–12)[b] 3.15±1.56	6 (4–13)[c] 7.42±3.95	0.000 84.131
ISS Score	2 (0–25)[a] 3.32±3.35	9 (1–54)[b] 8.51±6.78	25 (8–75)[b] 32.42±23.07	0.000 95.873
RTS Score	12 (11–12)[a] 11.98±0.10	12 (11–12)[a] 11.95±0.21	12 (3–12)[b] 10.28±3.30	0.000 37.728
TRISS Score	98.16 (88.68–98.44)[a] 97.81±1.02	96.75 (41.03–98.31)[b] 95.90±6.34	88.68 (1.85–97.01)[b] 61.35±43.52	0.000 82.950
Length of stay in the Emergency Department	2 (0–27) 2.85±2.47	3 (0–48) 3.98±4.92	2 (1–6) 2.85±1.86	0.007 9.871

Kruskal Wallis test and post hoc Tamhane T2 test were used. There is a significant correlation between those with different letters.

have revealed that the mortality rate in the elderly trauma population varies between 10 and 34%[7]. In the study by Ümit İ. Güneytepe et al., this ratio was reported to be 9.6%[1]. In our study, this ratio was 2.4%, which was quite low compared to the literature. The hospital where the study was conducted is a tertiary-level university hospital where advanced examinations and treatments are performed. We believe that the mortality rate is low since our study is up-to-date, our hospital is more equipped in terms of technology and information, advanced examinations and treatments can be performed in

Table 3. Comparison of trauma scores.

	Age	GCS	Lactate	AIS Score	ISS Score	RTS Score	TRISS Score	Length of Stay in the Emergency Department
Age (years)	1							
GCS	-0.195**	1						
Lactate	0.247**	-0.606**	1					
AIS	0.138*	-0.394**	0.289**	1				
ISS	0.141*	-0.413**	0.409**	0.842**	1			
RTS	-0.208**	-0.914**	-0.603**	-0.432**	-0.438**	1		
TRISS	-0.154**	0.690**	-0.600**	-0.613**	-0.819**	0.725**	1	
Length of stay in the Emergency Department	0.146*	0.036***	0.025***	0.009***	-0.020***	0.041***	0.046***	1

*p<0.05, **p<0.01, ***p>0.05.

our hospital, and physicians who are experts in their fields are easily accessible.

GCS, RTS, AIS, ISS, TRISS scores, and blood lactate level are parameters that can be helpful in predicting conditions such as triage and prognosis in geriatric trauma patients[2,8]. GCS is a physiological scoring system and is used to evaluate the severity of critical neurological status and traumatic brain injury. However, only the severity of head trauma can be evaluated with GCS in multiple trauma patients, and the measurement of other physiological parameters is insufficient, especially in multiple trauma patients. Therefore, AIS, ISS, RTS, and TRISS scoring systems overcome the GCS. On the contrary, these scoring systems also include the GCS during measurement.

In the study by Seda et al., the mortality rate was found to be higher in the group with low RTS scores[2]. While it was observed in the study by Akkose that GCS, RTS, and ISS scores were lower in exitus patients, it was observed in the study by Orhon that RTS and TRISS scores were significantly lower in patients with mortality[9,10]. In the study in which Watt et al. investigated the effects of trauma scores on predicting mortality and length of hospital stay in geriatric patients, it was demonstrated that ISS and RTS were better predictors of mortality than predicted ones; however, they had a limited correlation with the length of hospital stay[11]. Eryılmaz et al. found that the RTS values were lower in exitus patients compared to living patients[12]. RTS is an important physiological scoring system in showing survival when it is used alone, and RTS provides a high rate of observation and compliance in predicting the risk of mortality and associating it with survival[13].

In our study, AIS and ISS scores of the patients hospitalized in the intensive care unit were found to be significantly higher; however, their TRISS scores were significantly lower. The Glasgow coma scores of the patients whose prognosis resulted in exitus were found to be significantly lower compared to those with sequelae and discharge. Patients with exitus had significantly higher lactate levels at admission compared to patients with sequelae and discharge. The AIS and ISS scores of the patients who died were found to be significantly higher compared to those who resulted in sequelae and discharge. The RTS and TRISS scores of the patients who resulted in exitus were found to be significantly lower compared to the patients who were discharged and/or resulted in sequelae. It was determined that the AIS, ISS, and blood lactate levels increased as the Glasgow coma score decreased. The value of the TRISS score also increased as the Glasgow coma score increased. As the patients' admission lactate levels increased, the AIS and ISS values increased; however, RTS and TRISS values decreased. As the AIS scores of the patients increased, the ISS scores also increased; but the RTS and TRISS scores decreased. RTS and TRISS scores decreased as ISS scores increased. As the RTS scores of the patients increased, the TRISS scores also increased significantly.

It was observed that the prognosis of patients with high blood lactate level and high AIS and ISS scores was poor and that the morbidity/mortality rate was high. It was determined that the prognosis was poor and the morbidity/mortality rate was high in patients with low GCS, RTS, and TRISS scores.

CONCLUSION

GCS, RTS, TRISS, AIS, ISS scores, and blood lactate levels are important parameters that can be used during admission

to the emergency department for early detection of high-risk patients in geriatric trauma, the prevention of mortality by better stabilizing these patients with appropriate treatment, and the evaluation of the condition/prognosis of patients with geriatric trauma. We think that the use of trauma scores in geriatric trauma patients will contribute to the triage, diagnosis, follow-up, treatment, and prognosis of the patients.

AUTHORS' CONTRIBUTIONS

HH: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **MSG:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **RAO:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **AİK:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **YS:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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ORIGINAL ARTICLE

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Screening for coronavirus disease 2019 in pregnant women admitted for delivery: an observational study

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SUMMARY

OBJECTIVE: The aim of this study was to examine the impact of symptom-based screening on the prevalence and outcomes of neonatal coronavirus disease 2019 in pregnant women admitted for delivery.

METHODS: A retrospective observational study was conducted from June to August 2020 at Gonzaga Mota of Messejana Hospital, Fortaleza, CE, Brazil. All pregnant women were screened for coronavirus disease 2019 based on symptoms. Reverse transcription-polymerase chain reaction or immunology assays for severe acute respiratory syndrome coronavirus 2 were performed when a patient reported a symptom. All newborns of symptomatic patients were submitted for Reverse transcription-polymerase chain reaction. Newborns were divided into groups according to the Reverse transcription-polymerase chain reaction results to identify the relationship between maternal symptoms and neonatal coronavirus disease 2019.

RESULTS: A total of 55 (55/1,026, 5.4%) and 50 (50/1,026, 4.8%) pregnant women reported symptoms and had a positive confirmatory test, respectively. The most common symptom of coronavirus disease 2019 among the pregnant women with positive confirmatory test was cough (n=23, 46%). Seven newborns (7/50, 14%) of symptomatic mothers had positive Reverse transcription-polymerase chain reaction. Upon birth, no newborn had serious complications.

CONCLUSION: Universal screening of pregnant women admitted for delivery can reduce the perinatal transmission of coronavirus disease 2019. Symptom-based screening can be an alternative for regions with a low prevalence of the disease where a better allocation of financial resources is necessary.

KEYWORDS: COVID-19. Obstetric delivery. Newborn. Perinatal care. SARS-CoV-2. Infectious disease transmission.

INTRODUCTION

The first case of a severe acute respiratory syndrome caused by a new coronavirus, later called severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was identified in December 2019 in Wuhan, Hubei Province, China. Coronavirus disease 2019 (COVID-19) has spread rapidly across the world, prompting the World Health Organization (WHO) to declare a pandemic status on March 11, 2020[1,2]. At the end of January 2021, COVID-19 infected more than 98 million people and was responsible for more than 2.1 million deaths worldwide[3].

Clinically, COVID-19 has a wide range of disease manifestations that have been identified in five different cases: asymptomatic (1.2%), mild to moderate (80.9%), severe (13.8%), critical (4.7%), and mortality (2.3%). Young adults of reproductive age are the most affected, but the highest mortality occurs in the elderly population and those with comorbidities such as obesity, hypertension, diabetes, and other chronic diseases[4]. The number of COVID-19 cases among pregnant women is still uncertain. Studies performed with pregnant women at the time of delivery revealed that the prevalence of COVID-19 ranged from 0.43 to 19.8%[5-10]. This large variation in the prevalence of SARS-CoV-2 infection at the time of delivery depends on the area studied, type of screening performed, number of tests performed, period studied, and gestational risk[11].

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The impact of COVID-19 on pregnant women is still poorly understood. Initially, based on the respiratory physiology of a pregnant woman, maternal immune response during pregnancy, and experience of other severe acute respiratory syndromes, it was believed that SARS-CoV-2 infection in the perigestational period could lead to more serious cases[12]. Current evidence indicates that the morbidity and mortality of COVID-19 among pregnant women are similar to those of non-pregnant women. However, some authors have identified high mortality among pregnant women with COVID-19, suggesting that factors such as a higher prevalence of comorbidities, ethnic differences, and poor quality of obstetric care may contribute to a more severe development of the disease[13-15].

The risk of neonatal COVID-19 is due to possible vertical transmission, maternal transmission after delivery, or even transmission from the health team involved in the care of the mother-child binomial. Current knowledge about neonatal COVID-19 is still limited to a small number of cases, which indicate a favorable clinical course with few symptoms and low morbidity and mortality. However, identification of pregnant women infected with SARS-CoV-2 at the time of delivery is important in order to enforce safety and isolation measures[16].

The goal of this study was to evaluate the impact of a symptom-based screening protocol on the prevalence and outcomes of neonatal COVID-19.

METHODS

This was a retrospective observational study conducted from June 2020 to August 2020 at Gonzaga Mota of Messejana Hospital, Fortaleza, CE, Brazil. The inclusion criterion was admission for delivery during the study period. Exclusion criteria were loss of follow-up and non-agreement to participate in the study. During this period, all pregnant women admitted for delivery were screened for COVID-19 clinical symptoms such as cough, dyspnea, abnormalities in smell and taste, hyporexia, fever, diarrhea, and headache at any time during pregnancy. Pregnant women with a history or occurrence of any symptoms were immediately isolated and referred for laboratory investigation at the time of childbirth. A single swab collection from the posterior oropharynx and nasopharynx was performed when the pregnant woman was between the 3rd and 10th days after the onset of symptoms, and then a reverse transcriptase polymerase chain reaction (RT-PCR) assay was conducted for SARS-CoV-2 (Bio-Manguinhos, Rio de Janeiro, RJ, Brazil; Instituto de Biologia Molecular do Paraná, Curitiba, PR, Brazil; Mobius Life Science, Pinhais, PR, Brazil). On the contrary, the immunology assay (rapid test) was performed from the 7th day after the onset of symptoms (Wondfo One Step COVID-19 rapid test kits, Guangzhou, China).

In all newborns of symptomatic pregnant women, clinical conditions at birth were assessed, and, if possible, prompt clamping of the umbilical cord within 1 min of life was suggested, while skin-to-skin contact was not indicated. The nasopharyngeal swab to perform RT-PCR was conducted in children after initial care at birth or up to 48 h of life[17]. Bathing a newborn was done if birth was by vaginal delivery. After birth, asymptomatic newborns of symptomatic pregnant women with favorable maternal conditions stayed isolated with the mother in the rooming-in until hospital discharge, with maternal hygiene care guidelines and by wearing a mask and maintaining 2 m between the binomials. If the newborns had symptoms (fever, cough, difficulty in breathing and/or tachypnea, hypoactivity, and food refusal), they were referred to the neonatal unit in intensive or intermediate care and remained in isolation until symptoms had been resolved and the mothers could be accommodated in room[18].

Newborns of symptomatic pregnant women were divided into two groups based on the result of the RT-PCR after birth (RT-PCR SARS-CoV-2 positive and RT-PCR SARS-CoV-2 negative) to identify the possible relationship between any maternal symptom and a positive neonatal COVID-19.

Data were analyzed using IBM SPSS Statistics for Windows, version 25.0 (Armonk, NY: IBM Corp.). In addition, descriptive statistics were performed on symptomatic pregnant women. The mean (± standard deviation) and median (interquartile ranges) were used for continuous variables. Differences in the distribution of maternal and neonatal characteristics were assessed using the chi-square or Fisher's exact tests for categorical variables and the Mann-Whitney U test for continuous variables. p=0.05 was found to be statistically significant. The study was approved by the Ethic Committee of the University of Fortaleza (CAAE number 34594620.9.0000.5052/approval number 4.147.638) and conducted within ethical principles and rules, with a free and informed consent form signed by all participants.

RESULTS

During the time of the study (June to August 2020), 1,026 pregnant women were admitted for delivery. A total of 55 (55/1,026, 5.4%) pregnant women reported a history or presence of symptoms related to COVID-19. On the contrary, 50 (50/1,026, 4.8%) pregnant women had a positive confirmatory test (Figure 1). The median age of these symptomatic pregnant women was 23 years (interquartile range [IQR]: 20 to 28.2

years). The most common symptom of COVID-19 among the 50 pregnant women with positive confirmatory tests was cough (n=23, 46%), followed by coryza (n=16, 32%), ageusia (n=16, 32%), anosmia (n=15, 30%), headache (n=12, 24%), fever (n=11, 22%), sore throat (n=11, 22%), myalgia (n=9, 18%), nasal obstruction (n=8, 16%), diarrhea (n=8, 16%), fatigue (n=5, 10%), dyspnea (n=4, 8%), and adynamia (n=2, 4%) (Table 1).

Of the total symptomatic pregnant women submitted to the laboratory, 31 tested positive for COVID-19 (31/50, 62%) (Figure 1). Based on the interval between the onset of symptoms and admission for delivery, 26 patients were investigated using RT-PCR, with 5 positive tests (5/26, 19.2%), and 40 patients underwent immunological testing, with 28 positive cases (28/40, 70%). Moreover, 7 of the 50 newborns from symptomatic mothers had a positive RT-PCR for SARS-CoV-2, and 6 swabs were obtained immediately at birth and 1 after 36 h of life. Five were neonates of mothers with positive COVID-19 laboratory tests (two positive RT-PCR and three positive immunologic tests), and two were neonates of pregnant women with negative COVID-19 laboratory tests (one negative RT-PCR and one negative RT-PCR and immunological test) (Figure 1 and Table 2).

The overall median birth weight was 3,290 g (IQR: 3,037–3,650 g) and the median APGAR score at the 1st and 5th min was 8 (IRQ: 8–9) and 9 (IRQ: 9–9), respectively. There was no substantial difference between maternal and neonatal variables compared to groups of positive and negative newborns for SARS-CoV-2 RT-PCR (Table 1). Upon birth, no newborn had serious complications. One newborn had mild respiratory distress, while another (male) had mild respiratory distress associated with fever at 36 h of life, and bilateral hydronephrosis was diagnosed and transferred to a tertiary hospital during the investigation (Table 2).

Table 1. Characteristics of pregnant women with symptoms for coronavirus disease 2019 and their newborns.

Variables	RT-PCR SARS-CoV-2 positive (n=7)	RT-PCR SARS-CoV-2 negative (n=43)	p
Maternal age, median (IQR 25;75)	20 (19–34)	23 (20–28)	0.661[a]
Gestational age, median (IQR 25;75)	39 (38–40)	39 (38–40)	0.410[a]
Gravidity, median (IQR 25;75)	2 (1–3)	2 (1–3)	0.848[a]
Parity, median (IQR 25;75)	2 (1–2)	2 (1–2)	0.913[a]
Miscarriages, median (IQR 25;75)	0 (0-0)	0 (0–1)	0.565[a]
Cough, n (%)	03 (42.9)	20 (46.5)	1.000[b]
Coryza	00 (00)	16 (37.2)	0.159[b]
Ageusia, n (%)	01 (14.3)	15 (34.9)	0.485[b]
Anosmia, n (%)	01 (14.3)	14 (32.6)	0.496[b]
Headache, n (%)	03 (42.9)	09 (20.9)	0.433[b]
Fever, n (%)	01 (14.3)	10 (23.3)	1.000[b]
Sore throat, n (%)	01 (14.3)	10 (23.3)	1.000[b]
Myalgia	00 (00)	09 (20.9)	0.417[b]
Nasal obstruction	00 (00)	08 (18.6)	0.640[b]
Diarrhea, n (%)	01 (14.3)	07 (16.3)	1.000[b]
Fatigue	01 (14.3)	04 (9.3)	0.616[b]
Dyspnea, n (%)	00 (00)	04 (9.3)	1.000[b]
Adynamia	01 (14.3)	01 (2.3)	0.370[b]
Pregnant COVID-19 test positive, n (%)	05 (71.4)	26 (60.4)	0.695[b]
Birth weight median (IQR25;75)	3,345 (3,250–3,555)	3,250 (3,010–3,655)	0.546[a]
APGAR score 1st minute (median, IQR)	8 (8–9)	9 (8–9)	0.870[a]
APGAR score 5th minute (median, IQR)	9 (9–9)	9 (9–9)	0.935[a]

Miscarriages: number of pregnancy losses before 20 weeks of gestation. Differences in the distribution of maternal and neonatal characteristics were assessed using the chi-square or Fisher's exact tests for categorical variables and Mann-Whitney U test for continuous variables; SD: standard deviation; IQR: interquartile range; p=0.05 was considered statistically significant. Note: [a]p=Mann-Whitney U test; [b]p=Fisher's exact test.

DISCUSSION

The effect of COVID-19 on pregnant women and newborns is still poorly understood. Apparently, vertical transmission is an unusual phenomenon. However, there is a risk of transmission during the neonatal period, particularly during breastfeeding and in the care of the newborn by healthcare professionals and family members. Some studies suggest that pregnant women should be universally screened at the time of admission for delivery. Some authors recommend screening based on symptoms or pregnancy risk[19,20].

The main findings of our study showed that at the beginning of the COVID-19 pandemic and in the face of financial and laboratory constraints, symptom-based screening of pregnant women admitted to a low-risk hospital for delivery was a decision that did not seem to worsen perinatal outcomes.

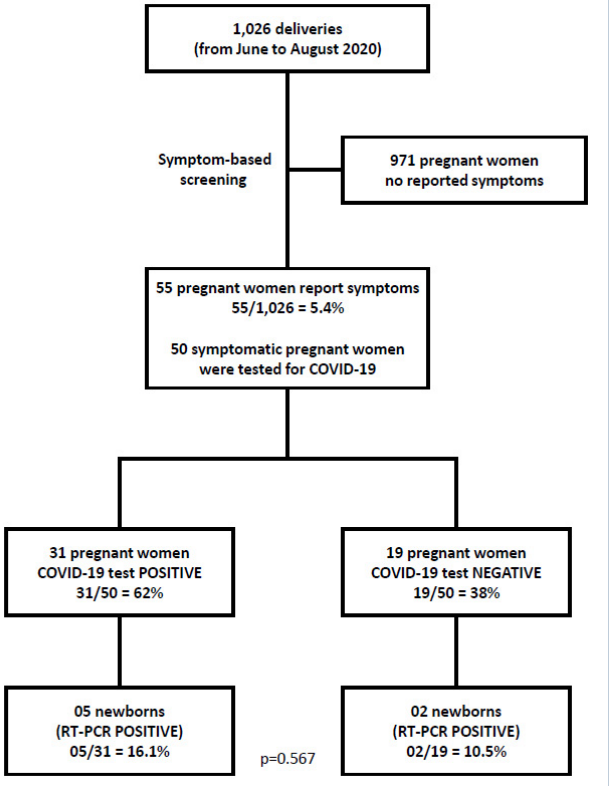

Figure 1. Patients' flowchart. From June to August 2020, 1,026 pregnant women were admitted for delivery. A total of 55 pregnant women reported a history or symptoms related to coronavirus disease 2019. Additionally, 50 pregnant women had a positive confirmatory test. Thirty-one pregnant women tested positive for coronavirus disease 2019. Moreover, 7/50 newborns from symptomatic mothers had a positive reverse transcriptase polymerase chain reaction for severe acute respiratory syndrome coronavirus 2. Five were neonates of mothers with positive coronavirus disease 2019 laboratory investigation, and two were neonates of pregnant women with negative coronavirus disease 2019 laboratory investigation. There was no significant difference between these groups in the prevalence of neonatal coronavirus disease 2019.

However, our study has several limitations. First, the lack of a control group that underwent universal screening did not allow comparison of perinatal outcomes with symptom-based screening. The other limitation was the lack of diagnostic criteria for perinatal SARS-CoV-2 transmission, which were not defined at the time of the pandemic.

At the beginning of December 2020, the countries with the highest number of confirmed cases of COVID-19 were the USA (15.4 million), India (9.7 million), and Brazil (6.6 million), with a total of 46,435, 7,022, and 31,099 cases, respectively, per million inhabitants[3]. The overall prevalence of symptomatic SARS-CoV-2 infection has remained uncertain. Oran et al.[21] estimated that about 55–60% of people infected with SARS-CoV-2 had any symptoms. However, the authors highlighted the inconsistency of the data in the literature, as the percentage of symptomatic patients in the studies ranged from 7 to 93.7%.

Universal screening showed that about one-third of pregnant women with COVID-19 were symptomatic when they were admitted for delivery. However, the proportion of pregnant women infected with SARS-CoV-2 with symptoms was highly variable. A study conducted in Chile between April and June 2020 found that 56.8% of pregnant women (37/583) with COVID-19 at delivery were symptomatic[5], while another four studies, carried out in three different countries [Japan, Spain, and the USA (Southern California and Brooklyn, New York, NY)] with fewer participants, found no symptomatic pregnant women with RT-PCR for SARS-CoV-2[10].

Also, the Centers for Disease Control and Prevention (CDC) described that the most commonly reported signs and symptoms were cough (50.3%), headache (42.7%), muscle aches (36.7%), and fever (32%) among pregnant women with COVID-19. In the review study by Amaral et al.[22], cough (44.4%) and nausea (10.2%) were the most frequent symptoms among pregnant women affected by COVID-19, while in the study by Elshafeey et al.[23], these symptoms were fever (67.3%) and cough (65.7%). In our study, we found that, in addition to cough and coryza, taste and smell abnormalities were often reported by pregnant women with COVID-19.

The rate of perinatal transmission at our institution was 16.1% (5/31), similar to that described by Biasucci et al.[6] (13.3%), and higher than that described by Corvillon et al.[5] (5.4%). Shah et al.[23] described a classification system and case description for SARS-CoV-2 infection in pregnant women, fetuses, and neonates. Based on the proposal by Shah et al.[23] 6/7 cases of COVID-19 in our observational study can be categorized as neonatal infection acquired intrapartum (detection of the virus by PCR in a nasopharyngeal swab at birth and

Table 2. Clinical and laboratory characteristics of newborns with reverse transcriptase polymerase chain reaction positive for coronavirus disease 2019.

Case #	Maternal age (years)	Gestational age (weeks)	Mode of delivery (reason)	Gender	Weight (g)	APGAR score (1–5 min)
1	35	38	C-section (gestational diabetes)	Female	3,555	8–9
2	24	42	Vaginal	Male	3,140	9–9
3	17	39	Vaginal	Female	3,390	8–9
4	20	37	C-section (breech presentation)	Male	3,325	9–9
5	19	42	C-section (postterm pregnancy)	Male	3,560	8–9
6	20	39	Vaginal	Male	3,345	8–9
7	34	40	C-section (previous C-section)	Male	3,250	9–9

RT-PCR: reverse transcriptase polymerase chain reaction; NA: not available; NS: no symptom; CRP: C-reactive protein; ALT: alanine aminotransferase; AST: aspartato aminotransferase; LDH: lactic acid dehydrogenase. Case #6: this newborn was transferred to tertiary hospital due to the diagnosis of bilateral hydronephrosis and urinary infection.

not at 24–48 h). Interestingly, there were two cases of perinatal COVID-19 in neonates of mothers who tested negative for SARS-CoV-2, which can be due to a mother's false-negative RT-PCR or neonatal transmission shortly after delivery.

Current data indicate an association between COVID-19 and a risk of preterm birth and low birth weight[14,15,22,24,25]. However, in our study, all newborns with perinatal COVID-19 were born at term and with adequate weight. This result is justified by the fact that our maternity care serves low-risk pregnancies. All neonates evolved without serious complications. Two newborns developed respiratory distress, which may be due to COVID-19 or adaptation of the fetal circulation to neonatal or newborn transient tachypnea, in addition to neonatal infection, as one of these children had a positive urine culture and was diagnosed with bilateral hydronephrosis.

CONCLUSION

Universal screening of pregnant women admitted for delivery is the best way to reduce the risk of perinatal transmission of COVID-19. At the beginning of the COVID-19 pandemic, still in a scenario without vaccination coverage and with financial and laboratory restrictions, as well as the low morbidity of COVID-19 in the neonatal period, it was necessary to carry out a selective screening. The observed results suggest that screening only symptomatic pregnant women at the time of admission for delivery in low-risk maternity was the correct alternative for regions with a low prevalence of the disease and where a better allocation of financial resources was needed.

AUTHORS' CONTRIBUTIONS

ANMC: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Validation, Visualization, Writing – original draft. **RLFA**: Conceptualization, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Writing – review & editing. **DNO**: Formal Analysis, Investigation, Methodology, Visualization, Writing – review & editing. **DML**: Formal Analysis, Investigation, Methodology, Visualization, Writing – review & editing. **CTMBC**: Formal Analysis, Investigation, Methodology, Visualization, Writing – review & editing. **LVST**: Formal Analysis, Investigation, Methodology, Visualization, Writing – review & editing. **RPA**: Formal Analysis, Investigation, Methodology, Visualization, Writing – review & editing. **RPGM**: Formal Analysis, Investigation, Methodology, Visualization, Writing – review & editing. **EAJ**: Formal Analysis, Investigation, Methodology, Visualization, Writing – review & editing. **MBC**: Conceptualization, Formal Analysis, Investigation, Methodology, Visualization, Writing – review & editing.

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ORIGINAL ARTICLE

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Role of magnetic resonance imaging in the differentiation of mucinous ovarian carcinoma and mucinous borderline ovarian tumors

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SUMMARY

OBJECTIVE: This study was carried out to investigate the differentiation of mucinous borderline ovarian tumor from mucinous ovarian carcinoma using magnetic resonance imaging.

METHODS: We evaluated 77 women patients who underwent abdominal magnetic resonance imaging due to pelvic mass. magnetic resonance imaging was reviewed by an experienced radiologist. A total of 70 women patients were included in the study. The magnetic resonance imaging features were retrospectively evaluated and compared between the two pathologies.

RESULTS: There was no difference between the two groups in terms of maximum tumor size. Age at diagnosis was 56.29±11.92 in the mucinous ovarian carcinoma group and 44.74±13.60 in the mucinous borderline ovarian tumor group ($p<0.05$). A significant difference was found between the two groups, and it was observed that mucinous borderline ovarian tumors appeared in the younger age group compared to mucinous ovarian carcinomas. Presence of ascites, peritoneal dissemination, lymphadenopathy, and mural nodules was found significantly more frequently in mucinous ovarian carcinomas than in mucinous borderline ovarian tumors. Honeycomb appearance was found more frequently in mucinous borderline ovarian tumor patients than in mucinous ovarian carcinoma patients.

CONCLUSION: magnetic resonance imaging findings of these two pathologies overlapped considerably. Compared with mucinous borderline ovarian tumors, mucinous ovarian carcinomas frequently had mural nodules larger than 5 mm, larger tumor size, peritoneal dissemination, and abnormal ascites.

KEYWORDS: Magnetic resonance imaging. Mucinous carcinoma. Ovary. Epithelial ovarian cancer.

INTRODUCTION

Mucinous ovarian neoplasms constitute 10–15% of epithelial ovarian neoplasms[1]. mucinous cystadenomas constitute 80% of ovarian mucinous neoplasms, while MBOTs and MOCs constitute about 16–17% and 3–4% of them, respectively[1]. Borderline tumors were first described as semi-malignant mass lesions[2]. They are histopathologically malignant but do not show invasive features, and their clinical course is quiescent[3]. Mucinous neoplasms can appear morphologically as giant multicystic masses. Therefore, magnetic resonance imaging (MRI) findings may be similar between these three subtypes[4].

Mucinous-derived lesions can show multiloculations. The formation of different signals in T1- and T2-weighted sequences of such loculations is described as "stained glass appearance." This finding is accepted as one of the characteristic MRI findings of mucinous neoplasms[5,6].

According to the generally accepted view, a contrasting solid component or thick septa in a cystic mass in epithelial ovarian tumors is considered significant for malignancy[7-10]. In addition, in previous studies on mucinous neoplasms, honeycomb-shaped loculations, T1 hyperintense, T2 hypointense intracystic signal, thick septa, and a cyst wall thicker than 5 mm were observed significantly more frequently in MBOTs than MOCs[7-10].

As most of the patients with MBOT are young, fertility-sparing surgery can be considered in this patient group. However, in patients with carcinoma, the main treatment is surgery and adjuvant or neoadjuvant chemotherapy[11]. Therefore, we aimed to describe, compare, and find differences in MRI findings

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between MBOT and MOC in our patients to improve the precision of the preoperative diagnosis.

METHODS

Patients

In our retrospective study, 45 patients with MBOT and 32 patients with MOC, diagnosed between February 2011 and September 2022, were included after approval from the ethics committee of our hospital. Two patients with MBOT and three patients with MOC were excluded from the study because their MR images could not be accessed. Another two patients with MOC were also excluded because their MR images were of poor technical quality.

Magnetic resonance imaging technique

MRI examinations were performed with the standard protocol using a 1.5 T MRI system (Siemens Avanto, Siemens Aera, GE Optima360) with a pelvic phased-array coil. The protocol included sagittal, axial, and coronal T2-weighted images without fat saturation, axial T2-weighted fat-saturated images, and axial T1-weighted fat-saturated gradient-echo images before and after intravenous contrast administration (Gadoteric acid, Dotarem®, Guerbet, Paris, 0.1 mmol/kg).

Image analysis

As MRI findings, among morphological features, tumor diameter, T2 hypointense component, mural nodule (MN), number of septa, thick septa (5 mm), honeycomb appearance, stained glass appearance, presence of ascites, and peritoneal spread were evaluated.

A solid component adhering to the septa or cyst wall was described as a mural nodule, and the evaluation was made with T2 and/or contrast-enhanced images. The number of loculations was evaluated quantitatively as 1–10, 10–20, and 20–30. Septa thickness of 5 mm and above was described as thick septa. The presence of multiple cysts, 5–10 mm in size and located in close proximity to each other, was evaluated as honeycomb appearance. Stained glass appearance was evaluated according to the different signal formations of intralesional loculations in T1- and T2-weighted sequences. T2 hypointense cyst was considered isointense or slightly hyperintense on T2-weighted sequences when compared to adjacent muscle tissue. While assessing the ascites, the presence of ascites exceeding the level of uterine fundus and/or filling the pelvic cavity was evaluated as a positive finding, and fluid at the Douglas level was counted as physiological. All findings were reviewed by an experienced abdominal radiologist.

Statistical analysis

Statistical analyses were performed using SPSS 24.0 (IBM Corp., Armonk, NY, USA) program. Normality tests, skewness-kurtosis values, and histogram graphs were used to determine whether the numerical variables were normally distributed. Student's t-test was used to determine whether numerical variables differed significantly between groups, and the χ^2 test was used to determine the differences between categorical variables between groups. Variables that differed between the two groups were included in the logistic regression analysis. The variables determining the differences between the groups were investigated with the logistic regression analysis applied with the "Enter" method. A p-value of <0.05 was considered significant in all statistical analyses.

RESULTS

In our study, 27 patients with MOC and 43 patients with MBOT were included. Age at the time of diagnosis was 56.29±11.92 in the MOC group and 44.74±13.60 in the MBOT group ($p<0.05$). There was no significant difference between the two groups regarding maximum tumor size. The mean tumor size was 203.55+79.66 mm in the MOC group and 175.11+88.93 in the MBOT group ($p<0.180$).

Categorical variables are summarized in Table 1. According to these findings, ascites, peritoneal carcinomatosis, lymphadenopathy (LAP), and mural nodules were observed significantly more frequently in MOCs than in MBOTs. In addition, honeycomb appearance was more commonly seen in MBOT patients than in MOC patients. The number of loculi, the presence of the T2 hypointense component, and stained glass appearance were emphasized and studied in previous research. However, in our study, there was no significant difference between the groups regarding those parameters.

The variables that differed between these two groups were included in the logistic regression analysis afterward. We identified the variables that had a direct and independent effect on the determination of the groups. As shown in Table 2, "honeycomb appearance" and "thick septa" have determined the difference between groups independently and directly. Beta value was positive in the presence of honeycomb sign and was negative in the presence of thick septa. This finding was interpreted as the "honeycomb sign" directly predicts the presence of MBOT and is considered to have diagnostic significance, while "thick septa" is found to predict the MOC group directly.

Table 1. Comparison of qualitative variables between groups.

Variables	MOC (n=27)	MBOT (n=43)	Statistical analysis*	
	n (%)	n (%)	χ^2	p-value
Abnormal ascites	9 (33.3)	5 (11,6)	4.88	**0.027**
Peritoneal carcinomatosis	9 (33.3)	0 (0)	16.44	**<0.0001**
Mural nodule (>5 mm)	17 (63.0)	10 (23.3)	11.03	**0.001**
Honeycomb sign	10 (37.0)	32 (74.4)	9.65	**0.002**
Septa (>5 mm)	26 (96.3)	26 (61.9)	10.46	**0.001**
Stained glass appearance	7 (25.9)	15 (34.9)	0.61	0.432
T2 hypointense cyst	11 (40.7)	12 (27.9)	1.23	0.266
Loculi (10–20)	4 (14.8)	9 (20.9)	0.41	0.522
Loculi (20–30)	1 (3.7)	8 (18.6)	3.28	0.070
Loculi (>30)	22 (81.5)	26 (60.5)	3.39	0.065
Lymphadenopathy	5 (18.5)	0 (0)	8.57	**0.003**

*Chi-square test. MOC: mucinous ovarian carcinoma; MBOT: mucinous borderline ovarian tumor. Statistically significant values are indicated in bold.

Table 2. Results of logistic regression analysis.

	B	SE	Exp (B)	p-value	95%CI
Constant	2.397	1.066	10.985	0.025	-
Abnormal ascites	0.121	1.139	1.129	0.864	0.121–10.529
Peritoneal carcinomatosis	-21.194	11405.94	0.000	0.999	0.000
Mural nodule (>5 mm)	-0.335	0.850	0.715	0.694	0.135–3.787
Honeycomb sign	3.306	1.208	27.286	**0.006**	2.559–290.970
Septa	-4.335	1.579	0.013	**0.006**	0.001–0.289
Lymphadenopathy	-17.253	14704.46	0.000	0.999	0.000

Dependent variable encoding (0: MOC; 1: MBOT). Nagelkerke R^2: 0.661. MOC: mucinous ovarian carcinoma; MBOT: mucinous borderline ovarian tumor. The bolded values show that the p value is stastically significant. The honeycomb sign and the septa are predictive features for the mucinous borderline tumors.

DISCUSSION

As observed within the spectrum of mucinous neoplasms, MOCs can develop from MBOTs after going through multiple stages of carcinogenesis[12-14]. Histopathologically, invasive carcinoma and areas showing borderline features can be simultaneously observed in the same mass. Intraoperative consultation/frozen results may not be definitive in terms of diagnosis due to the large size of mucinous tumors and heterogeneity in the epithelial ovarian tumors. Consequently, understaging has been observed in approximately one-third of mucinous ovarian tumor cases[15,16]. Therefore, it is important to distinguish between MBOT and MOC preoperatively to determine the surgical approach.

In our study, the presence of ascites, MN>5 mm, peritoneal involvement, thick septa>5 mm, and the presence of LAP were observed to be significantly higher in MOC cases than in MBOT cases (Figure 1). Honeycomb appearance was found to be more significant in the MBOT group. The aim was to identify the variables that have a direct and independent effect on determining the groups when the variables that differ between two groups are included in the logistic regression analysis. The results showed that "honeycomb appearance" and "thick septa" determine the difference between groups independently and directly (Table 2). Beta value was positive in the presence of honeycomb sign and was negative in the presence of thick septa. This finding was interpreted as the "honeycomb sign" directly predicts the presence of MBOT and is considered to have diagnostic significance, while "thick septa" is found to predict the MOC group directly (Figure 2).

In previous studies[17,18], MBOT is shown to be encountered in a wide age group (13–88 years) and the mean age of the patients was 40–49 years, while the mean age in the MOC

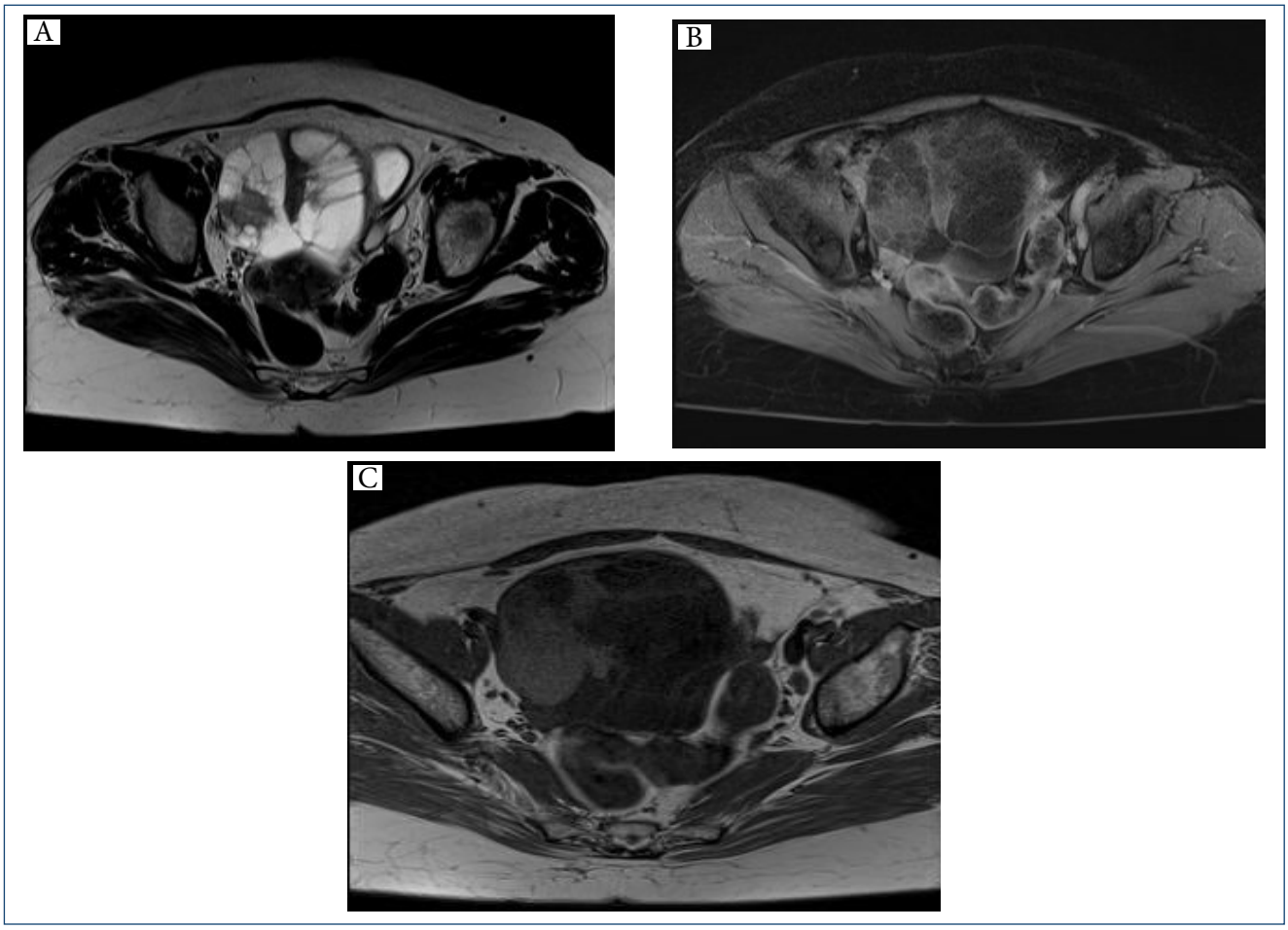

Figure 1. A 42-year-old woman with mucinous borderline tumor. **(A)** Axial T2-weighted image shows multilocular cystic tumor with stained glass appearance (arrow) and honeycomb sign (arrowhead). **(B)** T1-weighted image shows multilocular cystic tumor with stained glass appearance. **(C)** Contrast-enhanced T1-weighted image shows enhanced multiple thin septa.

group was 53 years. Consistently, the MOC group has been observed to consist of older patients compared to the MBOT group in our study as well. Our results revealed no significant difference between MBOT and MOC cases in terms of tumor size, though Kaga et al.[19] reported that tumor size in MOC cases was larger than in MBOT cases. In our experience, evaluating the tumor size alone may not be sufficient to distinguish carcinomas from borderline lesions, as all lesions within the spectrum of mucinous neoplasms (including cystadenomas) often present as large masses.

A solid component adhering to the septa or cyst wall was described as a mural nodule and evaluated accordingly. The presence of solid components may suggest malignancy as per the generally accepted view. In our study, the presence of MN was observed significantly more in the MOC group. However, a solid component was also detected in a substantial number of MBOT cases. Yang et al.[20] observed that the maximum size of the solid component is significantly larger in the MOC group when compared to the MBOT group. Upon reviewing the literature, we have come across a report of an MBOT case with a large solid component, published by Kozawa et al.[21], pointing out that evaluating the solid component alone can make the differential diagnosis process harder. Hence, the necessity of evaluating all parameters and the importance of synthesizing all of the findings to make differential diagnoses have arisen.

Among other findings, the presence of thick septa and ascites has been observed more in MOC cases than MBOT cases in our study. In Yang et al.'s[20] study, the presence of ascites was observed to be significantly more frequent in carcinoma cases. Similar statistically significant results have been noted in our study, as nine of our MOC patients and five of our MBOT patients had ascites. However, in our present sample, peritoneal washing cytology specimens of both groups were hypocellular and were negative for malignancy. The large size of mucinous neoplasms may create pressure, resulting in

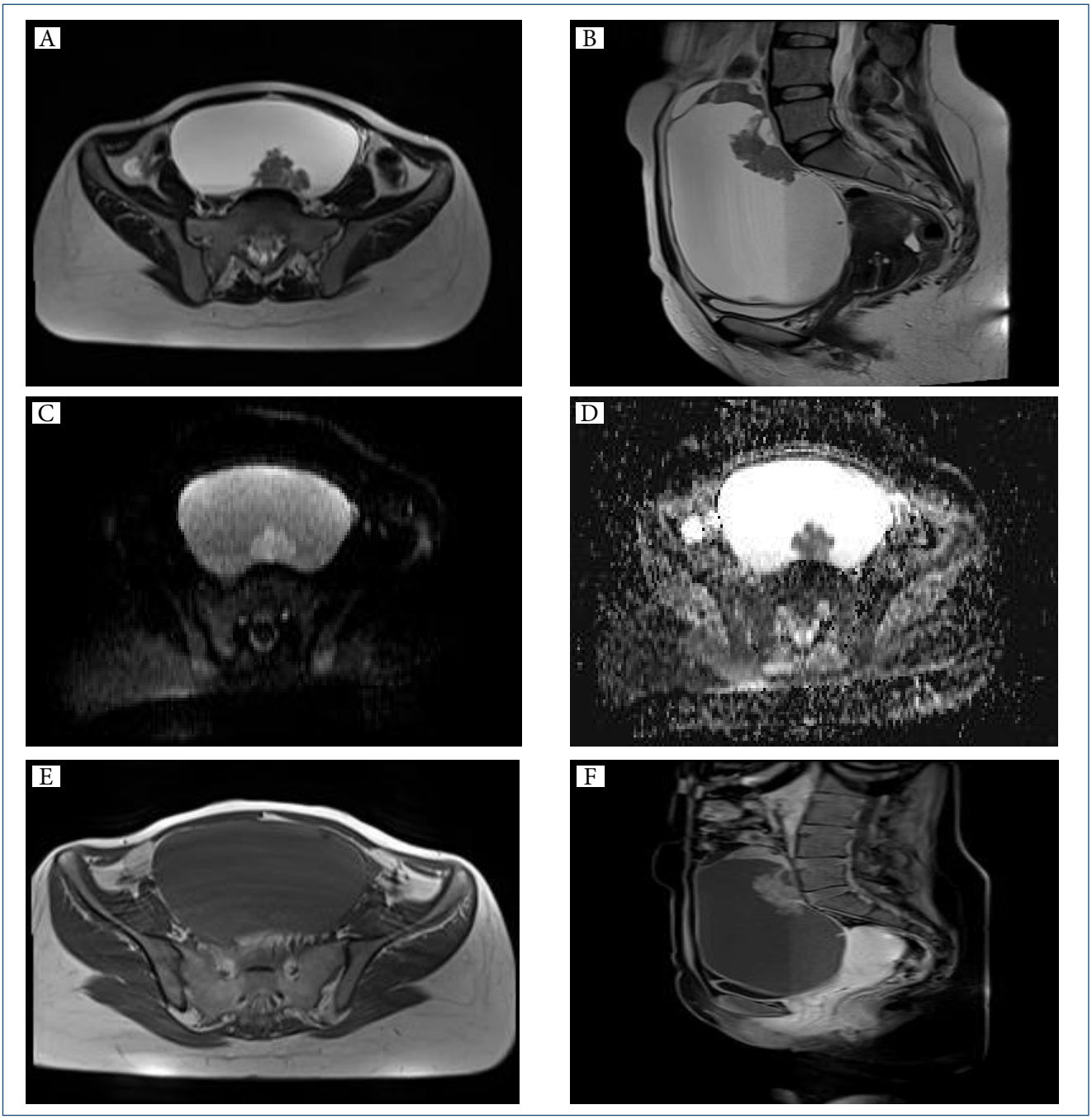

Figure 2. A 53-year-old woman with mucinous carcinoma. **(A and B)** Axial and sagittal T2-weighted image shows a gross multilocular cystic tumor with a mildly hyperintense mural nodule larger than 5 mm (arrow). **(C)** Diffusion-weighted image shows a hyperintense mural nodule (arrow). **(D)** Apparent diffusion coefficient (ADC) map shows a low ADC value. **(E)** T1-weighted image shows a hypointense mural nodule (arrow). **(F)** Contrast-enhanced T1-weighted image shows a moderately enhanced mural nodule (arrow).

congestion findings on the peritoneum and possible development of ascites.

Due to the proliferation rate of malignant tumor cells, MBOTs appear as masses with heterogeneous internal structures with thicker walls and septum, harboring more solid components (Figure 1).

There are some limitations to our study. First of all, our study is retrospective, and the number of patients with carcinoma is low. It was difficult to detect the thickest septa during the measurement of "thick septa" because mucinous neoplasms are known to have a multiseptal appearance. As a result, the contribution of "apparent diffusion coefficient (ADC)" values

to the diagnosis could not be examined either. We think that ADC measurement from the level of the septa or millimeter-sized mural nodule may be misleading, especially at these small sizes.

CONCLUSION

The MRI findings of MOCs and MBOTs are similar. MOCs tend to have larger tumor sizes and larger mural nodules, and the development of ascites is observed to be more frequent. "Honeycomb sign" can be used as a specific MRI finding for MBOT cases.

INSTITUTIONAL REVIEW BOARD STATEMENT

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee of our hospital.

DATA AVAILABILITY STATEMENT

There are no publicly stored datasets associated with this paper. Data are available upon request, from the corresponding author.

INFORMED CONSENT STATEMENT

Informed consent was not obtained because the study was in a retrospective design.

AUTHORS' CONTRIBUTIONS

EH: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Visualization, Writing—original draft, Writing – review & editing. **GG:** Data curation, Resources. **BGO:** Writing—review & editing. **MS:** Conceptualization, Data curation, Resources, Supervision.

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ORIGINAL ARTICLE

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Evaluation of the efficacy of systemic inflammatory indices in determining mortality in very low birth weight infants

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SUMMARY

OBJECTIVE: In our study, we aimed to investigate whether systemic inflammatory indices could be an indicator of mortality in very low birth weight (<1,500 g) preterm infants.

METHODS: Very low birth weight preterm infants were included in our study, and patient data were recorded retrospectively. Neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio, monocyte-to-lymphocyte ratio, systemic immune-inflammation index, pan-immune-inflammation value, and systemic inflammation response index were calculated and recorded. The survivors and infants who died were compared for systemic inflammatory indices.

RESULTS: A total of 1,243 very low birth weight infants were included in the study. Of the patients, 1,034 survived and 209 died. Neutrophil-to-lymphocyte ratio, monocyte-to-lymphocyte ratio, platelet-to-lymphocyte ratio, pan-immune-inflammation value, systemic immune-inflammation index, and systemic inflammation response index were found to be statistically significantly lower in the mortality group than those in the survivor group (p=0.039, p=0.001, p<0.001, p<0.001, p<0.001, and p=0.002, respectively). According to the receiver operating curve analysis, systemic immune-inflammation index with the highest area under the curve (0.844) was found to be the most effective systemic inflammatory indices in predicting mortality with a cutoff level of ≤28.87 (p=0.0001). Multiple regression analysis showed that a lower level of systemic immune-inflammation index (≤28.87) was independently associated with mortality (OR: 1.677, 95%CI 1.061–2.685, p=0.001).

CONCLUSION: We have shown that low systemic immune-inflammation index value in very low birth weight preterm infants may be a novel systemic inflammatory index that can be used to predict mortality.

KEYWORDS: Mortality. Infant. Inflammation. Very low birth weight.

INTRODUCTION

The most important factors affecting neonatal mortality are gestational week (GW) and birth weight (BW)[1]. Preterm infants may require a variety of diagnostic and therapeutic interventions depending on their GW, BW, and medical conditions[2]. Determining the risk factors that affect mortality and taking precautions for these risk factors help to reduce mortality[3]. Moreover, it is clear that, besides reducing mortality, effective/reliable parameters to predict mortality can provide valuable information about the prognosis of preterm infants to pediatricians, neonatal specialists, and families. Therefore, the search for new markers that can predict the prognosis of the preterm infant still continues[2-4].

Systemic inflammatory indices are calculated by numeric ratios of cells derived from complete blood count parameters. It has been reported that some systemic inflammatory indices can be an important determinant of mortality and a predictor of the prognosis in adult patients[5-7]. There is very less information available about systemic inflammatory indices in newborns[8,9]. Especially in preterm infants, it is still unknown whether systemic inflammatory indices can be used as indicators of mortality. In this study, which we designed based on these possible advantages, we aimed to evaluate whether systemic inflammatory indices could be an indicator of mortality in very low BW (VLBW) preterm infants.

METHODS

Study design

All VLBW (<1,500 g) infants who were admitted to the neonatal intensive care unit between January 2019 and April 2022 were retrospectively analyzed. Preterm infants with major congenital anomalies and BW ≥1,500 g were excluded from the study. Ethical approval was obtained from the local ethics committee (dated April 11, 2019; decision no. 47/2019), and the study followed the tenets of the Declaration of Helsinki.

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Demographical features and clinical outcomes

The data related to GW, BW, antenatal steroid, male gender, cesarean section, Apgar scores at 5th minute, early onset sepsis (EOS), late onset sepsis (LOS), respiratory distress syndrome (RDS), intraventricular hemorrhage (IVH), necrotizing enterocolitis (NEC), bronchopulmonary dysplasia (BPD), patent ductus arteriosus (PDA), retinopathy of prematurity (ROP), and mortality were recorded.

Complete blood count analysis

Blood samples were taken from all VLBW premature babies within the first hour after birth and placed in ethylenediaminetetraacetic acid tubes for complete blood count. Complete blood count was performed using Cell-Dyn 3700 automatic hemocytometer (Abbott, Abbott Park, IL, USA). Leukocyte count (10^3 μ/L), platelet (P) count (10^3 μ/L), neutrophil (N) count (10^3 μ/L), monocyte (M) count (10^3 μ/L), and lymphocyte (L) count (10^3 μ/L) values were recorded.

Calculation of systemic inflammatory indices

N, M, L, and P counts were used to calculate systemic inflammatory indices. Neutrophil-to-lymphocyte ratio (NLR)=N/L, platelet-to-lymphocyte ratio (PLR)=P/L, monocyte-to-lymphocyte ratio (MLR)=M/L, pan-immune-inflammation value (PIV)=P×N×M/L, systemic immune-inflammation index (SII)=P×N/L, and systemic inflammation response index (SIRI)=N×M/L values were calculated using the mentioned formulations[10].

Patients with and without mortality were compared in terms of demographic features and clinical outcomes, preterm morbidities, complete blood count, and systemic inflammatory indices.

Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS), version 20.0 (SPSS Inc., Chicago, IL, USA) analysis program. Histogram and Kolmogorov-Smirnov test were used to analyze the distribution of the data. Fisher's exact test or Pearson chi-square test was used for the analysis of categorical variables, and t-test or Mann-Whitney U test was used in the analysis of continuous variables. Normally distributed continuous variables were presented as mean±standard deviation, and non-normally distributed variables were presented as median and interquartile range (IQR). The results of categorical variables were presented as frequency. The receiver operating characteristics (ROC) analysis was carried out to evaluate the significance level of the variables. The area under the curve (AUC) and cutoff values were calculated using the ROC analysis. COX regression analysis was performed for investigating the association between the survival time of patients and one or more predictor variables. Odds ratios (ORs) and 95% confidence intervals (CI) were defined. A p-value of <0.05 was considered significant.

RESULTS

During the study period, 1,243 preterm infants were included in the study. Of these patients, 1,034 survived and 209 died. The mortality rate in VLBW infants was 16.8%. The GW (28.2±1.2 weeks) and BW (1,091±222 g) of preterm infants in the survivor group was significantly higher than the GW (27.5±1.1 weeks) and BW (911±227 g) of the infants in the mortality group (p<0.001 and p<0.001, respectively). The frequency of antenatal steroid administration and Apgar scores at the 5th minute were found to be significantly lower in the mortality group than those in the survivor group (p=0.006 and p<0.001, respectively). The frequency of RDS and IVH was significantly higher in the mortality group than that in the survivor group (p<0.001 and p<0.001, respectively). Gender, cesarean section, and the frequency of EOS, LOS, PDA, NEC, ROP, and BPD were found to be similar in both groups (p>0.05) (Table 1).

Table 1. Demographic characteristics and clinical outcomes in relation to mortality in very low birth weight infants.

Characteristics	Survivors (n=1034)	Mortality (n=209)	p-value
Gestational age, weeks[a]	28.2±1.2	27.5±1.1	<0.001*
Birth weight, g[a]	1091±211	911±227	<0.001*
Antenatal steroid[b]	715 (69.1)	128 (61.2)	0.006*
Male gender[b]	511 (49.4)	113 (54.1)	0.221
Apgar 5th minute[c]	8 (1)	7 (3)	<0.001*
EOS[b]	111 (10.7)	29 (13.8)	0.102
LOS[b]	227 (21.9)	53 (25.3)	0.445
RDS[b]	604 (58.4)	173 (82.7)	<0.001*
IVH[b]	67 (6.5)	44 (21.1)	<0.001*
PDA[b]	383 (37.0)	84 (40.2)	0.397
NEC[b]	23 (2.2)	3 (1.4)	0.427
ROP[b]	102 (9.8)	16 (7.65)	0.124
BPD[b]	204 (19.7)	31 (14.8)	0.215

[a]Mean±standard deviation. [b]n (%). [c]Median (interquartile range). *p<0.05 was considered statistically significant. BPD: bronchopulmonary dysplasia; EOS: early onset sepsis; IVH: intraventricular hemorrhage; LOS: late-onset sepsis; NEC: necrotizing enterocolitis; PDA: patent ductus arteriosus; RDS: respiratory distress syndrome; ROP: retinopathy of prematurity.

Platelet and neutrophil counts were significantly lower in the mortality group than those in the survivor group (p<0.001 and p=0.031, respectively). There was no difference between the groups in terms of leukocyte, monocyte, and lymphocyte counts (p=0.294, p=0.153, and p=0.551, respectively). NLR, MLR, PLR, PIV, SII, and SIRI were significantly lower in the mortality group than those in the survivor group (p=0.039, p=0.001, p<0.001, p<0.001, p<0.001, and p=0.002, respectively) (Table 2 and Figure 1).

ROC analysis was performed for systemic inflammatory indices that were statistically significant in terms of mortality. The AUC value of NLR was 0.568, and the cutoff

Table 2. Systemic inflammatory indices in very low birth weight infants.

Laboratory parameters	Survivors (n=1034)	Mortality (n=209)	p-value
Leukocyte count (10^3 μ/L)[a]	10.90 (8.32)	12.30 (11.00)	0.294
Platelet count (10^3 μ/L)[a]	232.00 (101.25)	198.00 (87.50)	<0.001*
Neutrophil count (10^3 μ/L)[a]	2.25 (2.62)	2.21 (2.01)	0.031*
Monocyte count (10^3 μ/L)[a]	0.66 (0.58)	0.66 (0.80)	0.153
Lymphocyte count (10^3 μ/L)[a]	7.05 (5.75)	8.56 (7.29)	0.551
NLR[a]	0.33 (0.37)	0.27 (0.32)	0.039*
MLR[a]	0.09 (0.06)	0.08 (0.07)	0.001*
PLR[a]	35.34 (31.55)	21.65 (17.76)	<0.001*
PIV[a]	48.91 (100.64)	35.39 (65.29)	<0.001*
SII[a]	82.81 (94.65)	17.48 (41.52)	<0.001*
SIRI[a]	0.20 (0.32)	0.18 (0.33)	0.002*

[a]Median (interquartile range). *p<0.05 was considered statistically significant. MLR: monocyte-to-lymphocyte ratio; NLR: neutrophil-to-lymphocyte ratio; PIV: pan-immune-inflammation value; PLR: platelet-to-lymphocyte ratio; SII: systemic immune-inflammation index; SIRI: systemic inflammation response index.

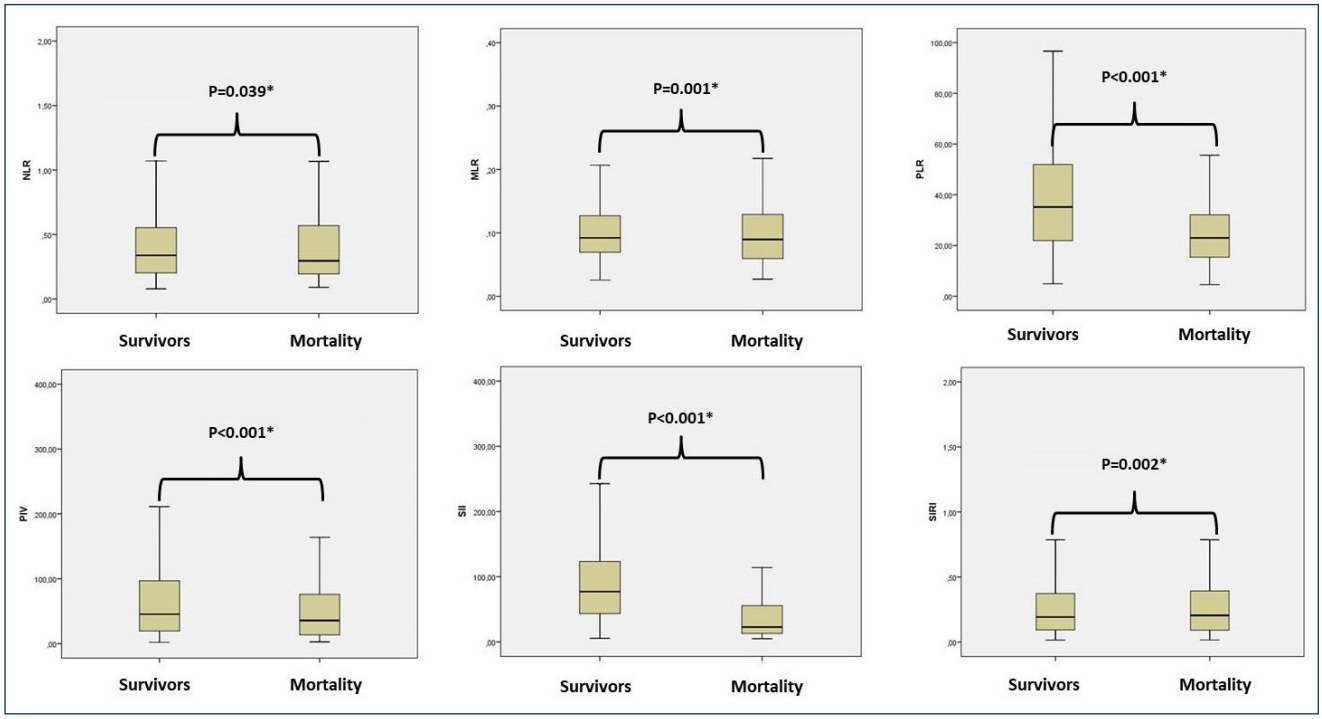

Figure 1. Box plot of systemic inflammatory indices for mortality in preterm infants. *p<0.05 was considered statistically significant. MLR: monocyte-to-lymphocyte ratio; NLR: neutrophil-to-lymphocyte ratio; PIV: pan-immune-inflammation value; PLR: platelet-to-lymphocyte ratio; RDS: respiratory distress syndrome; SII: systemic immune-inflammation index; SIRI: systemic inflammation response index.

level was ≤0.33 for the estimation of mortality. The AUC value of MLR for the predictivity of mortality was 0.546, and the cutoff level was ≤0.06. The AUC value of PLR for the predictivity of mortality was 0.702 and the cutoff level was ≤27.46. PIV had an AUC of 0.577 and a cutoff level of ≤66.65 for the predictive of mortality. The AUC of SII for predictivity of mortality was 0.844, and the cutoff level was ≤28.87. The AUC value of SIRI for the predictivity of mortality was 0.541, and the cutoff level was ≤0.10 (p=0.0013, p=0.0330, p=0.0001, p=0.0002, p=0.0001, and p=0.0430, respectively). The ROC graph for SII is presented in Figure 2.

COX regression analysis was performed for investigating the association between the survival time of patients and one or more predictor variables. The potential confounder risk factors, including GW, BW, and antenatal steroid administration, were subsequently entered into the multivariable COX regression model. It was found that the risk of mortality was independently associated with antenatal steroid administration of completed doses (OR: 1.323, 95%CI: 1.112–1.575, P=0.001), GW (OR: 1.878, 95%CI: 1.777–1.991, P=0.001), and BW (OR: 1.997, 95%CI: 1.897–2.219, P=0.001). Multiple Cox analysis showed that a lower level of SII (≤28.87) was independently associated with mortality (OR: 1.677, 95%CI 1.061–2.685, P=0.001).

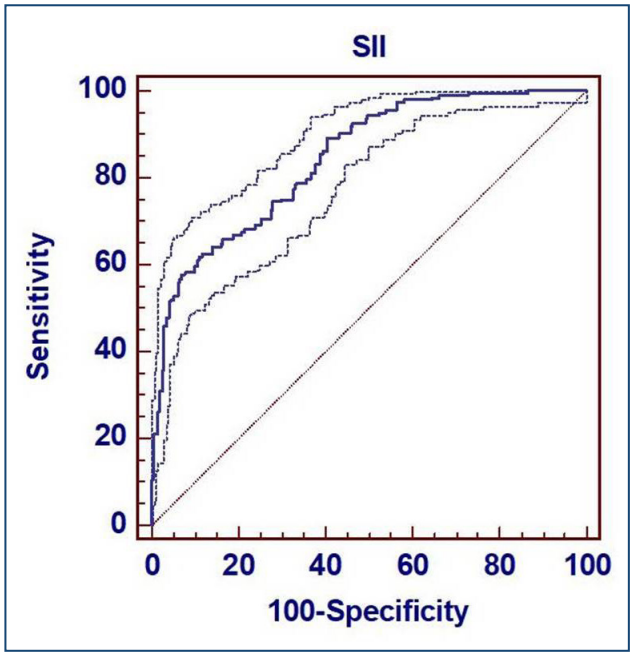

Figure 2. Receiver operating characteristic curves for mortality in very low birth weight infants.

DISCUSSION

In this study, we evaluated the relationship between mortality and systemic inflammatory indices in VLBW infants, including NLR, MLR, PLR, PIV, SII, and SIRI. We determined that all mentioned systemic inflammatory indices were lower in patients who died. When the predictivity significance level of mortality of the six systemic inflammatory indices was evaluated, SII was the most effective systemic inflammatory index among others. SII values of ≤28.87 were related to the predictivity of mortality. Moreover, GW and BW were lower in VLBW infants who died, while RDS and IVH were found to be more frequent[2].

The most important factors determining morbidity and mortality in premature infants are their GW and BW. As GW and BW decrease, morbidities and mortality increase inversely. If antenatal steroid administration decreases, the mortality rate may increase even more, as in our results. The use of Apgar scores to evaluate mortality and clinical outcomes in preterm infants is limited. The use of laboratory parameters in addition to clinical scores may be more useful in predicting mortality, especially in VLBW infants[2-4]. For this purpose, systemic inflammatory indices were evaluated in our study in order to assist in the early and effective estimation of mortality, especially in VLBW infants who were at higher risk for mortality.

Low platelet count in newborn infants may be associated with increased mortality. The main relationship between increased mortality and decreased platelet count is due to increased inflammation, which decreases platelet production and circulating megakaryocyte progenitors. In our results, although the platelet count decreased in the mortality group, a decrease in neutrophil count seems to be evidence of higher inflammation. However, low platelet count alone may not always be an indicator of mortality. Therefore, it may be more beneficial to use markers that can be recognized as indicators of inflammation as a predictor of mortality. In this respect, in our study, we showed, for the first time, that among the six systemic inflammatory indices, the marker with the highest predictive value for mortality was the SII value[11].

It has been shown that higher NLR, MLR, PLR, PIV, SII, and SIRI are positively associated with the severity of the disease and the mortality of the patients in oncology, patients and those with sepsis[5,12]. Particularly, SII has been reported to be closely associated with the prognosis of cancer, multiple sclerosis, coronary artery bypass surgery, and pulmonary embolism compared to other systemic inflammatory indices[6,7,13]. As can be seen from this information, according to the results of these studies in adults, the effectiveness and significance level of systemic inflammatory indices may vary depending on the type of disease.

Based on the results of studies conducted on adults, the relationship between systemic inflammatory indices and neonatal

disease has recently been investigated. When maternal systemic inflammatory indices were examined, it was reported that the NLR value of the systemic inflammatory indices was the most effective indicator of preterm delivery in mothers who gave preterm delivery[14]. It has been reported that higher NLR, MLR, and PLR may be an indicator in the diagnosis of preterm morbidities[8,9]. Six systemic inflammatory indices have been evaluated for the diagnosis of HIE in preterm infants. Higher NLR, PIV, SII, and SIRI and lower MLR and PLR have been reported to be useful markers for the diagnosis of HIE. Among these systemic inflammatory indices, NLR and SII, which have the highest AUC values for the diagnosis of HIE, are reported to be the most effective diagnostic markers. The cutoff value of SII for the diagnosis of HIE is found to be >410, and the AUC value is 0.763[10]. In our results, the AUC value of SII for the predictivity of mortality in VLBW infants was 0.844, and the cutoff value was ≤28.87. It has been reported that an SII level ≥78.2 after birth may be a risk factor for the development of RDS in preterms ≤32 weeks of gestation[15]. It was found that the high SIRI value could predict moderate to severe BPD in preterm infants[16]. According to these results, when using systemic inflammatory indices in the evaluation of neonatal diseases, it seems to be a more accurate approach to evaluate each index specific to the diseases. To the best of our knowledge, in this study, for the first time, SII was found to be the most effective parameter in the predictivity of mortality in VLBW infants among the six systemic inflammatory indices.

One question is why the most effective parameter among these indices for mortality indicator was found to be SII. The neutrophil, lymphocyte, and platelet values used in the SII formulation may help us understand the relationship between mortality and systemic inflammatory indices. Neutrophils and platelets used in the SII formulation decreased significantly in VLBW infants who died. There was no significant difference between our groups in terms of lymphocyte count. However, when lymphocyte counts were used together with neutrophils and platelets in the formulation of SII, it could be interpreted that the significance level of SII increases as an indicator of mortality. Additionally, each disease occurs with its own different pathophysiological mechanisms. The immune response to any disease may vary according to the patient's postnatal age and GW[17]. According to our results of the study group consisting of preterm infants, it seems that adequate neutrophil and platelet cell response did not occur in patients who died. Moreover, outcomes seemed to be reflected as mortality in the clinical follow-up of these patients and as a lower SII value in the laboratory follow-up.

Both scoring systems and laboratory parameters were evaluated for the indicator of mortality in newborn infants. However, the immaturity of preterm infants limits the use of these tools for mortality. Therefore, the search for additional indicators continues. The ideal tool for demonstrating mortality should be a powerful parameter that is quickly available and does not create additional costs for the clinician to predict mortality. With the parameters that meet these features, the clinician will have more reliable information about the patient's prognosis. In addition, it will provide more reliable information about the prognosis of the preterm infant to both parents and other doctors who treat the patient. Thus, the SII value can be a new and active parameter. As the SII value was effective, rapid, and readily available obtained from blood counts in VLBW infants, it may also provide an advantage as it can show mortality without additional costs[2-4].

The main strengths of our study are as follows: a large case series was studied with 1,243 VLBW infants. In addition, there are currently no strong enough parameters to indicate mortality in these highly frail infants. According to our results, it was shown, for the first time, that the SII value among the parameters evaluated based on inflammation could be a safe indicator for predicting mortality in VLBW infants. Finally, the SII value could be an easy-to-use parameter as it did not require additional costs and extra time to predict mortality.

Although the large number of patients was the strength of our study, there were also some limitations. Our main limitation was that the study was conducted retrospectively in a single center. The inability to monitor the systemic inflammatory indices serially on postnatal days and not being able to compare them with the values in term babies could be counted among our other limitations.

CONCLUSION

The research for an effective parameter that can be used for the predictivity of mortality in preterm infants is still ongoing. Our results determined, for the first time, that six systemic inflammatory indices were decreased in preterm infants resulting in mortality compared to survivors. In addition, among these six indices, SII was found to be the most effective systemic inflammatory indices for predicting mortality. Another important advantage of SII was that it was cheap, simple, fast, and easily accessible. Further prospective studies conducted with larger case series should be warranted.

AUTHORS' CONTRIBUTIONS

UC: Conceptualization, Methodology, Writing – original draft.
AUT: Data curation, Formal Analysis, Investigation, Validation.
CT: Supervision, Writing – review & editing.

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ORIGINAL ARTICLE

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Effects of closed and open kinetic chain exercises on pain, muscle strength, function, and quality of life in patients with knee osteoarthritis

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SUMMARY

OBJECTIVE: Therapeutic exercises are well documented for the treatment of osteoarthritis; there is less evidence on what the effect of closed kinetic chain exercises is for knee osteoarthritis. The aim of this study was to investigate the effects of open kinetic chain exercises and closed kinetic chain exercises on pain, muscle strength, functional status, and quality of life in patients with knee osteoarthritis.

METHODS: The study included a total of 60 patients with primary unilateral knee osteoarthritis grade I and II. The patients were categorized into three groups as open kinetic chain exercises (n=20), closed kinetic chain exercises (n=20), and control group (n=20). The outcome measures, including pain, isokinetic muscle strength, functional status, and quality of life, were collected at baseline and at the end of 6 and 12 weeks.

RESULTS: Closed kinetic chain exercises and open kinetic chain exercises had significant improvement in pain, muscle strength, WOMAC, and SF-36 scores after the treatment and at their 6th and 12th week follow-ups compared to their baseline values and compared to the control group ($p<0.05$).

CONCLUSION: The changes in all outcome measures were similar between closed kinetic chain exercises and open kinetic chain exercises ($p>0.05$). Closed kinetic chain exercises and open kinetic chain exercises were similar for knee osteoarthritis grade I and II. Closed kinetic chain exercises could be safely added to the exercise programs of patients with low-grade knee osteoarthritis.

KEYWORDS: Knee osteoarthritis. Weight bearing exercise program. Joint pain. Muscle strength. Functional status.

INTRODUCTION

Knee osteoarthritis (OA) is an important and painful health problem as it leads to functional disability and reduced quality of life (QoL)[1]. Furthermore, knee OA is a significant cause of disability and accounts for 3% of all disability causes[2]. As OA leads to disability and consequent labor and economic loss[2], its treatment is of great importance. Studies have shown that knee OA is characterized by inadequacy and pain associated with decreased quadriceps muscle strength. Strengthening training and has been shown to have positive effects on OA[3]. OA treatment is classified under three headings as follows: pharmacological methods, non-pharmacological methods, and surgical methods[4]. There are several studies showing the effectiveness of exercise training as a non-pharmacological method[5] as it is an easy and low-cost method that can be done for a long time. Exercise training is more effective than other treatment methods in terms of increasing the physical activity level of patients and enhancing physiological improvements such as increased muscle strength, flexibility of soft tissues, and ROM[6].

In spite of numerous studies reporting the importance of different types of exercise in the treatment of knee OA, the literature on exercise programs with optimal gains for knee OA has not yet been established[7]. The focus of knee and hip rehabilitation exercises for degenerative diseases has gradually shifted from open kinetic chain exercises (OKCE) to closed kinetic chain exercises (CKCE), which are more functional and could be applied safely and effectively. In addition to increasing muscle strength, CKCE could also facilitate joint position sense[8]. Nevertheless, it seems that researchers frequently prefer OKCE to decrease symptoms of hip or knee OA instead of CKCE[9].

It is well known that CKCE increase muscle strength and improve proprioceptive function by activating more muscle spindle and joint proprioceptors, consequently preparing the patient for daily living activities as they simulate some activities such as walking, climbing stairs, or standing up from a chair[10]. In addition, CKCE allow early weight bearing and mobilization and are usually performed after anterior or posterior cruciate ligament injuries or reconstruction surgeries[11]. Some researchers emphasize that CKCE cause axial loading and consequently increase compressive and destructive stress on the joint structures particularly on cartilage tissue. Therefore, the results of studies about the effects of CKCE on OA are

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controversial because some predict that CKCE cannot be easily tolerated or may increase symptoms in patients with hip or knee OA. The aim of this study was to investigate the effects of CKCE on the severity of knee pain and stiffness, isokinetic muscle strength, WOMAC functional scores, and SF-36 QoL scores in patients with knee osteoarthritis and compare them with the effects of OKCE.

METHODS

Participants

This study was carried out with a total of 60 patients with knee OA grade I and II according to the Kellgren-Lawrence classification. The participants, diagnosed with knee OA according to the radiographic assessment of their tibiofemoral joints by the same orthopedist, were randomly assigned to one of the three groups using an online random allocation software program.

The inclusion criteria were as follows: age between 45 and 75 years, diagnosed with unilateral knee OA grade I or II, and ongoing pain for at least 3 months. Patients with active synovitis, those who had participated in physiotherapy, conservative therapy, or regular exercise programs in the last 6 months, those who had undergone orthopedic surgeries in their knees, and those who were under medication (pain killers or NSAIDs) during the study were excluded from the study. Exclusion due to health problems at 6 and 12 weeks reveals other systemic health problems not related to exercise.

This study was approved by the Institutional Ethics Committee for Non-Invasive Human Research (ethical protocol number 05/2017), and in accordance with the policies and procedures of the Declaration of Helsinki, each participant provided verbal and written informed consent after being informed about the study.

Interventions

The participants were randomly categorized into three groups: CKCE group (n=20), OKCE group (n=20), and control group (CG) (n=20). Patients in the CKCE and OKCE groups did exercises three times per week for a total of 12 weeks (the first 6 weeks under supervision and the second 6 weeks as progressive home exercise program). During the last 6 weeks, the patients in the CKCE and OKCE groups visited the physiotherapy department every 2 weeks for their exercise control and progression. Patients in the control group were asked to apply the home program three times per week for 12 weeks. Prepared by researchers, this program included standard OA exercises and was handed to the CG patients as printed brochures. As a home program, both the OKCE and CKCE groups were used together with the control group; ankle dorsiflexion/plantar flexion, active knee extension, hip adduction, heel slide, pelvic elevation, and hip adduction. Participants in the control group were checked for their participation in the exercise program via telephone.

CKCE: The patients in this group performed progressive "sit to stand, mini squat, anterior lunge and three-side step-up" exercises in a closed-chain position.

OKCE: The patients in this group performed "terminal knee extension, concentric quadriceps, and four-way straight leg rise" exercises as hip flexion, extension, abduction, and adduction. The exercise session lasted an average of 45 min including the warming and cooling phases.

Outcome measures

Pain severity: Affected knee pain at rest was measured using visual analog scale (VAS)[12].

Isokinetic muscle strength: Although the isokinetic test is an open kinetic chain measurement, it is the gold standard in measuring muscle strength in patients with knee osteoarthritis[13]. For quadriceps and hamstring muscles, five repeated isokinetic muscle strength tests were performed at angular velocities of 90°/s, 120°/s, and 180°/s using an isokinetic testing device (Cybex System 4 Pro).

Functional level: As a valid and commonly used evaluation index in OA, the WOMAC index was used to evaluate the functional level of the patients[14].

Joint stiffness: The WOMAC index was used to evaluate the stiffness of the joints.

Quality of life: The Turkish version of the SF-36 was used to assess the QoL of the patients. This form includes 36 items and provides 8 dimensional measurements[15].

Statistical analyses

Two-way ANOVA (mixed-model, 3 (time)×3 (group), repeated measures) was used to determine changes in dependent variables from baseline to posttreatment measurements.

RESULTS

The study was completed with 60 patients. All groups had similar demographic and anthropometric characteristics ($p>0.05$) and baseline outcome measurements ($p>0.05$) (Table 1).

Pain severity

For pain intensity, there was a significant effect of time observed in all groups according to statistical analysis ($p<0.05$). Post hoc

analyses revealed a significant difference in change for pain at rest between baseline values and after 6th and 12th week values for both intervention groups (p<0.05). But there was no significant difference between the OKCE and CKCE groups at 6 and 12 weeks (p>0.05) (Table 2).

Isokinetic knee extension and flexion muscle strength

Knee extension and flexion muscle strength increased at the 6th and 12th week evaluations in both kinetic-chain groups. Yet, there was no change in the control group (p>0.05). For knee flexion and extension muscles isokinetic strength, there was a significant effect of time, with all groups showing an increase. There was also a significant time-group interaction. There was a significant difference between groups when groups were compared across various time points (p<0.05) (Table 3).

Functional level

For WOMAC scores (pain, stiffness, and physical function), there was a significant effect of time, with all groups showing a decrease (lower values indicate improvement). There was also a significant time-group interaction. There was a significant difference between groups when the groups were compared across various time points (p<0.05) (Table 3).

Quality of life

Post-intervention at the 6th and 12th week evaluations, no difference was found between OKCE and CKCE for pain severity, muscle strength, WOMAC scores, and SF-36 score (p>0.05). But there were significant differences between the control group and intervention groups after the 6th and 12th week values (p<0.05) (Tables 2 and 3).

DISCUSSION

To conduct this study, 12-week-long CKCE and OKCE protocols were prepared for the patients with knee osteoarthritis. These two exercise protocols were investigated in terms of their effects on pain, isokinetic muscle strength, WOMAC functional scale, and QoL. The results of this study demonstrated that both protocols reduced pain and joint stiffness and improved isokinetic knee muscle strength as well as WOMAC and SF-36 scores after 6 and 12 weeks. In addition, these two types of kinetic-chain exercises were found to have similar effects on all outcome measures.

Pain

Patients in the CKCE and OKCE groups, but not those in the control group, had a significant decrease in their knee pain by the end of the 6 and 12 weeks. In this study, the exercises in the closed kinetic chain position, which were performed with body weight from the first day, were carried out under a certain plan for 12 weeks and performed under supervision. Pain intensity in our patients in the CKCE group decreased in the third month at a similar rate to the OKCE group patients. The fact that the results of the CKCE program and the OKCE group were close suggested that the exercises given were aimed at the lower extremity, especially the muscles around the knee, and that the improvements in muscle strength and joint stability were similar.

Table 1. Comparison of groups' descriptive and demographic characteristics.

	OKCE (n=20) X±SD	CKCE (n=20) X±SD	Control (n=20) X±SD	p-value
Age (years)	53.05±10.88	54.40±7.92	56.10±12.73	0.667**
Height (cm)	163.00±9.85	162.55±7.04	160.25±12.03	0.641**
Body weight (kg)	79.40±13.35	75.85±13.49	76.40±12.98	0.410**
BMI (kg/m^2)	30.05±5.55	28.93±6.27	29.45±6.28	0.854**
		n (%)	n (%)	n (%)	p-value
Gender	Female	11 (55)	10 (50)	9 (45)	0.819*
	Male	9 (45)	10 (50)	11 (55)	
Affected leg	Left	10 (50)	10 (50)	9 (45)	0.935*
	Right	10 (50)	10 (50)	11 (55)	
Radiological assessment	Grade I	3 (15)	10 (50)	8 (40)	0.057*
	Grade II	17 (85)	10 (50)	12 (60)	

OKCE: open kinetic chain exercise group; CKCE: closed kinetic chain exercise group; BMI: body mass index; *Chi-square test; **Kruskal-Wallis test; X: mean; SD: standard deviation.

Table 2. Two-way mixed model ANOVA post hoc multiple comparison of participants' pain and muscle strength.

Variable	Time frame	OKCE (n=20) Mean±SD	CKCE (n=20) Mean±SD	Control (n=20) Mean±SD	Time		Group × time	
					F	p-value	F	p-value
Pain (VAS) 0–10	Baseline	5.31±0.86	4.87±1.24	5.26±1.13	81.075	<0.001*	18.248	<0.001*#
	6th week	2.07±1.22	2.55±1.61	5.10±1.75				
	12th week	2.01±1.14	1.76±1.07	5.08±1.37				
90°/s Extension (Nm)	Baseline	62.02±14.31	57.59±14.83	63.46±10.94	189.817	<0.001*	597.661	<0.001*
	6th week	76.57±15.59	69.67±16.56	64.06±11.03				
	12th week	76.79±15.24	70.17±16.39	64.48±11.72				
120°/s Extension (Nm)	Baseline	51.19±8.31	54.65±10.13	51.52±7.95	70.693	<0.001*	21.546	<0.001*x
	6th week	59.08±9.99	61.14±11.19	51.21±8.25				
	12th week	58.34±10.76	60.72±11.75	50.98±8.56				
180°/s Extension (Nm)	Baseline	36.08±16.02	37.08±13.04	36.83±12.02	120.811	<0.001*	28.964	<0.001*
	6th week	48.24±16.42	46.73±13.95	36.90±11.75				
	12th week	48.47±17.12	47.23±13.86	37.32±12.64				
90°/s Flexion (Nm)	Baseline	46.47±10.60	43.35±12.13	43.64±11.21	28.105	<0.001*	13.522	<0.001*
	6th week	52.78±11.54	47.87±12.40	42.49±11.37				
	12th week	51.98±11.40	47.79±12.64	42.58±11.52				
120°/s Flexion (Nm)	Baseline	56.67±11.40	53.50±9.39	53.86±8.89	33.361	<0.001*	17.209	<0.001*x
	6th week	64.61±12.90	57.83±10.06	53.26±9.16				
	12th week	63.92±11.54	56.72±10.58	57.62±11.44				
180°/s Flexion (Nm)	Baseline	61.44±13.68	58.82±15.26	57.57±12.66	2.080	N.S.	5.416	<0.001*
	6th week	64.24±17.16	60.59±16.86	56.18±13.05				
	12th week	64.74±18.00	59.89±17.24	55.21±14.63				

*Significant difference; CG: control group; N.S.: not significant; #significant difference with the control group; xSignificant difference between OKCE and CKCE.

Muscle strength

There are several studies in the literature about progressive resistive exercises and muscle strength in knee OA, due to the fact that knee extensor and flexor muscle weakness increases the risk of knee osteoarthritis[16]. Although it is well known that CKCE and OKCE are both effective in improving quadriceps muscle strength in knee OA, there is no consensus regarding the comparative effectiveness of these two types of kinetic-chain exercises[17]. The effects on muscle strength can differ among studies depending on the duration, intensity, or number of sets of the exercises. In some studies, it has been shown that both types of exercises have similar efficacy; the others, however, report that CKCE has superior effects on muscle strength as it improves electromyographic activities on muscle fibers, particularly type IIB, and it also has large neural adaptive responses[18,19]. In contrast, there are a small number of studies about CKCE and knee or hip OA and research focusing on the effectiveness of CKCE in ligamentous injuries of the knee, particularly in young patients with ACL injuries or after reconstruction and patellofemoral pain syndromes[20-22]. Additionally, unlike CKCE, OKCE and combined exercises (OKCE and CKCE) have been well documented to improve quadriceps muscle strength in knee OA[19]. Similar to the results of our study, Olagbegi et al. reported that OKCE, CKCE, and combined exercises are similarly effective in improving quadriceps muscle strength in grade II knee OA.

In this study, 12-week-long OKCE and CKCE progressive exercise programs were planned for each patient with OA. Particular attention was paid to activate same muscle groups to keep the two exercise programs homogenous. As a result of the study, in the OKCE and CKCE groups, significant increases in muscle strength were observed in both knee flexors (hamstring muscles) and knee extensors (quadriceps muscles) compared to the control group. However, comparing the effects of OKCE and CKCE showed that the increase in muscle strength was similar at all angular velocities. Our patients with knee OA grade I and II could benefit from participation in exercise programs because their pain severity was moderate and not too high. These 12-week-long regular and supervised

Table 3. Two-way mixed model ANOVA post hoc multiple comparison of participants' physical function and quality of life scores.

Variable	Time frame	OKCE (n=20) Mean±SD	CKCE (n=20) Mean±SD	Control (n=20) Mean±SD	Time		Group × time	
					F	p-value	F	p-value
WOMAC Pain	Baseline	9.70±4.15	8.90±4.27	10.15±3.66	27.984	<0.001*	10.519	<0.001*#
	6th week	6.65±3.66	5.80±5.27	10.80±4.26				
	12th week	6.65±4.35	6.00±5.43	10.30±4.49				
WOMAC Stiffness	Baseline	3.95±1.35	4.15±1.08	3.90±1.11	51.510	<0.001*	12.275	<0.001*#
	6th week	2.30±1.08	2.50±1.31	3.85±1.42				
	12th week	2.35±1.38	2.55±1.27	3.90±1.88				
WOMAC Physical function	Baseline	33.20±9.32	33.55±10.31	32.90±8.76	115.030	<0.001*	24.471	<0.001*
	6th week	23.65±9.61	24.05±9.94	32.60±8.42				
	12th week	23.00±9.59	24.15±10.58	32.65±8.59				
SF-36 Physical Functioning	Baseline	44.00±4.75	44.30±4.56	45.25±5.25	13.639	<0.001*	5.377	<0.001*#
	6th week	52.75±11.41	54.80±12.57	44.50±9.58				
	12th week	52.00±14.81	55.05±15.71	43.25±11.72				
SF-36 Role Limitations (Physical)	Baseline	11.50±11.36	16.00±11.42	13.00±9.23	56.00	<0.001*	114.00	<0.033*#
	6th week	26.00±9.94	22.50±11.18	14.00±10.46				
	12th week	28.00±10.05	24.00±12.73	13.50±12.25				
SF-36 Pain	Baseline	30.75±16.74	30.25±13.76	31.12±17.61	14.057	<0.001*	4.313	<0.003*#
	6th week	49.50±25.75	49.00±19.62	30.05±20.04				
	12th week	48.25±23.84	49.62±27.91	29.25±21.38				
SF-36 General Health Perception	Baseline	53.50±22.48	51.75±22.95	51.75±19.68	54.891	<0.001*	14.932	<0.001*
	6th week	71.25±20.25	69.25±21.23	51.00±20.65				
	12th week	70.75±20.14	68.75±22.64	51.00±22.10				
SF-36 Mental Health	Baseline	56.40±13.35	58.80±13.11	59.00±15.94	10.211	<0.001*	3.310	<0.001*#
	6th week	73.20±9.97	69.40±9.20	59.20±6.43				
	12th week	68.80±11.50	62.20±14.76	58.80±7.00				

*Significant difference; CG: control group; #significant difference with the control group.

exercise programs led to favorable physiological responses[23,24] and created positive changes on both neural, cellular, and hormonal elements, causing a similar increase in muscle strength.

Functional level

Studies investigating the effects of OKCE and CKCE programs on functional levels, evaluated using the WOMAC index, have reported inconsistent results; while the effects of these two exercise types tend to be similar in some studies, others report benefits in favor of either of the programs.

Several studies in the literature report that exercise programs increase functional level in patients with knee OA[7,25]. According to the results of some studies, CKCE might be slightly more effective than the other therapeutic exercises and practices, since the CKCE exercises include weight transfer and are similar to functional daily activities such as sitting and stair climbing[9,26,27].

In this study, OKCE and CKCE had similar effects on the patients' functional levels. This may be because one of these programs was composed of resistive exercises, which—if performed regularly—can lead to improvements in neuromuscular system and proprioceptive structures by stimulating mechanoreceptors. The other reason can be the fact that OKCE and CKCE programs can reduce knee extensor limitation and increase quadriceps muscle strength, which decrease joint stiffness, knee flexion contracture, or imbalance of the knee muscle strength.

Quality of life

OA plays a dramatic role in decreasing the quality of the patients' lives as it causes physical, psychological, and social impairments associated with inactivity and pain. Although exercise programs for knee OA have been reported to improve QoL by improving variables such as pain, stiffness, joint stability, or

the functional status of patients, many studies have not evaluated QoL. The other power of this study was that we did assess QoL using SF-36 scale, and we wanted to determine whether CKCE or OKCE could change physical, mental, and social health parameters or not.

There is no consensus regarding the exercise type that could improve all parameters of QoL in patients with knee OA.

Each subparameter of the SF-36 questionnaire was assessed separately within and between groups. Regular and supervised OKCE and CKCE programs increased the QoL in patients with knee OA. Both exercise groups improved in the subparameters of "physical function," "pain," "general health perception," and "mental health," but not in the subparameter of "role limitations." Changes in the musculoskeletal and neuromuscular system and a decrease in pain intensity increased the physical capabilities of the patient, and this may have been reflected in some subparameters of QoL.

Clinical implication of this study

The results of the present study indicated that, similar to OKCE or combined exercise programs, progressive resistive CKCE can be safely applied in patients with grade I and II knee OA. This study also suggests that, similar to OKCE programs, CKCE programs can also be easily tolerated by the patients. Both types of kinetic-chain exercise regimes decrease knee pain and stiffness and improve muscle strength, functional status, and QoL of the patients. A key strength of the current study was including a control group that was also evaluated at baseline, 6th week, and 12th week. It helped to clearly demonstrate the pure effects of the CKCE and OKCE programs by eliminating control group effects.

Limitations and strengths of the study

Patients with knee OA tend to discontinue their exercise programs particularly when their pain decreases or their daily living activities improve. Similarly, this study was limited by the absence of long-term follow-ups such as 6 months or 1 year. Another limitation of the present study was that the physiotherapists—as the evaluators—were not blind to the groups, although the researchers are academic staff and did their best to minimize assessment-related bias.

On the contrary, the strongest aspect of our study is that both middle-aged and older patients with knee OA in our CSCE and CSCE program groups complete regular, supervised programs for the first 6 weeks, frequent control for the last 6 weeks, and exercise progression, with a daily appointment system. In addition, exercise programs were created homogeneously for all groups.

CONCLUSION

This study demonstrates that in patients with knee OA, both OKCE and CKCE are effective in reducing knee pain and stiffness and in improving isokinetic muscle strength, WOMAC, and SF-36 scores. It also indicates that the beneficial effects of regular and supervised CKCE or OKCE programs outweigh those of unsupervised home exercise programs. It is recommended that future studies investigate the effects of the CKCE and OKCE programs over a longer period of time.

AUTHORS' CONTRIBUTIONS

AÖ: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Resources, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. **NG:** Conceptualization, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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ORIGINAL ARTICLE

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Comparison of orlistat and orlistat plus metformin therapy between diabetic and nondiabetic groups

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SUMMARY

OBJECTIVE: The objective of this study was to examine the effects of orlistat use on metabolic control and weight loss in diabetic and nondiabetic patients.
METHODS: A total of 119 patients with body mass index≥40 kg/m^2 and receiving orlistat therapy, who applied to the Endocrinology polyclinic between January 2016 and October 2019, were included. The patients' weight changes and biochemical values (i.e., fasting glucose, HbA1c, ALT, creatinine, and lipid parameters) were evaluated at the drug beginning and the last polyclinic control. The patients were divided into groups, whether they had diabetes or used metformin, and compared.
RESULTS: The mean age of the 119 patients in the study was 45.3±11.5 years. A total of 94.1% of the patients were females and 5.9% were males. A total of 38.7% of the patients had diabetes and 29.4% had prediabetes. When the patients were compared to whether they had diabetes or used metformin, there was a statistically significant difference between the groups according to weight loss. The mean weight change of patients without diabetes and receiving metformin and orlistat was statistically significantly higher than that of patients with diabetes and receiving metformin and orlistat.
DISCUSSION: It was determined that the weight loss effect of orlistat in obesity was seen in all groups, but this effect decreased in the diabetic group.
KEYWORDS: Obesity. Diabetes mellitus. Orlistat.

INTRODUCTION

Nowadays, obesity has become one of the most critical health problems. Obesity prevalence has tripled in the United States since 1975[1]. Diabetes, metabolic syndrome, cardiovascular diseases, and malignancies such as the esophagus and colorectal cancer, and the mortality rate are related to obesity[2,3].

In our country, orlistat is the only oral agent used in the medical treatment of obesity. Orlistat is a pancreatic lipase inhibitor that temporarily inhibits fat absorption from the gastrointestinal tract and can be used safely for decades without serious side effects[4]. In a meta-analysis of 12 studies involving patients with and without diabetics, lifestyle changes with orlistat treatment resulted in significant weight loss compared to the placebo[5].

Metformin is one of the most commonly used oral antidiabetic agents in treating type 2 diabetes, and it can also be used in prediabetic individuals to retard diabetes progression. Some studies demonstrate that metformin positively affects weight loss due to its mechanism, and some demonstrate a neutral effect[6]. In overweight and obese diabetic patients, 5–10% weight loss improves glycemic control and reduces the need for antidiabetic drugs[7]., although providing weight loss is problematic in diabetic patients due to polypharmacy, weight gain side effects of drugs, and glycemic fluctuations[8].

Our study aimed to examine the effects of orlistat on metabolic control and weight loss in diabetic and nondiabetic patients. However, the comparison of the effects of orlistat plus metformin treatment in prediabetic patients and diabetic patients on weight loss was examined.

METHODS

Study design

In the patients with body mass index (BMI)≥40 kg/m^2 who applied to Karadeniz Technical University Endocrinology and Metabolism Diseases polyclinic between January 2016 and October 2019, those who were received on orlistat (3×120 mg/day) to lose weight were retrospectively screened, and 119 patients were included in the study. Patients with diabetes

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whose treatment was changed after starting orlistat therapy and receiving the treatment for less than 3 months were excluded.

It was recorded whether the patients were with or without diabetes. An oral glucose loading test was performed in patients with Hba1c between 5.7 and 6.4% or FG above 100 mg/dL. Patients with impaired FG (100–125 mg/dL) and impaired glucose tolerance (IGT) (140–199 mg/dL) were accepted as prediabetes, and FG≥126 and PPG≥200 were accepted as type 2 diabetes. At the beginning of the treatment, the height (m^2) and weight (kg) of the patients were recorded, and BMI (kg/m^2) was calculated. Weight was measured at the last polyclinic follow-up of the patients. The drug's beginning time and the patients' biochemical values at the last polyclinic follow-up were evaluated (i.e., FG, HbA1c, ALT, creatinine, and lipid parameters). The changes in biochemical and hematological parameters with the treatment were evaluated comparatively at the beginning and the last follow-up.

It was examined whether orlistat use changed in terms of FG, Hba1c, lipid profile, and weight change between the diabetic and nondiabetic groups. The patients were compared by dividing them into three groups according to drug use and the presence of diabetes. Group 1 does not have T2DM but receives orlistat only, Group 2 has T2DM-receiving metformin (2×1,000 mg/day) and orlistat, and Group 3 does not have T2DM but receives metformin and orlistat. It was compared whether the presence of diabetes and the drug use combination made a difference in weight change between the groups.

Biochemical measurement methods

Biochemical parameters were analyzed from plasma samples. Plasma glucose values were measured via hexokinase, which is an enzymatic reference method (Beckman Coulter AU5800). Hba1c was tested via high-performance liquid chromatography (HPLC) and mass spectroscopy (Premier HB9210). Low-density lipoprotein (LDL) was evaluated via the enzymatic calorimetric method (Beckman Coulter AU5800).

Statistical analysis

The SPSS 22.0 statistical package program was used to analyze the data. Descriptive statistics of results are numbers and percentages for categorical variables, namely, mean, standard deviation, median, and minimum-maximum for numerical variables. The Kolmogorov-Smirnov test was used to determine the conformity of the groups to the normal distribution. In comparing numerical variables between two independent groups, the Student's t-test was used when the normal distribution condition was met, and the Mann-Whitney U test was used when it was not. In comparing numerical variables between two dependent groups, the t-test in the dependent groups was used when the normal distribution condition was met, and the Wilcoxon test was used when the normal distribution condition was not met. In comparing three or more independent groups, one-way ANOVA was used when the normal distribution condition was met, and the Kruskal-Wallis test was used when it was not. The chi-square test was used to compare qualitative data. The value $p<0.05$ was considered statistically significant.

RESULTS

The mean age of the 119 patients included in the study was 45.3±11.5 years. A total of 94.1% (n=112) of the patients were females and 5.9% (n=7) were males. The mean baseline BMI was 46.7±6.1. Considering the patients' chronic diseases, 38.7% (n=46) had T2DM, and 50.4% had hypertension. A total of 29.4% (n=35) of the patients received metformin treatment due to prediabetes. The mean duration of drug use of the patients was 7.6±3.4 months. The demographic and clinical characteristics of the patients are given in Table 1.

Table 1. Baseline participant characteristics.

Age (years)	45.3±11.5
Gender	
Female (n%)	112 (94.1)
Male (n%)	7 (5.9)
Weight (kg)	119.4±17.1
BMI (kg/m^2)	46.7±6.1
Diabetes, Yes (n%)	46 (38.7)
Prediabetes, Yes (n%)	35 (29.4)
Prediabetes, No (n%)	38 (31.9)
Hypertension	
Yes (n%)	60 (50.4)
No (n%)	59 (49.6)
Diet compliance	
Complete (n%)	52 (43.7)
Partial (n%)	57 (47.9)
None (n%)	10 (8.4)
Exercise frequency	
More than 3 days a week (n%)	21 (17.7)
Less than 3 days a week (n%)	60 (50.4)
Not exercising (n%)	38 (31.9)
Using metformin	
Yes (n%)	68 (57.1)
No (n%)	51 (42.9)
Drug usage period (months)	7.6±3.4

When the biochemical parameters between the beginning of the treatment and the end of the treatment were examined in all patients, a statistically significant decrease was found in the measured alanine transaminase (p<0.001), triglyceride (TG) (p=0.031), and HbA1C (p<0.001) values (Table 2). Weight changes (pre-post 116–109) and BMI (pre-post 44.9–42.1 kg/m^2) were also found to be significant (p<0.001) (Table 2).

In Table 3, the patients were compared by grouping according to whether they had diabetes and whether they received metformin. Considering the age, there was a statistically significant difference between the groups in terms of mean age (p<0.001). The mean age of patients with diabetes who received metformin and orlistat was significantly higher than in the other groups. In terms of weight change, there was a statistically significant difference between the groups (p=0.030). The mean weight change of patients without diabetes who received metformin and orlistat was statistically significantly higher than that of patients with diabetes who received metformin and orlistat. There was no difference between the groups in biochemical parameters (p>0.05 for each).

A total of 119 patients were included in the study, of whom 94 continued treatment for 6 months, 18 for 9 months, and 36 for 12 months. In all, 29.4% (n=35) of the patients continued the drug for 12 months, 28.6% (n=34) thought it was ineffective, 26.9% (n=32) had problems with drug supply, and 15.1% (n=18) could not use the drug because of side effects (i.e., abdominal pain, nausea, fecal incontinence, etc.)

DISCUSSION

Obesity creates a major metabolic disorder in patients and is a public health problem[8,9]. Our study aimed to compare the effects of orlistat, the only oral obesity drug in our country, in patients with and without diabetes and to understand the effect of adding metformin on weight loss. As a result, it was determined that metformin plus orlistat treatment provided more weight loss in the prediabetic group than in the diabetic group.

In the XENDOS study, which is one of the most extensive studies conducted with orlistat, 3,305 patients with normal FG or IGT were included and lasted for 4 years. In this study, weight loss was significantly higher in patients who received orlistat after treatment. However, the rates of weight loss were similar between groups[10]. In our study, when the results of the 119 patients were evaluated, a significant decrease was found in ALT, TG, and HbA1c after treatment. Body weight and BMI were also decreased from baseline (p<0.05 for each).

When the patients were divided into prediabetic (n=35), diabetic (n=33), and nondiabetic (n=38) groups and compared, weight loss was higher in the prediabetic group receiving orlistat plus metformin treatment compared to the other two groups. Weight loss was statistically higher in the prediabetes group, especially compared to the diabetic group. In addition, 13 patients with diabetes were not received metformin in our study. Weight loss was found to be relatively less in these patients compared to the prediabetic and diabetic groups who received metformin. It demonstrates that orlistat plus metformin treatment significantly affects weight loss in our study. When the ages of the groups were evaluated, it was determined that the diabetic group was older (p<0.05). Although there was no statistically significant difference, the continuation of orlistat was lower in the diabetic group than in the other groups due to side effects, drug supply, and regard as ineffective. Weight loss in diabetic patients is more complicated than in other patients. Although

Table 2. Biochemical changes at the beginning and end of the treatment (all patients).

	At the beginning of treatment	At the end of treatment	p-value
Glucose (mg/dL)	100 (71–305)	99 (70–265)	0.109*
Albumin (g/dL)	4.1 (3.6–5.1)	4.1 (3.4–5.0)	0.501*
Creatinine (mg/dL)	0.7 (0.4–1.8)	0.7 (0.4–1.9)	0.797*
ALT (U/L)[a]	22 (6–153)	19 (5–90)	**<0.001***
Total cholesterol (mg/dL)[b]	199 (92–367)	195 (98–400)	0.668*
TG (mg/dL)[c]	138 (41–768)	124 (48–984)	**0.031***
HDL-C (mg/dL)[d]	45 (29–83)	47 (27–90)	0.369*
LDL-C (mg/dL)[e]	121.6±4.4	119.9±40.7	0.585**
HBA1C (%)[f]	5.9 (4.6–11.7)	5.8 (4.6–11.0)	**<0.001***
Weight (kg)	116 (87–169)	109 (81–170)	**<0.001***
BMI (kg/m^2)[g]	44.9 (40–64.8)	42.1 (30–64)	**<0.001***

*Wilcoxon test and **paired t-test. [a]Alanine transaminase, [b]total cholesterol, [c]triglyceride, [d]high-density lipoprotein cholesterol, [e]low-density lipoprotein cholesterol, [f]hemoglobin A1C, and [g]body mass index. Bold indicates statistically significant values (p<0.05).

Table 3. Evaluation of the change between groups according to drug use status.

	Group 1 (without diabetes, no metformin, only receiving orlistat) (n=38)	Group 2 (with diabetes, receiving metformin and orlistat) (n=33)	Group 3 (without diabetes, receiving metformin and orlistat) (n=35)	p-value
Age (years)	41.6±10.2	51.9±10.2	42.7±11.2	**<0.001** Post hoc: 1-2, 2-,3**
Gender				
Female (n%)	36 (94.7)	32 (97.0)	32 (91.4)	0.682***
Male (n%)	2 (5.3)	1 (3.0)	3 (8.6)	
Glucose (mg/dL)	-1 (-32 to 40)	6 (-80 to 184)	2 (-15 to 37)	0.320*
Albumin (g/dL)	0 (-0.4 to 0.5)	0 (-0.6 to 1)	0 (-0.3 to 0.8)	0.878*
Creatinine (mg/dL)	0 (-0.2 to 0.2)	0 (-0.5 to 0.3)	0 (-0.1 to 0.3)	0.297*
ALT (U/L)[a]	1 (-27 to 42)	4 (-17 to 112)	3 (-37 to 46)	0.419*
Total cholesterol (mg/dL)[b]	9 (-151 to 170)	-7 (-101 to 111)	2 (-106 to 117)	0.178*
TG (mg/dL)[c]	7 (-240 to 140)	19 (-513 to 170)	-2 (-98 to 129)	0.360*
HDL-C (mg/dL)[d]	0.9±6.6	-2.6±9.4	-2.4±6.4	0.088**
LDL-C (mg/dL)[e]	5 (-49 to 143)	-7 (-55 to 102)	1 (-95 to 93)	0.205*
HBA1C (%)[f]	0 (-0.6 to 0.7)	0.2 (-1.8 to 3.4)	0.1 (-0.8 to 0.6)	0.129*
Weight change (kg)	9.2±7.9	6.6±6.0	11.5±8.3	**0.030** Post hoc: 2-3**
Reasons for drug discontinuation (n%)				
Continued	12 (31.6)	7 (21.2)	12 (34.3)	0.305***
Adverse effect	6 (15.8)	7 (21.2)	1 (2.9)	
Providing the drug	10 (26.3)	8 (24.2)	12 (34.3)	
Regarding as ineffective	10 (26.3)	11 (33.3)	10 (28.6)	

*Kruskal-Wallis test, **One-way ANOVA test, and ***Chi-square test. [a]Alanine transaminase, [b]total cholesterol, [c]triglyceride, [d]high-density lipoprotein cholesterol, [e]low-density lipoprotein cholesterol, and [f]hemoglobin A1C. Bold indicates statistically significant values (p<0.05).

the underlying causes are unclear, drugs for blood sugar regulation decrease calorie deficit by reducing glucosuria. At the same time, these drugs themselves (e.g., sulfonylurea, insulin, and beta-blocker) can induce weight gain. Genetic factors of diabetic patients and insulin resistance caused by abdominal obesity complicate weight loss[11]. The multiple drug use to treat diabetes and psychological factors also affect this situation[12]. Although weight loss is difficult, weight loss achieved with orlistat therapy in diabetic patients improves metabolic parameters. In the study conducted by Kelley et al., improvement in glycemic parameters and cardiovascular risk factors was achieved after 1 year of orlistat treatment in patients with type 2 diabetes[13].

While some studies demonstrate that metformin is neutral on weight loss, others state that it has positive aspects. The metformin effect on weight is low in obese patients, such as PCOS with glucose disturbance[14]. The main reasons why metformin causes weight loss are that it increases insulin sensitivity and decreases hepatic gluconeogenesis. In addition, it is thought to have a suppressive effect on the hypothalamic appetite center[15]. The use of metformin in obese patients with insulin resistance reduces hunger by reducing the frequency of postprandial hypoglycemia. At the same time, it increases energy metabolism and physical activity by providing phosphorylation of the AMP protein kinase pathway[16]. In addition, it benefits the anorectic effect by increasing leptin sensitivity; the decrease in ghrelin and the increase in GLP 1 suggest this situation. This increase in GLP 1 contributes to the weight loss effect[17].

Preprandial metformin treatment also increases GLP 1 in diabetic patients, but variations in glucose and insulin levels in the diabetic group affect GLP 1 change[18]. In the 10-year Diabetes Prevention Program Outcome Study (DPPOS), 2,155 patients were randomized, and the effects of metformin on weight change and diabetes occurrence were evaluated. In this study, weight loss was significantly higher than placebo, independently of drug compliance[19]. In another retrospective study, which included 6-month and 1-year follow-ups of diabetic or euglycemic patients, it was

observed that metformin had a weight loss effect[20]. In a study by Sarı et al., it was found that orlistat plus metformin treatment did not provide an additional benefit to only orlistat treatment on weight loss. However, the small number of patients and the 3-month short follow-up period are the negative aspects of the study[21]. In our study, it was concluded that metformin made an additional contribution to orlistat treatment.

CONCLUSION

This study found that the weight loss effect of orlistat in obesity was observed in all groups, but this effect decreased in the diabetic group. The decrease in the weight loss effect of this combination in the diabetic group can be explained by the drug compliance difficulty due to polypharmacy in diabetic patients and resistance that may have developed in the GLP 1 response to metformin. Due to the decrease in the effect of orlistat in the diabetic patient group, another alternative weight loss treatment should be considered in this group. As it is a rare study comparing orlistat and orlistat plus metformin treatment in prediabetic and diabetic populations, it is thought to contribute to the literature.

LIMITATIONS

The study's limitations are retrospective design, its inability to reflect the general population due to the large female population, and the lack of 12-month follow-ups of all patients.

AUTHORS' CONTRIBUTIONS

YEG: Data curaion, Formal Analysis, Resources, Visualization, Writing – original draft. **SVK:** Formal Analysis, Resources, Supervision, Visualization. **SK:** Formal Analysis, Resources, Supervision, Visualization. **DT:** Formal Analysis, Resources, Supervision, Visualization. **ASD:** Formal Analysis, Resources, Supervision, Visualization. **HC:** Conceptualization, Formal Analysis, Methodology, Project administration, Supervision, Writing – original draft. **IN:** Conceptualization, Formal Analysis, Methodology, Project administration, Supervision, Writing – original draft. **MK:** Conceptualization, Formal Analysis, Methodology, Project administration, Supervision, Writing – original draft. **HOE:** Conceptualization, Formal Analysis, Methodology, Project administration, Supervision, Writing – original draft.

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ORIGINAL ARTICLE

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Evaluation of sexual function and depression in female patients with fibromyalgia

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SUMMARY

OBJECTIVE: Fibromyalgia is one of the most important problems, especially for women. Studies point to disorders in the sexual functions of fibromyalgia patients that reduce their quality of life. The aim of this study was to investigate sexual dysfunction and its relationship with disease severity and depression in women with fibromyalgia.

METHODS: This study included 98 female patients diagnosed with fibromyalgia and 54 healthy women. The Female Sexual Function Index was used to assess sexual dysfunction. Fibromyalgia disease severity was measured with the Fibromyalgia Impact Questionnaire. Hamilton Depression Scale was filled in to evaluate the depression status of the patients.

RESULTS: According to the female sexual function index data, female sexual dysfunction was found in 78 (79.6%) patients with fibromyalgia and only in 12 (22.2%) controls. When the female sexual function index scores of fibromyalgia patients with and without depression were compared, patients with additional depression had lower female sexual function index scores, and this difference was statistically significant (p=0.002). In the correlation analysis, the female sexual function index score showed a significant negative correlation with the hamilton depression scale (rho=-0.235, p=0.020) and fibromyalgia impact questionnaire (rho=-0.215, p=0.033) scores.

CONCLUSION: This study highlights the high prevalence of sexual dysfunction in female fibromyalgia patients and the significant correlation between sexual dysfunction and both disease severity and depression.

KEYWORDS: Fibromyalgia. Sexual dysfunction, psychological. Depression.

INTRODUCTION

Fibromyalgia (FM) is a chronic condition of uncertain origin, deemed the most prevalent cause of widespread pain among middle-aged women. It is a complex disorder, characterized by the presence of widespread pain in the muscles and joints, in addition to other symptoms including poor sleep quality, persistent fatigue, comorbid cognitive difficulties, and a diverse array of somatic and psychiatric symptoms, which all contribute to a decline in the patients' overall quality of life[1,2]. Besides the primary complaints of tenderness and pain, individuals diagnosed with FM have also reported other symptoms such as chronic fatigue, sleep disturbance, anxiety, depression, cognitive difficulties, numbness/tingling, headaches, and sexual disturbances[3,4].

Medications used to treat depression have also been frequently associated with sexual dysfunction (SD). There are numerous reports of high occurrences of SD among women diagnosed with panic disorder, anxiety disorders, and depression. These problems may include issues related to sexual desire and arousal or pain during sexual activity[3-5].

The aim of this study was to examine the frequency of SD and its association with the severity of illness and depression among a group of female FM patients seeking treatment at our clinic using a cross-sectional design.

METHODS

This study included 98 female patients diagnosed with FM and 54 healthy women. Patients were selected from consecutive patients diagnosed with FM according to the American College of Rheumatology criteria who applied to our outpatient clinic. The control group consisted of hospital staff and patient relatives who volunteered among married women in the same age range as the patients.

Considering the possible effects of medical treatments used in FM treatment on sexual function, only newly diagnosed patients who have not been treated yet were included in the study. Furthermore, history of psychiatric illness that may affect sexual function or psychological state, use of antidepressant/

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antipsychotic medication, smoking, presence of urogenital disease, systemic disease (autoimmune, inflammatory, neurological, and endocrine), pregnancy, postmenopausal women, or women who did not have sexual activity in the last month were excluded from the study. At the end of the evaluations, 98 married women aged 23–51 (mean 38.47±6.72) years with FM and 54 healthy relatives aged 25–52 (mean 36.94±6.55) years without FM complaints were included in the study.

Demographic characteristics such as age, educational level, marital status, employment, and body mass index (BMI) were recorded. The Turkish-approved version of the 19-item "Female Sexual Function Index" (FSFI), which evaluates sexual function in the last 4 weeks, was used to evaluate sexual functions. FSFI total score less than ≤26.5 was considered SD[1]. The Turkish version of the Hamilton Depression Scale (HDS), which was previously validated in Turkish, was used to assess depression. FM disease severity was measured with the Fibromyalgia Impact Questionnaire (FIQ). A higher score on the FIQ indicates a more severe disease, with a maximum score of 100 points.

Statistical analysis

Data were abbreviated as mean±standard deviation (minimum-maximum) or percentage and number. The normality of the numerical variables was determined through the application of the Shapiro-Wilk test of normality. To compare scale scores for two-category variables, an independent sample t-test was utilized, while chi-square tests were employed to compare groups with categorical variables. Significance was set at a p-value of less than 0.05.

RESULTS

The age of the participants ranged from 25 to 56 years, with a mean±standard deviation of 38.49±6.68. A total of 72 patients and 32 controls were not working, while 26 patients and 22 controls were actively employed. The mean BMI was 26.38±4.34 in patients and 24.88±3.42 in controls. The results indicate that there is no correlation between the patient's BMI, employment status, disease severity, SD, or depression status (Table 1).

HDS scores ranged from 5 to 20 in patients (11.55±3.76) and 1 to 9 in controls (4.30±2). FIQ scores ranged from 32 to 96 in patients (71.45±21.69) and between 5 and 41 in controls (18.43±8.84). The mean FSFI scores were 23.80±2.68 in patients and 29.15±2.38 in controls. The mean HDS and FIQ score for the patients were significantly higher than the controls ($p<0.001$, $p<0.001$) and the mean FSFI score was significantly lower than the scores for the controls ($p<0.001$) (Table 2).

Based on the overall FSFI score, SD was present in 78 (79.6%) patients with FM and only in 12 (22.2%) controls. The most common sexual problem was satisfaction in patients

Table 1. Comparison of descriptive features by groups.

Characteristics	Groups		Test statistics	
	Control group n=54	FM patients n=98	Test value	p-value
Age			-1.350‡	0.179
Mean±SD	36.94±6.55	38.47±6.72		
M (min–max)	36 (25–52)	39 (23–51)		
BMI			-1.927‡	0.054
Mean±SD	24.88±3.42	26.38±4.34		
M (min–max)	24.4 (18.2–33.2)	25.7 (18.1–37.9)		
Occupation			3.254†	0.071
Not working	32 (59.3%)	72 (73.5%)		
Working	22 (40.7%)	26 (26.5%)		
Educational level			1.152†	0.764
Illiterate	3 (5.6%)	4 (4.1%)		
Primary school	16 (29.6%)	37 (37.8%)		
Secondary school	23 (42.6%)	39 (39.8%)		
Higher education	12 (22.2%)	18 (18.4%)		

BMI: body mass index, ‡independent sample t-test (t); †Chi-square test (χ^2). Summary statistics are given as mean±standard and median (minimum-maximum) for numerical data and number (percentage) for categorical data.

(n=79, 80.6%) and orgasm in controls (n=15, 27.8%). The least detected sexual problem was pain (n=45, 45.9%) in patients and lubrication (n=4, 7.4%) in controls. When the FSFI subdomain scores were compared, no significant difference was found in the "Desire" domain of FSFI between patients and controls (p>0.05). The difference in all domains except the "Desire" domain was statistically significantly higher in favor of the control group (p<0.001) (Table 2).

According to the FSFI results, the mean score of the patients was 23.80±2.68, and the mean score of the controls was 29.15±2.38 (p<0.001). In addition, the difference between the mean FIQ score of the patients (71.45±21.6) and the mean FIQ score of the controls (18.43±8.8) was statistically significant (p<0.001) (Table 2).

HDS results show that 75 (76.5%) of the patients had depression. When the FSFI scores of FM patients with and without depression were compared, patients with additional depression had lower FSFI scores, and this difference was statistically significant (p=0.002).

No significant correlation was found between sociodemographic characteristics such as age, BMI, occupation, education and depression, SD, or disease severity (Table 3).

Table 2. Comparison of scales and subscales according to groups.

Characteristics	Groups		Test statistics	
	Control group n=54	FM patients n=98	Test value	p-value
FIQ score				
Mean±SD	18.43±8.84	71.45±21.69	-17.171	**<0.001**
M (min–max)	17 (5–41)	81 (32–96)		
HDS				
Mean±SD	4.30±2.00	11.55±3.76	-13.175	**<0.001**
M (min–max)	4 (1–9)	12 (5–20)		
FSFI				
Mean±SD	29.15±2.38	23.80±2.68	12.254	**<0.001**
M (min–max)	29.4 (24.8–33.6)	23.8 (18.2–29.5)		
FSFI desire				
Mean±SD	4.61±0.89	4.41±1.17	1.076	0.284
M (min–max)	4.7 (3–6)	4.2 (1.8–6)		
FSFI arousal				
Mean±SD	4.86±0.74	3.73±0.84	8.291	**<0.001**
M (min–max)	4.8 (3.6–6)	3.6 (2.1–6)		
FSFI lubrication				
Mean±SD	4.84±0.77	3.65±0.70	9.678	**<0.001**
M (min–max)	4.8 (3.6–6)	3.6 (2.4–6)		
FSFI orgasm				
Mean±SD	4.71±0.98	3.80±0.94	5.572	**<0.001**
M (min–max)	4.6 (3.2–6)	3.6 (1.6–6)		
FSFI satisfaction				
Mean±SD	5.06±0.93	3.58±0.58	12.041	**<0.001**
M (min–max)	5.2 (3.2–6)	3.6 (2.8–5.6)		
FSFI pain				
Mean±SD	5.07±0.57	4.62±0.78	3.775	**<0.001**
M (min–max)	5.2 (4–6)	4.8 (3.2–6)		

FSFI: Female Sexual Function Index; Mean±SD: mean±standard deviation; HDS: Hamilton Depression Scale score, independent sample t-test (t). Summary statistics are given as mean±standard and value. The values denoted in bold are statistically significant (p<0.05).

Table 3. Comparison of measurements according to sexual dysfunction status in fibromyalgia patients group.

FM patients	Sexual dysfunction		Test statistics	
	No n=20	Yes n=78	Test value	p-value
Age				
Mean±SD	38.60±6.64	38.44±6.79	0.097‡	0.923
M (min–max)	38.5 (23–51)	39 (25–56)		
Occupation				
Not working	12 (60%)	60 (76.9%)	2.339†	0.126
Working	8 (40%)	18 (23.1%)		
Educational level				
Illiterate	2 (10%)	2 (2.6%)		
Primary school	6 (30%)	31 (39.7%)	2.883†	0.410
Secondary school	9 (45%)	30 (38.5%)		
Higher education	3 (15%)	15 (19.2%)		
BMI				
Mean±SD	24.78±3.42	26.79±4.47	-1.871‡	0.064
M (min–max)	24.39 (21–31)	26.41 (18–38)		

BMI: body mass index, ‡independent sample t-test (t); †Chi-square test (χ^2). Summary statistics are given as mean±standard and median (minimum, maximum) for numerical data and number (percentage) for categorical data.

DISCUSSION

FM is a widely debated disorder characterized by widespread musculoskeletal pain and believed to affect a significant portion of the global population, ranging from 2 to 4%[6]. In addition to the primary symptoms of pain and tenderness, patients with FM also reported SD and cognitive disorders such as depression, anxiety, memory, and concentration difficulties[2,5,7].

Previous research has demonstrated that sexual difficulties are prevalent among both men and women, with reported rates of occurrence ranging from 10 to 52% in males and 25 to 63% in females[8,9]. There is a significant body of literature indicating that the prevalence of SD is elevated among individuals with FM in comparison to the general population[1,3-5,7]. The results of our study indicate that SD is a common problem among FM patients, with a high prevalence of 79.6%. This is consistent with previous studies, which have reported a prevalence of SD in FM patients ranging from 54 to 97%[1,7,10].

In our study, when the subfield scores of the FSFI scale were evaluated, the most common sexual problems in FM patients were satisfaction (n=79, 80.6%) and orgasm (n=15, 27.8%) in the control group. Pain was the least detected sexual problem in FM patients (n=45, 45.9%). Aydın et al.[7] found that the most common sexual disorder among the subscales in their study involving 48 FM patients was a lack of desire (n=30, 62.5%). Additionally, the study found a prevalence of SD of 54.2%, a frequency that is less than the one reported in our study. We think that this difference is due to the fact that Aydın et al.[7] took the cutoff value of 22.7 instead of 26.55 for FSFI, unlike other studies[11]. In our study, there was a statistically significant difference in favor of the control group in all domains except the "Desire" domain in the FSFI subdomain scales. There was a difference in favor of the control group in the "Desire" subdomain scores, but this difference was not statistically significant. This may be due to our sample size not being large enough. Overmeire et al.[4] also reported the frequency of decreased sexual desire in women with FM but suggested that this was not related to FM severity but to depression and antidepressant drugs. In our study, although none of the patients used antidepressants, there was a significant relationship between SD and both depression and FM disease severity.

Past studies have established a correlation between FM and a high prevalence of both anxiety and depression[12,13]. Additionally, it has been established that both conditions are associated with decreased sexual function[7,14,15]. However, studies on the specific impact of depression on SD in FM patients have yielded inconsistent results. Our study demonstrated that 76.5% of the FM patients had depression. These rates were significantly higher than those in the control group (p<0.001). Tikiz et al.[5] examined the SD of 40 female patients with FM, and similarly showed that 67.5% of these patients also had depression. In the same research,

there was no considerable difference found between the FSFI scores of FM and FM plus depression patients. On the contrary, Yılmaz et al.[16] reported that patients with higher Beck Depression Inventory scores had lower FSFI scores and concluded that depression aggravated FM-related female SD. Our study revealed that individuals with depression had notably lower FSFI scores in comparison to those without depression (p=0.002), aligning with the results reported by Yılmaz et al[16].

In the correlation analysis, the FSFI score was found to have a strong inverse relationship with the HDS (rho=-0.235, p=0.020) and FIQ (rho=-0.215, p=0.033) scores. There was a statistically significant positive correlation between HDS and FIQS (rho=0.227, p=0.024). These findings suggest that as HDS increases, disease severity scores increase and SD scores decrease. Although Aydın et al.[7] reported a negative correlation between depression and FSFI scores, similar to our results, they did not report a result related to FIQ scores since they did not measure the severity of the disease in their study. Tıkız et al.[5], on the contrary, reported that FSFI scores decreased significantly in FM and FM plus depression groups compared to healthy controls, but depression did not make an additional contribution to SD, and they did not detect a correlation between FIQ scores and FSFI scores. In their study, Yılmaz et al.[16] reported a strong inverse relationship between the total FSFI score and both FIQ and depression scores in women with FM. Our findings align with those reported by Yılmaz et al.[16].

SD still represents a major clinical challenge, as the diagnosis and treatment of SD are limited by several factors. In their study of 106 female subjects with hypoactive SD, Lerner et al.[17] showed that group cognitive-behavioral group therapy can be an effective option for the treatment of female SD, which can have an effect on most of the possible factors and have a positive effect on female SD.

One of the limitations of our study is that it was a cross-sectional study and conducted on a limited number of population, and that it could not give an idea about the status of sexual function after FM treatment was started. In addition, the fact that the patients did not receive any treatment that could affect sexual functions, including antidepressants, is one of the strengths of our study in terms of showing the simple relationship between SD and FM.

CONCLUSION

Our study highlights the high prevalence of SD in female FM patients, as well as a significant correlation between SD and both disease severity and depression. These findings emphasize the importance of addressing SD in the management of FM and the need for further research in this area.

ETHICAL APPROVAL

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Istanbul Training and Research Hospital Institutional Clinical Research Ethics Committee (Date: 30.11.12/No. 209).

AUTHORS' CONTRIBUTIONS

İHE: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. **FU**: Data curation, Formal Analysis, Investigation, Resources, Writing – review & editing.

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ORIGINAL ARTICLE

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Comparison of pain levels of traditional radial, distal radial, and transfemoral coronary catheterization

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SUMMARY

OBJECTIVE: The aim of our study was to compare the traditional radial artery, distal radial artery, and transfemoral artery, which are vascular access sites for coronary angiography, in terms of pain level using the visual analog scale.

METHODS: Between April 2021 and May 2022, consecutive patients from three centers were included in our study. A total of 540 patients, 180 from each of the traditional radial artery, distal radial artery , and transfemoral artery groups, were included. The visual analog scale was applied to the patients as soon as they were taken to bed.

RESULTS: When the visual analog scale was compared between the groups, it was found to be significantly different (transfemoral artery: 2.7±1.6, traditional radial artery: 3.9±1.9, and distal radial artery: 4.9±2.1, respectively, p<0.001). When the patients were classified as mild, moderate, and severe based on the visual analog scale score, a significant difference was found between the groups in terms of body mass index, process time, access time, and number of punctures (p<0.001). Based on the receiver operating characteristic analysis, body mass index>29.8 kg/m^2 predicted severe pain with 72.5% sensitivity and 73.2% specificity [(area under the curve: 0.770, 95%CI: 0.724–0.815, p<0.0001)].

CONCLUSION: In our study, we found that the femoral approach caused less access site pain and a high body mass index predicts severe pain.

KEYWORDS: Visual analog scale. Coronary angiography. Radial artery. Femoral artery.

INTRODUCTION

Due to the lack of patient comfort in transfemoral artery (TFA), the development of complications related to bleeding at the vascular access site, the need for long-term follow-up and bed rest, and alternative intervention methods have come to the fore[1]. The radial approach appears to be a safe method, and many randomized clinical trials have shown that the transradial approach is more advantageous than TFA, with excellent success rates and very low complication rates in elective and acute procedures[2-4]. In addition, it was stated that, because of traditional radial artery (TRA), the patients' discomforts such as long-term bed rest and vascular compression due to the procedure were reduced[5]. Most operators prefer the right TRA as they work on the right side of their patients. However, right TRA occlusion, underdeveloped right TRA, excessive curvature, sclerosis, calcifications, arteria lusoria, and use of right TRA as a free arterial graft in the past or future cause operators to prefer left TRA[6]. Left TRA catheterization has a similar anatomical course to transfemoral access and is suitable for patients after coronary artery bypass grafting requiring left internal mammary artery angiography. However, access to the left TRA can be somewhat difficult as the operator has to lean over the patient to place the sheath on the left TRA. This unpleasant position may make the catheterization procedure inconvenient. An alternative way to maintain a comfortable position for both patient and operator is to access the distal radial artery (DRA) located in the anatomical snuffbox or the "fossa radialis" on the dorsal side of the hand[6]. If any obstruction occurs in the anatomical snuffbox area, antegrade flow continues through the superficial palmar arch and collaterals, thus preventing tissue ischemia[7].

One of the main challenges during TRA is radial artery spasm (RAS), which can reduce the success rate of the procedure. The small diameter of the radial artery may complicate

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the procedure, and multiple cannulation attempts may increase the risk of RAS[8]. In addition, moderate-to-severe pain during radial artery cannulation may precipitate the incidence of RAS[9]. There are less data in the literature on pain in the vascular access area associated with the radial and femoral approach.

Therefore, our aim in this prospective and randomized study was to compare the vascular access sites of TRA, DRA, and TFA for coronary angiography in terms of pain level using the visual analog scale (VAS).

METHODS

Patients from three centers were included in our prospective and randomized study. Patients were selected consecutively between April 2021 and May 2022. The patients were divided into three groups, namely, DRA, TRA, and TFA. A total of 540 patients, 180 in each group, were included. Patients with acute ST-elevation myocardial infarction, cardiogenic shock, hemodynamic instability, use of catheters other than 6 French, patient rejection, and patients over 75 years of age were excluded from the study. As the use of VAS in elderly patients is not reliable enough, we did not include patients over 75 years of age. The procedures were performed by experienced interventional cardiologists at each center. The choice of approach is left to the discretion of the operator.

Written informed consent for inclusion in the study was obtained from all patients. The study approval was obtained by the local ethics committee (Diyarbakır Gazi Yaşargil Training and Research Hospital Ethics Committee, date and number: 30/12/2022-303). The study was conducted in accordance with the Declaration of Helsinki (2013).

Procedures

Transfemoral access

After local anesthesia with 15–20 mL of lidocaine, the right femoral artery was entered using the Seldinger technique with an 18 g needle. 6-F Judkins right and left catheters were used for diagnostic angiography. After angiography, the sheath was removed immediately in patients who did not undergo PCI and after 4–6 h in patients who did. After the sheath was removed, hemostasis was achieved with manual compression for 15–20 min. Afterward, a sandbag was placed instead of the sheath, and compression was applied for 4–6 h.

Traditional radial access

The arm was placed on a board at an angle of 60–70° relative to the body. The radial artery was punctured at an angle of 30–45°, 1 cm proximal to the radial styloid process. A 6-French radial hydrophilic sheath was placed on the patients. Afterward, 2,500 units of unfractionated heparin and 200 µg nitrate were administered through the sheath. After the procedure, the sheath was removed immediately and hemostasis was achieved using a transradial band.

Distal radial access

The deep palmar artery point between the first metacarpal bone and the second metacarpal bone was determined as the entry site. After local anesthesia with 2–3 mL of lidocaine was applied to the inlet, the needle was directed toward the point where the pulse was strongest. Afterward, a 6 French radial hydrophilic sheath was placed. All patients were given 2,500 units of unfractionated heparin (50 IU/kg) and 200 µg nitrate over the sheath. Subsequently, coronary angiography was performed.

Visual analog scale

The VAS is a vertical line between 0 and 10 cm, in which 0 represents no pain and 10 represents the most severe pain imaginable. Pain levels of all patients were evaluated with VAS. Each patient indicated the severity of pain by choosing a point on the line. This scale was applied as soon as the patients were taken to bed after the procedure. In addition, patients were classified as mild (0–3), moderate (4–7), and severe (8–10) based on the VAS score.

Statistical analysis

Analyses were performed using the SPSS 25.0 (Armonk, NY: IBM Corp.) statistical analysis software. Kolmogorov-Smirnov test was used to determine whether each variable showed a normal distribution. Normally distributed continuous variables were defined as mean±standard deviation. One-way analysis of variance (ANOVA) test was used to compare more than two groups for normally distributed continuous variables. Categorical variables were given as numbers and percentages and compared using the Pearson chi-square test. p-value<0.05 was considered significant. BMI cutoff value was estimated by receiver operating characteristic (ROC) curve analysis to predict severe pain (>7 points on VAS) with corresponding sensitivity and specificity.

RESULTS

While 434 of the patients presented with stable angina pectoris, 106 patients presented with myocardial infarction without ST elevation. A total of 172 patients underwent percutaneous coronary intervention (PCI). The main clinical

features are summarized in Table 1. Age distribution between the groups was found to be significantly different (TFA: 64.2±11.0, TRA:58.4±9.8, and DRA: 58.6±11.0, respectively, p<0.001). Especially the radial group was younger than the transfemoral group.

VAS between the groups was found to be significantly different when compared (TFA: 2.7±1.6, TRA: 3.9±1.9, and DRA: 4.9±2.1, respectively, p<0.001) (Figure 1). The procedure time did not differ between groups (TFA: 39.3±11.5, TRA: 41.4±12.0, and DRA: 40.7±12.4, respectively, p=0.249). The duration of access was found to be the shortest TFA and the longest DRA, and it was significantly different between the groups (TFA: 38.1±7.0, TRA: 41.8±12.6, and DRA: 53.0±16.4, respectively, p<0.001). The number of punctures was significantly different between the groups (TFA: 1.4±0.5, TRA: 1.6±0.6, and DRA: 1.7±0.7, respectively, p<0.001). There was no difference between the groups in terms of PCI (TFA: 57 (31.7%), TRA: 63 (35%), and DRA: 52 (28.9%), respectively, p=0.460). Only one patient in the TRA group developed mortality.

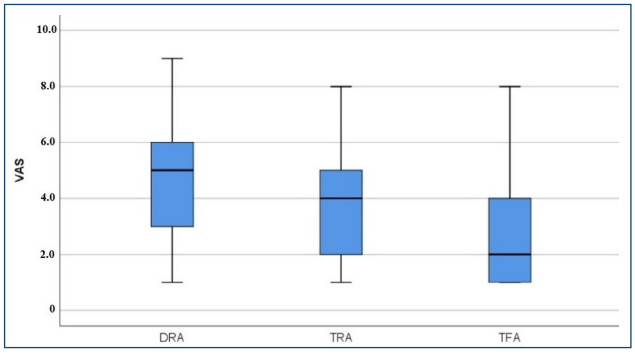

Figure 1. Box plots of visual analog scale scores of access groups.

Table 1. Baseline characteristics of the study population and comparison of pain groups.

Baseline characteristics	TFA (n=180)	TRA (n=180)	DRA (n=180)	p-value
Gender (female), n (%)	65 (36.1)	59 (32.8)	53 (29.4)	0.403
Age (years)	64.2±11.0	58.4±9.8	58.6±11.0	**<0.001**
Body mass index (kg/m²)	28.2±3.9	28.2±4.0	27.7±4.1	0.414
HT, n (%)	62 (34.4)	58 (32.2)	65 (36.1)	0.738
DM, n (%)	55 (30.6)	64 (35.6)	55 (30.6)	0.503
HPL, n (%)	7 (3.9)	10 (5.6)	10 (5.6)	0.704
CRF, n (%)	45 (25)	49 (27.2)	55 (30.6)	0.494
Smoker, n (%)	8 (4.4)	9 (5)	8 (4.4)	0.959
EF (%)	48.2±10.7	49.1±10.8	48.9±10.8	0.708
VAS	2.7±1.6	3.9±1.9	4.9±2.1	**<0.001**
Processing time (min)	39.3±11.5	41.4±12.0	40.7±12.4	0.249
Access time (s)	38.1±7.0	41.8±12.6	53.0±16.4	**<0.001**
Number of punctures	1.4±0.5	1.6±0.6	1.7±0.7	**<0.001**
PCI, n (%)	57 (31.7)	63 (35)	52 (28.9)	0.460
Mortality, n (%)	0 (0)	1 (0.6)	0 (0)	0.367
Comparison of pain groups	**Mild pain (n=264)**	**Moderate pain (n=236)**	**Severe pain (n=40)**	**p-value**
Body mass index (kg/m²)	28.1±4.0	27.6±4.1	30.8±1.8	**<0.001**
Processing time (min)	36.3±7.3	42.5±12.6	56.2±16.5	**<0.001**
Access time (s)	40.8±9.5	44.6±13.1	65.8±23.3	**<0.001**
Number of punctures	1.4±0.5	1.6±0.6	2.5±0.8	**<0.001**
Access zone, n(%)	TFA: 130 (49.2)	TFA: 47 (19.9)	TFA: 3 (7.5)	**<0.001**
	TRA: 78 (29.6)	TRA: 97 (41.1)	TRA: 5 (12.5)	
	DRA: 56 (21.2)	DRA: 92 (39.0)	DRA: 32 (80)	

TFA: transfemoral access; TRA: traditional radial access; DRA: distal radial access; HT: hypertension; DM: diabetes mellitus; HPL, hyperlipidemia; CRF: chronic renal failure; EF: ejection fraction; VAS: Vascular Analog Scale; PCI: percutaneous coronary ıntervention. Bold indicates statistically significant values.

In addition, when the patients were classified as mild, moderate, and severe according to the VAS score, a significant difference was found between the groups in terms of body mass index, processing time, access time, and number of punctures (p<0.001) (Table 1). Severe pain was detected in 40 patients and was more especially in the DRA (n:32, 80%) group. Based on the ROC analysis, BMI>29.8 kg/m^2 predicted severe pain with 72.5% sensitivity and 73.2% specificity [(area under the curve (AUC): 0.770, 95%CI: 0.724–0.815, p<0.001)] (Figure 2).

DISCUSSION

Coronary interventional procedures are performed by the radial and femoral routes. There are two different approaches to radial access, namely, TRA and DRA. Many studies have been done comparing these access routes[2-4,10]. However, there are limited data in the literature comparing these three entry points according to pain levels. To the best of our knowledge, our study is the first to compare the pain levels of all three access routes according to the VAS. In our study, pain levels were determined by VAS as soon as the patients were taken to bed immediately after the procedure. Pain levels were the lowest in the TFA group and the highest in the DRA group. Long process and access time, high BMI, and high number of punctures were found to increase the severity of pain.

Many studies have shown that transradial access can address many of the deficiencies in femoral access. Transradial access has a lower complication rate. In addition, mortality and major adverse cardiac events are less in STEMI patients[11]. The radial artery is more superficial than the femoral artery and can be compressed more easily. The DRA and the deep palmar branch of the ulnar artery form a deep palmar arch with abundant collateral circulation. Therefore, the incidence of Ischemia in the hand after radial artery puncture is low[12]. However, the standard radial artery approach also has a disadvantage, such as radial artery occlusion (RAO). DRA, on the other hand, increases the comfort of the procedure by providing a more comfortable position to the operator during the procedure. In addition, this technique has a shorter hemostasis time and less RAO rate.

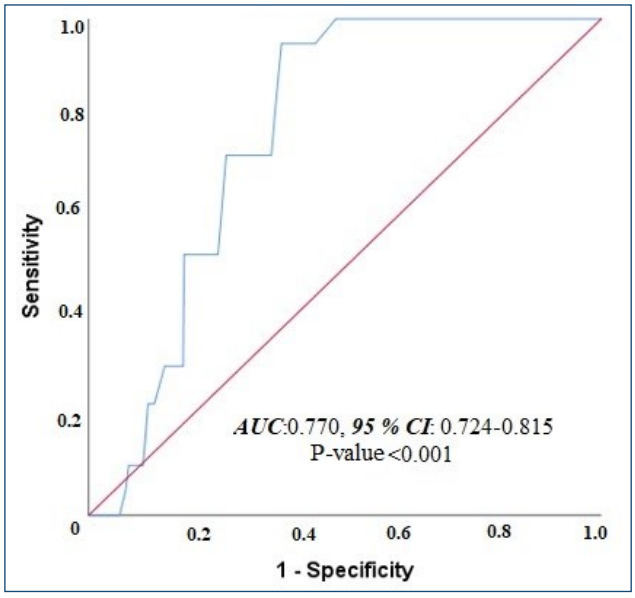

Figure 2. Receiver operating characteristic curve analysis of body mass index to predict severe pain.

In our study, the highest level of pain was in the DRA access route. It was followed by TRA and TFA, respectively. The anatomical snuffbox, in which the DRA is located, is between two tendons, namely, extensor pollicis longus and extensor pollicis brevis. This region contains the radial artery, the radial nerve (superficial branches), and the cephalic vein. Injury to the superficial branch of the radial nerve can cause pain and paresthesia in this area[13]. Robson et al. found a close relationship between the radial nerve and the radial artery[14]. In addition, more punctures in the DRA group in our study may explain the high VAS score. With increasing experience, the number and duration of punctures will be improved. A randomized trial comparing TRA and DRA showed a high rate of cannulation failure of 30% in the DRA group versus only 2% in the TRA group (p<0.001)[15]. The authors cited the reasons for this low success rate as the smaller diameter of the radial artery in the anatomical snuffbox with an increased risk of vasospasm, increased curvature that causes the wire to fail to advance at this level, and a longer learning curve. Lee et al. showed in a large prospective study that the learning curve for the puncture time stabilized after about 150 cases[16]. Aktürk et al. compared the pain level between TRA and TFA[17]. Consistent with our study, they found lower pain levels in the TFA group. The low VAS scale in the TFA group can be attributed to reasons such as wider vessel diameter, less number of punctures, and shorter access time.

According to the VAS scale, the pain felt by the patients was classified as mild, moderate, and severe. BMI, access time, process time, and number of punctures were found to be higher in patients in the severe pain class. A BMI>29.8 kg/m^2 predicted severe pain. In patients with high BMI, the need for more local anesthetic drugs, the difficulty of reaching the artery due to the increase in adipose tissue, the increase in the number of punctures, and the prolongation of the procedure may explain the severe pain in this patient group. Aktürk et al., consistent

with our study, found that BMI>37 kg/m^2 was associated with the severity of pain in patients who underwent femoral angiography[17]. They attributed this to hematomas and soft tissue hemorrhages in the procedure area.

LIMITATIONS

Although this trial included three centers, it was a regional study and the number of patients was relatively low. Patients with high post-procedure VAS scores were not routinely evaluated by ultrasound examination. The diagnosis of RAS was made according to subjective criteria. Although these procedures are performed in experienced centers, we cannot completely exclude the impact of operator experience on results.

CONCLUSION

We found that although the radial artery has many advantages over the femoral artery in coronary angiography, it is associated with higher pain severity. In addition, a high BMI is also a factor that increases the severity of pain. Although radial artery interventions (DRA and TRA) seem advantageous and popular, femoral intervention should not be ignored in suitable patients.

AUTHORS' CONTRIBUTIONS

RK: Conceptualization, Formal Analysis, Investigation, Methodology, Resources, Supervision, Visualization, Writing – original draft, Writing – review & editing. **TG:** Data curation. **AA:** Validation. **BA:** Software. **MA:** Investigation. **SG:** Investigation. **MZK:** Project administration.

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ORIGINAL ARTICLE

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Video-based digital platforms as an educational resource for the surgical preparation of orthopedic surgeons

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SUMMARY

OBJECTIVE: The aim of the study was to research the video-based digital platforms that orthopedic specialists in Turkey use as an educational resource in their surgical preparations that they have not seen or done before, the frequency of their use of these platforms, and their trust in these platforms, with a survey study.

METHODS: The importance of video-based digital platforms in surgical preparations that surgeons have not seen or done before was measured using the data obtained from 181 orthopedic specialists using a survey prepared on an Internet-based server (docs.google.com).

RESULTS: Orthopedists used video-based digital platforms with a ratio of 38.7% among the educational resources in their surgical preparations that they have not seen or done before. There was no significant difference between the specialists with a surgical experience of 1–10 years and more than 10 years of experience in terms of using video-based digital platforms in surgical preparation ($p>0.05$). A total of 81.2% of the participants used only video-based digital platforms in the preparation of a surgical procedure they have never seen before. The most frequently used digital platform was YouTube, and 62% of the participants considered these platforms reliable.

CONCLUSION: Orthopedic specialists in Turkey primarily and frequently use video-based digital platforms as a training resource in their preparations for surgery that they have not seen or done before. The establishment or support of platforms with evidence-based content with references from official orthopedic institutions and organizations can increase the trust of orthopedic specialists in these platforms.

KEYWORDS: Learning. Audio-video demonstration. Teaching materials.

INTRODUCTION

The master-apprentice model that has been traditionally used in the training of orthopedic surgeons is a matter of debate at present. The training concepts of the past are based on the blending of theoretical knowledge acquired from textbooks and medical journals with practical training in the operating theater[1]. However, the increased use of portable electronic devices and easier access to the Internet have made it possible for the field of Internet-based surgical learning to expand.

The accelerating effect of the coronavirus 2019 (COVID-19) pandemic led to a new transformation in the models for training orthopedic residents with online training resources as an alternative to face-to-face training[2]. Video-based digital platforms (VBDPs) are an important component in online training resources, and they make a positive contribution to the understanding and practical performance of residents, especially at the stage of surgical preparation[3]. It is already known that not only residents preparing to be surgeons but also orthopedic specialists on a lifelong learning path benefit from YouTube, VuMedi, and similar VBDPs[4]. However, the main prediction requiring investigation is that the "see one, do one, teach one" principle of Sir William Halsted, which has traditionally been one of the foundation stones of surgical training, has been changed to the current interpretation of "watch one, do one"[5]. In this respect, it can be considered worth investigating the tendency of orthopedic specialists to benefit from VBDPs in surgical preparation for procedures they have not seen or done before.

The continuous development of new techniques and indications for minimally invasive orthopedic surgery, primarily arthroscopy, has resulted in a knowledge burden that is difficult to cope with[6]. The sharing on digital platforms of high-quality videos taken in the operating theater provides the opportunity for cases to be seen from the hands of surgeons who are experienced and knowledgeable on the subject[7]. Some of these VBDPs undoubtedly include evidence-based, high-quality training videos, which can show the source[8]. However, there are also VBDPs hosting videos of surgical interventions that have a high number of views but require the indications and reliability to be confirmed.

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The aim of this questionnaire-based study was to investigate which training sources are used by orthopedic specialists in Turkey to prepare for a surgery that they have not seen or performed before and, especially, how often they use VBDPs and the reliability of the VBDP sources.

METHODS

Permission to conduct this questionnaire-based study was obtained from the Ethics Committee of Izmir Katip Celebi University. A questionnaire was prepared with 14 closed-ended items and 14 forced and multiple-choice items using a free Internet-based resource (www.docs.google.com). To reach the maximum possible number of participants, the link to this questionnaire was sent to an e-mail group that included only orthopedic specialists and orthopedic residents (turk-ortopedi@googlegroups.com). To prevent the participants from completing the questionnaire more than once, the Internet protocol (IP) was restricted.

The first section of the questionnaire recorded the demographic data of age, the institution where they are working, and the duration in the profession (i.e., resident, specialist for 1–10 years, and specialist for >10 years). In the following section, questions related to the training sources they tended to use in preparation for surgeries they had rarely or never performed before, and the reliability of the VBDP were asked.

After the termination of the data collection procedure, the opinions of 235 orthopedic specialists and residents who agreed to participate in the study and who worked in university hospitals, state hospitals, or private hospitals in Turkey were recorded. The data of 54 residents were excluded from the analysis, and 181 orthopedic specialists were included in the analysis.

Statistical evaluation

Data obtained from the respondents were analyzed statistically using the SPSS version 20.0 software. Continuous data were presented as mean±standard deviation (SD) values and categorical data were presented as numbers (n) and percentages (%). The χ^2 test was used in the comparisons of categorical data, and Fisher's exact test was used when the data did not meet the χ^2 test requirements. A value of $p<0.05$ was considered statistically significant.

RESULTS

The evaluation was made from the data of 181 orthopedic specialists with an average age of 43±8.7 years. As the database was recorded after the full completion of the questionnaires by the respondents, the response rate was 100%. Of the total respondents, 53.6% (n=97) worked in university and training hospitals, 27.6% (n=50) in state hospitals, and 18.2% (n=33) in private hospitals. The duration of working in the profession as an orthopedic specialist was reported to be 1–10 years as a specialist by 44.8% (n=81) of the respondents and >10 years as a specialist by 55.2% (n=100).

The resources from which the respondents benefitted when preparing for a surgery that they had rarely or never seen or performed before are shown in Figure 1. The orthopedic specialists reported that they first benefitted from VBDPs (n=70, 38.7%), surgical approach books (n=49, 27.1%), textbooks (n=31, 17.1%), and literature research (n=15, 8.3%). Verbal consultations were defined as a priority by 16 (8.8%) respondents.

The data related to the frequency that the respondents used training resources on the subject before cases of orthopedic surgery that had not seen/performed before are shown in Table 1. Of the total respondents, 66.3% (n=120) benefitted from VBDPs, 65.2% (n=118) from surgical approach books, 57.5% (n=104) from textbooks, 45.3% (n=82) from literature research, and 30.4% (n=55) from verbal consultations before each surgery they had not seen/performed before. The rates of taking no benefit from these training resources varied between 1 and 9%. The specialists with 1–10 years of experience benefitted from literature research, and those with more than 10 years of experience took relatively more benefit from textbooks and surgical approach books, but the difference was not statistically significant ($p>0.05$). No statistically significant difference was determined between the two groups of specialists according to experience in respect of the rates of benefit taken from VBDPs and verbal consultations ($p>0.05$).

Of the total orthopedic surgeons in the study, it was reported by 81.2% (n=147) of surgeons who had never seen a surgical intervention and by 66.9% (n=121) who had seen a

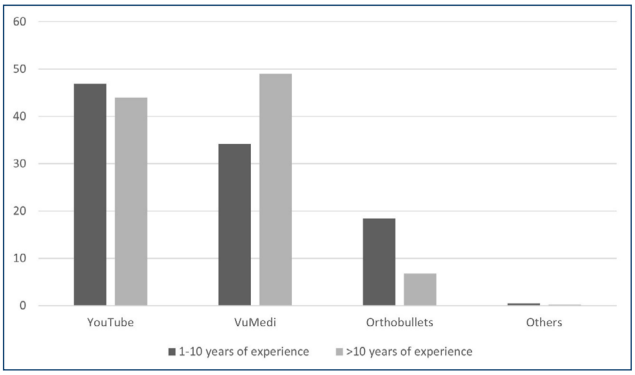

Figure 1. The percentages of resources from which the respondents benefitted when preparing for a surgery that they had rarely or never seen or performed before.

Table 1. The frequency of training resources that the respondents used on the subject before cases of orthopedic surgery that had not seen/performed before.

Educational resources	Average number of years in practice	Never n (%)	Sometimes n (%)	Always n (%)	p-value	Cramer's V
Textbooks	1–10 years	3 (3.7)	36 (44.4)	42 (51.9)	0.235	0.127
	>10 years	1 (1)	37 (37)	62 (62)		
Literature search	1–10 years	6 (7.4)	32 (39.5)	43(53.1)	0.165	0.141
	>10 years	9 (9)	52 (52)	39 (39)		
Approach books	1–10 years	4 (4.9)	26 (32.1)	51 (63)	0.269	0.120
	>10 years	1 (1)	32 (32)	67 (67)		
Verbal consultation	1–10 years	2 (2.5)	52 (64.2)	27 (33.3)	0.545	0.0819
	>10 years	5 (5)	67 (67)	28 (28)		
Video-based digital platforms	1–10 years	2 (2.5)	24 (29.6)	55 (67.9)	0.877	0.0381
	>10 years	2 (2)	33 (33)	65 (65)		

surgical intervention but not performed it themselves that they performed such a surgical intervention only with the benefit taken from VBDPs. The digital platform most often used in surgical preparation by all the specialists was determined to be YouTube at the rate of 45.3% (n=82). Between the two groups according to experience, VuMedi was used significantly more (49%) by the surgeons with more than 10 years of experience, and Orthobullets was used significantly more (18.5%) by the surgeons with 1–10 years of experience ($p<0.05$) (Figure 2).

Of the videos they watched on VBDPs with the aim of surgical preparation, 62% of the respondents reported that they considered the videos reliable, 28.5% stated that they were not much reliable, and 3.4% found them unreliable. In response to the question of the need for surgical training videos on the popular social/medical digital platforms of national and international orthopedic professional associations, 80.7% (n=146) of the respondents definitely agreed that they were necessary, 16% (n=29) stated that they may be necessary, and 2.2% (n=4) considered that they were not necessary.

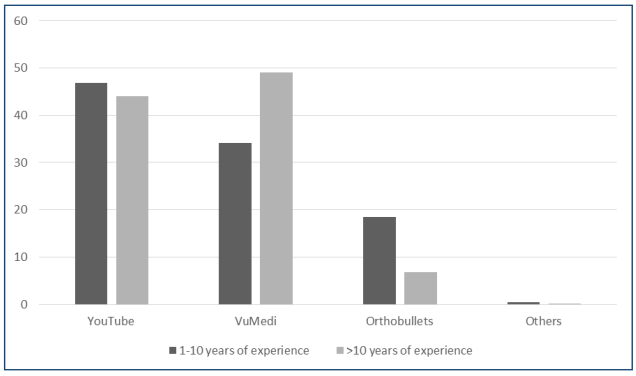

Figure 2. The usage of digital platforms according to experience of orthopaedic surgeons benefitted when preparing for a surgery that they had rarely or never seen or performed before.

DISCUSSION

The results of this study clearly showed that VBDPs are one of the most important training resources from which orthopedic surgeons in Turkey benefit when preparing for surgical procedures that they have not previously seen or performed. Moreover, the training resource from which surgeons with different durations of professional experience benefitted from most often as a priority when preparing for surgery that they had previously not seen or performed was again these platforms. That the VBDPs were preferred first in surgical preparation is because these resources have different advantages as a component of the electronic learning model[9]. Due to the printing process of printed materials, textbooks and surgical approach books tend to have the problem of not being up-to-date in surgical training, which is an area that undergoes continuous development and change. In contrast, VBDPs can be rapidly updated and accessed. With the combination of written, visual, and audio stimuli, a surgical training video can present a procedure in a more understandable way[8].

It has been previously shown in the literature that video-based learning is more effective than print media for better learning and understanding of complex surgical procedures[10]. It is also known that watching a surgical training video before performing a surgical procedure for the first time significantly reduces error rates and shortens the operating time[11,12]. In the development of the surgical knowledge and skills of orthopedic specialists, there are various alternative and effective programs such as training courses and industry-funded events[13]. However, personal participation in these programs has disadvantages in

the sense of both time and money, whereas training videos prepared in these programs, especially surgical preparation related to cadaver dissection that the surgeons have not previously seen or performed, can be accessed through VBDPs at a more convenient time and place.

The benefit of surgical preparation from literature resources such as systematic reviews or meta-analyses, or primary, focused research articles remains as important and frequent as the benefit from VBDPs[14]. However, according to the results of this study, more than half of the orthopedic specialists do not always research the literature on surgical preparation for a procedure they have not previously seen or performed. Although literature resources provide undoubtedly valuable theoretical information about the indications of a disease, the surgical procedure, and outcomes, the lack of video content related to the surgical procedure in most of them may lead to insufficient demand for these resources. Verbal consultations are often used, but this is a training model about which there has been very little examination in the literature[15]. Social media has become a more effective and easy-to-use means of communication for many surgeons from the same branch to participate in verbal consultations[16]. However, according to the results of this study, surgeons do not use this training model as a priority when preparing for surgeries that have not previously seen or performed. Although the recommendations from experienced surgeons in these groups are valuable for surgical preparation, a surgical procedure that is to be performed for the first time can probably be learned more easily from a video.

Supporting the results of this study, YouTube is known to be the most frequently used VBDP in surgical preparation throughout the world[4]. However, as videos can be uploaded to this platform with open access without showing the source, this creates a huge doubt in respect of the provision of current surgical standards. It has been previously shown that the educational value of videos on this platform is low for both patient information and medical training related to the field of orthopedics[17-19]. Although there are recommendations for showing the source of videos or for the development of a scoring system for reliability, it is clear that the current scoring system does not go beyond the common sense of the viewer[4]. In this study, the surgeons did not give high-reliability scores when asked about if the reliability of the VBDPs supports this disadvantage.

Despite the high response rates and consistent results obtained from the orthopedists, there were some limitations to this study. There may have been some bias due to the respondents marking different response options for a question they did not wish to answer. Moreover, when the number of respondents is taken into consideration, the results may not represent all orthopedic specialists either in Turkey or throughout the world.

CONCLUSION

The results of this study demonstrated that orthopedic specialists in Turkey primarily and frequently use VBDPs as a training resource in preparation for surgical procedures that they have not previously seen or performed. Similar to that in other educational models in medicine, platforms with evidence-based content and showing the source, which are established or supported by official orthopedic institutions and associations, would be beneficial to disseminate accurate and reliable up-to-date information.

ETHICAL APPROVAL

File Number: 20.10.2022/0458.

AUTHORS' CONTRIBUTIONS

HZ: Conceptualization, Data curation, Formal Analysis, Writing – original draft. **HC:** Formal Analysis, Writing – review & editing. **AIK:** Data curation, Writing – review & editing.

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ORIGINAL ARTICLE

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Opinions of female academicians on oocyte freezing: a qualitative study

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SUMMARY

OBJECTIVE: The aim of this study was to evaluate the opinions of female academicians about oocyte freezing.

METHODS: This qualitative study included 12 single academic women who had not yet entered menopause, did not have children, and were continuing their doctoral education in Istanbul, Turkey, between August and September 2022. Data were collected with semi-structured interviews and evaluated by content analysis.

RESULTS: Three main themes were "Difficulty of fertility in academics," "Advantages of oocyte freezing," and "Concerns about oocyte freezing." Participants mostly had positive attitudes about the advantages of oocyte cryopreservation, but they had concerns about pregnancies obtained with frozen oocytes.

CONCLUSION: The academic women attributed fertility as an obstacle to their career and experienced anxiety about fertility. They were aware of the advantages of oocyte cryopreservation; however, they defined the pregnancy with oocyte freezing as artificial.

KEYWORDS: Oocyte. Cryopreservation. Fertility. University.

INTRODUCTION

Social oocyte freezing is the retrieval of oocytes followed by cryopreservation without a medical indication, only because of women's preference for postponing childbearing until later ages[1]. The optimum age for freezing is below 35 years, and the number and quality of oocytes, the used technique, and frozen periods may influence the success of pregnancy with cryopreservatived oocytes[1-3].

Women who prefer freezing eggs are commonly between 36 and 40 years of age, with higher education and professional employment[3,4]. Economic reasons and the challenging conditions brought by busy working life have striking effects on postponing fertility[5]. The most common reasons to delay fertility are associated with higher education, professional career, and financial independence[1].

Becoming an academician usually requires long years of professional education and work[6]. Despite the increase in the number of female academics over the years, motherhood continues to be an important task in their lives. However, many academic women feel that their productivity and success are negatively impacted. The unequal impact of parenthood in academia and the challenges faced by mother academicians are well established in the literature[6,7]. Oocyte cryopreservation can be an option for academic women to preserve their fertility potential until later ages.

No previous qualitative studies have been found on oocyte freezing in academic women. This study will contribute to the limited literature[6,8,9] on this issue with qualitative data. In addition, understanding the opinions of academic women with a high risk of age-related infertility, about their fertility intentions, desire for delaying birth, and oocyte freezing option, will guide health professionals in counseling those women to develop realistic expectations regarding the advantages and disadvantages of oocyte cryopreservation.

This study aimed to evaluate the opinions of female academicians about oocyte freezing.

METHODS

The sample consisted of 12 academic women who had not yet entered menopause, had no children, were married or single, and were continuing at least their doctoral education in Istanbul, Turkey, between August and September 2022. Participants were determined by purposive and snowball sampling methods. In qualitative studies, the interviews are continued until a saturation point when no new/different data are obtained from subsequent participants[10-12]. As no new themes were emerging from the interviews after 12 women, it was assumed that the data had reached a saturation point and the interviews were ended.

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The researchers contacted potential participants by telephone. Agreed participants completed a written informed consent form by the guidelines of the Declaration of Helsinki before the interview. Data were collected through online interviews using an introductory information form about personal characteristics and a semi-structured interview form. Participants consented to audio/video recording. The semi-structured interview form included six questions (Table 1). The interviews lasted approximately 20 min.

Two researchers conducted the interviews, of them one asked the questions and the other reported the interview. The data were collected under subheadings by transcribing the most common and different points. Researchers evaluated the documents, which were summarized by the content analysis method, and transformed them into a written report. The data were coded line by line by the researchers and subjected to content analysis. The themes and sub-themes were then finalized after the researchers came together, re-evaluated, and reached a common opinion. In order to ensure the validity and reliability of the research, the percentage of consistency between the codes and themes determined by three researchers was calculated. The value of 0.85 was evident that the categories were consistent[12].

Ethical aspects: The ethics committee decision (Date: 28.06.2022/Decision No: 584) was obtained from the Medipol University Non-Interventional Clinical Research Ethics Committee for the research.

Findings

The participants were between 26 and 37 years of age (mean age 31.25±3.95 years), all of them were single, 83.3% of them had an income equal to their expenses, and 58.3% of them had information about egg freezing (Table 2). Participants gave an average answer of 7.08±1.62 out of 10 on the importance of having a child and 41.17±2.88 on the average for the age limit for natural conception.

In line with the interviews, three main themes, one subtheme, and 29 codes were determined as "Difficulty of fertility in academicians," "Advantages of oocyte freezing," and "Concerns about oocyte freezing" (Table 3).

Central Theme 1: Difficulty of fertility in academics: Ten codes were found (Table 3). Most participants stated that it is challenging to be a mother in academics and that would cause them to pause and work with lower productivity.

Participant 2 (aged 27 years): "Academics differ from other professions to a certain extent. We must constantly improve ourselves; of course, we can spare less time for home life than other professional groups. At this point, of course, the childbearing age may be delayed. The time allocated for children may decrease. Academics can be a disadvantage in terms of childbearing."

Participant 7 (aged 37 years): "It will affect the time of having children in academics because it is unrealistic to have children at this intensity anyway. However, this is my preference; other people do this, and they have children well. I preferred not to have them all on top of each other like this, but to spend that process by enjoying the child more, you know, not to have a child in the stress of doctoral education."

Central Theme 2: Advantages of oocyte freezing: Nine codes were found (Table 3). Most participants stated that they had a positive attitude toward the advantages of oocyte cryopreservation.

Participant 5 (aged 27 years): "Because the risk of childbirth increases with age. Naturally, if it is a method that reduces its effects, I would consider egg freezing from that point of view because I think freezing an egg that is fertile at the moment. I mean, also think about it to reduce the psychological pressure on me right now, the pressure of society, to say that I have already taken precautions."

Participant 6 (aged 27 years): "As a woman, we can all experience uneasiness from time to time due to the biological clock or the early menopause stories we see around us. If I have frozen eggs, I feel more secure, and I can make the decision of motherhood entirely according to my own life, and I am not interested in the biological clock."

Central Theme 3: Concerns about oocyte freezing: Ten codes were found (Table 3). Participants' concerns about oocyte freezing were generally about pregnancies obtained with frozen oocytes. The statements of a few participants are shared:

Table 1. Semi-structured ınterview questions.

1. How do you think your academic career plan will affect the decision/timing of having children in the future? Explain your reasons.
2. Would you consider freezing your eggs or embryos to postpone having children?
3. How would it make you feel if you froze your eggs?
4. Do you have any concerns about your future pregnancy with your frozen eggs/embryos? What about (advanced-age pregnancy, complications, etc.)?
5. If you do not need to use your frozen eggs/embryos in the future, what would you prefer to do with them? Why? (Destroyed/donated to those in need/donated for research.)
6. What do you think about the financing of egg freezing, and who do you think should provide it?

Table 2. Descriptive characteristics of the participants.

Participant	Age (years)	Profession	Title	Partner presence	Thinking about having children in the future	Importance of having children
K1	34	Psychology	Research Assistant/Instr.	No	I think	7
K2	27	Social Service	Research Assistant/Instr.	Yes	Undecided	6
K3	30	Child Development	Research Assistant/Instr.	Yes	I think	9
K4	26	Speech and Language Therapy	Research Assistant/Instr.	No	Undecided	10
K5	27	Speech and Language Therapy	Research Assistant/Instr.	Yes	I think	5
K6	27	Logistics	Research Assistant/Instr.	Yes	I think	6
K7	37	Nurse	Research Assistant/Instr.	Yes	Undecided	6
K8	36	Business	Prof. Dr. Lecturer	No	I think	5
K9	31	Electronic engineering.	Research Assistant/Instr.	No	I think	8
K10	33	Tourism	Research Assistant/Instr.	No	I think	7
K11	36	Nurse	Prof. Dr. Lecturer	Yes	I think	7
K12	31	Biochemistry	Prof. Dr. Lecturer	No	I think	9

Instr.: instructor.

Participant 11 (aged 36 years): "After all, it is not spontaneous; it feels like something artificial in a laboratory environment. So maybe I would be a little worried."

Participant 3 (aged 30 years): "After a certain age, I may not be able to achieve a healthy enough level to raise a living creature in it, when biologically my body is barely enough to even for me. For this reason, I have concerns."

DISCUSSION

Being a mother was highly important for most of the participants, and they were planning to become parents in the future. No matter how educated a woman is, her internalization of society's norms causes her to see herself as an "incomplete woman" when she is not fertile. In a previous qualitative study in Turkey, it was emphasized that women with higher education froze oocytes to meet social norms[13].

Gaining a professional career usually takes place during women's most fertile years, when women may need to postpone fertility. Fertility is interpreted as a significant detriment to the professional development of academic women because of the necessity of advancement in academic life, the need to work outside working hours, and the patriarchal social culture. The lack of childcare facilities in the workplace is another reason why women delay childbearing. In studies with women academics, women have reported difficulties balancing childcare with their academic careers[7,14]. Women generally have more childcare responsibilities than men and are therefore less likely to meet the idealistic view of academics[6]. Balancing research and other responsibilities in academia with motherhood can be difficult. Being a mother is scary for a woman who is caught between two dilemmas[7,15].

The women in this study described motherhood as a pause or loss in their career plans. Most of the participants were at the beginning of their careers and were younger. They may therefore have been less eager to become mothers. A small number of female academics in this study said that fertility was not a barrier to their career. However, these women had advanced in their careers. Therefore, they may have been more interested in becoming a mother and said it was not an obstacle in their career. In a study of highly educated women, half of the participants reported that they were concerned about their future fertility[16]. Oocyte cryopreservation can improve women's reproductive autonomy or be a chosen strategy against age-related infertility[1,17].

In a study of women who froze oocytes, a high proportion (88%) of women cited lack of a partner as a reason, while very

Table 3. Analysis of central themes and codes.

	Codes	n
Central Theme 1. The difficulty of fertility in academia	In between	2
	Engel	1
	Pause	1
	Disappear	1
	Difficult	2
	Reducing productivity	1
	Harmful	1
	Disadvantage	1
	Postpone	1
	Not a barrier	3
Central Theme 2. Advantages of oocyte cryopreservation	No information	4
	Reliable	1
	Guaranteeing	1
	Healthy	3
	Precaution	1
	Happy	1
	Trust	5
	At ease	3
	Guarantee	1
Central Theme 3: Concerns about oocyte cryopreservation	Unnatural	1
	Expensive	1
	Moving away from feminine energy	1
	Concern	1
Central Theme: Concerns about the upcoming pregnancy	Concern	8
	Artificial	1
	Anxiety	1
	Guilt	1
	On Trial	1
	I am not pessimistic	1
	I relax	2

few (19%) women cited a demanding work schedule at work[4]. Many educated women are willing to postpone fertility and opt for oocyte freezing in order to find the right partner to share their lives with[18]. However, half of the women in this study had a partner, but they mostly had planned to postpone fertility. We cannot say that this plan was due to the absence of a partner in academics.

Although women said they would consider oocyte cryopreservation, many of them said they might be worried about pregnancy with frozen oocytes. The thought of this pregnancy being artificial made them uneasy. They even felt that they would be distancing themselves from feminine energy. However, the current literature emphasizes that pregnancies with vitrified oocytes are mostly safe[18,19]. Due to the novelty of the oocyte cryopreservation procedure in Turkey, many women have inadequate knowledge about the safety of the procedure, including the possible complications of pregnancy at an advanced age. In a previous study of undergraduate students in Turkey, female students were more likely to say that they would consider freezing their oocytes if it was medically necessary[20].

Social oocyte cryopreservation is privately funded in Turkey, as in many countries worldwide[7]. Participants in this study were also undecided because of the high cost of freezing, and about half of the participants said that the financing should be within the government. More women would be able to consider oocyte cryopreservation with government funding.

In conclusion, the academic women attributed fertility as an obstacle to their career. They valued the advantages of oocyte cryopreservation; however, they had concerns about pregnancy with frozen oocytes. The fertility intentions of women at high risk of age-related infertility, such as academics, should be questioned, and if interested, they should be counseled about the advantages and disadvantages of oocyte freezing.

Strengths of the study

This study provides in-depth qualitative data to the limited literature about academicians' views on oocyte freezing, with high consistency between the themes determined by researchers. The study was carried out in Istanbul, where the number of female academicians observing the effects of parenting in academia is high.

Limitations

The participants were mostly doctoral research assistants in their early 30s, the most suitable group for oocyte freezing. The results do not reflect the academicians with higher degrees. In addition, the use of the purposive sampling method may be a limitation.

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AUTHORS' CONTRIBUTIONS

ÖT: Conceptualization, Methodology, Writing – original draft, Writing – review & editing. **GK:** Conceptualization, Methodology, Writing – original draft, Writing – review & editing. **İGS:** Conceptualization, Formal Analysis, Methodology, Supervision, Writing – review & editing.

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ORIGINAL ARTICLE

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Sclerostin and TNF-related weak inducer of apoptosis: can they be important in the patients with glomerulonephritis?

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SUMMARY

OBJECTIVE: Sclerostin is a protein produced by osteocytes, kidneys, and vascular cells and has many effects on kidney and vascular structures. Soluble TNF-related weak inducer of apoptosis is a proinflammatory cytokine that may cause glomerular and tubular injury and increase sclerostin expression. This study aimed to investigate serum sclerostin and soluble TNF-related weak inducer of apoptosis levels in patients with glomerulonephritis and the effects they may be associated with.

METHODS: This cross-sectional study included 93 patients, 63 of whom were glomerulonephritis and 30 were healthy controls. Serum sclerostin, soluble TNF-related weak inducer of apoptosis, and 24-h urinary protein excretion were measured, and pulse wave velocity was calculated for arterial stiffness.

RESULTS: Serum sclerostin and soluble TNF-related weak inducer of apoptosis were higher in glomerulonephritis patients than in the control group, and serum sclerostin and soluble TNF-related weak inducer of apoptosis levels were correlated with both proteinuria and pulse wave velocity. In addition, in the regression analysis, serum sclerostin and soluble TNF-related weak inducer of apoptosis levels were found to be independent predictors of proteinuria in patients with glomerulonephritis.

CONCLUSION: This is the first study to show that serum sclerostin and soluble TNF-related weak inducer of apoptosis are elevated in glomerulonephritis patients, and these two markers correlate with arterial stiffness and proteinuria in these patients. Considering the effects of sclerostin and soluble TNF-related weak inducer of apoptosis in patients with glomerulonephritis, we think these mechanisms will be the target of both diagnosis and new therapies.

KEYWORDS: Glomerulonephritis. Pulse wave velocity. Proteinuria. Vascular stiffness.

INTRODUCTION

Glomerulonephritis (GN) is glomerular inflammation caused by immune- or non-immune-mediated injury. In GN patients, cardiovascular disease (CVD) risk increases due to systemic inflammation[1]. There are many pathogenetic mechanisms in GN, and many new mechanisms have been discovered recently.

Sclerostin is a protein produced by osteocytes, kidneys, and vascular cells. As kidney functions decrease, serum sclerostin levels increase[2]. One of the critical mechanisms by which sclerostin affects bone regulation is the modulation of the Wnt/β-catenin pathway. Sclerostin is a Wnt signaling pathway antagonist that causes suppression of osteoblast differentiation and proliferation[3]. Wnt pathway, inhibited by sclerostin, plays a central role in bone turnover and remodeling. Sclerostin is also involved in the pathophysiological process of atherosclerosis, due to its roles in the regulation of endothelial inflammation, vascular calcification (VC), and mesenchymal stem cell differentiation[4]. These pathways have roles in various pathological processes such as renal fibrosis, podocyte injury, proteinuria, and chronic kidney disease (CKD)-related vascular injury. While inhibition of the Wnt/β-catenin pathway by sclerostin inhibits osteoblast differentiation and proliferation, the effects of this inhibition on VC development and its renal effects are still complex[3,4].

Soluble TNF-related weak inducer of apoptosis (sTWEAK) is a proinflammatory cytokine belonging to the TNF ligand superfamily that may cause glomerular and tubular injury[5]. It regulates pathways with potential pathophysiological implications for kidney injury, such as the induction of inflammatory cytokines[5]. sTWEAK also increases the expression of sclerostin[6]. Fibroblast growth factor inducible-14 (Fn14) is a TWEAK receptor, and its expression is induced secondary to inflammatory events. Activating the Fn14 receptor by TWEAK contributes to glomerular and tubulointerstitial injury[7].

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Pulse wave velocity (PWV) is the gold standard for measuring arterial stiffness. Recently, the relationship between sclerostin and PWV has been investigated in different patient groups, but this relationship has not been revealed in GN patients[8,9]. This relationship becomes more intriguing as new information emerges regarding the roles of TWEAK and sclerostin in the VC process.

TWEAK and its associated receptors are involved in pathogenetic processes that affect overall and renal survival in GN patients through inflammatory and non-inflammatory pathways. Both the renal and vascular effects of sclerostin become evident day by day. Anti-sclerostin treatments have become popular in recent years. Based on these ideas, we aimed to detect serum TWEAK and sclerostin levels in GN patients and their relationship with outcomes that may affect the prognosis of the disease.

METHODS

This cross-sectional study includes 63 GN patients and 30 healthy matched controls. The study protocol was approved by our institution. Informed consent was obtained from all subjects. A review of medical records (including information on age, sex, weight, medications, and duration of the disease) was recorded from hospital system.

Inclusion criteria were as follows: (1) patients diagnosed with GN confirmed by kidney biopsy; (2) patients who aged 18–70 years; and (3) patients who gave written informed consent. Exclusion criteria were as follows: (1) patients who have other diseases, including diabetes mellitus, osteoporosis, malignities, and active infectious diseases; (2) patients with estimated glomerular filtration rate (e-GFR) below 60 mL/min; (3) patients who do not feel willing to participate in the study; (4) patients who have parathyroidectomy; (5) patients with a history of CVD in the past 3 months; (6) post-menopausal women; (7) patients who have received immunosuppressive therapy for any disease; and (8) presence of emergency medical (such as respiratory failure due to interstitial disease) status.

Diagnosis of glomerulonephritis

Patients who were previously diagnosed with GN by kidney biopsy and had no history of GN treatment were included in the study. The distribution of GN patients included in the study was as follows: membranous nephropathy (n=23, 37%), IgA nephritis (n=17, 27%), lupus nephritis (n=16, 25%), and pauci-immune GN (n=7, 11%).

24-h urine proteinuria measurements

Samples for 24-h urinary protein excretion were collected before renal biopsy. Total protein concentration levels were measured by a turbidometric assay using benzethonium chloride. The results were expressed as mg/day.

Serum TNF-related weak inducer of apoptosis measurements

Serum sTWEAK levels were measured by a commercially available kit based on an enzyme-linked immunosorbent assay (eBiosience, Human sTWEAK Instant Elisa, Cat No.: BMS2006INST). The results were expressed as pg/mL. The calculated overall intra-assay coefficient of variation was 7.8%.

Serum sclerostin measurements

Concentrations of human sclerostin were measured with enzyme-linked immunosorbent assay (ELISA) kits (Biomedical Medizinprodukte GmbH & Co. KG, Vienna, Austria) according to the manufacturer's instructions. Intra- and inter-assay coefficients of variation were 5 and 4%, respectively, for sclerostin. The results were expressed as pg/mL.

Pulse wave velocity measurements

The Arterio Vision device (OSACHI Co. Ltd.) simultaneously measured brachial-ankle pulse wave velocity (baPWV; units: m/s) on each side of the body, arterial stiffness index (units: arbitrary) in each limb, and blood pressure and heart rate in each limb. Measurements were made in duplicate, separated by 1 min. If the second blood pressure value was >5 mmHg to the first measurement, a third measure was collected, and the closest two measures were averaged. The baPWV was calculated by dividing the arterial path length by the pulse transit time between the brachial and ankle arterial segments.

Statistical analyses

Clinical data were analyzed using Statistical Package for Social Sciences for Windows version 21.0 (SPSS Inc., Chicago, Illinois, USA). Descriptive statistics for each variable were determined. Data were expressed as mean±standard deviation. Results for continuous variables without normal distribution were presented as median [interquartile range (IQR)]. A statistically significant difference between the groups was determined by the χ^2 test for categorical variables. Nonparametric statistics (Mann-Whitney U test) and parametric statistics (independent sample t-test) were all used for continuous variables. Associations between the variables were explored using Spearman's rho test. A linear regression analysis was also performed to define variables associated with proteinuria. A statistically significant difference was considered when $p \leq 0.05$.

RESULTS

Demographic, clinical characteristics, and biochemical parameters of 63 patients with GN and 30 healthy subjects are depicted in Table 1. The control group had significantly lower serum urea and uric acid levels, while serum albumin levels were significantly higher in this group. When 24-h proteinuria levels were compared, the median proteinuria level was higher in the GN group as expected (GN: 752 (1401) mg/day; control group: 72 (73) mg/day (p=0.008)) (Table 1).

Serum sclerostin and soluble TNF-related weak inducer of apoptosis levels

The median sTWEAK level of the GN group was 77.95 (7.3) pg/mL, and the control group was 57.02 (1.94) pg/mL, and the difference was statistically significant (p=0.001). Serum sclerostin was higher in the GN group [2322.92 (1857.11) pg/mL vs. 470.21 (685.65) pg/mL (p=0.003), respectively].

Factors associated with proteinuria in patients with glomerulonephritis

Factors associated with proteinuria were evaluated in patients with GN. There was a positive correlation between proteinuria and sTWEAK (r_s=0.409; p=0.021), sclerostin (r_s=0.426; p=0.013), and PWV (r_s=0.342; p=0.007) (Table 2).

Evaluation of arterial stiffness in patients with glomerulonephritis

The effect of inflammatory markers on arterial stiffness in GN patients was investigated. Brachial PWV was used for arterial stiffness measurement. PWV was significantly higher in GN patients compared to healthy controls (7.71±1.45 vs. 8.57±1.75; p=0.043). Both sTWEAK and serum sclerostin levels were correlated with PWV. The correlation with both sTWEAK (r_s=0.260, p=0.043) and sclerostin was statistically significant (r_s=0.310, p=0.015, respectively).

Table 1. Demographic, clinical characteristics, and biochemical parameters of patients with glomerulonephritis and healthy subjects.

Parameters	Healthy subjects (n=30) Mean±SD or Median (IQR)	Patients with glomerulonephritis (n=63) Mean±SD or Median (IQR)	p-value
Age (years)	38.8±9.22	43.59±13.92	0.231
Female/male	15/15	29/34	0.828
Glucose (mg/dL)	94.5±13.15	94.79±17.53	0.996
Urea (mg/dL)	28 (10.3)	38 (44.3)	**0.048**
Serum creatinine (mg/dL)	0.83 (0.17)	0.92 (0.11)	0.235
e-GFR (mL/min)	97.88 (22.21)	89.63 (17.92)	0.352
White blood count	7.87±1.47	8.26±2.65	0.651
Hemoglobin (g/dL)	13.87±1.92	12.94±2.24	0.223
Platelet count (10^3/μL)	266.9±56.68	290.46±115.12	0.317
Uric acid (mg/dL)	4.82±1.28	6.39±1.68	**0.009**
Albumin (g/L)	4.5±0.49	4.09±0.56	**0.037**
ALT (U/L)	18 (10.3)	14.5 (9.3)	0.507
Calcium (mg/dL)	9.45 (0.63)	9.25 (0.82)	0.481
Phosphorus (mg/dL)	3.65±05	3.79±0.94	0.641
24-h proteinuria (mg/day)	72 (73)	752 (1401)	**0.008**
CRP (mg/L)	2.4 (5.15)	2.1 (6.03)	0.284
PWV (m/s)	7.71±1.45	8.57±1.75	**0.043**
sTWEAK (pg/mL)	57.02 (1.94)	77.95 (7.3)	**0.001**
Sclerostin (pg/mL)	470.21 (685.65)	2322.92 (1857.11)	**0.003**

E-GFR: estimated glomerular filtration rate; CRP: C-reactive protein; ALT: alanine aminotransferase; PWV: pulse wave velocity. Bold values indicate statistical significance at the p<0.05 level.

Predictors of proteinuria in patients with glomurlonephritis

In patients with GN, traditional and correlated factors were included in the regression model to determine independent predictors of proteinuria. sTWEAK and sclerostin were identified as independent predictors of proteinuria (Table 3).

DISCUSSION

In this study, we found three essential conclusions in GN patients. First, serum sclerostin and sTWEAK were higher in GN patients than in the healthy group. Second, proteinuria was correlated with serum sclerostin, sTWEAK, and arterial stiffness. Another significant result was that serum sclerostin and sTWEAK were independent predictors of proteinuria. This is the first study that shows the correlation of serum sclerostin and sTWEAK with the PWV in GN patients.

Especially below GFR 60 mL/min, as kidney function declines, serum sclerostin increases[10]. As GFR decreases, urinary sclerostin excretion increases. This indicates increased serum sclerostin in CKD is due to increased osteocyte-mediated production rather than reduced urinary excretion[11]. In our study, although e-GFR was similar between the two groups, the serum sclerostin levels were higher in GN group. Apart from decreased GFR and increased uremia in patients with GN, additional factors arising from the primary disease may increase sclerostin or cause insufficient suppression of sclerostin levels. sTWEAK contributes to kidney inflammation by promoting the production of cytokines in kidney cells, and as a result, kidney injury is increased[7]. TWEAK is also an essential inducer of sclerostin[6]. Studies investigating serum sclerostin and sTWEAK levels in patients with GN are insufficient. A few studies have shown that sclerostin and sTWEAK levels increase in GN patients[12-14]. These markers have been shown to correlate with the severity of renal involvement in patients with lupus and IgA nephritis. TWEAK induces a local inflammatory environment and plays a crucial pathogenic role in developing GN[5]. Therefore, high serum sclerostin and sTWEAK in our GN patient group are expected depending on the underlying inflammatory response. But, the overexpression of Fn14, which is a TWEAK receptor, has also been shown in immune and non-immune glomerulopathies. This suggests that the role of TWEAK in the mechanism of kidney injury is not through immune effects alone[7]. Our study is valuable because it is the first to show that both sclerostin and TWEAK increase together in GN patients.

We found that, in GN patients, serum sclerostin and sTWEAK were correlated with proteinuria, and these markers were independent predictors of proteinuria. Increased Wnt expression causes proteinuria secondary to podocyte injury[15]. WNT activity increases rapidly in the early stages of proteinuria and tubular injury and decreases as the injury progresses. In addition, the development of apoptosis in renal cells following decreased WNT activity suggests that the WNT pathway has protective effects on renal cells[16,17]. However, if the activity in this pathway persists, it causes cellular injury[17]. The Wnt-β-catenin pathway can be considered both a cause and a result of increased proteinuria. It is a pathway that should be considered as a treatment target

Table 2. Bivariate correlations between 24-h proteinuria and other parameters in glomerulonephritis patients.

Parameters	r_s	p-value
sTWEAK (pg/mL)	0.409	**0.021**
Sclerostin (pg/mL)	0.426	**0.013**
PWV (m/s)	0.342	**0.007**

PWV: pulse wave velocity. Bold values indicate statistical significance at the p<0.05 level.

Table 3. Independent variable of proteinuria in glomerulonephritis patients.

Parameters	Standardized beta	t	p-value	95%CI
Step 1				
BMI (kg/m²)	0.057	0.420	0.677	-48.09 to 73.35
Creatinine (mg/dL)	-0.027	-0.209	0.835	-183.03 to 148.64
CRP (mg/L)	-0.082	-0.568	0.573	-10.24 to 5.74
PWV (m/s)	0.026	0.184	0.855	-163.56 to 196.41
sTWEAK (pg/mL)	0.429	3.185	0.003	5.341–23.84
Sclerostin (pg/mL)	0.3	2.266	0.029	0.048–0.82
Step 5				
sTWEAK (pg/mL)	0.424	3.435	**0.001**	5.97–22.9
Sclerostin (pg/mL)	0.307	2.483	**0.017**	0.085–0.811

BMI: body mass index; CRP: C-reactive protein; PWV: pulse wave velocity; CI: confidence interval. Bold values indicate statistical significance at the p<0.05 level.

in patients with proteinuria in the future since its long-term activation facilitates the progression to renal fibrosis[18]. In GN patients, increased TWEAK levels may cause an increase in sclerostin and proteinuria. Urinary sclerostin excretion was increased due to increased serum sclerostin, and proteinuria was correlated with urinary sclerostin excretion[11,19]. Although increased urinary sclerostin excretion contributes little to total proteinuria, this is one of the mechanisms that can explain the correlation between serum sclerostin levels and proteinuria in GN patients. Our study found that both serum sclerostin and sTWEAK levels were correlated with proteinuria in GN patients.

This is the first study to show the correlation of serum sclerostin and sTWEAK levels with PWV in GN patients. Recently, it has been shown that sclerostin is associated with arterial stiffness in different patient groups[8,9,20-22], but it is still unclear whether the role of sclerostin in VC development is to increase or inhibit VC[20,21]. Due to the similarities between osteogenesis and VC, the Wnt-β-catenin pathway may also play an important role at this point[23]. Moreover, increased Wnt signaling activity and sclerostin expression during calcification of vascular smooth muscle cells draw attention to the role of this pathway in increased VC. However, some studies still claim that sclerostin preserves vascular smooth muscle integrity. Also, TWEAK promotes angiogenesis by increasing the proliferation and migration of endothelial cells[24]. As can be seen, the relationship between sclerostin TWEAK and VC is still a subject with many unknowns. In our study, both TWEAK and sclerostin are related to PWV in GN patients and draw attention to the possible roles of these molecules in the increased CVD risk in GN patients.

Our study has some limitations. First, our patient group was insufficient to perform subgroup analysis for each GN subtype. This limitation prevents us from direct propositions about a specific GN type. Second, the fact that the entire patient is Turkish makes our results not be applicable to all patients due to the differences between nationalities.

CONCLUSION

This is the first study showing that serum sclerostin and sTWEAK levels are increased together in GN patients, and it also demonstrated for the first time that these two markers were correlated with arterial stiffness and proteinuria in GN patients. Considering the effects of sclerostin and TWEAK in patients with GN, we think our study will guide the development of new treatment strategies.

AUTHORS' CONTRIBUTIONS

HO: Conceptualization, Data curation, Investigation, Methodology, Resources, Software, Supervision, Validation, Writing – original draft, Writing – review & editing. **İB:** Conceptualization, Data curation, Formal Analysis, Methodology, Software, Supervision, Validation, Writing – original draft, Writing – review & editing. **TA:** Conceptualization, Data curation, Investigation, Resources, Visualization. **MAD:** Data curation, Investigation, Resources, Visualization. **FHYA:** Formal Analysis, Methodology, Validation. **KT:** Formal Analysis, Project administration, Supervision, Writing – original draft, Writing – review & editing.

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ORIGINAL ARTICLE

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Coronavirus disease-2019 and heart: assessment of troponin and cardiovascular comorbidities as prognostic markers in patients hospitalized with coronavirus disease-2019 in a tertiary center in Brazil

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SUMMARY

OBJECTIVE: Our study aimed to evaluate the correlation of cardiac troponin T levels with comorbidities and in-hospital outcomes in patients with coronavirus disease-2019 in Brazil.

METHODS: Data from a cohort of 3,596 patients who were admitted with suspected coronavirus disease-2019 in a Brazilian tertiary center, between March and August 2020, were reviewed. A total of 2,441 (68%) patients had cardiac troponin T determined in the first 72 h of admission and were stratified into two groups: elevated cardiac troponin T (cardiac troponin T >0.014 ng/mL) and normal cardiac troponin T. Associations between troponin, comorbidities, biomarkers, and outcomes were assessed. Regression models were built to assess the association of several variables with in-hospital mortality.

RESULTS: A total of 2,441 patients were embraced, of which 924 (38%) had normal cardiac troponin T and 1,517 (62%) had elevated cardiac troponin T. Patients with elevated cardiac troponin T were older and had more comorbidities, such as cardiovascular disease, hypertension, diabetes, arrhythmia, renal dysfunction, liver disease, stroke, cancer, and dementia. Patients with abnormal cardiac troponin T also had more altered laboratory parameters on admission (i.e., leukocytes, C-reactive protein, D-dimer, and B-type natriuretic peptide), as well as more need for intensive care unit, vasoactive drugs, mechanical ventilation, dialysis, and blood transfusion. All-cause mortality was markedly higher among patients with increased cardiac troponin T (42 vs. 16%, P<0.001). Multiple regression analysis demonstrated that in-hospital mortality was not independently associated with troponin elevation.

CONCLUSION: This study showed that cardiac troponin T elevation at admission was common and associated with several comorbidities, biomarkers, and clinical outcomes in patients hospitalized with coronavirus disease-2019, but it was not an independent marker of in-hospital mortality.

KEYWORDS: COVID-19. Troponin. Cardiovascular disease. Myocardial ischemia.

INTRODUCTION

Coronavirus disease-2019 (COVID-19) is a global pandemic with diverse clinical severity, ranging from asymptomatic disease to fatal cases. On risk stratification, cardiac troponin (cTn) has proved to be a useful tool, along with many other biomarkers and comorbidities, especially those related to the cardiovascular (CV) system[1-3]. Elevated serum cTn reflects myocardial injury, which may occur in COVID-19 due to a myriad of mechanisms[4,5]. Systemic events, such as generalized inflammation, hemodynamic instability, hypoxemia, and pulmonary embolism, are likely involved in the majority of cases. Local mechanisms, such as myocardial

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infarction, stress cardiomyopathy, and myocarditis, have also been described but they occur less frequently. Elevated cTn in COVID-19 patients has been previously associated with older age, higher prevalence of CV comorbidities, higher serum biomarkers, and worse outcomes, including higher rates of mechanical ventilation requirement, acute kidney injury, and in-hospital mortality[1-3,6-8]. Meta-analyses showed that cTn is an accurate prognostic tool to predict critical outcomes and mortality in COVID-19[9,10]. Dynamic changes in serial cTn measures are also associated with higher mortality[11]. Combining cTn with natriuretic peptides further improves risk prediction[12].

Most studies on COVID-19 risk assessment were conducted among Asian, European, and North American populations. Brazil, along with other South American Countries, was severely affected by the pandemic, with more than 20 million cases and more than 500,000 deaths by the end of August 2021[13]. In Brazil, a multicentric study evaluated a high CV risk population and found cTn to be an independent predictor of in-hospital mortality[7]. The patients included in this study had elevated cardiac biomarkers, abnormalities in electrocardiogram or echocardiogram, or clinically relevant cardiac manifestations. So far, however, there are no studies on cTn prognostic value in a more varied population hospitalized with COVID-19 in Brazil, including lower CV risk patients. These patients are less prone to having direct cardiac impairment by COVID-19 on admission, and the impact of cTn elevation needs better understanding.

We conducted a retrospective observational study that assessed the correlation of cTn elevation at admission with CV comorbidities, biomarkers, and in-hospital outcomes in patients hospitalized with suspected COVID-19.

METHODS

Study design and participants

We analyzed data from all patients (≥14 years) with suspected COVID-19 who were admitted for at least 24 h to Hospital das Clínicas, Faculdade de Medicina, Universidade de São Paulo (HCFMUSP) between March 30 and August 31, 2020. Patients with nosocomial COVID-19 infection were excluded. HCFMUSP is a tertiary teaching hospital in São Paulo, Brazil, which is dedicated to treating high-complexity cases, and it comprises eight specialized institutes accounting for approximately 2,200 beds and 20,000 healthcare personnel. Between March and August 2020, the HCFMUSP designated 900 beds for COVID-19 patients, including 300 ICU beds[14].

Suspected cases of COVID-19 were defined according to the evaluation of the attending physicians. Confirmed COVID-19 was defined as a positive reverse-transcriptase polymerase chain reaction (RT-PCR) for SARS-CoV-2 on Abbott m200RT (Abbott Laboratories, Chicago, IL, USA) established at the Central Laboratory Division of HCFMUSP on swab, collected from nasopharyngeal and/or oropharyngeal samples, at admission with a minimum of 3 days of symptoms and, if negative, repeated after 48 h[15]; or a positive test by chemiluminescent immunoassays on Liaison XL analyzer (DiaSorin S.p.A., Saluggia, Italy) to detect serum antibodies against SARS-CoV-2, performed for highly suspect cases with at least two RT-PCR negative samples after 7 days of the onset of symptoms or in subjects with high clinical suspicion for whom an RT-PCR test was not available up to the 10th day of symptom onset[16]. Likely COVID-19 cases were defined as clinical and lung computer tomography (CT) signs highly suggestive of COVID-19 with negative RT-PCR tests and lack of serum antibody confirmation. Non-likely COVID-19 cases were defined as suspected COVID-19 with no RT-PCR or serum antibody confirmation who were later reviewed by an infectious diseases' specialist team as having a more plausible alternative diagnosis.

This study was approved by Hospital das Clinicas' Ethics Review Board under the registry number CAAE: 32037020.6.0000.0068. No informed consent was necessary because we acquired all data retrospectively. In this description, we sought to conform to the STROBE guidelines[17].

Data collection

Data from routine hospitalized clinical care were extracted from patients' electronic health records and organized into standardized forms in the RedCap system by trained extractors. We retrospectively collected information from all patients, including demographic data, clinical characteristics, laboratory parameters, and outcomes. Patients were stratified into two groups according to cardiac troponin T (cTnT) levels: elevated (cTnT>0.014 ng/mL, the upper normal limit) and normal.

Statistical analysis

Descriptive statistics include frequency analysis (percentages) for categorical variables and mean±standard deviation (SD) or median and interquartile range (IQR) for continuous variables. Comparisons were determined by the t-test or Mann-Whitney U test for continuous variables, as appropriate, and by the χ^2 test or Fisher's exact test for categorical variables. The level of statistical significance was set at 0.05 (two-tailed). Regression models were constructed using the stepwise backward method to consider the risk of fatal outcome as the dependent variable

to demonstrate the effects of cTnT elevation and CV comorbidities. Statistical analyses were carried out using IBM SPSS Statistics for Windows v. 22.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

We screened 3,596 eligible patients with a suspected diagnosis of COVID-19, of whom 2,441 patients (68%) had cTnT determined in the first 72 h of admission and were included in this study (mean admission troponin interval of 20.2±11.5 h). Of them, 2,042 patients (83.7%) were classified as confirmed cases of COVID-19, 215 patients (8.8%) as likely COVID-19 cases, and 184 patients (7.5%) as non-likely COVID-19 cases. The study flowchart is shown in Figure 1.

Included patients had a mean age of 59±17 years and 1,342 (55%) were males. Notably, 971 (38%) patients had normal admission cTnT and 1,517 (62%) had elevated cTnT (cTnT>0.014 ng/mL). The baseline clinical characteristics are described in Table 1. Patients with an elevated cTnT were more likely to be older (67±16 years vs. 52±15 years, p<0.001) and to have a history of CV disease, hypertension, diabetes, arrhythmia, kidney dysfunction, liver disease, stroke, alcohol drinking, cancer, dementia, and hypothyroidism.

The baseline laboratory parameters are described in Table 2. Patients with abnormal cTnT were more likely to have significantly (p<0.001) worse laboratory parameters at admission (i.e., leukocytes, C-reactive protein, D-dimer, and BNP).

Clinical outcomes during the hospitalization are reported in Figure 2. Patients with elevated cTnT had more need for ICU, vasoactive agents, mechanical ventilation, dialysis, and blood transfusion. All-cause mortality was markedly higher among patients with increased cTnT than those with normal levels (42.66 vs. 16.3%, p<0.001). To better understand the relationship between troponin levels at admission and mortality, patients were stratified into three subgroups: normal, up to three times, and above three times the upper limit of normal (Figure 3). Higher levels of cTnT were associated with higher mortality (p<0.001).

Multiple regression analysis (Table 3) demonstrated that in-hospital mortality was independently associated with hypertension (p<0.004), dialytic kidney dysfunction (p<0.001), age>70 years (p=0.003), and absence of obesity (p=0.021). Elevated troponin did not meet statistical significance for independent association with mortality on the regression analysis.

DISCUSSION

This study demonstrated that elevated cTnT is frequently observed and correlates with multiple comorbidities, biomarkers, and adverse outcomes, including in-hospital mortality, in patients hospitalized with COVID-19. Yet, elevated cTnT was not independently associated with in-hospital mortality after multivariable analysis.

Myocardial injury was more frequent in our report than that previously reported in China or in the United States[2,3,18-20]. Guimaraes et al.[7] selected a high-risk population in Brazil and found that a total of 54.2% of patients presented troponin elevation[7]. Among our patients, 62% had elevated cTnT. Similar to other reports, in our study, patients with myocardial injury

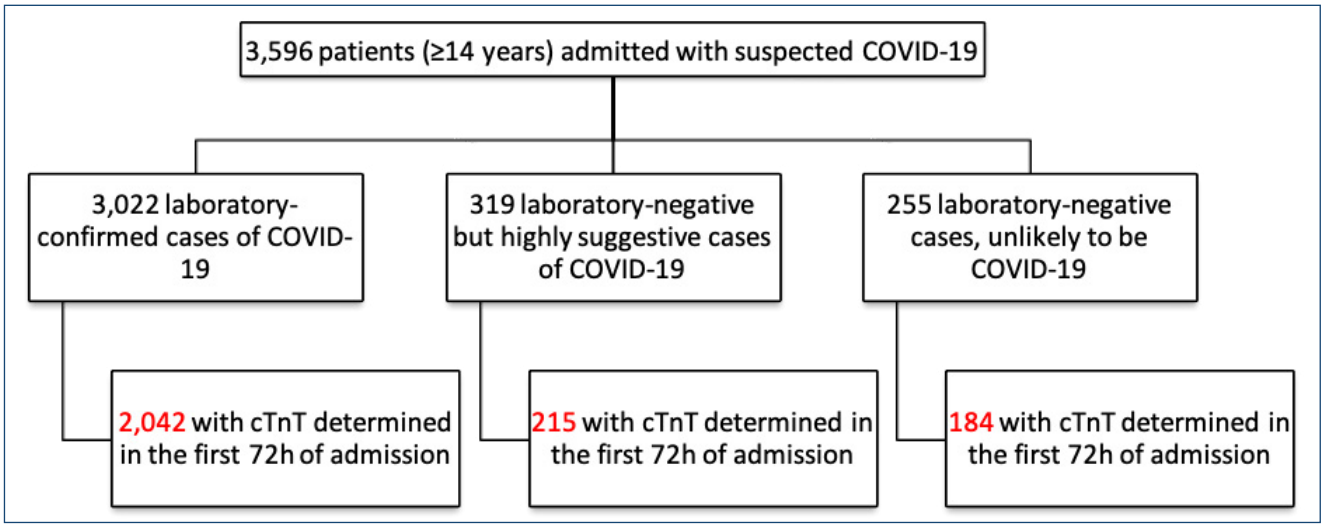

Figure 1. Flowchart showing the distribution of consecutive patients (≥14 years) admitted for at least 24 h as inpatients to Hospital das Clínicas, Faculdade de Medicina, Universidade de São Paulo with suspected coronavirus disease-2019, between March and August 2020.

tended to be older and have a history of CV diseases or CV comorbidities such as hypertension, diabetes, smoking, and kidney dysfunction[2,3,19].

Our results are also in accordance with respect to the association between cTnT elevation and poor outcomes previously demonstrated[2,3,19]. Lala et al.[19] stratified troponin levels into

Table 1. Population baseline characteristics.

	Normal troponin	Elevated troponin	p-value
Age (mean±SD)	52.2±15.4	63.6±15.8	<0.001
Male (n, %)	458 (49.6)	884 (58,3)	<0.001
BMI (mean±SD)	31.5±11.9	29±12	<0.001
Race (n, %)			
White	541 (60.7)	976 (66)	0.005
Black	61 (6.8)	114 (7.7)	
Mixed race	282 (31.6)	369 (24.9)	
Asian	8 (0.9)	20 (1.4)	
Time (days) between symptom onset and hospital admission (mean±SD)	8.7±4.9	8.8±6.9	0.689
Cardiovascular disease (n, %)	81 (8.8)	427 (28.2)	<0.001
Hypertension (n, %)	430 (46.5)	1029 (67.9)	<0.001
Arrhythmia (n, %)	22 (3.4)	110 (9.2)	<0.001
Obesity (n, %)	163 (17.6)	163 (10.7)	<0.001
Diabetes (n, %)	279 (30.3)	660 (43.5)	<0.001
Dyslipidemia (n, %)	56 (20.3)	93 (22.8)	0.436
Smoker (n, %)	57 (6.2)	126 (8.3)	0.052
Former smoker (n, %)	191 (20.7)	326 (21.6)	0.613
Alcoholism (n, %)	32 (8.9)	72 (13.3)	0.043
CPOD (n, %)	44 (4.8)	128 (8.5)	<0.001
Asthma (n, %)	46 (5)	53 (3.5)	0.072
Chronic kidney disease (n, %)	21 (2.3)	184 (12.2)	<0.001
Dialysis (n, %)	4 (0.4)	78 (5.1)	<0.001
Liver disease (n, %)	14 (1.5)	54 (3.6)	0.003
Stroke (n, %)	30 (3.2)	151 (10)	<0.001
Epilepsy (n, %)	15 (6)	23 (6.4)	0.812
Dementia (n, %)	9 (1)	55 (3.6)	<0.001
Rheumatologic disease (n, %)	31 (3.4)	35 (2.3)	0.122
Hematologic disease (n, %)	38 (6.7)	92 (8.2)	0.276
Peripheral artery disease (n, %)	16 (2.6)	81 (7.1)	<0.001
Solid organ transplant (n, %)	13 (5.3)	67 (17)	<0.001
Cancer (n, %)	42 (5.2)	129 (9.2)	<0.001
Hematologic cancer (n, %)	14 (2.2)	39 (3.4)	0.167
Previous thrombotic event (n, %)	20 (7.9)	39 (10.9)	0.228
HIV (n, %)	20 (2.2)	11 (0.7)	0.002
Hypothyroidism (n, %)	58 (20.1)	124 (28.6)	0.011

BMI: body mass index; COPD: chronic obstructive pulmonary disease. The t-test was used to compare numeric variables between groups, and the chi-square test was used to compare qualitative variables between groups.

Table 2. Laboratory characteristics at admission.

Biomarkers (mean±SD or median±IQR)	Normal troponin	Elevated troponin	p-value
Leukocyte (cells/mm^3)	8,200±4,910	9,800±7,050	<0.001*
Neutrophil (cells/mm^3)	6,400±4,850	8,000±6,560	<0.001*
Lymphocyte (cells/mm^3)	1,000±740	800±680	<0.001*
Hemoglobin (g/dL)	12.6±1.9	11.7±2.4	<0.001
Platelet (cells/mm^3)	253,400±104,800	231,200±104,800	<0.001
C-reactive protein (mg/L)	131.8±100.8	159.8±120.9	<0.001
Lactate dehydrogenase (U/L)	401.7±213.2	529.6±483.9	<0.001
Aspartate aminotransferase (U/L)	36±30	40±39	<0.001*
Alanine aminotransferase (U/L)	34±31	30±32	0.01*
D-dimer (ng/mL)	1,134.5±1,536	2,073.5±5,052.5	<0.001*
Prothrombin time (seg)	13.3±5.6	14.6±8.4	<0.001
Activated partial thromboplastin time (seg)	30.3±6.4	33±14.9	<0.001
Fibrinogen (mg/dL)	596.2±182.2	523.5±201.5	<0.001
Creatine phosphokinase (U/L)	89.5±154.25	147±431.5	<0.001*
Lactate (mg/dL)	12.0±7.0	14.0±8.0	<0.001*
Urea (mg/dL)	37.7±27.3	80.1±59.1	<0.001
Creatinine (mg/dL)	0.81±0.38	1.35±1.58	<0.001*
Sodium (mEq/L)	139±4.5	139.8±6.1	<0.001
Potassium (mEq/L)	4.22±0.66	4.45±0.92	<0.001
Ionic calcium (mg/dL)	4.74±0.34	4.63±0.47	<0.001
Magnesium (mg/dL)	2.1±0.35	2.13±0.43	0.079
N-terminal B-type natriuretic peptide – NT-proBNP (pg/mL)	183.5±388.5	2,056±6,040.25	<0.001*

The t-test was used for all comparisons, except N-terminal B-type natriuretic peptide – NT-proBNP (pg/mL), for which *Mann-Whitney U test was used.

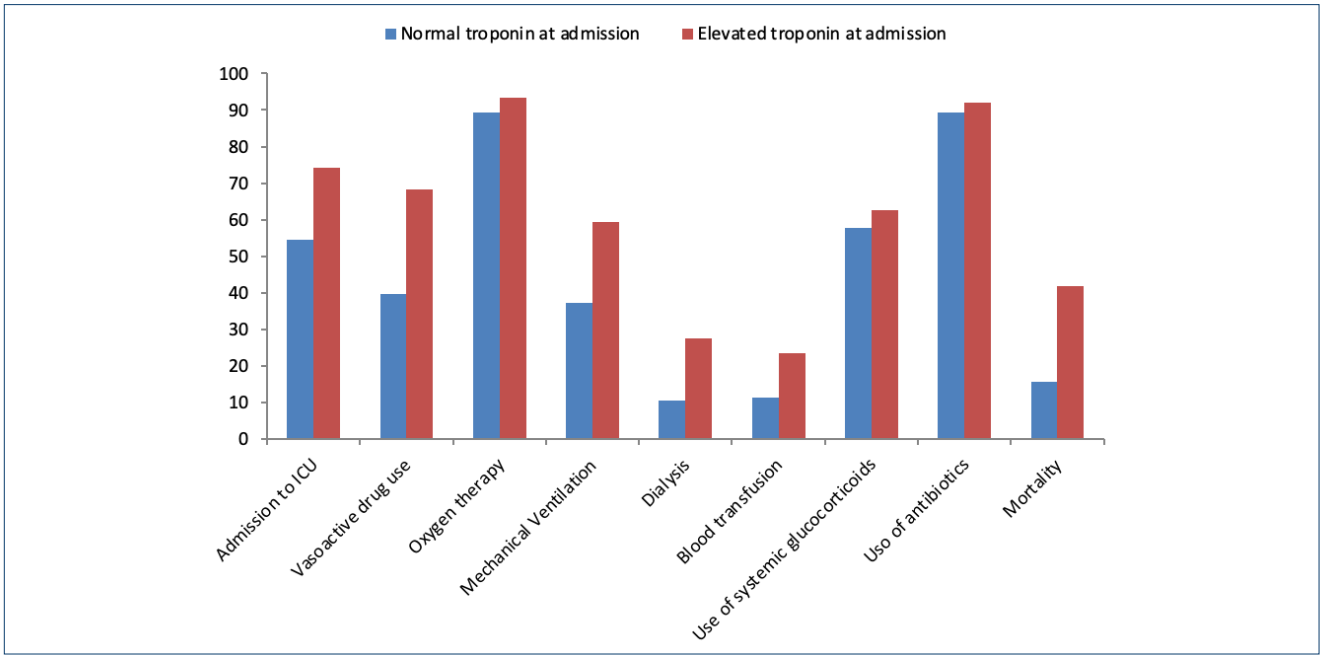

Figure 2. Outcomes during the hospitalization. ICU: intensive care unit. p<0.001 for all comparisons between normal and elevated troponin levels at admission.

normal, mildly elevated, and elevated and found increased mortality related to higher troponin levels[19].

The mechanism that links troponin elevation and higher rates of adverse outcomes in COVID-19 is not entirely elucidated. As observed in our cohort, the troponin rise occurs concomitantly with the increase in other inflammatory biomarkers, such as D-dimer, leukocytes, C-reactive protein, procalcitonin, ferritin, interleukin-6, and lactate dehydrogenase[3,20], which suggests that this reflects cytokine storm and critical illness more than the direct myocardial injury itself.

In this report, elevated troponin was not independently associated with mortality after logistic regression. A previous study demonstrated that myocardial injury was significantly associated with death, even after adjusting disease severity and relevant clinical factors[19]. Shi et al.[2] also observed a higher risk of death in patients with elevated troponin after adjusting for age, previous comorbidities, ARDS, creatinine levels, and NT-proBNP levels[2,19]. On the contrary, Metkus et al.[21] found results that are similar to our study. Mortality was greater with higher troponin levels, but the association between myocardial injury and mortality was not statistically significant after adjusting for age, sex, and multisystem organ dysfunction[21]. A Norwegian study points in the same direction, suggesting the limited role of troponin in prognostic assessment[21,22]. These findings corroborate the hypothesis that myocardial injury is reflective of baseline risk and comorbidities, especially underlying multisystem organ dysfunction, and troponin values reduce its prognostic importance when clinical severity features are included in the regression analysis.

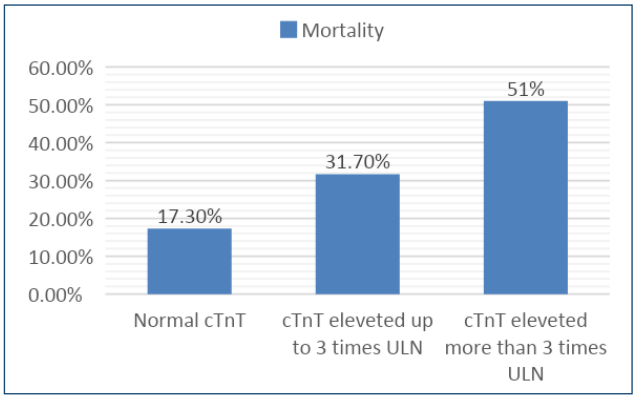

Figure 3. Association between troponin levels at admission and mortality. cTnT: cardiac T troponin; ULN: upper limit of normal. p<0.001 for all comparisons between individual groups.

Limitations

As an observational study, it presents a risk of selection bias. Our data are drawn from a single academic health institution, dedicated to treating high-complexity cases, which could influence the findings and limit its generalizability. Also, the retrospective nature of the study is another limitation, since the exams were collected at the discretion of the treating physician and the results were retrieved by the investigator from the patients' records. The admission troponin was not available in 32% of patients, which could represent a lower-risk population. Another limitation is that we did not assess secondary diagnoses that could contribute to troponin elevation, such as myocarditis or acute coronary syndrome.

Similar to other studies, we evaluated troponin only at admission, while serial troponin measurements during hospital stay were not available. Dynamic changes in troponin during hospitalization could provide better prognostic information. Zhou et al.[20] showed that troponin levels increased progressively among non-survivors, whereas they did not change significantly among survivors. These results were corroborated by other authors as well[3,19].

Table 3. Multiple regression analysis for prediction of in-hospital death—final model after backward stepwise method.

	OR	95%CI		p-value
		Inferior	Superior	
Hypertension	2.23	1.28	3.89	0.004
Obesity	0.45	0.23	0.89	0.021
Cancer	4.58	1.00	21.05	0.050
Use of vasoactive drugs	20.18	5.75	70.83	<0.001
Dialysis	4.15	2.38	7.26	<0.001
Use of ACEi	0.32	0.14	0.70	0.004
Use of ARB	0.13	0.05	0.32	<0.001
Age above 70 years	2.29	1.31	3.98	0.003

CONCLUSION

Our study showed that cTnT elevation at admission was common and associated with mortality, but it was not an independent marker of in-hospital mortality in patients with COVID-19. Some comorbidities, such as hypertension (OR 2.23) and age>70 years (OR 2.29), were strongly associated with mortality in these patients. Further research is needed to fully understand the prognostic role of troponin in COVID-19.

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AUTHORS' CONTRIBUTIONS

CVSJ: Conceptualization, Funding acquisition, Methodology, Software, Supervision, Validation, Visualization, Writing – review & editing. **HTP**: Conceptualization, Data curation, Formal Analysis, Methodology, Project administration, Software, Writing – original draft, Writing – review & editing. **FRG**: Data curation, Formal Analysis, Writing – original draft. **BRSM**: Data curation, Writing – original draft. **EGL**: Formal Analysis, Methodology, Validation, Writing – review & editing. **CLG**: Formal Analysis, Methodology. **RKF**: Resources, Supervision, Validation, Visualization.

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ORIGINAL ARTICLE

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Expression of cytotoxic T lymphocyte-associated antigen 4, CD44, and E-cadherin in the microenvironment of breast carcinomas

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SUMMARY

OBJECTIVE: The expression of cytotoxic T lymphocyte-associated antigen 4, E-cadherin, and CD44 in the area of tumor budding was investigated in breast carcinomas in our study.

METHODS: Tumor budding was counted at the invasive margins in 179 breast carcinomas. To understand the microenvironment of tumor budding, we examined the expression status of the immune checkpoint molecules such as cytotoxic T lymphocyte-associated antigen 4, E-cadherin, and CD44.

RESULTS: Tumors were separated into low (≤5) and high tumor budding groups (>5) based on the median budding number. Lymphovascular, perineural invasion, and the number of metastatic lymph nodes were significantly higher in high-grade budding tumors (p=0.001, p<0.001, and p=0.019, respectively). Tumor-infiltrating lymphocytes were significantly higher in tumors without tumor buddings (p<0.001). When the number of budding increases by one unit, overall survival decreases by 1.07 times (p=0.013). Also, it increases the risk of progression by 1.06 times (p=0.048). In high tumor budding groups, the cytotoxic T lymphocyte-associated antigen 4 staining percentage of lymphocytes was significantly higher (p=0.026). With each increase in the number of buds, an increase in the percentage of cytotoxic T lymphocyte-associated antigen 4 staining was seen in lymphocytes in the microenvironment of TB (p=0.034).

CONCLUSION: Tumor budding could predict poor prognosis in breast carcinomas, and anti-cytotoxic T lymphocyte-associated antigen 4 immunotherapies may be beneficial in patients with high tumor budding tumors.

KEYWORDS: Breast neoplasms. CD44 protein, mouse. CTLA-4 antigen. E-cadherin. Tumor microenvironment.

INTRODUCTION

TB is considered the histological reflection of epithelial-mesenchymal transition (EMT)[1]. Loss of E-cadherin expression in the EMT area disrupts cell-cell interaction and causes an increase in the invasion capacity of the tumor[2,3]. CD44, a cell surface transmembrane glycoprotein, plays an important role in tumor invasion, metastasis, and EMT[4,5]. Cytotoxic T lymphocyte-associated antigen 4 (CTLA-4), one of the immune checkpoint molecules, is a receptor that plays an important role in the regulation of T cell activation and the maintenance of self-tolerance[6]. It contributes to escape from immune surveillance by suppressing the immune response against the tumor. This may facilitate TB.

We aimed to reveal TB in breast carcinomas (BCs), the relationship between TB and the microenvironment, and clinicopathological prognostic factors. Treatments targeting immune checkpoints, such as CTLA-4, may be a patient-specific treatment option for patients with BCs.

METHODS

Definition and assessment of tumor-budding and tumor-infiltrating lymphocytes

From 2011 to 2018, 179 cases operated in our hospital were evaluated retrospectively. The definition of "isolated single cancer cell or cluster of less than 5 cancer cells" was accepted for TB. TB evaluation was performed at 200× magnification (BX51, 200×, field size 0.95 mm^2) in the most invasive area. Tumor ınfiltrating lymphocyte (TIL) was evaluated using the method proposed by the International TILs Working Group 2014 in BC[7].

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Immunohistochemical staining and evaluation

The sections of 162 cases with TB were stained with antibodies, including mouse anti-human E-cadherin (NCH-38, ready-to-use kit, Dako, California), anti-human CD44 (MRQ-13, 1:100, Cell Marque California), mouse anti-human CTLA-4 (F8, 1:100, Santa Cruz, Texas), and mouse anti-human Ki67 (MIB-1, 1:200, DakoCytomation).

E-cadherin and CD44 were evaluated in tumor and TB areas with 200× magnification. Membranous staining of 90% and above for E-cadherin and 10% and above staining for CD44 was considered positive (Figure 1).

CTLA-4 was evaluated at 400× magnification in the buds and lymphocytes in the bud microenvironment. Both staining percentages and staining intensity were evaluated. CTLA-4 was divided into four groups according to the staining intensity. If there was no cytoplasmic-membranous staining, the score was 0. Weak staining was scored as 1 point, moderate staining as 2 points, and strong staining as 3 points. Those with no staining and mild staining (scores 0 and 1) were included in the negative group and those with moderate and strong staining (scores 2 and 3) in the positive group (Figure 2).

Statistics

The Mann-Whitney U test was used for comparing two independent groups. The Kruskall-Wallis test was used for comparing more than two independent groups. For comparisons between categorical variables, the Pearson χ^2 test was used in 2×2 tables, and Fisher's exact test was used in cross tables. Immunohistochemical staining differences between tumor and TB were compared with categories, groupings, and the McNemar test. For statistical significance, type 1 error level is used as 5%. In the survival analysis, the Kaplan-Meier analysis used the log-rank test for the comparison of survival curves.

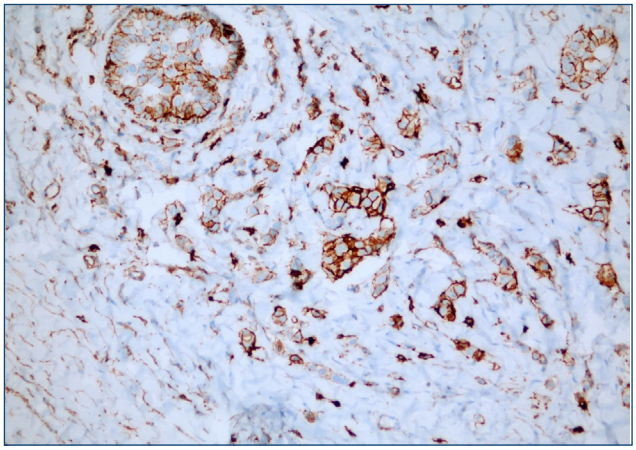

Figure 1. CD44 positivity in the tumor (CD44, 200´ magnification).

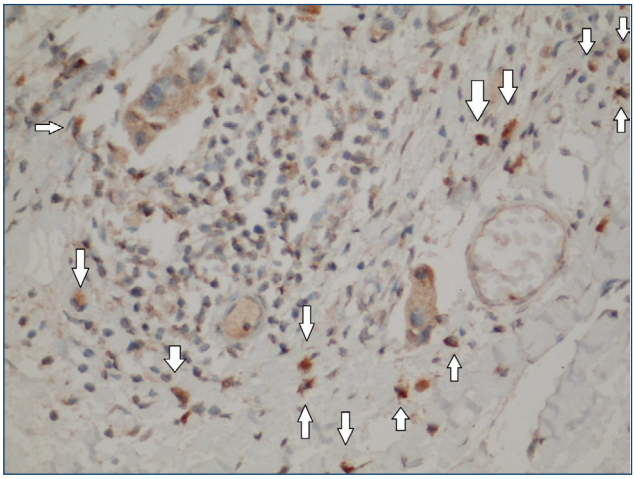

Figure 2. Cytotoxic T lymphocyte-associated antigen 4 evaluation according to the percentage of staining in lymphocytes around the tumor bud 13% (cytotoxic T lymphocyte-associated antigen 4, 400´ magnification).

The cutoff value was considered the median value. The significance level was considered $p<0.05$ in the statistical analysis.

Ethical approval

Our study's ethics committee approval was obtained from the University of Health Science Bagcılar Training and Research Hospital, non-interventional clinical research ethics committee chairmanship. According to the Declaration of Helsinki and the ethical standards of the institutional research committee, the study was conducted.

RESULTS

General characteristics and findings in cases with high and low buds

TB was not observed in 17 of the patients. Of the patients, 64 (35.7%) were at pT1, 93 (52%) at pT2, and 22 (12.3%) at pT3. A total of 13 (7.3%) were in Grade 1, 66 (36.9%) in Grade 2, and 100 (55.8%) in Grade 3.

The number of TB ranges from 0 to 35. The average number of buds was determined as 6±5.1. The value "5," which is the median of the bud numbers, was determined as the cut-off score. A total of 96 cases (53.6%) with ≤5 buds were categorized as low TB, and 83 cases (46.4%) with >5 buds were divided into high TB (Table 1).

Survival analysis

Progression was observed in 35 (19.6%) of the cases. Of the 35 patients with progression, 19 of them died from the disease.

Table 1. Significant results of tumor budding.

	Tumor budding present	No tumor budding	p-value	High-tumor budding	Low-tumor budding	p-value
Grade						
G1	10 (6.2%)	3 (17.65%)	0.155	5 (6%)	8 (8.3%)	0.798
G2	62 (38.3%)	4 (23.52%)		32 (38.6%)	34 (35.4%)	
G3	90 (55.5%)	10 (58.83%)		46 (55.4%)	54 (56.30%)	
Mean tumor size	3.1±2.2 cm	2.1±1.8 cm	0.075	3.3±2.2	2.8±2.1	0.133
Mean number of metastatic lymph nodes	3.9±6.6	0.3±0.6	0.024*	4.8±7.9	2.6±4.4	0.019*
N stage						
N0	58 (35.8%)	13 (76.5%)	0.006*	23 (27.7%)	48 (50%)	0.008*
N1	49 (30.2%)	4 (23.5%)		26 (31.3%)	27 (28.1%)	
N2	33 (20.4%)	0 (0%)		22 (26.5%)	11 (11.5%)	
N3	22 (13.6%)	0 (0%)		12 (14.5%)	10 (10.4%)	
Lymphovascular invasion						
Negative	99 (61.1%)	3 (17.6%)	0.001*	24 (28.9%)	53 (55.2%)	0.001*
Positive	63 (38.9%)	14 (82.4%)		59 (71.1%)	43 (44.8%)	
Molecular subgroup						
Luminal	97 (59.9%)	5 (29.4%)	0.031*	52 (62.7%)	50 (52.1%)	0.114
Nonluminal	65 (40.1%)	12 (70.6%)		31 (37.3%)	46 (47.9%)	
Tumor-infiltrating lymphocytes						
Low	101 (62.3%)	7 (41.2%)	0.01*	53 (63.9%)	54 (56.2%)	0.354
Intermediate	39 (24.1%)	4 (23.5%)		20 (24.1%)	23 (24%)	
High	22 (13.6%)	6 (35.3%)		10 (12%)	19 (19.8%)	

*Indicate statistical significance at the p<0.05 level.

One unit increase in the number of buds increases the risk of progression 1.06 times (1.00–1.13, p=0.048). Also, one unit increase in the number of buds decreases the overall survival (OS) by 1.07 times (1.01–1.12, p=0.013).

In a multivariate analysis including bud number, tumor size, Ki-67 groups, pT, pN, molecular groups, PR, necrosis, LVI, PNI, and neoadjuvant therapy, the number of buds independently affected disease-free survival (DFS).

There was no significant difference in 5-year OS and DFS between cases with and without TB and between high- and low-bud groups (p>0.05).

E-cadherin, CD44, and cytotoxic T lymphocyte-associated antigen 4 expression in budding cells

In the IHC study conducted in 162 cases with TB, loss of staining with E-cadherin was detected in 21 (13%) of the tumors and in 131 (81%) of the tumor buddings (p=0.7, p>0.9).

CD44 was stained in 50 (63%) of the low-bud tumors. CD44 staining percentage was significantly higher in low-bud tumors (p=0.026). CD44 was stained in 88% (54.3%) of TB. There was no significant difference between the two groups in terms of CD44 staining of TB (p=0.3).

The percentage of CTLA-4 staining of lymphocytes in the microenvironment of TB ranged from 0 to 100, with an average of 12±12.643%. While the percentage of CTLA-4 in the lymphocytes in the microenvironment of cases with high buds was found to be 13.82% on average, it was observed to be 10.48% in those with low buds. The percentage of CTLA-4 in lymphocytes in the bud microenvironment was found to be significantly higher in the high-bud group compared to the low-bud group (p=0.026). Each increase in the number of buds correlates with an increase in the percentage of CTLA-4 staining in lymphocytes in the tumor microenvironment (rho=0.17, p=0.034).

According to the staining intensity score, 62 (38.3%) of the buds were stained and 100 (61.7%) were not stained with CTLA-4. As homogeneous staining was observed in all of the stained TB, the staining percentage was accepted as 100%. There was no significant difference between the bud groups and lymphocytes in the bud microenvironment in terms of CTLA-4 staining intensity (p>0.05).

DISCUSSION

TB is the histological reflection of a dynamic process that determines the potential of tumor invasion[2]. The increased migration and invasion capacity of budding cells facilitate the spread to lymphatics and lymph nodes. These results suggest that TB can be used as a parameter to predict possible lymph node metastasis and a poor prognostic factor in BCs. Studies also support that TB is a poor prognostic factor for survival independent of other prognostic parameters[8-11]. The loss or decrease of E-cadherin expression is by the interaction of signal pathways and transcription factors during EMT. It is considered that the separation of TB from the main tumor mass with loss of connections between cells, increased mobility, and invasion capacity is thought to represent EMT[3]. In our study, the loss of E-cadherin expression was determined as 81% in both high- and low-bud areas, and this indicates that E-cadherin decreases in the bud area regardless of the number of buds. As a result, the loss of E-cadherin seen in the bud areas in BCs supports EMT.

Molecular studies show that high CD44 expression is associated with cancer stem cell characteristics and EMT and demonstrated that it contributes to tumor invasion, metastasis, recurrence, and drug resistance[12-14]. Therefore, an increase in CD44 expression is expected in the TB area, which is thought to be the histological reflection of EMT and shows stem cell characteristics. Gurzu et al.[15] found an increase in CD44 staining in the bud area in their study on colorectal carcinomas[15]. Similarly, an increase in CD44v6 expression was observed in the budding area in the study of Masaki et al.[16]. In our study, CD44 staining was significantly higher in low-bud tumors, supporting studies showing good prognosis in CD44-positive tumors. However, no relationship was found with CD44 in bud groups. This suggests that the relationship between basic cell biology and clinical behavior is complex, and extensive studies are needed on CD44 expression in tumors and buds.

In our study, TILs were found to be significantly higher in tumors with no TB. It can be thought that the high immune response in the tumor stroma prevents the increase in the invasive potential of the tumor. Gujam et al.[8] found that high TB was associated with a lower inflammatory response, according to Klintrup-Makinen's grade[8]. In our study, although TIL was detected less frequently in high TB, no significant difference was found between them. According to the TIL evaluation recommended by ITILWG in breast cancers, we evaluated TIL in the entire tumor stroma[7]. However, evaluating only the invasive margin of stroma in the Klintrup-Makinen grading may have more clearly demonstrated the relationship between the number of TB and TIL in their study.

CTLA-4 is an immune checkpoint molecule that plays an important role in regulating T-cell activation and maintaining self-tolerance[17,18]. Paulsen et al.[19] evaluated CTLA-4 expression in tumoral cells in lymph node metastases in non-small cell lung carcinomas[19]. Yu et al. associated high CTLA-4 expression and low tumor CTLA-4 expression in lymphocytes in the interstitial area around the tumor with a good prognosis[20]. This finding suggests that EMT suppresses the antitumor immune response. In our study, the average CTLA-4 percentage in lymphocytes in the high budding area reflecting EMT was found to be significantly higher. It can be considered that patients with high-bud tumors may benefit greatly from anti-CTLA-4 antibodies.

Three main results were found in our study. First, in BCs, TB can be considered a poor prognostic factor alone as it predicts lymph node metastasis, LVI, and PNI. Second, the density of tumor-infiltrating lymphocytes may play a role in the prevention of TB by the antitumor immune response. Third, in tumors with high TB, significantly higher staining of CTLA-4 is observed in lymphocytes around the TB; thus, CTLA-4 may promote TB by inhibiting the antitumor immune response. If supported by comprehensive studies, it is thought that anti-CTLA-4 therapy may be beneficial in patients with high TB tumors.

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AUTHORS' CONTRIBUTIONS

TBS: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. **TCS:** Conceptualization, Formal Analysis, Investigation, Validation, Writing – review & editing. **HEP:** Conceptualization, Formal Analysis, Project administration, Supervision, Visualization, Writing – original draft, Writing – review & editing. **AM:** Data curation, Funding acquisition, Resources. **MT:** Resources. **ÇÖ:** Methodology, Supervision, Writing – review & editing.

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ORIGINAL ARTICLE

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Association between ABO blood groups and mortality in upper gastrointestinal bleeding

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SUMMARY

OBJECTIVE: Gastrointestinal bleeding is an important part of gastrointestinal emergencies. This study aimed to examine the association between ABO blood groups and mortality in patients who were admitted to the emergency department and diagnosed with upper gastrointestinal bleeding.
METHODS: The patients with upper gastrointestinal bleeding in the emergency department of a tertiary hospital in Turkey and the data of healthy blood donors were studied. The data of these patients were analyzed, and it was determined that the primary outcome was in-hospital mortality.
RESULTS: The study was completed with 274 patients and 274 control group. The mean age of these patients was 65.1±18.2 years, and 64.2% of patients were males. It was found that the in-hospital mortality rate of patients with O blood group (16.2%) was statistically significantly higher than non-O blood group (7.5%) (p:0.032).
CONCLUSION: The study concluded that the mortality rate of gastrointestinal bleeding patients with O blood group was higher compared to patients with other blood groups. Physicians can use ABO blood groups to predict mortality risk in gastrointestinal bleeding.
KEYWORDS: ABO blood-group system. Gastrointestinal hemorrhage. Mortality.

INTRODUCTION

Upper gastrointestinal (GI) bleeding occurs with bleeding from anywhere between the proximal esophagus and the ligament of Treitz into the lumen. This disease is a serious cause of mortality and morbidity, and therefore, early diagnosis and appropriate treatment are necessary[1-3]. In the United States, more than half a million patients are hospitalized annually due to GI bleeding, and 80% of patients diagnosed with GI bleeding who visit emergency departments (EDs) are admitted to the hospital[4,5].

International guidelines for the management of patients with GI bleeding recommend assessing the risk using prognostic tools that can be obtained early in the course of the disease[6]. To achieve this, various risk-scoring systems and patient-related factors have been investigated. ABO blood group is one of these factors because the relationship between some blood groups and diseases is a known fact. For instance, O blood group is known to have a risk factor for peptic ulcer disease, which is one of the significant causes of upper GI bleeding. Similarly, A blood group is known to have a risk factor for gastric cancer, which is another cause of upper GI bleeding[7]. Previous studies have shown that patients with O blood group experience longer time bleeding and have lower plasma levels of factor VIII or von Willebrand factor (vWF) than patients with non-O blood group. Therefore, it has been concluded that patients with O blood group bleed more easily[8,9]. Additionally, it is known that platelet function is more limited in patients with O blood group than in patients with other blood groups, which increases the potential for bleeding[10]. In the literature, studies have reported that patients with O blood group have more bleeding complications than those with other blood groups in various diseases such as variceal GI bleeding, postpartum hemorrhage, and severe traumatic bleeding[11-13].

This study aimed to determine the association between blood groups and mortality in Turkish patients diagnosed with upper GI bleeding who were admitted to the ED.

METHODS

The study was carried out in the ED of a tertiary hospital located on the European side of Istanbul, Turkey, with a bed capacity of 650 and an annual ED visit of 223,069. This examination was held as a retrospective observational study. The scientific and ethical suitability of the study has been confirmed by the local ethics committee with decision number 2,235 (date: 22.02.2023). Due to the retrospective nature of the study, informed consent forms from patients were not required.

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The study included patients aged 18 years and older who visited the ED and were hospitalized with a diagnosis of upper GI bleeding between January 1, 2022, and January 1, 2023. The medical records of patients were obtained through the hospital-based electronic automation system using ICD (International Statistical Classification of Diseases) codes. Only the first ED visit of patients with multiple ED visits due to GI bleeding was included in the study. Patients with diagnoses other than GI bleeding, those for whom blood group data could not be obtained, those who left the hospital without permission, and foreign patients were not included in the study. The demographic information, vital signs, comorbidities, blood groups, and mortality status of the included patients were recorded in a data set. The analysis of ABO blood groups was performed using the gel centrifugation method with Erytra Eflexis (Eflexis, Grifols, Barcelona, Spain). The data of healthy blood donors in the same region where the study was conducted were used as the control group. The study revealed a primary outcome as all-cause in-hospital mortality.

Statistical analysis

For the statistical analyses, the SPSS 22.0 (SPSS Inc., IL, Chicago) software was used. The p-value of <0.05 was used as the level of significance. All patients included in the study were categorized into two groups for analysis, namely, O blood group and non-O blood group. Descriptive statistics were presented as mean and standard deviation and percentage distribution. The normal distribution of the data was monitored using the Kolmogorov-Smirnov test. To compare the distribution of sociodemographic, clinical, and vital signs characteristics between O blood group and other blood groups, Pearson's chi-square analysis was operated. To compare continuous variables, Student's t-test was operated.

RESULTS

Overall, 4 patients with foreign nationality, 12 patients whose blood group data could not be reached, and 1 patient who left the hospital without permission were excluded from the study. A total of 274 patients and 274 control group and their data were analyzed. The mean age of these patients was 65.1±18.2 years, and 64.2% of them were males. It was found that the in-hospital mortality rate of patients with O blood group (16.2%) was statistically significantly higher than those with non-O blood group (7.5%) (p=0.032) (Table 1).

The distribution of blood groups in patients with GI bleeding was analyzed, 24.5% of patients had A blood group, 26.3%

Table 1. Comparison of patient characteristics and mortality status between O and non-O blood groups.

	Non-O blood group n (%) Mean±SD	O blood group n (%) Mean±SD	Total n (%) Mean±SD	p-value
Gender				
Female	73 (36.5)	25 (33.8)	98 (35.8)	0.677
Male	127 (63.5)	49 (66.2)	176 (64.2)	
Age (years)	65.3±17.1	64.6±20.9	65.1±18.2	0.759
Systolic blood pressure (mmHg)	120.2±19.1	125.7±18.0	121.7±18.9	**0.031**
Diastolic blood pressure (mmHg)	73.5±14.1	76.3±13.5	74.2±13.9	0.139
Heart rate (bpm)	98.6±17.3	96.1±11.9	97.9±16.0	0.263
Congestive heart failure	25 (12.5)	8 (10.8)	33 (12.0)	0.703
Peripheral vascular disease	7 (3.5)	4 (5.4)	11 (4.0)	0.476
Cerebrovascular disease	15 (7.5)	8 (10.8)	23 (8.4)	0.380
Hypertension	15 (7.5)	7 (9.5)	22 (8.0)	0.596
Peptic ulcer disease	23 (11.5)	9 (12.2)	32 (11.7)	0.880
Diabetes mellitus	42 (21.0)	18 (24.3)	60 (21.9)	0.555
Chronic kidney disease	15 (7.5)	7 (9.5)	22 (8.0)	0.596
Length of the hospital stay (day)	6.2±2.8	6.3±2.7	6.2±2.8	0.689
In-hospital mortality	15 (7.5)	12 (16.2)	27 (9.9)	**0.032**

χ^2/Student's t-test. Bold values indicate statistical significance at the p<0.05 level.

of patients had B blood group, 22.2% of patients had AB blood group, and 27.0% of patients had O blood group (Figure 1). In the control group, the distribution of blood groups was as follows: 35.8% of patients had A blood group, 23.8% of patients had B blood group, 19.4% of patients had AB blood group, and 21.0% of patients had O blood group.

Regarding the distribution of in-hospital mortality status by blood group, 10.4% of patients with A blood group, 3.3% of patients with B blood group, 8.3% of patients with AB blood group, and 16.2% of patients with O blood group had mortality (Figure 2).

DISCUSSION

Blood group distributions can vary across regions and races. This study found that in the Turkish population of patients with GI bleeding, patients with O blood group were found to be higher mortality rates compared to other blood groups.

GI bleeding is a disease with a wide clinical spectrum, ranging from a self-limiting condition to mortality. Many international guidelines recommend using scoring systems or prognostic tools to predict poor outcomes and identify low- and high-risk patients in these patients[14].

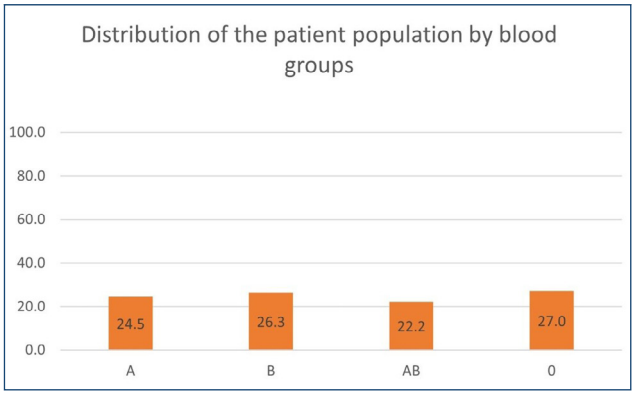

Figure 1. Distribution of ABO blood groups in the patient group.

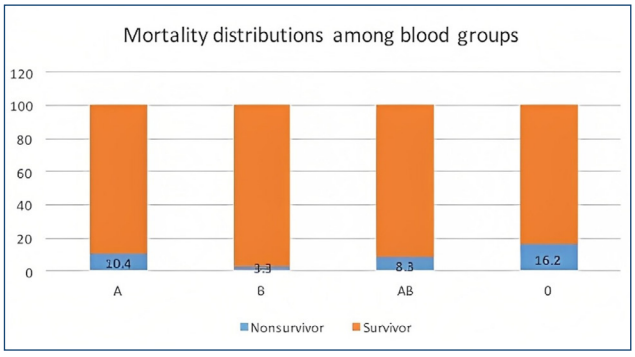

Figure 2. Distribution of in-hospital mortality of the patient group according to ABO blood groups.

In 1900–1901, Karl Landsteiner discovered that some red blood cells agglutinated when mixed with serum samples from another patient. He recorded agglutination parameters and classified blood into different groups, creating the ABO blood group system. He was awarded the Nobel Prize for this study. Landsteiner identified three different groups, namely, A, B, and C (later renamed as O), and in 1902, De Castello and Sturli identified the fourth blood group, i.e., AB[15]. Since that time, the relationship between blood groups and diseases has attracted the attention of scientists, and numerous studies have been conducted. There are some studies in the literature stating an association between blood groups and diseases such as coronary heart disease, gastric cancer, pancreatic cancer, malaria, *Helicobacter pylori* infection, and Crimean-Congo hemorrhagic fever[16].

In a study carried out in Iran, data from 513 patients with GI bleeding and 520 controls were analyzed. It was found that O blood group was more prevalent in Iranian patients with GI bleeding than in healthy blood donors. In addition, it was concluded that there was a higher rate of re-bleeding in patients with O blood group within the first 72 h of hospital admission[17]. In the study by Bayan et al., data from 364 patients with GI bleeding and 734 blood donors were examined. While emphasizing that O blood group was more prevalent in the patient group, it was reported that the rate of re-bleeding and mortality was similar among blood groups[18].

As previously mentioned, although it is not clearly defined why O blood group is more prone to GI bleeding and has a worse prognosis, there are some views on that. One possibility is that patients with O blood group have a higher prevalence of duodenal ulcers and *H. pylori* infection. Additionally, it is believed that O blood group may be found to be more predisposed to bleeding, which could contribute to the higher rate of GI bleeding[19]. However, further research is needed to fully understand the mechanisms behind this relationship.

In our study, the most common was A blood group with a prevalence of 35.8%, which is compatible with studies in the literature. In a study carried out in Istanbul, the distribution of blood groups in 123,900 blood donors was determined, and it was conducted that A blood group was the most common blood group with a prevalence of 43.8%[20]. In Turkey as a whole, it has been found that the most common is A blood group with a prevalence of 42.8%[21].

One of the main limitations of this study is its retrospective and single-center design. Therefore, the results of the study

cannot be generalized to the population. Thus, there is a need for multicenter and prospective studies.

CONCLUSION

GI bleeding is a significant causality of morbidity and mortality among patients visiting EDs. In this study, it was determined that patients with O blood group who had GI bleeding had a higher mortality rate than patients with other blood groups. Physicians can use ABO blood groups to predict mortality risk in patients with GI bleeding.

ETHICAL APPROVAL

This study was approved by Şişli Hamidiye Etfal local ethics committee (ethics committee ruling number: 2,235, date: 22.02.2023).

AUTHORS' CONTRIBUTIONS

HA: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **MK**: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Writing – original draft.

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Labor analgesia and its impact on the maternal and perinatal outcomes

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SUMMARY

OBJECTIVE: This study aimed to assess adverse maternal and perinatal outcomes in parturients undergoing labor analgesia.

METHODS: This was a retrospective cohort study in parturients who underwent labor analgesia. Parturients were categorized into three groups: Group 1 (n=83)—analgesia performed with cervical dilatation ≤4.0 cm; Group 2 (n=82)—analgesia performed with cervical dilatation between 5.0 and 8.0 cm; and Group 3 (n=83)—analgesia performed with cervical dilatation ≥9.0 cm.

RESULTS: Analgesia in parturients with cervical dilatation ≥9.0 cm showed a higher prevalence and a 3.86-fold increase (OR 3.86; 95%CI 1.50–9.87; p=0.009) in the risk of forceps delivery. Analgesia in parturients with cervical dilatation ≤4.0 cm showed a higher prevalence and a 3.31-fold increase (OR 3.31; 95%CI 1.62–6.77; p=0.0016) in the risk of cesarean section. Analgesia in parturients with cervical dilatation ≥9.0 cm was associated with a higher prevalence of fetal bradycardia (20.7%), a need for neonatal oxygen therapy (6.1%), and a need for admission to a neonatal intensive care unit (4.9%). Analgesia in parturients with cervical dilatation ≤4 cm was associated with a higher prevalence of Apgar score <7 at 1st minute (44.6%).

CONCLUSION: Performing labor analgesia in parturients with cervical dilatation ≤4.0 or ≥9.0 cm was associated with a higher prevalence of adverse maternal and perinatal outcomes.

KEYWORDS: Delivery room. Analgesia. Perinatal mortality.

INTRODUCTION

One of the main concerns among parturients regarding vaginal delivery is the pain experienced during labor. Labor pain is a complex phenomenon influenced by anatomical and physiological characteristics along with psychosocial and cultural factors. Regarding pain intensity, the scores of labor pain are comparable with those of other clinical conditions, such as non-terminal cancer, acute myocardial infarction, renal colic, and burns. There are biochemical and neurophysiological evidences that maternal pain during labor results in deleterious consequences for the parturient and fetus[1].

Epidural analgesia is the most frequently used treatment modality during labor. Effective labor analgesia controls maternal pain and anxiety, benefiting the maternal-fetal binomial with effective pain relief using low anesthetic doses, without significant motor block. Moreover, there is a possibility of analgesic complementation through a catheter[2]. However, some undesired effects are still associated with this technique. The risks include arterial hypotension, prolonged labor, labor instrumentation, the need for oxytocin, and adverse fetal outcomes[3].

In general, epidural analgesia at an early stage of labor in patients with a cervical dilatation of <4.0 cm could be associated with higher rates of cesarean sections, which would relatively contraindicate this procedure during this period. However, a systematic review showed that there was no difference in the rate of cesarean sections between parturients who underwent epidural analgesia in the early active phase and those who underwent analgesia in the late active phase of the first stage of labor. The study also showed that the appropriate time for analgesia depends on maternal demand[4].

Pain relief during labor has received considerable attention, which is aimed at maternal well-being, reducing the stress caused by pain, and reducing its consequences for the fetus. However, there are controversies regarding the possibility that analgesia interferes with the progress of labor and fetal vitality. This study aimed to evaluate adverse maternal and perinatal outcomes in parturients undergoing labor analgesia.

METHODS

This was a retrospective cohort study conducted at the Mário Palmério University Hospital in Uberaba, State of Minas Gerais,

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Brazil, through an active search using the SOUL MV system (MV Informática Nordeste Ltda., Recife, Brazil) in the electronic medical records of parturients who underwent analgesia during labor between August 2014 and October 2021. This study was approved by the Research Ethics Committee of the University of Uberaba (CAAE Nº. 52405921.6.0000.5145).

The parturients were categorized into three groups: Group 1—analgesia performed with cervical dilatation ≤4.0 cm; Group 2—analgesia performed with cervical dilatation between 5.0 and 8.0 cm; and Group 3—analgesia performed with cervical dilatation ≥9.0 cm. The inclusion criteria were as follows: primigravidas of single fetuses who underwent epidural analgesia at a gestational age of ≥37 weeks; those admitted during the active phase of the first stage of labor; and those with no fetal malformations, chromosomal disorders, or Doppler changes in the umbilical artery, middle cerebral artery, or ductus venosus.

The decision of the parturient was respected for the indication of labor analgesia. Contraindications included refusal, infection or tumors at the puncture site, coagulation disorders, changes in consciousness, sepsis, known allergies to the administered drugs, and hemodynamic instability. In the absence of contraindications, once the diagnosis of labor was confirmed, analgesia was performed by a resident physician and supervised by an anesthesiologist experienced in labor. In this hospital, epidural analgesia was performed by administering 0.2% ropivacaine and 50 µg of fentanyl in a total volume of 10 mL.

The variables analyzed in this study were age, body mass index (BMI), hypotension, nausea, vomiting, pruritus, respiratory depression, cervical dilatation at the time of analgesia, use of oxytocin, the need for labor instrumentation, the presence of fetal bradycardia after analgesia, birth weight, Apgar score at 1st and 5th minutes, the need for neonatal oxygen therapy, and the need for admission to a neonatal intensive care unit (ICU).

The GPower 3.1 software (Heinrich-Heine-Universität, Düsseldorf, Germany) was used to calculate the sample size. According to the analysis, the study required a sample size of 248 patients who underwent analgesia during labor. The sample size analysis was based on a *w* effect of 0.25, α error probability of 0.05, and a power (1-β error probability) of 0.95, with two degrees of freedom.

Data were transferred to an Excel 2019 spreadsheet (Microsoft Corp., Redmond, WA, USA) and analyzed using SPSS version 20.0 (IBM Corporation, Armonk, NY, USA) and Prism version 7.0 (GraphPad Software, San Diego, CA, USA). Quantitative variables were analyzed using a normality test (D'Agostino-Pearson), and those with a normal distribution were presented as means and standard deviations. Variables with a non-normal distribution were presented as medians and minimum and maximum values. Categorical variables were described based on absolute and percentage frequencies and are represented as tables. To study the difference between categorical variables and their proportions, the Chi-square test was used. Analysis of variance (ANOVA) was used for normally distributed variables to study the difference between continuous variables. The Kruskal-Wallis test was used for non-normally distributed variables. Dunn's post hoc test was used for pairwise comparison. The significance level for all tests was set at $\alpha<0.05$.

RESULTS

Overall, 247 parturients undergoing labor analgesia were evaluated and were categorized into three groups: Group 1 (n=83), Group 2 (n=82), and Group 3 (n=82), as described above. The characteristics of the study population are summarized in Table 1.

The different degrees of cervical dilatation were negatively correlated (r=-0.78) with the time to delivery (Figure 1) and showed a linear relationship. The model's coefficient of determination (R^2=0.71) indicated that 71.0% of the variation in

Table 1. Characteristics of the study population.

	Group 1 (n=83)	Group 2 (n=82)	Group 3 (n=82)	p-value
Age (years)	18.0 (16.0–27.0) [A,B]	22.0 (20.7–26.0)	25.0 (21.0–30.0)	<0.0001†
BMI (kg/m²)	32.3 (28.6–33.9)	30.8 (28.6–32.4)	30.9 (29.0–32.9)	0.05†
Gestational age (weeks)	40.1 (39.0–40.5)	39.3 (38.1–40.2)	39.6 (37.7–40.7)	0.05†
Birth weight (g)	3180.0 (474)	3207 (439)	3139 (543)	0.605ƒ
Apgar score at 1st minute	8 (6–8)[A,B]	8 (8–8)	8 (8–8)	0.0004†
Apgar score at 5th minute	9 (9–9)	9 (9–9)	9 (9–9)	>0.9999†

Group 1: analgesia performed in patients with cervical dilatation ≤4.0 cm; Group 2: analgesia performed in patients with cervical dilatation between 5.0 and 8.0 cm; Group 3: analgesia performed in patients with cervical dilatation ≥9.0 cm. BMI: body mass index; Kruskal-Wallis †median (interquartile range); ANOVA ƒmean (standard deviation); [A]Group 1 vs. Group 2; [B]Group 1 vs. Group 3.

the time to delivery was linearly related to cervical dilatation at the time of analgesia, and the remaining 29.0% of the variation results from other factors that are not considered in the model. Increase in this dilatation by 1.0 cm at the time of labor analgesia reduced the time to delivery by 0.94 h.

A statistically significant association was observed between the need to use oxytocin and the use of analgesia in parturients with smaller cervical dilatation (p<0.001). Parturients who underwent analgesia with cervical dilatation ≤4.0 cm had a higher prevalence of the need to use oxytocin than those who underwent analgesia with cervical dilatation between 5.0 and 8.0 cm (89.2 vs. 59.8%, p<0.0001) and ≥9.0 cm (89.2 vs. 52.4%, p<0.0001). Parturients who used oxytocin presented a 2.6 times (OR 2.67; 95%CI 1.31–5.57; p=0.0125) greater probability of progressing to vaginal delivery than to cesarean section.

A significant association was observed between cervical dilatation at the time of analgesia and the need for instrumentation during delivery (p=0.010). Parturients who underwent analgesia with cervical dilatation ≥9.0 cm had a higher prevalence of forceps delivery than those with cervical dilatation ≤4.0 cm (14.6 vs. 2.4%, p=0.005). Patients with cervical dilatation of ≥9.0 cm showed a 3.86-fold increase (OR 3.86; 95%CI 1.50–9.87; p=0.009) in the risk of forceps delivery. Alternatively, parturients with cervical dilation ≤4.0 cm showed a reduction of 79.0% (OR 0.21; 95%CI 0.04–0.89; p=0.040) in the risk of forceps delivery.

Parturients who underwent analgesia with cervical dilatation ≤4.0 cm had a higher prevalence of cesarean sections than those with cervical dilatation between 5.0 and 8.0 cm (24.1 vs. 11.0%, p=0.039) and ≥9.0 cm (24.1 vs. 6.1%, p=0.0019). Parturients with cervical dilatation of ≤4.0 cm showed a 3.31-fold increase (OR 3.31; 95%CI 1.62–6.77; p=0.0016) in the probability of progression to cesarean section. Alternatively, parturients with dilation between 5.0 and 8.0 cm and ≥9.0 cm showed reductions of 74.0% (OR 0.26; 95%CI 0.12–0.56; p=0.0003) and 29.0% (OR 0.29; 95%CI 0.12–0.78; p=0.010), respectively, in the probability of progression to cesarean section.

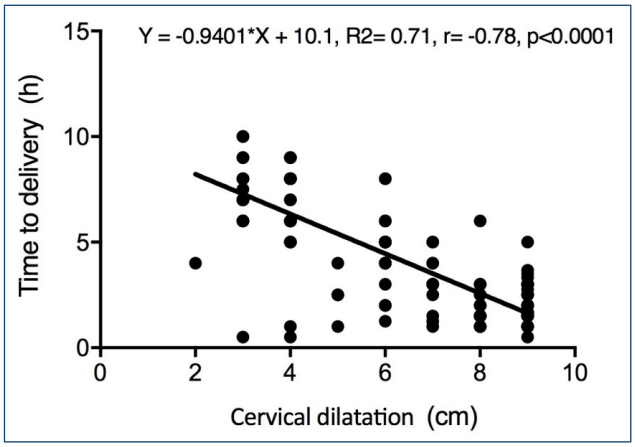

Figure 1. Correlation between the degree of cervical dilatation at the time of analgesia and the time to delivery (Spearman's correlation test, p<0.05).

There were no cases of arterial hypotension, nausea, vomiting, pruritus, or respiratory depression in the three groups. Analgesia in parturients with cervical dilatation ≥9.0 cm was associated with a higher prevalence of fetal bradycardia (20.7, 9.6, and 8.5% for Groups 1, 2, and 3, respectively). The neonates of Group 3 had a higher prevalence of the need for neonatal oxygen therapy (6.1, 0.0, and 0.0% for Groups 3, 1, and 2, respectively) and the need for neonatal ICU admission (4.9, 0.0, and 0.0% for Groups 3, 1, and 2, respectively) than the neonates of the other groups. Labor analgesia in parturients with cervical dilatation ≤4.0 cm was associated with a higher prevalence of Apgar score <7 at the 1st minute (44.6, 17.1, and 22.0% for Groups 1, 2, and 3, respectively) than in the parturients of the other groups (Table 2).

DISCUSSION

In the USA, 61% of women who had a single-fetus vaginal delivery underwent epidural analgesia in 2008[5]. According to the American College of Obstetricians and Gynecologists, in the absence of a medical contraindication for analgesia, a request from the mother is a sufficient medical indication for pain relief during labor. A woman who requests epidural analgesia during labor should be allowed to undergo this procedure irrespective of her health insurance status[6].

Epidural analgesia involves the use of low doses of local anesthetics along with opioids. Initial indications were based on maternal chronic diseases that could decompensate during the second stage of labor due to sympathetic stimulation caused by the pain and Valsalva efforts. Currently, these indications have expanded to include several high-risk conditions of the fetus as well as preeclampsia[7].

However, epidural analgesia poses several risks to the mother and fetus. These risks include respiratory depression in the newborn associated with the use of fentanyl, which reaches the maternal circulation and crosses the placenta[8]. Other complications of epidural analgesia include intrapartum maternal fever and sepsis in the newborn[9].

In a systematic review of five randomized clinical trials comprising 879 parturients undergoing epidural anesthesia, the standing and reclining positions were compared during the second stage of labor, and no statistical difference was observed between the groups in terms of operative delivery rates (cesarean

Table 2. Side effects and adverse maternal and perinatal outcomes.

	Group 1 (n=83)	Group 2 (n=82)	Group 3 (n=82)	p-value[§]
Arterial hypotension	0% (0/83)	0% (0/82)	0% (0/82)	*
Nausea	0% (0/83)	0% (0/82)	0% (0/82)	*
Vomiting	0% (0/83)	0% (0/82)	0% (0/82)	*
Pruritus	0% (0/83)	0% (0/82)	0% (0/82)	*
Respiratory depression	0% (0/83)	0% (0/82)	0% (0/82)	*
Fetal bradycardia	9.6% (8/83)	8.5% (7/82)	20.7% (17/82)	0.036
Apgar score <7 at 1st minute	44.6% (37/83)	17.1% (14/82)	22.0% (18/82)	<0.001
Need for neonatal oxygen therapy	0% (0/83)	0% (0/82)	6.1% (5/82)	0.016
Need for neonatal ICU	0% (0/83)	0% (0/82)	4.9% (4/82)	0.017

Group 1: analgesia performed in patients with cervical dilatation ≤4.0 cm; Group 2: analgesia performed in patients with cervical dilatation between 5.0 and 8.0 cm; Group 3: analgesia performed in patients with cervical dilatation ≥9.0 cm. ICU: intensive care unit; Chi-square [§]percentage (n/N); p<0.05. *It was not possible to calculate the p-value due to the absence of at least three cases in each group.

section or instrumental)[10]. In the present study, the association between patients' positions during labor and the type of delivery was not assessed. It is known that epidural analgesia affects labor progression and is associated with higher rates of vacuum- and forceps-assisted delivery[11]. Additionally, epidural analgesia leads to difficulty in standing and walking, and this has been shown to reduce the duration of the first stage of labor as well as the rates of cesarean sections[12].

In the present study, epidural analgesia in patients with a cervical dilatation of ≥9.0 cm was associated with adverse perinatal outcomes, such as fetal bradycardia, the need for neonatal oxygen therapy, and the need for neonatal ICU admission. Alternatively, epidural analgesia in patients with a cervical dilatation ≤4.0 cm was associated with a higher prevalence of Apgar score of <7 at the 1st minute. Shiro et al.[13] evaluated 138 parturients who received epidural analgesia and categorized them into two groups according to cervical dilatation (≤3.0 cm and ≥4.0 cm). In nulliparous women, no differences were noted in perinatal outcomes, except for a longer duration of the first stage in the ≤3.0 cm group. Similarly, in multiparous women, no differences were observed in perinatal outcomes, except for a higher proportion of Apgar scores of <7 at the 1st minute in the ≤3.0 cm group, which is in agreement with the results of the present study. Kumar et al.[8] evaluated neonates at an age of ≥34 weeks who developed respiratory distress within the first 24 h and required oxygen therapy at ≥2 h and/or positive-pressure ventilation in a neonatal ICU. They observed a significant association between epidural analgesia and respiratory distress in newborns (OR 1.75; 95%CI 1.03–2.99; p=0.04). However, discontinuation of epidural analgesia did not reduce adverse perinatal outcomes, such as lower rates of instrumental births, as demonstrated in a systematic review of five studies comprising 462 women[14].

In the present study, parturients who underwent epidural analgesia with a cervical dilatation of ≤4.0 cm had a higher prevalence of the need for oxytocin than those in the other two groups. Additionally, parturients who were administered oxytocin presented a 2.6 times (OR 2.67; 95%CI 1.31–5.57; p=0.0125) higher probability of progressing to vaginal delivery. In a previous study, Shmueli et al.[15] evaluated 15,500 deliveries of full-term singletons and observed that the use of oxytocin was associated with a longer duration of the second stage of labor in nulliparous women, regardless of whether they underwent epidural analgesia. A systematic review of two studies comprising 319 parturients demonstrated that the use of oxytocin in women who underwent epidural analgesia did not reduce the rates of cesarean sections or instrumental deliveries. Furthermore, it did not reduce the adverse perinatal outcomes, such as Apgar scores of <7 at the 1st minute, admission to a neonatal ICU, uterine hyperstimulation, or postpartum hemorrhage[16].

CONCLUSION

In summary, performing labor analgesia in parturients with cervical dilatation of ≤4.0 cm or ≥9.0 cm was associated with a higher prevalence of adverse maternal and perinatal outcomes.

AUTHORS' CONTRIBUTIONS

ABP: Conceptualization, Formal Analysis, Methodology, Supervision, Visualization. **GDG:** Data curation, Visualization, Writing – original draft. **GAM:** Data curation, Visualization. **MCP:** Investigation, Project administration, Visualization. **EAJ:** Validation, Visualization, Writing – original draft, Writing – review & editing.

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REVIEW ARTICLE

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Chronic kidney disease and the severity of non-alcoholic fatty liver disease: a systematic review

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) has emerged as the leading cause of liver disease in developed countries, affecting more than one-third of the adult population[1]. NAFLD encompasses a spectrum of diseases, ranging from simple hepatic steatosis to non-alcoholic steatohepatitis (NASH), which can progress to liver cirrhosis and hepatocellular carcinoma[2].

NAFLD is now recognized as a multisystemic disease with extrahepatic involvement that affects the cardiovascular, endocrine, pulmonary, and renal systems. In the United States, NAFLD is expected to become the leading cause of liver transplantation in the next few years.

NAFLD is also associated with an increase in the incidence and prevalence of chronic kidney disease (CKD), which is defined as a glomerular filtration rate (GFR) <60 mL/min/1.73 m². These two diseases share several cardiometabolic risk factors, as well as profibrotic and proinflammatory molecular mechanisms[3]. Recent studies have reported CKD in 20–25% of individuals with NAFLD[4] essential to establish primary prevention strategies for CKD and to implement appropriate interventions to manage this condition in patients with NAFLD.

This systematic review aims to contribute to the ongoing discussion of the relationship and impact of NAFLD severity on the development of CKD.

METHODS

This systematic review was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statements. The study protocol was registered on the PROSPERO platform under the number CRD42022307980 and followed a predefined protocol based on the systematic reviews guideline[5].

To select articles, we conducted a literature search using the PUBMED, Web of Science, Scopus, Literatura Latino-americana e do Caribe em Ciências da Saúde (LILACS), and EMBASE databases. The search was conducted between May 2021 and September 2021.

The initial search was performed on PubMed to access the MEDLINE database, using the strategy defined below by the following descriptors (Non-alcoholic Fatty Liver Disease OR (non-alcoholic AND Fatty Liver AND disease) OR Nonalcoholic Steatohepatitis) AND "Renal Insufficiency, Chronic" OR "Chronic Renal Insufficiency."

The entire process was conducted by two independent authors, who searched the databases, read the titles and abstracts of the articles, and applied the inclusion and exclusion criteria. Disagreements were resolved through consensus, and when necessary, a third reviewer was consulted.

Based on the articles found, we conducted the study according to the PECOS strategy (Participants, Exposure, Comparison, and Outcomes) and followed the inclusion criteria described below:

Inclusion criteria: prospective and retrospective cohort and cross-sectional studies assessing the association between NAFLD and CKD in patients aged 18 years, and the studies that evaluated renal dysfunction in adult patients with NAFLD fibrosis progression with the risk of CKD.

Exclusion criteria: review articles, clinical trials, case reports, editorials, and experimental studies; studies in specific populations such as diabetics and individuals with CKD from dialysis centers; and studies in patients with NAFLD diagnosis by non-invasive markers such as the fatty liver index (FLI)[6] or by the parametric attenuation coefficient (PAC)[7].

To assess the quality of the selected studies, we used the Newcastle-Ottawa Scale, which employs a scoring system from

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0 to 9 in three domains: selection, comparability, and results. The higher score indicated better study quality, and a minimum score of 7 was established for the inclusion of the studies. The Rayyan Qari software was used to confirm the accuracy of the included articles.

CKD was diagnosed by estimating the GFR using the CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) method.

We conducted a sensitivity analysis of the studies based on several factors, including the research site, length of stay in the study, methodology used in NAFLD follow-up, and NAFLD severity assessed by non-invasive methods, such as the FIB-4[8-10] and NAFLD score[11,12], liver elastography, or histological analysis.

RESULTS

A PRISMA flowchart (Figure 1) shows the studies evaluated in this review. Initially, 431 articles were identified during the literature search, of which 52 duplicated articles were excluded. Based on the title and abstract, 365 articles were reviewed, and 19 studies were selected for full text assessment.

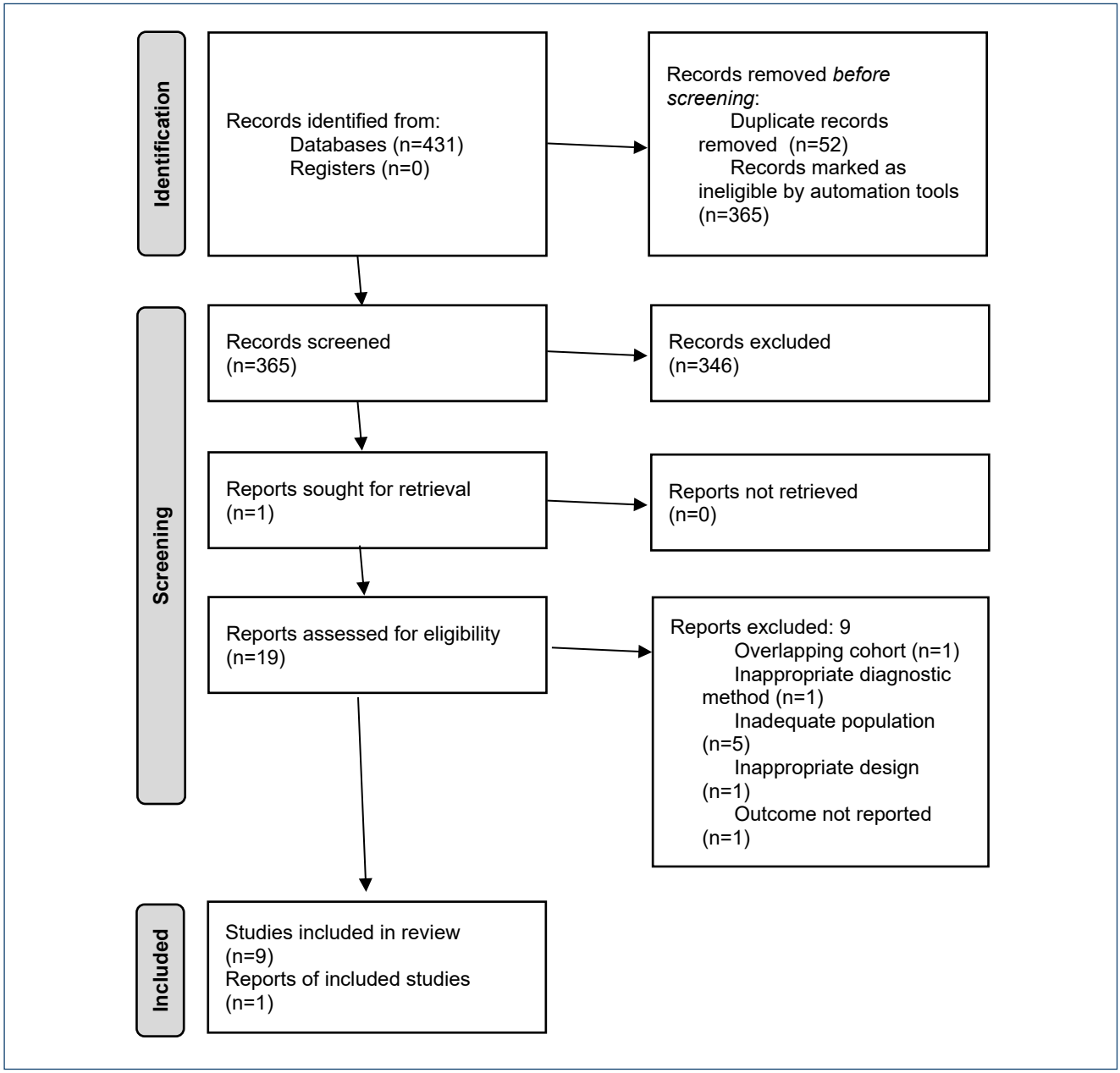

Figure 1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses flowchart of the studies evaluated.

After excluding articles with an inadequate population (n=5), inadequate diagnostic method of steatosis (n=1), overlapping cohort (n=1), unreported outcome of interest (n=1), and inadequate study design (n=1), a total of 10 articles were included in the final analysis, and their quality was assessed.

Table 1 presents the main characteristics of the included studies. The presence of NAFLD was found to be associated with the presence of CKD in four studies[13-16], and the development of NAFLD was found to increase the risk of CKD incidence in four additional studies[17-20]. In these eight studies[13-20], the severity of liver disease was associated with a reduction in kidney function, regardless of known risk factors. Two studies did not find an association between NAFLD and CKD nor an increase in the incidence of CKD after adjusting for metabolic syndrome elements.

Table 2 presents the studies that were excluded after evaluation. Three studies were excluded because they focused on a cohort of individuals with pre-existing CKD. Two studies focused solely on the molecular mechanisms involved in the condition being studied. Seven studies evaluated only specific populations of individuals. One study had a small sample size. One study examined only the effect of fibrosis. In one study, the outcome was not of interest to the researchers. Finally, one study used a non-imaging or biopsy-based diagnosis of steatosis, which may not be sufficiently reliable for this investigation.

DISCUSSION

In this systematic review of 10 observational longitudinal studies, comprising a total of 165,246 participants from different countries, we found a positive association between the presence of NAFLD and CKD stage ≥3, defined as the occurrence of eGFR <60 mL/min/1.73 m^2, with or without accompanying overt proteinuria. Moreover, the prevalence of CKD increased with the severity of NAFLD.

This systematic review is relevant because less is still known about the relationship between the evolution of NAFLD and the incidence of CKD. In recent years, this association has aroused increasing scientific interest, mainly due to the knowledge that NAFLD, in its broad spectrum, is associated with the development and progression of cardiovascular diseases.

There is now increasing evidence that the severity of NAFLD predicts the development and progression of CKD, suggesting that NAFLD-associated CKD might involve some unique mechanisms. Indeed, the hepatic and systemic vaso-regulatory changes seen in NAFLD may evoke the hepatorenal reflex and impair renal function[21].

According to the literature, the study developed by Targher et al.[15] demonstrated for the first time that individuals with NASH confirmed by liver biopsy had a moderate decrease in GFR and more frequent albuminuria than controls without NASH. This study was carried out with 80 non-obese patients, and adjustments were made for the main confounding factors such as age, gender, waist circumference, Homeostases Model Assessment-Insulin Resistance (HOMA-IR) score, systolic blood pressure, and triglycerides. Thus, it is possible to speculate that NASH is not associated with CKD because of shared cardiovascular risk factors, but NASH itself may contribute to the development of CKD.

Yasui et al.[16] studied 92 individuals with NASH and found a high prevalence of CKD (21%) in those with NASH when compared to control without NASH. The study also identified obesity and NASH as risk factors for CKD in NASH.

In addition, a cohort study by Sinn et al.[18] assessed the longitudinal association between NAFLD and the incidence of CKD over a 10-year period. The study concluded that CKD developed more frequently in participants with NAFLD compared to those without NAFLD. This association was not explained by the emergence of systemic arterial hypertension (SAH) and type 2 diabetes mellitus (T2DM) and persisted after adjustments for risk factors and potential metabolic mediators.

These findings further support the association between NAFLD and CKD and highlight the need for early detection and management of NAFLD to prevent the development and progression of CKD.

This cohort also observed the strong association between NAFLD and CKD in individuals with more advanced fibrosis, indicated by the high NAFLD fibrosis score (NFS) score[9], and that fibrosis markers bring additional risk stratification, being used to identify the patients with NAFLD at increased risk of renal complications.

The European Association for the Study of the Liver recommends the evaluation of serum markers of fibrosis in hepatic steatosis[22]. The NFS can identify patients at low risk of advanced fibrosis and has been externally validated in ethnically diverse populations with NAFLD[23].

Despite the literature being likely to understand that NAFLD would act directly in the pathogenesis of CKD, other divergent studies in the literature have emerged. Sirota et al.[24] developed a large cross-sectional study with 11,469 adults who participated in the National Health and Nutrition Examination Survey, 1988–1994 (NHANES III). The hypothesis was that NAFLD was associated with CKD and that the severity of NAFLD would bring a greater chance of CKD. They concluded that there is a positive association between the presence and severity of NAFLD and CKD, but this association was attenuated after adjusting for confounding factors.

There are multiple possibilities for such discordant results found in studies examining the association between NAFLD and CKD. Some studies did not adjust for important confounding factors,

Table 1. Overview of the included studies investigating the association between renal dysfunction in individuals with nonalcoholic fatty liver disease.

Author, year of publication	Country	Design of study	Number of subjects	Diagnosis of NAFLD	Setting	Definition of DRC	Statistical adjustment	NOS score
Targher, 2010[15]	Japan	Cross-sectional Case-control	160	Liver biopsy	01 medical center	GFR <60 mL/min or albumin-to-creatinine ratio >3 mg/mmol	Age, gender, BMI, waist circumference, smoking, systolic BP, HOMA-IR score, and TG	08
Yasui, 2011[16]	Japan	Cross-sectional Case-control	174	Liver biopsy	02 medical center	GFR <60 mL/min (Japanese Society of Nephrology)	BMI, SAH, age, sex, the presence of T2DM or dyslipidemia, levels of AST, ALT, or γ-GTP	08
Sinn, 2017[18]	South Korea	Cohort	41430	Ultrasonography	Samsung Medical Center	CKD-EPI <60 mL/min	Smoking, alcohol consumption, BMI, and estimated GFR at baseline, systolic blood pressure, HbA1c, LDL-C, use of diabetes, lipid lowering, and antihypertensive medications	
Sirota, 2012[24]	USA	Cross-sectional Case-control	11469	Ultrasonography	NHANES program	GFR <60 mL/min or GFR >60 mL/min with albuminuria	Model adjusted for age, sex and race (Model 1); and a model adjusted for history of hypertension, history of diabetes, systolic BP, waist circumference, TG, HDL-C, and HOMA-IR score	08
Wilechansky, 2019[25]	USA	Cohort	987	Tomography	Framingham Heart Study	CKD-EPI GFR <60 mL/min/1.73 m² and/or microalbuminuria (sex-specific urinary albumin-creatinine ratio)	Age, sex, smoking status, drinks per week, systolic blood pressure, diastolic blood pressure, use of antihypertensive medications, HDL-C, total cholesterol, regular aspirin use, and T2DM	08
Zhang, 2020[20]	USA and China	Cross-sectional	65087	Ultrasonography	NHANES and dataset chinese	CKD-EPI GFR <60 mL/min/1.73 m² and/or abnormal albuminuria and/or overt proteinuria	Age, sex, BMI, history of T2DM, and history of SAH	08
Liu, 2020[14]	Taiwan	Cross-sectional Case-control	37825	Ultrasonography	Taipei Tzu Chi Hospital	Proteinuria or GFR ≤60 mL/min/1.73 m²	Sex, age, current smoking, T2DM, SAH, low HDL-C, high TG, ALT, and systolic BP	07
Kaps, 2020[17]	Germany	Cohort	48057		Disease Analyzer Database		Diabetes, obesity, SAH, and ischaemic heart diseases	08
Zuo, 2021[19]	China	Cohort	4402	Ultrasonography	Medical center	Albumin-to-creatinine ratio >3 mg/mmol or GFR <60 mL/min/1.73 m²	Age, sex, smoking status, drinks, physical activity BMI, SBP, HbA1c, white blood cell count, UACR, eGFR, use of antidiabetic medications, antihypertensive, and use of lipid lowering medications	08
Cao, 2021[13]	China	Cross-sectional Case-control	3872	Ultrasonography	Medical center	Albumin-to-creatinine ratio >3 mg/mmol or GFR <60 mL/min/1.73 m²	Age, sex, and T2DM	07

CKD was defined as a urinary albumin-to-creatinine ratio (UACR) ≥30 mg/g, an estimated glomerular filtration rate (eGFR) <60 mL/min/1.73 m² or both. BMI: body mass index; HOMA-IR: homeostatic model of insulin resistance; HDL-C: high-density lipoprotein cholesterol; TG: triglycerides; LDL-C: low-density lipoprotein cholesterol; NOS: Newcastle Ottawa Scale; SAH: systemic arterial hypertension; T2DM: Type 2 diabetes mellitus; SBP: systemic blood pressure; HbA1c: hemoglobin A1C.

Table 2. Studies that were evaluated in full text and were excluded.

No.	First author	Year	Title	Reason for exclusion
1	Chinnadurai	2019	Non-alcoholic fatty liver disease and clinical outcomes in chronic kidney disease	Cohort of individuals who already had CKD
2	Mikolasevic	2013	Chronic kidney disease and nonalcoholic fatty liver disease proven by transient elastography	Cohort of individuals with CKD
3	Musso	2015	Emerging liver-kidney interactions in nonalcoholic fatty liver disease	Molecular study
4	Kasim	2019	Correlation between non-alcoholic fatty liver and chronic kidney disease	Small number of subjects in the study
5	Li	2014	Association between non-alcoholic fatty liver disease and chronic kidney disease in population with prediabetes or diabetes	Specific population with diabetes
6	Paik	2019	Chronic kidney disease is independently associated with increased mortality in patients with nonalcoholic fatty liver disease	Outcome was not of interest
7	Targher	2014	Nonalcoholic fatty liver disease is independently associated with an increased incidence of chronic kidney disease in patients with type 1 diabetes	Specific population with diabetes
8	Xu	2016	High FIB-4 index as an independent risk factor of prevalent chronic kidney disease in patients with nonalcoholic fatty liver disease	Associates only the effect of fibrosis
9	Targher	2008	Increased risk of CKD among type 2 diabetics with nonalcoholic fatty liver disease	Specific population
10	Aubert	2021	Role of non-alcoholic fatty liver disease in the evolution of renal function in patients with diabetes mellitus	Specific population
11	Targher	2012	Increased prevalence of chronic kidney disease in patients with Type 1 diabetes and non-alcoholic fatty liver	Specific population
12	Lu	2020	Non-alcoholic fatty liver disease increases the prevalence of maintenance haemodialysis in patients with chronic kidney disease	Specific population with chronic kidney disease
13	Machado	2012	Impaired renal function in morbid obese patients with nonalcoholic fatty liver disease	Specific population with obesity
14	Zeng	2017	Association between non-invasively diagnosed hepatic steatosis and chronic kidney disease in Chinese adults on their health check-up	Non-imaging or biopsy diagnosis of steatosis
15	Musso	2016	Fatty liver and chronic kidney disease: novel mechanistic insights and therapeutic opportunities	Molecular study
16	Chon	2020	Decrease in waist-to-hip ratio reduced the development of chronic kidney disease in non-obese non-alcoholic fatty liver disease	Specific population without obesity

such as antihypertensive drug use and smoking. Additionally, studies conducted in hospitals may have selected individuals with more advanced liver disease, including NASH and fibrosis[25]. It is possible that only those with more advanced NAFLD, particularly with NASH or liver fibrosis, may be at increased risk for CKD.

More recently, cross-sectional studies with a large number of individuals[20,14] and long-term cohorts[17] have confirmed that NAFLD is an independent risk factor for CKD. Zuo et al.[19] confirmed this hypothesis but suggested that the progression of liver fibrosis in individuals with NAFLD is a more significant predictor for the development of CKD than baseline levels of metabolic diseases.

The biological mechanisms underlying this association are still not well understood. Possible factors include the activation of the renin-angiotensin system, hepatic insulin resistance, atherogenic dyslipidemia, proinflammatory, procoagulants, and pro-oxidants mediators, as well as alterations in the intestinal microbiota[13].

This review has certain limitations inherent to the design of the studies included. First, the observational design of the

eligible studies prevents us from establishing causality. Second, although most of the eligible studies adjusted the results for age, sex, obesity, hypertension, and diabetes, residual confounding due to some unmeasured factors cannot be excluded. Additionally, none of the eligible studies provide a histological characterization of NAFLD-associated kidney disease.

CONCLUSIONS

This systematic review suggests a positive association between the presence and severity of NAFLD and CKD. However, further follow-up studies are needed to confirm these findings.

ETHICAL STATEMENT

The authors declare that all experiments were conducted in accordance with the Declaration of Helsinki.

AUTHORS' CONTRIBUTIONS

KSDC: Visualization, Writing – original draft. **CHCD**: Formal Analysis, Funding acquisition, Methodology, Project administration, Validation, Visualization, Writing – review & editing. **VAA**: Data curation, Investigation,. **RRS**: Investigation, Resources. **HPC**: Conceptualization, Methodology, Project administration, Supervision, Writing – review & editing.

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REVIEW ARTICLE

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Depression in women in climacteric period: a brief review

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INTRODUCTION

Women have evident and concrete milestones during their lives such as menarche and the last menstrual period, which are related to the menstrual cycle and signalize different phases or periods of life. The image of women is linked to the reproductive phase, as a result of their biological cycle, but it is important to emphasize that the period of transition to menopause and post-reproductive period stand out as population aging is a global reality related to increased life expectancy and decreasing fertility rates, leading to a demographic, social, and economic transition[1].

The Brazilian population over 50 years of age increased from 20.8 million people in 2010 to 30.1 million in 2020, with an estimated increase of more than 1 million annually, reaching 42.1 million in 2030. It is estimated that the female population will exceed 23 million in 2030, and thus a larger number of women will be experiencing the post-reproductive period and its transition, considering that the life expectancy for women in Brazil is 75.6 years[2].

These data are associated with the fact that the magnitude of the consequences for women's lives, such as menopausal symptoms, urinary symptoms, vaginal atrophy, sexual dysfunction, increased risk of cardiovascular disease, and osteoporosis, and the concomitant appearance of psychological manifestations, such as irritability, nervousness, depression, and anxiety, corroborate the classification of this phase of life as a public health problem[3].

The climacteric phase is defined as the period of transition from a woman's reproductive to non-reproductive life and extends from 40 to 65 years of age. It can be divided into two phases: transition to menopause and postmenopause[4].

Menopause is the event that occurs at the end of the transition to menopause, and the milestone for the beginning of postmenopause. It is recognized after 12 months of absence of menstrual cycles associated with permanent and physiological ovarian insufficiency. Worldwide, it occurs around 50 years of age, in Latin America at 47 years of age, and in Brazil between 48 and 50 years of age[5].

Postmenopause starts from the last menstrual period (menopause) and can be divided into early and late. Early postmenopause is defined as the period of 5 years from menopause, where the levels of the follicle-stimulating hormone remain high with a progressive decline of estradiol and greater representation of vasomotor symptoms, mood, and sleep changes. The late phase starts after 5 years and lasts until death with greater repercussions on bone and cardiovascular metabolism[4,6].

With increasing life expectancy, women spend approximately one-third of their lives in menopause. Therefore, the objective of this review was to identify the causes of the disorders that occur in this phase and their impact on the family, to help women increase their motivation and sense of self-efficacy and, consequently, to improve their quality of life as well as educational initiatives in health for this phase of women's lives.

CLIMACTERIC PERIOD AND DEPRESSION

Depression corresponds to a mood disorder, being two times as prevalent in women than in men, manifesting itself through the following symptoms: depressed mood, fatigue, reduced reasoning, decision-making capacity, and physiological manifestations, such as altered sleep, appetite, and sexual interest, in addition to the manifestation of social withdrawal behavior[7].

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During a woman's reproductive period, physiological changes occur that lead to mood swings, which, depending on the frequency and intensity, may be accompanied by the possibility of a diagnosis of depression[8].

Depressive disorders that occur before the climacteric period are called reproductive depression and are associated with endocrine changes rather than psychiatric. This occurs due to hormonal changes, such as in the days before the menstrual period (premenstrual syndrome) or in the weeks after pregnancy as postpartum depression and in the years approaching menopause as climacteric depression[9]; thus, the primary treatment would be hormonal rather than antidepressants.

The menopausal transition is a vulnerable period for the onset of depressive symptoms because women are more prone to depressed moods. Also, perimenopause is accompanied by physiological changes that include vasomotor symptoms, cognitive and metabolic decline, and somatic and psychological changes[3]. These changes are attributed to hormonal fluctuations that act on specific areas in the brain that operate as estrogen receptors that influence mood regulation, core body temperature, and cognitive function[10].

A study of the Center for Epidemiological Studies Depression (CES D) Scale demonstrated high scores in the symptoms of sadness, loss of interest, appetite, sleep, concentration, feeling of guilt, fatigue, agitation, and suicidal ideation. Regarding anxiety, women with both high and low levels of anxiety may feel anxiety and even reach high levels of anxiety during the climacteric period; thus, this period may be critical for women susceptible to depression and anxiety disorders[8].

Depression in perimenopause manifests itself through verbal outbursts, due to minor stress factors resulting from feelings associated with anger, irritability, and paranoia, and such attitude is not something inherent to the character of these women. Together with the psychological symptoms, cognitive declines, such as low concentration, reduced memory, anxiety, nervousness, irritability, low self-esteem, melancholy, and sadness, which are present mainly during the transition to menopause, are pointed out[11]. These may be associated with other symptoms such as depressed mood, hot flashes, fatigue, physical symptoms, use of medication, and other stressors common to women in this phase of life[3].

The literature shows that perimenopause corresponds to the period where mood disorders can appear with a fivefold increase in the risk of the first episode of depression in the transition to menopause. In the pre-menopausal phase, one of the factors to be considered is the past existence of depressive history that can result in changes[8].

During perimenopause and postmenopause, there is a higher risk of the reappearance of major depressive disorder in women with a previous history. The association of the risk profile with health factors and psychological symptoms favors depressive episodes in this period[8].

Furthermore, women who manifest depression during the transition to menopause present a higher risk of developing depression in postmenopause, with increased morbidity and mortality. The National Epidemiological Survey on Alcohol and Related Conditions carried out in the United States identified a risk factor for major depressive disorder at the age of 50 years and a history of previous depression, besides a greater susceptibility to chronic diseases, such as hypertension and diabetes in this period[12].

The overlap of the aging process with prolonged hypoestrogenism aggravates the risk of depression in women in the climacteric period and can double even with no history; this fact is linked to hormonal and psychosocial changes[13].

Thus, depressive episodes can occur during at least one-third of a woman's life cycle. In the climacteric period, the prevalence of these episodes is 9% and may be associated with the fear of aging, feeling of uselessness, affective needs, and unemployment. A depressive mood state added to a history of depression increases 1.5–2.0 times the risk of depression in menopause. Furthermore, women in the climacteric period with no history of depression diagnosis present an odds ratio of 2.50 for major depression[12], with the highest risk of a depressive episode occurring in perimenopause.

Thus, women in climacteric period may report depressed mood during follow-up, with loss of energy, libido, and confidence, and receive antidepressant indications. Professionals ignore the hormonal association of estrogen with the symptoms; this failure can have serious consequences. It should be considered that depressive disorders correspond to the second leading cause of disability throughout life[14], and these are associated with other comorbidities, such as cardiovascular diseases.

The literature demonstrates the positive effects of estrogen therapy in depressed perimenopausal women but no effects in postmenopausal women. Thus, there may be a window of opportunity for the treatment of depressive disorders during perimenopause. Estrogen therapy may benefit perimenopausal women without depression by improving their mood and well-being. Also, when indicated for other menopausal symptoms, it demonstrates an increased clinical response to antidepressants in both perimenopausal and postmenopausal women[15].

Perceived mood swings in the transition to menopause and postmenopause as well as depression are related to an increase in health care utilization and costs, and sick leave[16].

It is important to recognize that women with no family history of depression may be more vulnerable to the effects of

the menopausal transition than women with such a history and that this group of women may benefit from increased monitoring for signs of depression during this period. Even women who have not experienced a depressive episode during the menopausal transition are two to four times more likely to develop a depressive episode during the menopausal transition. Furthermore, there is a threefold greater risk of developing a major depressive episode[17].

Monitoring can lead to early interventions including pharmacological, behavioral, and psychological therapy that can prevent the progression of depressed mood to minor or major depression and is effective at other times in the life cycle. Interventions can also include brief counseling on how to cope with mood changes such as treatment of menopausal symptoms that can exacerbate or worsen depression and are unique to this period in a woman's life, such as vasomotor and genitourinary symptoms and difficulty in sleeping[3,15].

The study by Colvin et al. signals that major depression has a higher chance of being identified in late perimenopausal or postmenopausal women when compared to premenopausal or early perimenopausal women[12], and it is essential to ask and investigate mood swings in this phase of a woman's life. Yet cognitive decline, especially in verbal memory, can be accompanied by problems with organization and planning or concentration at this stage of life impairing the depressive state.

A change in lifestyle and behavior, including regular physical activity and a diet based on fresh and unprocessed foods, favors the reduction of menopausal symptoms and depressive symptoms, consequently improving the quality of health and quality of life as protective factors[18].

PREDICTORS FOR DEPRESSION DURING THE CLIMACTERIC PERIOD

Cultural factors, lifestyle, and sociodemographic aspects influence the quality of life of women in the climacteric period. Women with more anxiety traits, higher mean private self-consciousness, and lower mean optimism are more likely to develop anxiety disorder throughout life. Also, factors such as two or more medical conditions and prior use of psychotropic medications increase a woman's propensity to develop major depressive disorder over her lifetime[19].

In menopause, the increase in vasomotor symptoms and sleep disorders potentiate depression that can affect cognitive function, difficulty in sleeping, reduced quality of sleep with advancing age, in addition to climacteric symptoms from moderate to intense, and others such as the presence of arthritis, arthrosis, and/or rheumatism. Sleep problems, in turn, interfere with the cognitive function of women, such as attention, executive function, and episodic memory[6,10,20].

The Women's Healthy Aging Project (WHAP) identified that women around the age of 50 years who have negative attitudes toward aging, negative attitudes toward menopause, negative mood scores, and previous premenstrual complaints are susceptible to higher depressive symptom scores when they reach the age of 60 years[8].

Factors such as alcohol consumption, a sedentary lifestyle, and living without a partner can negatively impact emotion and mood in postmenopausal women. Barghandan et al.[19] demonstrated that the older the postmenopausal women are and the lower their level of physical activity, the more likely they are to develop depression[19].

CLOSING COMMENTS

The occurrence of menopause is a "window of vulnerability" for depression, as hypoestrogenism is associated with changes in neurotransmitter metabolism. The identification of risk predictors for the development of depressive symptoms such as previous depression will favor early behavioral and clinical therapeutic intervention.

The strategy of treating vasomotor symptoms, sleep disturbances, and urogenital symptoms ameliorates and positively impacts mood and cognition symptoms. Thus, it is important to extend knowledge, so that women can seek monitoring and develop coping strategies through lifestyle changes, including healthy habits.

There are controversies about the factors associated with the onset of mood swings, along with the high cost of treatment and the social and family repercussions associated with depression and anxiety. It is important to have a multidisciplinary team intervention taking advantage of a possible window of opportunity to monitor women in the climacteric period where prevention and early diagnosis can guide individualized and holistic treatment.

AUTHORS' CONTRIBUTIONS

JZR: Conceptualization, Writing – original draft, Writing – review & editing. **ICES:** Conceptualization, Writing – original draft, Writing – review & editing. **CMPR:** Writing – original draft. PCLB: Writing – original draft. **LMPRC:** Writing – original draft. **ECB:** Writing – original draft. **JMSJ** Conceptualization, Supervision, Writing – original draft, Writing – review & editing. **RDR:** Conceptualization, Supervision, Writing – original draft, Writing – review & editing.

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REVIEW ARTICLE

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Microglia role as the regulator of cognitive function

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INTRODUCTION

Microglial cells are classified as the resident immune cells of the central nervous system (CNS), and they have been pointed out as key players in the development of neurodegenerative diseases[1]. These cells were discovered in the late eighties and early nineties, through studying the mouse brain, and showing that microglia are mononuclear cells distributed throughout the brain and spinal cord, accounting for over 20% of the glial cell population in the brain parenchyma[2]. The microglial cells are the only immune defense in the brain parenchyma.

These immune vigilants of infections contribute and regulate innate and adaptive responses, being involved in many different roles, such as the formation of synapses and connections, neuronal proliferation and differentiation, and the maintenance of brain homeostasis in health and disease[3]. Usually, microglia will protect the brain under inflammatory conditions by activating a strong immune response and supporting tissue repair and remodeling[4].

Microglial cells respond effectively to pathogens and brain trauma by promoting morphological changes. They respond to pathogens and injury by migrating to the site where the infection or injury occurred, changing its morphology, and destroying the pathogens to remove damaged cells and debris[5,6]. These glial cells secrete cytokines, chemokines, reactive oxygen species, and prostaglandins as part of the immune response[7,8]. On the contrary, microglia can regulate and increase the damage to the CNS when overstimulated, which generates a condition named by many authors as a reactive gliosis[9,10]. Therefore, microglia responses have been studied in many diverse types of infections, brain traumas, neurodegenerative diseases, and several other conditions[11-14].

However, the terms "reactive gliosis," "activated microglia," or "overactivated microglia" may not be the best choices to represent a range of several morphological, physiological, and sex-specific differences related to the multiple states of microglia, which vary not only from one condition or disease to another, but also from one specific brain region to another. Therefore, microglia have a key role in the defense and maintenance of CNS. Microglia have a remarkable therapeutic potential as a target in neurological disorders and brain injury. Here, we review what makes microglia so interesting to be studied as a possible therapeutic target in different conditions by starting to analyze its origins, passing through a few different conditions/diseases, and then discussing the potential future directions in research and clinic.

ORIGINS OF MICROGLIA

Virchow was the first to describe the neuroglia in 1856, which would be related to astrocytes and oligodendrocytes, while the first description of microglial cells came from Franz Nissl in the late 19th century by describing them as reactive glial elements with migratory potential, phagocytic activity, and the capacity of proliferating, which were named rod cells[15]. Santiago Ramon y Cajal defined these cells as the third element because they were neither neurons nor part of the neuroglia, which comprises astrocytes and oligodendrocytes, and Pio Del Rio-Hortega introduced the term microglial cell to differentiate them from the other glial cells and neurons[15]. Many hypotheses were tested until the establishment of the nature of microglia.

There is a growing body of evidence that suggests microglial cells are originated hematopoietically and able to reach the CNS through the bloodstream[16,17]. Initially, the evidence for a yolk sac microglial origin was mixed until Takahashi and Naito described the development of immature macrophages of the yolk sac at embryonic day 9 in mouse and rat tissues[18,19]. Thus, microglia are derived from yolk sac progenitors showing expression of the transcription factor RUNX1

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and CD117, a tyrosine kinase receptor, but these cells do not express CD45, a leukocyte marker protein[20]. Microglia emerge from the fetal yolk sac macrophages, whereas other tissue macrophages emerge from precursors produced later in development[21].

Migration and colonization of the brain by the microglial progenitors occur during fetal development before the blood-brain barrier (BBB) is completely formed, and microglia have the ability to self-renew throughout life when occurs the brain maturation and its confinement by the fully developed BBB[22]. However, human microglial cells appear near the mesenchymal tissue capillaries before their appearance in neural tissue in the fourth gestational week, and are present in the neural tissue around the fifth gestational week[23]. Nevertheless, it is important to mention that after bone marrow transplantation, and under other pro-inflammatory conditions, there might occur the recruitment of monocytes or other bone marrow-derived progenitors, which may supplement microglia to some extent[3].

REACTIVE GLIOSIS: A TERM TO BE REVIEWED

Reactive gliosis is classified as a change that occurs in the glial cells' morphology and activity due to damage in CNS, and seems to be the most important pro-inflammatory mechanism in the development of many neurodegenerative diseases, such as Alzheimer's disease and others[24,25]. Even during infectious conditions caused by virus like Zika, a higher phagocytic activity that contributes to changes in behavior can be seen in microglial cells[26]. Microglial activation is the expansion of microglia during microgliosis, the first step in the reactive gliosis, and results mainly from the existing resident microglia expansion, which might be harmful to neurons and will contribute to the development of a pro- and harmful inflammatory state[22].

A reactive gliosis consists of different stages where the primary response is the migration of macrophages and microglia to the specific site of the injury, followed by the recruitment of oligodendrocytes, which should contribute to remyelination, and, finally, there would be the enhancement of astrocyte expression, which leads to the formation of glial scars, completing, then, all steps of a reactive gliosis[9]. Thus, microglia act primarily as a neuroprotective mechanism, and when overactivated, according to the classic concepts mentioned above, they can be harmful to the CNS.

Besides being extensively used in research and review papers[27,28], the term reactive gliosis starts facing a new concept about microglial morphology, and the fact is that this term may disappear soon. The reason for this is that recent and impeccable studies have shown that microglia cannot be classified as simple as "resting" or "activated" microglia due to the fact that these cells can present multiple different states, morphology, and physiological function, and assume different characteristics that might change according to the brain area, sex, species, and several other factors[29,30].

A recently published article analyzed microglial cells in multiple periods (p7, p15, p22, and adult), diverse brain regions (cerebellum, primary somatosensory cortex, substantia nigra, cochlear nucleus, dentate gyrus, and frontal cortex), and in several conditions (healthy, Alzheimer's disease, and ovactomerized) in mice[29]. The authors have shown that different brain regions present a well-differentiated microglial morphology in adult mice; besides microglial developmental trajectories are similar between brain regions, in neonates (p7) and weaning (p22), they present higher similarities to adult morphology; frontal cortex and dentate gyrus of the hippocampus are definitely the brain regions where we can identify the biggest changes in microglia; and that there is not only a specificity regarding the morphology according to the brain region, but there is a sexual dysmorphism, which affects the production of estrogen during puberty with consequences in adult life. All these results together shed light on reviewing the term reactive gliosis and re-evaluating microglial mechanisms according to this vast pool of possible phenotypes (Figure 1).

A simplistic view such as microglia is "resting" or "activated" is no longer the best way of referring to the multiple phenotypes seen in microglia morphology and function in different brain regions, across several different conditions, and when looking at sex-specific differences too.

MICROGLIA IN ALZHEIMER'S DISEASE AND RELATED CONDITIONS

A more specific and sensitive discussion about microglia biomarkers in Alzheimer's disease has emerged[31]. It is known that Alzheimer's disease is the most common neurodegenerative disease and type of dementia, being determined clinically and in research by the excessive aggregation of extracellular amyloid-beta (Aβ) peptide and by the presence of neurofibrillary tangles, which are formed due to the hyperphosphorylation of the Tau protein, and these two main features would contribute to a progressive cognitive decline with the development of memory loss in more advanced stages of the disease[32,33]. Genetic causes of Alzheimer's disease correspond to 5% of

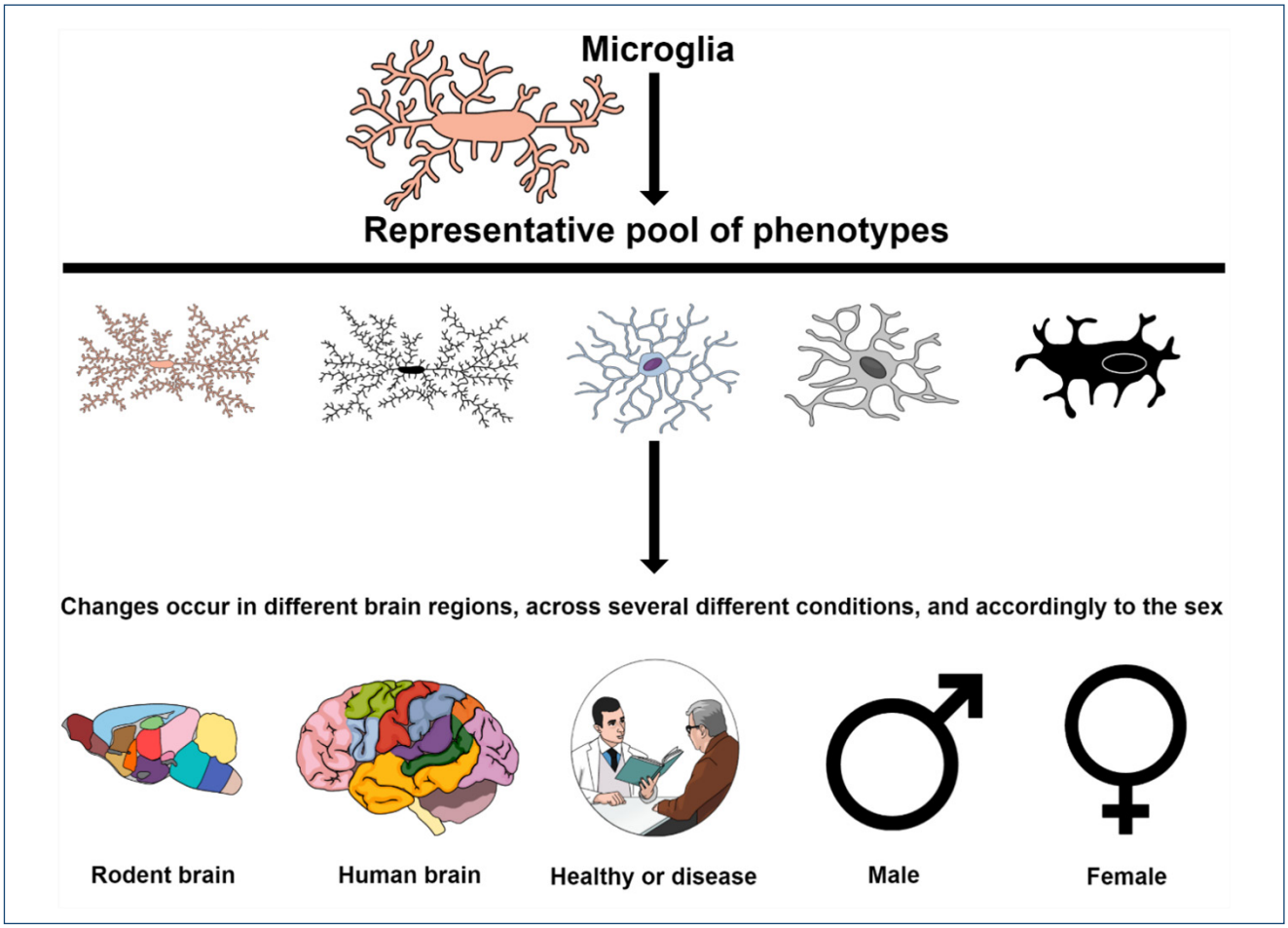

Figure 1. Representative pool of microglial phenotypes. Microglial cells present a vast pool of phenotypes that might change according to the species, brain region, condition, and sex.

the total cases, while the vast majority of the cases are related to the sporadic form of it. However, there is a common feature present in all types of dementia, that is, the presence of inflammation mediated by excessive activation of microglia and astrocytes[34].

This cognitive decline and memory loss mediated by brain inflammation is seen not only in Alzheimer's disease, but also in Alzheimer's-like pathologies, such as when there is excessive contact with air pollution[35], in type 2 diabetes mellitus[36], obesity[37,38], or even in the offspring born from gestational diabetes[39,40], among others. The fact is that a pro-inflammatory brain state is always present in cognitive impairment, with memory loss being the only common thing between all types of dementia and Alzheimer's-related pathologies. Thus, investigating the potential therapeutics of microglial interventions is essential.

CONCLUSION

Microglia are crucial for modulating cognition, memory, behavior, gene expression, oxidative stress, and inflammation. The vast pool of phenotypes exhibited by microglia brings new insights in finding specific pools of microglia that could be targeted into specific neurodegenerative diseases.

AUTHORS' CONTRIBUTIONS

RALDS: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **RCC:** Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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In the manuscript "Impressions of the chronic 900-MHz electromagnetic field in the prenatal period on Purkinje cells in male rat pup cerebella: is it worth mentioning?", https://doi.org/10.1590/1806-9282.20220893, published in the Rev Assoc Med Bras. 2022;68(10): 1383-1388, on page 1383:

Where it reads:
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https:// doi.org/10.1590/1806-9282.20221163ERRATUM

In the manuscript "Effects of hepatitis C virus genotypes and viral load on glucose and lipid metabolism after sustained virological response with direct-acting antivirals", https:// doi.org/10.1590/1806-9282.20221163, published in the Rev Assoc Med Bras. 2023;69(5):e20221163:

Pages 5 and 6 on Table 2, where it reads:

Table 2. Analysis of the homeostasis model assessment-insulin resistance index, homeostasis model assessment-β cell index, TyG index, and HbA1c in relation to viral characteristics.

	HOMA-IR pretreatment Mean (SD)	HOMA-IR pretreatment 95%CI	HOMA-IR SVR Mean (SD)	HOMA-IR SVR 95%CI	p-value		HOMA-β pretreatment Mean (SD)	HOMA-β pretreatment 95%CI	HOMA-β SVR Mean (SD)	HOMA-β SVR 95%CI	p-value
Genotypes (G)						**Genotype (G)**					
Genotype 1 (146)	2.42 (±1.76)	2.13–2.72	2.65 (±1.87)	2.37–2.99	0.079	Genotype 1 (146)	89.29 (±62.85)	79.42–99.37	103.97 (±79.97)	91.27–117.30	0.028
G1 a (67)	2.36 (±1.60)	2.02–2.79	2.53 (±1.83)	2.12–3.00	0.457	G1 a (67)	85.81 (±53.67)	73.25–99.82	98.27 (±91.03)	79.46–121.54	0.223
G1 b (78)	2.63 (±2.44)	2.14–3.19	3.06 (±3.76)	2.34–3.94	0.098	G1 b (78)	91.12 (±62.94)	77.56–105.43	104.16 (±61.73)	90.66–117.47	0.086
Genotype 2 (38)	2.75 (±2.03)	2.17–3.40	2.53 (±1.80)	2.00–3.10	0.323	Genotype 2 (38)	79.34 (±54.38)	63.93–99.16	72.78 (±43.84)	59.75–87.58	0.516
Genotype 3 (87)	2.56 (±2.33)	2.09–3.14	2.96 (±3.50)	2.34–3.80	0.117	Genotype 3 (87)	87.51 (±56.82)	76.00–101.42	99.61 (±57.16)	87.76–11.01	0.058
Viral load (VL)	2.51 (±1.80)	2.22–2.84	2.68 (±2.05)	2.36–3.06	0.300	**Viral load** (VL)	87.85 (±65.71)	76.86–99.40	98.62 (±79.30)	86.28–113.14	0.147
≥600,000 – High (132)	2.51 (±1.81)	2.22–2.85	2.69 (±2.06)	2.34–3.07	0.294	≥600,000 – High (132)	87.40 (±65.76)	77.61–98.48	99.23 (±79.29)	86.33–113.98	0.108
≤599,999 – Low (131)	2.50 (±1.87)	2.09–2.93	2.76 (±1.80)	2.33-3.16	0.239	≤599,999 – Low (131)	92.17±70.27	77.17–108.12	110.80±94.10	90.41–134.05	0.089
Genotype 1 (G1)	2.31 (±1.66)	1.96–2.77	2.52 (±1.98)	2.10–3.07	0.148	**Genotype 1 (G1)**	85.15±52.49	72.99–99.07	96.34±60.84	82.02–110.66	0.050
High VL (76)	2.54 (±1.74)	2.10–3.04	2.60 (±2.38)	2.38–3.29	0.813	High VL (76)	82.02±59.63	66.46–98.06	81.67±49.57	68.98–94.74	0.966
Low VL (66)	2.67 (±2.57)	2.11–3.32	3.00 (±3.55)	2.35–3.85	0.181	Low VL (66)	87.40±53.29	75.30–100.29	99.14±57.62	86.12–114.00	0.080
Non-1 Genotype (GN1)						**Non-1 Genotype (GN1)**					
High VL (55)						High VL (55)					
Low VL (70)						Low VL (70)					
	TyG pretreatment Mean (SD)	**TyG pretreatment 95%CI**	**TyG RVS Mean (SD)**	**TyG RVS 95%CI**	**p-value**		**HbA1c pretreatment Mean (SD)**	**HbA1c pretreatment 95%CI**	**HbA1c RVS Mean (SD)**	**HbA1c RVS 95%CI**	**p-value**
Genotypes (G)						**Genotypes**					
Genotype 1 (144)	4.56 (±0.26)	4.52–4.60	4.58 (±0.27)	4.53–4.62	0.510	Genotype 1 (146)	5.63 (±0.92)	5.49–5.80	5.54 (±0.78)	5.42–5.69	0.144
G1 a (66)	4.58 (±0.24)	4.51–4.63	4.56 (±0.31)	4.49–4.64	0.651	G1 a (68)	5.78 (±1.02)	5.54–6.04	5.69 (±0.98)	5.46–5.94	0.391
G1 b (74)	4.51 (±0.25)	4.46–4.57	4.57 (±0.30)	4.51–4.64	0.017	G1 b (78)	5.59 (±0.77)	5.43–5.79	5.47 (±0.66)	5.33–5.61	0.155
Genotype 2 (37)	4.63 (±0.31)	4.53–4.73	4.64 (±0.29)	4.54–4.74	0.771	Genotype 2 (38)	5.83 (±0.94)	5.56–6.15	5.91 (±1.05)	5.59–6.27	0.400
Genotype 3 (83)	4.48 (±0.23)	4.43–4.52	4.54 (±0.33)	4.48–4.62	0.024	Genotype 3 (87)	5.85 (±1.06)	5.65–6.11	5.54 (±1.08)	5.34–5.79	0.001
Viral load (VL)	4.55 (±0.27)	4.51–4.60	4.56 (±0.31)	4.51–4.62	0.639	**Viral load (VL)**	5.71 (±0.91)	5.57–5.88	5.61 (±1.02)	5.44–5.80	0.117
≥600,000 – High (128)	4.55 (±0.27)	4.50–4.60	4.56 (±0.31)	4.50–4.62	0.634	≥600,000 – High (132)	5.72 (±0.92)	5.56–5.88	5.61 (±1.03)	5.45–5.80	0.119
≤599,999 – Low (127)	5.56 (±0.26)	4.50–4.62	4.55 (±0.27)	4.49–4.61	0.813	≤599,999 – Low (131)	5.68 (±0.97)	5.47–5.92	5.53 (±0.88)	5.35–5.75	0.108
Genotype 1 (G1)	4.56 (±0.26)	4.50–4.63	4.59 (±0.26)	4.52–4.65	0.389	**Genotype (G1)**	5.58 (±0.83)	5.40–5.79	5.52 (±0.63)	5.37–5.67	0.462
G1+High VL (76)	4.54 (±0.28)	4.46–4.62	4.57 (±0.37)	4.47–4.67	0.323	G1+High VL (76)	5.78 (±0.84)	5.58–6.01	5.73 (±1.20)	5.44–6.05	0.609
G1+Low VL (66)	4.51 (±0.26)	4.45–4.57	4.57 (±0.29)	4.51–4.64	0.039	G1+Low VL (66)	5.90 (±1.14)	5.65–6.19	5.59 (±0.99)	5.38–5.85	0.005
Non-1 Genotype (GN1)						**Non-1 Genotype (GN1)**					
GN1+High VL (55)						GN1+High VL (55)					
GN1+Low VL (68)						GN1+Low VL (70)					

Continue...

Table 2. Continuation.

	HOMA-IR pretreatment Mean (SD)	HOMA-IR pretreatment 95%CI	HOMA-IR SVR Mean (SD)	HOMA-IR SVR 95%CI	p-value		HOMA-β pretreatment Mean (SD)	HOMA-β pretreatment 95%CI	HOMA-β SVR Mean (SD)	HOMA-β SVR 95%CI	p-value
Genotypes (G)						**Genotype (G)**					
Genotype 1 (146)	2.42 (±1.76)	2.13–2.72	2.65 (±1.87)	2.37–2.99	0.079	Genotype 1 (146)	89.29 (±62.85)	79.42–99.37	103.97 (±79.97)	91.27–117.30	0.028
G1 a (67)	2.36 (±1.60)	2.02–2.79	2.53 (±1.83)	2.12–3.00	0.457	G1 a (67)	85.81 (±53.67)	73.25–99.82	98.27 (±91.03)	79.46–121.54	0.223
G1 b (78)	2.63 (±2.44)	2.14–3.19	3.06 (±3.76)	2.34–3.94	0.098	G1 b (78)	91.12 (±62.94)	77.56–105.43	104.16 (±61.73)	90.66–117.47	0.086
Genotype 2 (38)	2.75 (±2.03)	2.17–3.40	2.53 (±1.80)	2.00–3.10	0.323	Genotype 2 (38)	79.34 (±54.38)	63.93–99.16	72.78 (±43.84)	59.75–87.58	0.516
Genotype 3 (87)	2.56 (±2.33)	2.09–3.14	2.96 (±3.50)	2.34–3.80	0.117	Genotype 3 (87)	87.51 (±56.82)	76.00–101.42	99.61 (±57.16)	87.76–11.01	0.058
Viral load (VL)	2.51 (±1.80)	2.22–2.84	2.68 (±2.05)	2.36–3.06	0.300	**Viral load** (VL)	87.85 (±65.71)	76.86–99.40	98.62 (±79.30)	86.28–113.14	0.147
≥600,000 – High (132)	2.51 (±1.81)	2.22–2.85	2.69 (±2.06)	2.34–3.07	0.294	≥600,000 – High (132)	87.40 (±65.76)	77.61–98.48	99.23 (±79.29)	86.33–113.98	0.108
≤599,999 – Low (131)	2.50 (±1.87)	2.09–2.93	2.76 (±1.80)	2.33-3.16	0.239	≤599,999 – Low (131)	92.17±70.27	77.17–108.12	110.80±94.10	90.41–134.05	0.089
Genotype 1 (G1)	2.31 (±1.66)	1.96–2.77	2.52 (±1.98)	2,10–3.07	0.148	**Genotype 1 (G1)**	85.15±52.49	72.99–99.07	96.34±60.84	82.02–110.66	0.050
High VL (76)	2.54 (±1.74)	2.10–3.04	2.60 (±2.38)	2.38–3.29	0.813	High VL (76)	82.02±59.63	66.46–98.06	81.67±49.57	68.98–94.74	0.966
Low VL (66)	2.67 (±2.57)	2.11–3.32	3.00 (±3.55)	2.35–3.85	0.181	Low VL (66)	87.40±53.29	75.30–100.29	99.14±57.62	86.12–114.00	0.080
Non-1 Genotype (GN1)						**Non-1 Genotype (GN1)**					
High VL (55)						High VL (55)					
Low VL (70)						Low VL (70)					
	TyG pretreatment Mean (SD)	**TyG pretreatment 95%CI**	**TyG RVS Mean (SD)**	**TyG RVS 95%CI**	**p-value**		**HbA1c pretreatment Mean (SD)**	**HbA1c pretreatment 95%CI**	**HbA1c RVS Mean (SD)**	**HbA1c RVS 95%CI**	**p-value**
Genotypes (G)						**Genotypes**					
Genotype 1 (144)	4.56 (±0.26)	4.52–4.60	4.58 (±0.27)	4.53–4.62	0.510	Genotype 1 (146)	5.63 (±0.92)	5.49–5.80	5.54 (±0.78)	5.42–5.69	0.144
G1 a (66)	4.58 (±0.24)	4.51–4.63	4.56 (±0.31)	4.49–4.64	0.651	G1 a (68)	5.78 (±1.02)	5.54–6.04	5.69 (±0.98)	5.46–5.94	0.391
G1 b (74)	4.51 (±0.25)	4.46–4.57	4.57 (±0.30)	4.51–4.64	0.017	G1 b (78)	5.59 (±0.77)	5.43–5.79	5.47 (±0.66)	5.33–5.61	0.155
Genotype 2 (37)	4.63 (±0.31)	4,53–4.73	4.64 (±0.29)	4.54–4.74	0.771	Genotype 2 (38)	5.83 (±0.94)	5.56–6.15	5.91 (±1.05)	5.59–6.27	0.400
Genotype 3 (83)	4.48 (±0.23)	4,43–4.52	4.54 (±0.33)	4.48–4.62	0.024	Genotype 3 (87)	5.85 (±1.06)	5.65–6.11	5.54 (±1.08)	5.34–5.79	0.001
Viral load (VL)	4.55 (±0.27)	4,51–4.60	4.56 (±0.31)	4.51–4.62	0.639	**Viral load (VL)**	5.71 (±0.91)	5.57–5.88	5.61 (±1.02)	5.44–5.80	0.117
≥600,000 – High (128)	4.55 (±0.27)	4,50–4.60	4.56 (±0.31)	4.50–4.62	0.634	≥600,000 – High (132)	5.72 (±0.92)	5.56–5.88	5.61 (±1.03)	5.45–5.80	0.119
≤599,999 – Low (127)	5.56 (±0.26)	4.50–4.62	4.55 (±0.27)	4.49–4.61	0.813	≤599,999 – Low (131)	5.68 (±0.97)	5.47–5.92	5.53 (±0.88)	5.35–5.75	0.108
Genotype 1 (G1)	4.56 (±0.26)	4.50–4.63	4.59 (±0.26)	4.52–4.65	0.389	**Genotype (G1)**	5.58 (±0.83)	5.40–5.79	5.52 (±0.63)	5.37–5.67	0.462
G1+High VL (76)	4.54 (±0.28)	4.46–4.62	4.57 (±0.37)	4.47–4.67	0.323	G1+High VL (76)	5.78 (±0.84)	5.58–6.01	5.73 (±1.20)	5.44–6.05	0.609
G1+Low VL (66)	4.51 (±0.26)	4.45–4.57	4.57 (±0.29)	4.51–4.64	0.039	G1+Low VL (66)	5.90 (±1.14)	5.65–6.19	5.59 (±0.99)	5.38–5.85	0.005
Non-1 Genotype (GN1)						**Non-1 Genotype (GN1)**					
GN1+High VL (55)						GN1+High VL (55)					
GN1+Low VL (68)						GN1+Low VL (70)					

HOMA-IR: homeostasis model assessment-insulin resistance; HOMA-β: homeostasis model assessment-β cell; TyG: product of triglycerides and glucose; HbA1c: glycated hemoglobin; SD: standard deviation; SVR: sustained viral response; CI: confidence interval.

Pages 5 and 6 on Table 2, it should read:

Table 2. Analysis of the homeostasis model assessment-insulin resistance index, homeostasis model assessment-β cell index, TyG index, and HbA1c in relation to viral characteristics.

	HOMA-IR pretreatment Média (SD)	HOMA-IR pretreatment IC 95%	HOMA-IR SVR Mean (SD)	HOMA-IR SVR IC 95%	*p-valor*		HOMA-β pretreatment Mean (SD)	HOMA-β pretreatment IC 95%	HOMA-β SVR Mean (SD)	HOMA-β SVR IC 95%	*p-valor*
Genotypes (G)						**Genotype (G)**					
Genotype 1 (146)	2.42 (±1.76)	2.13 – 2.72	2.65 (±1.87)	2.37 – 2.99	0.079	Genotype 1 (146)	89.29 (±62.85)	79.42-99.37	103.97(±79.97)	91.27-117.30	0.028
G1 a (67)	2.36 (±1.60)	2.02 – 2.79	2.53 (±1.83)	2.12 – 3.00	0.457	G1 a (67)	85.81 (±53.67)	73.25-99.82	98.27(±91.03)	79.46-121.54	0.223
G1 b (78)	2.63 (±2.44)	2.14 – 3.19	3.06 (±3.76)	2.34 – 3.94	0.098	G1 b (78)	91.12 (±62.94)	77.56-105.43	104.16(±61.73)	90.66-117.47	0.086
Genotype 2 (38)	2.75 (±2.03)	2.17 – 3.40	2.53 (±1.80)	2.00 – 3.10	0.323	Genotype 2 (38)	79.34(±54.38)	63.93-99.16	72.78(±43.84)	59.75-87.58	0.516
Genotype 3 (87)	2.56 (±2.33)	2.09 – 3.14	2.96 (±3.50)	2.34 – 3.80	0.117	Genotype 3 (87)	87.51(±56.82)	76.00-101.42	99.61(±57.16)	87.76-11.01	0.058
Viral load (VL)						**Viral load (VL)**					
≥ 600000 – High (132)	2.51 (±1.80)	2.22 – 2.84	2.68 (±2.05)	2.36 – 3.06	0.300	≥ 600000 – High (132)	87.85(±65.71)	76.86-99.40	98.62(±79.30)	86.28-113.14	0.147
≤ 599999- Low (131)	2.51 (±1.81)	2.22 – 2.85	2.69 (± 2.06)	2.34 – 3.07	0.294	≤ 599999- Low (131)	87.40(±65.76)	77.61-98.48	99.23(±79.29)	86.33-113.98	0.108
Genótipo 1 (G1)						**Genotype 1 (G1)**					
High VL (76)	2.50 (±1.87)	2.09 – 2.93	2.76 (±1.80)	2.33 -3.16	0.239	High VL (76)	92.17 ±70.27	77.17-108.12	110.80 ± 94.10	90.41-134.05	0.089
Low VL (66)	2.31 (±1.66)	1.96 – 2.77	2.52 (±1.98)	2,10 – 3.07	0.148	Low VL (66)	85.15±52.49	72.99 - 99.07	96.34 ± 60.84	82.02 –110.66	0.050
Non-1 Genotype (GN1)						**Non-1 Genotype (GN1)**					
High VL (55)	2.54 (±1.74)	2.10 – 3.04	2.60 (±2.38)	2.38 – 3.29	0.813	High VL (55)	82.02 ±59.63	66.46 - 98.06	81.67±49.57	68.98 – 94.74	0.966
Low VL (70)	2.67 (±2.57)	2.11 – 3.32	3.00 (±3.55)	2.35 – 3.85	0.181	Low VL (70)	87.40± 53.29	75.30– 100.29	99.14±57.62	86.12– 114.00	0.080
	TyG pretreatment Média (SD)	**TyG pretreatment IC 95%**	**TyG RVS Mean (SD)**	**TyG RVS IC 95%**	***p-valor***		**HbA1c pretreatment Média (SD)**	**HbA1c pretreatment IC 95%**	**HbA1c RVS Mean (SD)**	**HbA1c RVS IC 95%**	***p-valor***
Genotypes (G)						**Genotypes**					
Genotype 1 (144)	4.56(±0.26)	4.52 – 4.60	4.58 (±0.27)	4.53 – 4.62	0.510	Genotype 1 (146)	5.63 (±0.92)	5.49 – 5.80	5.54 (±0.78)	5.42 – 5.69	0.144
G1 a (66)	4.58 (±0.24)	4.51 – 4.63	4.56 (±0.31)	4.49 – 4.64	0.651	G1 a (68)	5.78 (±1.02)	5.54 – 6.04	5.69 (±0.98)	5.46 – 5.94	0.391
G1 b (74)	4.51 (±0.25)	4.46 – 4.57	4.57 (±0.30)	4.51 – 4.64	0.017	G1 b (78)	5.59 (±0.77)	5.43 – 5.79	5.47 (±0.66)	5.33 – 5.61	0.155
Genotype 2 (37)	4.63 (±0.31)	4,53 – 4.73	4.64 (±0.29)	4.54 - 4.74	0.771	Genotype 2 (38)	5.83 (±0.94)	5.56 – 6.15	5.91 (±1.05)	5.59 – 6.27	0.400
Genotype 3 (83)	4.48 (±0.23)	4,43 – 4.52	4.54 (±0.33)	4.48 – 4.62	0.024	Genotype 3 (87)	5.85 (±1.06)	5.65 – 6.11	5.54 (±1.08)	5.34 – 5.79	0.001
Viral load (VL)						**Viral load (VL)**					
≥ 600000 High (128)	4.55 (±0.27)	4,51 – 4.60	4.56 (±0.31)	4.51 – 4.62	0.639	≥ 600000 High (132)	5.71 (±0.91)	5.57 – 5.88	5.61 (±1.02)	5.44 – 5.80	0.117
≤ 599999 Low (127)	4.55 (±0.27)	4,50 – 4.60	4.56 (±0.31)	4.50 – 4.62	0.634	≤ 599999 Low (131)	5.72(±0.92)	5.56 – 5.88	5.61 (±1.03)	5.45 – 5.80	0.119
Genotype 1 (G1)						**Genotype (G1)**					
G1 + High VL (76)	5.56 (±0.26)	4.50 – 4.62	4.55 (±0.27)	4.49 – 4.61	0.813	G1 + High VL (76)	5.68 (±0.97)	5.47 – 5.92	5.53 (±0.88)	5.35 – 5.75	0.108
G1 + Low VL (66)	4.56 (±0.26)	4.50 – 4.63	4.59 (±0.26)	4.52 – 4.65	0.389	G1 + Low VL (66)	5.58 (±0.83)	5.40 – 5.79	5.52 (±0.63)	5.37 – 5.67	0.462
Non-1 Genotype (GN1)						**Non-1 Genotype(GN1)**					
GN1 + High VL (55)	4.54 (±0.28)	4.46 – 4.62	4.57 (±0.37)	4.47 - 4.67	0.323	GN1 + High VL (55)	5.78 (±0.84)	5.58 – 6.01	5.73 (±1.20)	5.44 - 6.05	0.609
GN1 + Low VL (68)	4.51 (±0.26)	4.45 – 4.57	4.57 (±0.29)	4.51 - 4.64	0.039	GN1 + Low VL (70)	5.90 (±1.14)	5.65 – 6.19	5.59 (±0.99)	5.38 – 5.85	0.005

HOMA-IR: homeostasis model assessment-insulin resistance; HOMA-β: homeostasis model assessment-β cell; TyG: product of triglycerides and glucose; HbA1c: glycated hemoglobin; SD: standard deviation; SVR: sustained viral response; CI: confidence interval.