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CONTENTS

CONTENTS
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ORIGINAL ARTICLE

Cardiology

Regulatory Synchronization of Hemodynamics of the Heart and Brain in Norm

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Abstract

By catheterization, the integral indicators of synchronization and interaction of blood flows, designated as "venous and arterial boluses," were obtained, studied and analyzed in healthy people on the pathway: right heart–lung–left heart. It has been confirmed that the complete CC of the BB from RA to the ejection from the LV has a length equal to two completed heart contraction cycles. Interaction of venous and arterial boluses, with differentiated external myocardial exposure, along the path "venous block of the heart–lung–arterial block of the heart," forms averaged (compensated by the flexible septum) variable pressure values between the stages of intracardiac routes of BBs (unidirectional, synchronous, but spread in the space). The complex of these pressure values creates an intracardiac pressure balance at the border of high- and low-energy processes of the heart. We defined the sequential dynamics of these values as CMIP. Our mathematical and graphical data demonstrate the presence of direct and inversecardio-cerebral wave connections, where the waveguides are the vessels of entry and exit from the skull. We believe that CMIP is a universal, central rhythmic process, a regulator that determines the sequence and intensity of the CC phases, HR, and synchronous nervous and wave effects on brain structures. The modulating effect of CMIP on brain structures, providing some sensory-motor reactions, behavioral functions and forms of behavior, occurs outside the realm of consciousness. Our data suggest that the modulating effect of CMIP on the brain is carried out not only along the neural pathways, but also by the vascular wave structures that combine the heart and brain into a single hydrodynamic structure with phase-varying volume and configuration, as well as variable patterns of regulatory impulses. (**International Journal of Biomedicine. 2019;9(4):281-286.**)

Key Words: cardiac cycle • hemodynamic parameters • cardio-cerebral synchronization • ECG

Abbreviations

Ao, aorta; **AV**, aortic valve; **ANS**, autonomic nervous system; **BB**, "bolus" of the blood; **BP**, blood pressure; **CaS**, carotid siphon; **CC**, cardiac cycle; **CS**, coronary sinus; **CMIP**, cardiac mean integral pressure; **DP**, diastolic pressure; **EF**, ejection fraction; **HR**, heart rate; **IJV**, internal jugular vein; **IVS**, interventricular septum; **LV**, left ventricle; **MV**, mitral valve; **PV**, pulmonary valve; **PPW,** peripheral pulse wave; PW, pulse wave; **PWV**, pulse wave velocity; **RV**, right ventricle; **RA**, right atrium; **RHV**, right hepatic vein*;* **SC**, systemic circulation; **SS,** sigmoid sinus; **SV**, stroke volume; **TV**, tricuspid valve.

Basic Part

The purpose of this study was to determine the mechanisms of CMIP formation and its interaction with brain structures.

In previous articles,[1-9] we have given the hemodynamic indices obtained by catheterization in various vascular areas, as well as the relationship between parameters of the cerebral, central and peripheral blood flow analyzed by methods of mathematical statistics. The results we present (Table 1) were obtained by synchronization of ECG, PPW, CMIP and SS data. The SS curve, in contrast to the previous ones (constructed by average values), is a real curve obtained during the examination of a 43-year-old male patient, the combined results of which made it possible to exclude the suspected diagnosis. The

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results were obtained with the subject in the supine position (in a position perpendicular to the vector of gravity when its influence in all parts of the venous system is equal) and on exhalation (elimination of the effect of changes in intrathoracic pressure). The hemodynamic and metabolic parameters obtained by catheterization through a probe that was wedged in the upper bulb of IJV are the wave and metabolic parameters of the sigmoid venous sinus of the brain (SS).

The information thesaurus of the SS venous blood flow does not contain extracranial blood admixture but contains, along with other parameters, hemodynamic parameters and metabolites of blood flows from the cavernous sinus (including the dynamics of CaS impulses), transverse sinus, and straight sinus (vein collector from deep brain structures), and pressor (wave) effect of the dynamics of the pial chamber. Note that for fast processes that exceed the inertial characteristics of the outgoing flows, the cranial cavity is considered as a sealed formation.

We showed the presence of direct synchronized hemodynamic cardio-cerebral connections in previous[1,3,6-8] articles. It was established that the active processes in LV and Ao (systolic and pulse pressure) have no relationship to SS pulse pressure, providing perfusion dynamics of the brain starting from the Ao mean pressure. SS pressure levels are linked (Table 1)[1] with the LV ejection period, the achievement of maximum pressure in the LV-Ao system at the opening of AV, and the formation of a maximum pressure at the vascular entrances into the cranial cavity. As is known, LV systole creates a pulse dissipative wave structure in the aortic trunk, reaching all points of the body before the closing of AV.[10] We believe that the negative correlation between the pressure levels in SS, LV and Ao is explained by the damping effect of CaSs (located in the cavernous sinus), which change the internal lumen under the influence of the incoming Ao wave impulse.

CaSs are conductors of the wave structures of the heart (informational characteristics of different levels of control) interfering with the cavernous venous sinus of the brain. In norm, the changes in the internal lumen of CaSs eliminate pressure values, which exceed the Ao mean pressure, providing optimal levels of perfusion pressure in the brain.. The excess pressor effect of intravascular pressure is utilized by the ligamentous apparatus of CaSs and transfer pressor effect on the venous blood of the cavernous sinus (connected through the sinus petrosus superior and inferior with SS), which we recorded at the exit from the venous system of the brain as the SS wave synchronous with "X"— collapse of the RA (i.e., LV systole) (Table Norm).[6] In other words, this is the energetic, active phase, during which the synchronized wave impulses of LV and intracranial venous pressure have a unidirectional vector opposite to the vector of the venous blood flow dynamics (IJV-RA).

Perfusion dynamics of the brain is provided starting of the Ao mean pressure level (Table Norm)[1], positively conjugated to SS from the DP level, which is an indicator of the formation of a single hydrodynamic system in the phase of diastolic evolution (from the medium to diastolic pressure): Ao—vessels entering the skull—SS-IJV-RA (Table Norm[1]). This explains the positive conjugation of the diastolic evolution

of the intracranial venous bed with aortic diastolic evolution. Achieving the minimum values of the Ao-SS-RA gradient is replaced by LV systole. During one CC, the relationship Ao-SS pressure goes through a linking cycle: "-", "0", "+".

Thus, the fixation point of the parameters of intracranial venous blood flow (SS), which includes the information wave resources from the actual LV systole (CASs and pial chamber) and venous outflow from the brain, is a stage of a single hemodynamic pathway (without valves all along the way from the metabolic fields of the brain to the tricuspid valve), regulated by wave impulses of vessels of the inflow and outflow of the brain. We gave a preliminary analysis of the formation and interaction of BBs (IC-1, IC-2 and CMIP) in previous works.[6-9]

Minimal CMIP, influencing the formation of the RV spheroid, determines the pressure level of the "trigger point,"[9] which forms the level of the PV valve opening pressure and RVEF into the lung system, where there is a final transformation (hemodynamic and metabolic) of the "venous bolus" of the right heart into an "arterial bolus" passing into the left parts of the heart. Maximal CMIP determines the pressurized level of the opening of AV and LVEF going to the exchange zones. The indicated sequentially synchronized stage of CC (Ao-RV) constitutes a pathway, which a pulse wave impulse passes: LV-Ao-CASs, pial chamber-SS-SJV-RA-RV (with opened TV and closed MV).

Thus, during one phase of CC (LV contraction), the pulse wave passes through the hemodynamic pathway: LV–RV. CMIP is simultaneously 1) an integral derivative of the total interaction between all hemodynamic paths (incoming, transit and outgoing) and the structural elements of the heart; and 2) a derivative of the pressor factor of the myocardium, which regulates the conditions of formation, interaction, synchronization, and vectors of passage with BBs of the right and left parts of the heart of the corresponding tracts.

We believe that the primary link in the sequence of intracardiac transformations of BBs is the pressor effect of functional syncytium, involving the cascade dynamics of the synchronous interaction of different zones of the myocardium, valve, and fibrous and ligamentous apparatus of the heart, creating an extended synchronized pressor-depressive effect of the myocardium as a whole on the contents of the heart chambers during CC. With the trigger type of response, syncytium has synchronized differences in the developed power and the sequence of contraction in different areas of the myocardium. The result is differences in the initial pressor parameters for the formation of BBs, giving them inertia and a vector. Boluses 1 and 2, simultaneously and unidirectionally passing intracardiac tracts, have differences in the power of the pressor effect of the myocardium of the venous and arterial parts of the heart (the value that forms the «bolus» of pressure), as well as other systematic coordinate differences (density, viscosity, gas composition, electromagnetic properties, etc.). Boluses 1 and 2 interact through anatomically separated formations with different plasticity properties: a section of the upper RA wall (which is not an interatrial septum) and the ascending part of Ao; RA and LA through the oval window and atrial septum; RA with LV through the membranous

portion of IVS; RV with LV through IVS and others. As a result, throughout CC in the zones of contact of BBs on the path "venous block of the heart–lung–arterial block of the heart," averaged (compensated by plastic septa) variable pressure values are formed between synchronous, but spatially separated stages of intracardiac traces "venous and arterial boluses" (between the right and left chambers of the heart). Their combination creates an intracardiac pressure balance that determines the state of "current equilibrium" on the border between high- and low-energy processes of the heart (right and left parts), the sequential dynamics of which are indicated on the graph: CMIP, in combination with BBs.[9]

We believe that at the borders and in the interaction zones, the combined pressor and other parameters of BBs, with the external influence of the myocardium, form a single regulatory structure that balances the intracardiac pressor imbalance. This structure is CMIP—a systemic, intracardiac hemodynamic regulator, which is an integral function of myocardial dynamics and BBs. We believe that the echocardiographic methods, visualized the amplitude fluctuations of the intracardiac formations, reflect fragments of the structure that forms the CMIP. In other words, the phase architectonics of the vibrating flexible, structural heart formations forms the intracardiac structure of a variable configuration, which is the physical basis for the formation of integral CMIP.

In our opinion, CMIP is a nonlinear, multidimensional regulatory structure of the heart as a whole, which distributes and differentiates in intensity and duration components (pressor, electrical, magnetic, rheological, and other), the changes of which inevitably cause a cascade of changes in the subsystems. CMIP, as a system regulator of a higher regulatory level, the first in a hierarchy of regulators, exerts a dominant regulatory influence on parameters (pressure, speed, vector, etc.), ensuring the synchronism of the dynamics of Boluses 1 and 2.

The critical threshold value of the change in CMIP parameters— derived from the dynamics of the myocardium as a whole and the whole set of qualities of the hemodynamic flows of the heart, including the total information thesaurus of the large and small circles of the systemic blood flow— requires a significantly larger number of changes in the input parameters to change systemic stability than each bolus separately; systemic stability is an integral indicator (and regulator) of the next lower level, the second, in the hierarchy of systemic regulatory levels (Boluses 1 and 2). In other words, CMIP, as a system, has greater stability than each component controlled by it, determining their parametric stability and intrasystem balance. The intensity of the effect of the CC phases on the modulation of the activity of brain structures, due to the afferentation level from mechanoreceptors and baroreceptors of the heart and blood vessels,[11] in our opinion, also depends on the direct action of the LV-Ao wave pulses (information patterns consisting of frequency and intensity parameters) on brain structure. We consider direct evidence of this relationship are the results of our correlation analysis and graphical indicators of the ratio of CMIP and SS (Table 1), which coincide in the main peak values and

profiles of CMIP (integral indicator of cardiac activity) and SS, the information thesaurus of which includes the final hemodynamic and metabolic indicators of brain metabolism. Organ hemodynamics (the third regulatory level), the blood flows of which are combined into RA, transforms into a single hemodynamic formation a venous blood bolus, the total thesaurus of which includes the entire total information volume (hemodynamic and metabolic) of SC. We consider it necessary to note that in addition to metabolites, all flows entering RAs include hormones from the endocrine glands (IJV from the pituitary and thyroid glands; IVC from the adrenal glands, genital organs, etc.; RHV in transit through the liver from the pancreas, etc.), where they are mixed with myocardial metabolites from the actual systole (CS), structuring the venous bolus.

The combination of metabolites, gas functional[4] and hormones from the exchange zones is an informational result of the current state of homeostasis, changing in accordance with the needs of the mechanisms of homeostatic adaptation (i.e., status and needs of homeostasis). In this paper, we do not consider in detail the mechanisms of intracardiac transformation of BBs. BB, undergoing the transformation of the complete CC—the right heart ("venous bolus"), the lung exchange field (including the thesaurus of the pulmonary circulation), the left heart ("arterial bolus")—is a formed structure of the second regulatory level and goes during the LV systole into the vascular bed of the exchange fields, as the managing homeostatic complex (wave and metabolic). We believe that differences in the interpretation of the scatter of results obtained when determining SV (55ml-90ml) and LVEF (50%-80%)[12] are explained by a homeostatically adaptive contractile function of the heart. In other words, each myocardial contraction has its own initial regulatory mechanism: an integral variable 3rd regulatory level (current status of the homeostasis) that forms the venous bolus of RA. The set of its parameters forms the first regulatory level (CMIP) of the heart as a whole, which determines ("calibrates") the variation in the composition and volume of the second regulatory level (BBs of both circles of blood circulation) adequate to homeostatic needs, which goes to the exchange fields of large and small circles of blood flow. CMIP, composing an integral derivative of hemodynamics of the large and small circles of blood flow with the dynamics of constructive formations of the heart, and being the first hierarchical regulatory level, affects the third-order regulators by the second-order regulator: LV systolic BB (EF) and pulse wave variations. Thus is formed a closed cycle of direct and reverse wave relationships of the first through third hierarchical levels of the regulatory central (cardiac) and peripheral (organ) controls of the wave patterns of the body as a whole.

The idea of a central rhythmic process that determines the heart rhythm and synchronous behavioral (and electrophysiological) equivalents was advanced by Velden and Juris in 1975.[13] A distinction was made between the systolic and diastolic evoked potentials on the EEG[14,15] with the difference in the leads for the hemispheres of the brain and differences in synchronization with the pulse wave of the carotid artery. Many studies have shown the dependence

of behavioral reactions and EEG on HR, as well as the dependence of the sensorimotor reaction time on the CC phase,[15] including in patients with an artificial pacemaker.[16] The possibility of a direct effect of heart pulsations on brain tissue was emphasized,[15] the direct connection of which, through the influence of CC on intracranial venous pressure, is shown in Table 1. With a direct effect on brain tissue, in our opinion, rheological blood parameters are very significant (density and viscosity—functions of volume and fluidity), in the absence of the receptor apparatus, which we assigned to the primarily regulated functions of the homeostatic control.[1] Significant inhibition of cortical activity in the systole phase with hypertension was noted, with a decrease in the level of perception and cognitive abilities,[15] which Sandman et al. associated with "bar-receptor inhibition." At the same time, it was found that rapid responses of cerebral vessels to auditory signals were accompanied, depending on the CC phase, by changes in the intracranial blood volume—in response to stimuli given during the diastole.[17]

Many studies[18,19] have found changes in the cortical potentials of EEG caused by heart contraction under the influence of a conscious change in the vector of voluntary attention and additional motivation. In other words, cardio-cerebral synchronous processes, the initiator of which is CC, are registered. The interpretation of these relationships is based on ideas about the phase effect on cortical activity from baroreceptors and mechanoreceptors of the heart and blood vessels. The totality of our data, both mathematical and graphic, demonstrating the coincidence and mutual influence of the hemodynamic parameters of the heart and brain, allows us to supplement the concept of "adaptive modulation of the activity of mental functions with afferentation from the cardiovascular system" [11,20-22] with the fact of the presence of direct and reverse regulatory hemodynamic connections of the heart and brain. We consider the totality of these connections to be an autonomous (partially duplicating nervous regulation), direct, and feedback-wave regulatory channel. As an additional argument in favor of the double (nervous and wave) effect of CC on the modulation of brain structures (sensory and motor reactions), we consider changes in the motor response of the heart to an external impulse with a denervated heart,[23] as well as a change in the motor response of a transplanted (denervated) human heart to physical activity: increase (twice) in cardiac output and SV (>40%). Motor changes in the reaction of a denervated heart, devoid of afferent and efferent connections with the brain, in our opinion, confirm the presence of two partially independent, complementary pathways of the regulatory interaction of the heart and brain: hemodynamic and nervous channels. These changes indicate a deficiency of regulatory functions in combination: 1) autonomous cardiac and 2) wave (hemodynamic) regulatory mechanisms, in the absence of 3) central nervous influence, under the conditions of additional external stimuli.

In a previous publication,[9] we presented our ideas about the mechanism of autonomous regulation of CC through the pressor effect of intracardiac hemodynamics on the sinoatrial node. ANS, which provides sensory afferentation from the heart and blood vessels, has a low speed of nerve impulses

(the thinnest myelinated fibers of A-type, B-type and non-myelinated C-fibers): from 0.5 m/s to 18 m/s. PWV, which in contrast to the linear blood flow velocity increases with distance from the pulse generator (heart), is from 4 m/s to 12 m/s.[24] Thus, the rate of afferentation of nerve impulses from the baro- and mechanoreceptors of the heart and blood vessels along the ANS trunks is comparable to the speed of wave hemodynamic impulses, PW from the heart to the structures of the brain (and other organs). In other words, we establish the presence of two (duplicating and mutually complementary) ways of regulatory influence on the brain structures initiated by CC through the nervous and wave (hemodynamic) structures.

We believe that the data we have provided allow us to supplement and expand the semantic, theoretical, and practical content of Lacey's theory with new arguments. In particular, an increase in HR and BP, leading to an increase in afference from arterial baroreceptors, prevents the receipt of information from the environment into the projection zones of the cortex of the brain, thereby contributing to the optimization of cognitive processes.

Reduced baroreceptor inhibition (decreased HR and BP) leads to improved perception of external signals ("intake"). We attach particular importance to the synchronization of the CC phases and the alpha rhythm of the brain detected in depressive syndromes,[15] as well as their synchronization in the cerebral cortex of the occipital zones, which have no confirmed connection with the regulation of cardiac activity.[25,26] The aforementioned testifies, in our opinion, to a much larger, in comparison with existing views, range of influence of CC on the activity of the brain and mental derivatives.

We believe that a targeted study of the synchronization of the CC phases with variations in the rhythmic processes of brain activity (magnetograms, alpha rhythm and other waves) across the entire spectrum of mental norm and pathology is promising. The relevance of this vector of research is confirmed by the results of many works, in particular, the synchronization of the alpha rhythm with the CC phases, the differentiation of the phases and the CC rhythm in affective disorders, vital and neurotic depressions,[13,15,27] accounting for 30%-57% of visits to cardiologists, accompanied by sensations (often treated as senestopathies) in the projection zones of the heart, subjectively formulated as "pain, heaviness in the soul." It should be noted that other emotional states—emotional uplift, enthusiasm and many others, formulated as "connected with the soul"—are subjectively (both normal and pathological) also felt in these projection zones.

Conclusion

Thus, CMIP (multidimensional integral indicator of pressor dynamics of the myocardium during a complete CC) is a universal central rhythmic process, a regulator of the first hierarchical level, determining the sequence and intensity of the CC phases, HR and synchronous nervous and wave effects on brain structures. The action of this systemic regulator occurs outside the realm of consciousness, exerting a modulating effect on brain structures that provide some sensory-motor reactions, behavioral functions and conscious forms of behavior.
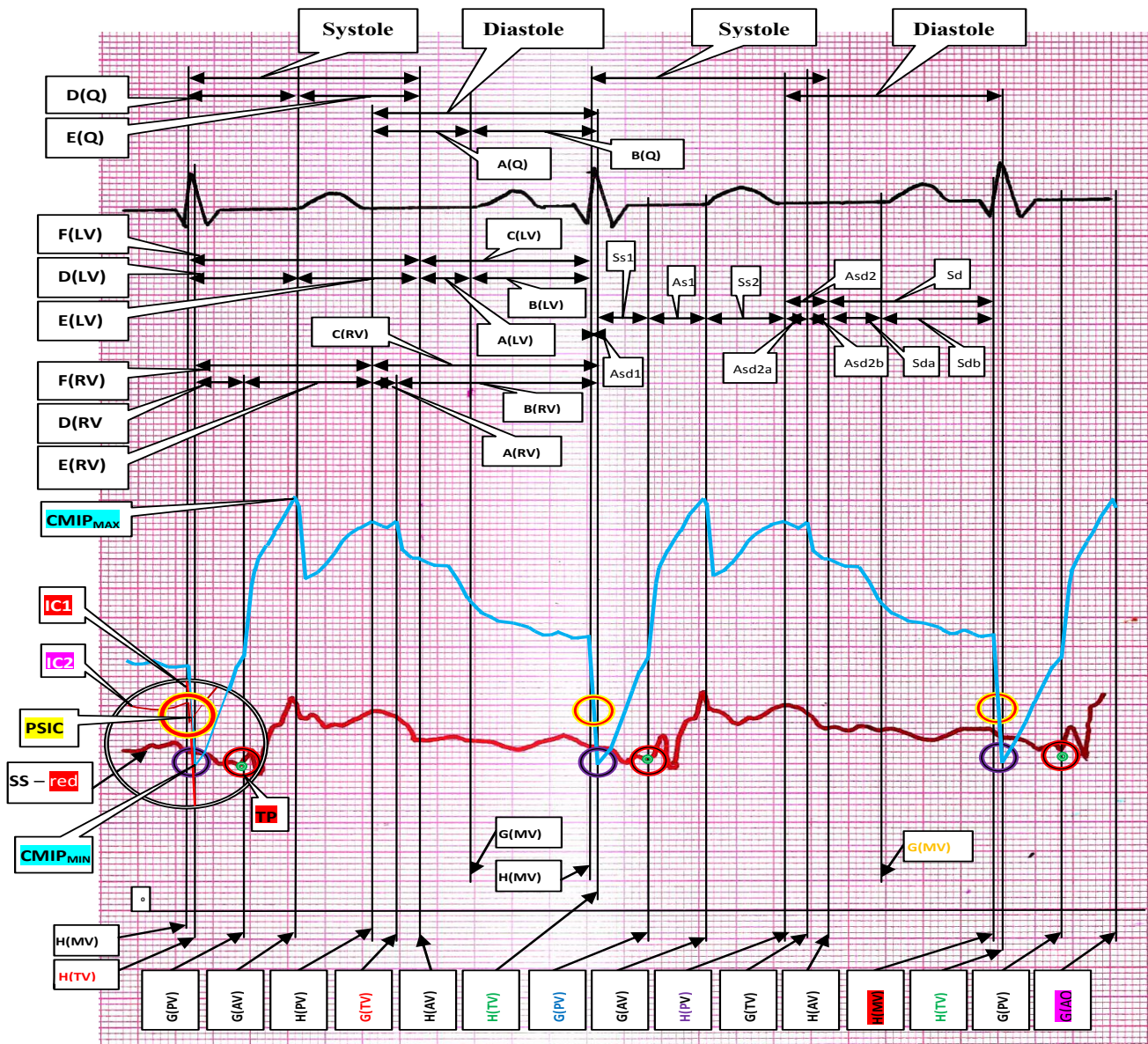
Table 1. Synchronization of ECG, CMIP and SS data in Norm

A(Q) – isometric ventricular relaxation
B(Q) – actual ventricular diastole
C(LV) – LV diastole
A(LV) – isometric LV relaxation
B(LV) – actual LV diastole
C(RV) – RV diastole
A(RV) – isometric RV relaxation
B(RV) – actual RV diastole
D(Q) – isometric ventricular contraction
E(Q) – actual ventricular systole
F(LV) – LV systole
D(LV) – isometric LV contraction
E(LV) – actual LV systole
F(RV) – RV systole
D(RV) – isometric RV contraction
E(RV) – actual RV systole
G(AV) – opening of AV
H(PV) – closing of PV
G(TV) – opening of TV
H(AV) – closing of AV

G(MV) – opening of MV
H(TV) – closing of TV
H(MV) – closing of MV
G(PV) – opening of PV
H(PV) – closing of PV
Acd1 – asynchronous period of ventricular systole-diastole -1
Ss1 – synchronization period of isometric ventricular contraction -1
As1 – asynchronous period of ventricular systole -1
Ss2 – synchronization of the actual ventricular systole -2
Asd2 – asynchronous period of ventricular systole-diastole -2
Asd2a – from closing of PV to opening of TV
Asd26 – from opening of TV to closing of AV
Sd – period of synchronization of ventricular relaxation
Sda – isometric relaxation of LV
Sdb – actual LV diastole
═ – integral curves (IC-1 and IC-2)
◯ – TP including ZTEP: SS-VH-SVC-CS-RV-LA
◎ – intersection point for ZTEP
◯ – point of "stabilization" of ICs of the right and left parts of the heart
— – CMIP

The modulating effect on brain structures that provide sensory-motor reactions and behavioral functions is carried out through two mutually complementary (duplicate) paths of synchronous regulation: 1) the neural tract—baroreceptors, mechanoreceptors of the heart and vascular bed, and 2) the vascular tract—wave hemodynamic structures having propagation velocities comparable to the nerve impulse. Waveguides are the vessels of entry and exit from the skull, providing direct and reverse control connections of the heart and brain, combining the heart and brain into a single hydrodynamic system with phase-changing volume, configuration and variable patterns of regulatory impulses.

Competing Interests

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Cardiology

Association of the *AGT* Gene M235T (rs699) Polymorphism with Arterial Hypertension and Metabolic Risk Factors in the Indigenous People of Yakutia

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Abstract

The research objective was to study the association of the *AGT* rs699 missense SNP with arterial hypertension (AH) and metabolic risk factors among indigenous people of the Arctic territory of Yakutia. The obtained data show that representatives of indigenous people of the Arctic territory of Yakutia with the homozygous GG genotype for the *AGT* SNP rs699 are characterized by high levels of systolic blood pressure. The carriage of the GG genotype in AH patients is associated with a high frequency of hypo-HDL cholesterolemia. The carriage of GG genotype in AH patients, compared to subjects without AH, is characterized by higher blood levels of total cholesterol, LDL-C, and triglycerides and is associated with a high frequency of abdominal obesity. Thus, the *AGT* rs699 missense SNP was found to be associated with metabolic risk factors in indigenous AH persons of the Arctic territory of Yakutia. (**International Journal of Biomedicine. 2019;9(4):287-291.**)

Key Words: *AGT* gene • polymorphism • arterial hypertension • risk factors • indigenous people • Yakutia

Abbreviations

AO, abdominal obesity; **AH**, arterial hypertension; **BP**, blood pressure; **FPG**, fasting plasma glucose; **GWAS**, Genome-wide association studies; **HDL-C**, high-density lipoprotein cholesterol; **HP**, hip circumference; **HWE**, Hardy-Weinberg equilibrium; **LDL-C**, low-density lipoprotein cholesterol; **TC**, total cholesterol; **TG**, triglycerides; **RAS**, renin–angiotensin system; **SBP**, systolic blood pressure; **WC**, waist circumference.

Introduction

Arterial hypertension (AH) is the leading risk factor for disability and premature mortality in the global population. As of 2010, 31.1% of the adult population of the world was suffering from hypertension (30.7% of men and 28.8% of women).[1] In Russia, according to an ESSE-RF epidemiological study, which was conducted in 12 regions, the prevalence of AH was 50.2% (51.1% in men, 49.7% in women).[2] AH is considered a multifactorial disease. From the early and negative GWAS for hypertension within the Wellcome Trust Case Control Consortium[3] to the more recent reports,[4-6] 10,280 genetic variants have been associated with risk of high BP. The gene encoding angiotensinogen (AGT) has been implicated in hypertension both through genetic linkage studies and by allelic association. The *AGT* rs699 missense SNP is a T to C substitution in the exon 2, resulting in a functional methionine (M) to threonine (T) substitution at codon 268 (M268T). Previously, rs699 was positioned to the amino acid 235, and the SNP is therefore referred to as M235T. The rs699 threonine variant is associated with higher plasma AGT levels and higher BP.[7] Despite numerous studies, the results are ambiguous and the degree and reliability of associations vary.

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The research objective was to study the association of the *AGT* rs699 missense SNP with AH and metabolic risk factors among indigenous people of the Arctic territory of Yakutia.

Materials and Methods

Material was collected under expeditionary conditions in the Arctic territory of Yakutia, including places of compact residence of the indigenous peoples (Nizhnekolymsky, Verkhnekolymsky and Tomponsky districts). A total of 348 indigenous people of Yakutia were examined. The sample consisted of an adult population aged between 20 and 70 years (225 women and 123 men). All subjects were divided into 2 groups: the Case group consisted of 175 patients with AH; the Control group included 173 people without elevated BP. The average age of hypertensive patients was 53.07±0.49 years, those without AH - 38.88±0.60 years.

The research program included the following sections:
• Anthopometrical reference data: the measurements of BMI (kg/cm²), WC (cm) and HC (cm)
• Assessment of BP by Korotkov's method. BP was measured twice with an OMRON automatic tonometer (Japan) on the right hand in a sitting position with the calculation of the average BP
• Assessment of FPG, OGTT, and blood levels of TC, TG, HDL-C, and LDL-C

Glucose and lipid metabolism disorders were diagnosed according to the Russian national recommendations (the All-Russian Scientific Society of Cardiologists [VNOK, 2009])[8] based on the IDF consensus criteria (2006)[9]: TG≥1.7 mmol/l; HDL-C<1.0 mmol/l in males and <1.2 mmol/l in females; LDL-C>3.0 mmol/l; FPG >6.1 mmol/l; IGT 2Hr PG ≥7.8 mmol/l and ≤11 mmol/l. Abdominal obesity (AO) was confirmed at WC ≥ 94 cm in males and ≥ 80 cm in females.

The diagnosis of AH was based on 2017 ACC/AHA Guideline for or the Prevention, Detection, Evaluation, and Management of High Blood Pressure in Adults.[10]

Genotyping of the AGT rs699 missense SNP was performed in the laboratory of molecular genetics at YSC CMP. From each patient, 2mL of peripheral blood were drawn into an EDTA tube. Genomic DNA was isolated from the peripheral blood leukocytes using standard phenol–chloroform extraction technique. Allelic variants of the AGT rs699 missense SNP were tested by real-time PCR on the «Real-time CFX96» amplifier (BioRad, USA) using Lytech kits (Lytech R&D LLC, Moscow) in accordance with the manufacturer's instructions. The actual SNP for M235T is a T→C substitution; however, the pyrosequencing was done by using the reverse complement strand, which resulted in an allele call of A→G. For quality control, 10% of samples were randomly repeated, with complete congruence.

The study was approved by the Ethics Committee of the Yakut Science Center of Complex Medical Problems. Written informed consent was obtained from each patient.

Statistical analysis was performed using SPSS (version 19.0). Baseline characteristics were summarized as frequencies and percentages for categorical variables and as mean±SEM for continuous variables. Means of 2 continuous normally distributed variables were compared by independent samples Student's t test. Mann-Whitney U test was used to compare means of 2 groups of variables not normally distributed. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated. Deviation from Hardy-Weinberg equilibrium and differences in allele distributions between the two groups were assessed by χ2- test with 1 degree of freedom (df). A probability value of *P<0.05* was considered statistically significant.

Results and Discussion

In the general population, the frequencies of the AA, AG and GG genotypes of the AGT rs699 SNP were 15.5% (n=54), 45.1% (n=157), and 39.4% (n=137), respectively, which corresponds to the HWE; the frequencies of the A allele and G allele were 38.1% (n=265) and 61.9% (n=431) (Table 1).

Table 1.

Frequencies of genotypes and alleles of the M235T polymorphism of the AGT gene and correspondence to Hardy-Weinberg equilibrium (HWE)

Genotype	Case	HWE	χ^2	P	Control	HWE	χ^2	P	Allele	Frequencies of alleles Case	Frequencies of alleles Control
AA	0.097	0.120			0.214	0.173			A	0.346	0.416
AG	0.497	0.452	1.71	0.19	0.405	0.486	4.85	0.03	G	0.654	0.584
GG	0.406	0.428			0.382	0.341					

In the general population, we found significant differences in average values of TC, LDL-C, and TG, depending on the carriage of genotypes of the AGT rs699 SNP. Thus, in carriers of the GG homozygous genotype, all values were higher than in AG heterozygotes and AA homozygotes. On the contrary, the average FPG level was significantly lower in GG carriers than in AG carriers and AA carriers. Carriers of all genotypes showed a high frequency of hypercholesterolemia, hyper-LDL cholesterolemia and hypo-HDL cholesterolemia without significant differences between genotypes. Thus, in the general population, the average frequency of hypercholesterolemia, hyper-LDL cholesterolemia, and hypo-HDL cholesterolemia was 45.8%, 63.3%, and 35.4%, respectively. The frequency of hypertriglyceridemia was as follows: AA carriers - 5.5% and GG carriers - 17.5% (*P*=0.033). The frequency of an increased FPG level was significantly higher in AG heterozygotes than in individuals with the mutant GG genotype (8.3% and 2.9%, respectively, *P*=0.048). In the general population, the frequency of AO in GG carriers, AG carriers and AA carriers was as follows: 60.6%, 59.2%, and 46.3%, respectively (*P*=0.174).

Considering the high frequency of AH in the general population (53.3%), we conducted a case-control study that included 175 AH persons (Case group) and 173 normotensive

persons (Control group) to determine the association of the *AGT* rs699 SNP with hypertension and metabolic risk factors among indigenous people of the Arctic territory of Yakutia.

We did not find statistically significant differences in the frequency distribution of the AG and GG genotypes between the Case group and the Control group. The frequency of the AA genotype was lower in the Case group than in the Control group: 9.7% and 21.4% (*P*=0.009). The distribution of the genotype frequency was not in HWE for the Control group (χ2=4.85, *P*=0.03) (Table 1). The occurrence of the departure from HWE in controls is probably due to population substructure.

We further used the two types of genetic models (Dominant and Recessive models of inheritance) to test the association between the *AGT* rs699 SNP and AH; the results are shown in Tables 2 and 3. We found a link between AH and the mutant homozygous GG genotype and the heterozygous AG genotype in the dominant model (OR=2.53, 95% CI=1.36-4.69, *P*=0.003). A number of studies have also found an association of the G allele and the GG genotype with the risk for developing hypertension.[11-16] The Russian study, which included 514 patients, found an association of the G allele with the risk for developing hypertension in men, with an odds ratio of 1.95 (*P*=0.003).[17] However, a number of studies did not find a reliable association of AG and GG genotypes with AH.[18-22]

Table 2.
Dominant model of inheritance (df = 1)

Genotype	Genotype frequencies		χ²	P	OR	95% CI
	Case (n=175)	Control (n=173)				
AG+GG	0.903	0.786	9.04	0.003	2.53	1.36-4.69
AA	0.097	0.214			0.40	0.21-0.73

Table 3.
Recessive model of inheritance (df = 1)

Genotype	Genotype frequencies		χ²	P	OR	95% CI
	Case (n=175)	Control (n=173)				
GG	0.406	0.382	0.21	0.64	1.11	0.72-1.70
AA+AG	0.594	0.618			0.90	0.59-1.39

Figure 1. *The frequency of dyslipidemia and carbohydrate metabolism in individuals with and without AH, depending on the carriage of genotypes of the AGT rs699 SNP.*

A-hypercholesterolemia; B-hyper-LDL cholesterolemia; C-hypo-HDL cholesterolemia; D-hypertriglyceridemia; E-increased FPG level; *- P<0.05

In Group 1 patients with AH, the average level of SBP in carriers of AA, AG and GG genotypes was 159.72±1.92 mmHg, 161.72±1.40 mmHg and 173.53±3.62 mmHg, respectively (F=6.6763, *P*=0.0016).

Table 4 presents the relationship between *AGT* genotype carriage and parameters of lipid and glucose metabolism in patients with and without AH. The TG level was significantly higher in the Case group regardless of the genotype carriage. In GG carriers, the blood levels of TC and LDL-C were significantly higher in the Case group than in the Control group. The AG carriers in the Case group had significantly lower HDL-C values than in the Control group. In AA and AG carriers, the FPG level was significantly higher in the Case group than in the Control group.

Table 4.
Mean levels of the parameters of lipid spectrum and blood glucose in indigenous people with and without AH depending on carriage of genotypes of the AGT rs699 SNP

Blood parameters	AA genotype			AG genotype			GG genotype		
	Case	P	Control	Case	P	Control	Case	P	Control
TC	5.16±0.11	<0.05	4.74±0.16	4.98±0.08	>0.05	4.78±0.10	5.29±0.07	<0.01	4.74±0.09
LDL-C	3.30±0.08	>0.05	3.06±0.12	3.17±0.06	>0.05	2.98±0.08	3.47±0.06	<0.01	3.04±0.08
HDL-C	1.32±0.06	>0.05	1.26±0.05	1.29±0.02	<0.05	1.40±0.04	1.22±0.02	>0.05	1.23±0.03
TG	1.17±0.08	<0.02	0.90±0.06	1.14±0.03	<0.01	0.87±0.04	1.29±0.05	<0.05	1.05±0.06
FPG	5.37±0.24	<0.01	4.43±0.14	5.10±0.15	<0.01	4.15±0.10	4.40±0.10	>0.05	4.24±0.10

In the Case and Control groups, we found a significantly higher incidence of hypo-HDL cholesterolemia in GG carriers than in carriers of the AA and AG genotypes (Fig. 1). It should be noted that only a few studies confirm the association of the G allele with the presence of hypercholesterolemia.[23]

In the Case group, the highest incidence of AO was found in carriers of the AG and GG genotypes (76.1% and 83.9%). In the Control group, the frequency of AO varied from 28.6% in AG carriers to 43.9% in GG carriers. It should be noted that a contribution of the GG genotype to the development of metabolic syndrome was confirmed by a number of studies.[24,25]

Conclusion

The obtained data show that representatives of indigenous people of the Arctic territory of Yakutia with the homozygous GG genotype for the *AGT* SNP rs699 are characterized by high levels of SBD. The carriage of the GG genotype in AH patients is associated with a high frequency of hypo-HDL cholesterolemia. The carriage of GG genotype in AH patients, compared to subjects without AH, is characterized by higher blood levels of TC, LDL-C, and TG and is associated with a high frequency of AO. Thus, the *AGT* rs699 missense SNP was found to be associated with metabolic risk factors in indigenous AH persons of the Arctic territory of Yakutia.

Competing Interests

The authors declare that they have no competing interests.

Sources of Funding

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Comparative Analysis of Lipid Peroxidation System in Humans and Rats with Arterial Hypertension

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Abstract

The aim of this research was to study the concentration of lipid peroxidation (LPO) products and the activity of antioxidant defense (AOD) parameters in ISIAH rats in comparison with a group of adolescents with arterial hypertension (AH).

Material and Methods: We conducted the study on young (2.5-3 months) sexually mature male rats of the normotensive line (WAG) (n=20) (intact animals) and hypertensive line (ISIAH) (n=20), weighing 200-220 g. The data of 65 adolescents aged 13-17 years with AH (Group 1) were used for the clinical study (the age of adolescents is comparable to the biological age of the experimental animals used). The comparison group consisted of 65 normotensive adolescents of the same age and sex ("copy-pair" type) (Group 2). The plasma level of antioxidant parameters (total antioxidant activity [TTA], SOD activity, α-tocopherol and retinol) and primary/secondary products of LPO (conjugated dienes [CD], ketodienes and conjugated trienes [KD-CT], and thiobarbituric acid reactants [TBARs]) were determined using spectrophotometric and fluorometric methods.

Results: We found that the course of LPO reactions in animals and humans was similar, which was expressed by the activation of prooxidant factors and the insufficiency of antioxidant response. Species differences concerned the intensity and number of parameters involved in the pathological process. Thus, in ISIAH rats there was an increase in toxic TBA-active products and a decrease in TTA, α-tocopherol and retinol in comparison with normotensive animals; in AH adolescents there was an increase in the content of intermediate–KD-CT and final TBA-active products, and a decrease in the α-tocopherol level in relation to the comparison group.

Conclusion: Features of response of the LPO nonspecific system in hypertensive rats and humans allow using this line of rats for further study of adaptive mechanisms, and to extrapolate the received experimental data on humans, taking into account certain specific distinctions.(**International Journal of Biomedicine. 2019;9(4):292-296.**)

Key Words: arterial hypertension • rats, adolescents • lipid peroxidation • antioxidant defense

Abbreviations

AH, arterial hypertension; **AOD**, antioxidant defense; **BP**, blood pressure; **CDs**, conjugated dienes; **DB**, substrates with unsaturated double bonds; **FR**, free radicals; **ISIAH**, inherited stress-induced arterial hypertension; **KD-CT**, ketodienes and conjugated trienes; **LPO**, lipid peroxidation; **OS**, oxidative stress; **SOD**, superoxide dismutase; **TAA**, total antioxidant activity; **TBARs**, thiobarbituric acid reactants; **WAG**, Wistar Albino Glaxo.

Introduction

Arterial hypertension (AH) is one of the most common diseases of the cardiovascular system, characterized by a persistent increase in blood pressure.[1,2] AH often manifests itself in childhood and adolescence.[3,4]

Like any pathological condition, AH, in addition to pathological reactions, is accompanied by the inclusion of sanogenetic mechanisms reflecting the dynamic complex of defense and adaptive reactions arising under the influence of a pathogenic factor and aimed at restoring the disturbed self-regulation of the body.[5,6] Sanogenetic reactions at the cellular level consist in plastic rearrangements of cell membranes, intracellular structures, macromolecules and their medium.[7] In this case, the main role belongs to the LPO-AOD reactions, an important regulatory mechanism involved in maintaining cell homeostasis.[8-10] Its predominant role in modification of cell membrane structure, xenobiotic metabolism, immune response regulation, cell proliferation, vascular permeability, and receptor sensitivity is well known.[11] Currently, some poorly studied sanogenetic mechanisms of hypertension, in particular, the role of nonspecific reactivity of bodily reactions in the genesis of the disease, are also recognized. The lack of clear ideas causes greater interest among researchers in modeling this pathological condition, finding ways to correct it and developing new treatment methods.[12,13] At the same time, scientists know about different reactivity and types of adaptation strategies of animals and humans in response to the influence of disturbing factors.[14-16] In the context of the above, it is extremely interesting to compare the systems that indicate the development of OS in humans (adolescents with hypertension) and in animals with hereditary hypertension.

The aim of this research was to study the concentration of LPO products and the activity of AOD parameters in ISIAH rats in comparison with a group of adolescents with AH.

Material and Methods

We conducted the study on young (2.5-3 months) sexually mature male rats of the normotensive line (WAG) (n=20) (intact animals) and hypertensive line (ISIAH) (n=20), weighing 200-220 g. The animals were bred at the SPF-vivarium Center for collective use of the Federal research center "Institute of Cytology and Genetics," Siberian branch of RAS (Novosibirsk).[17] Animals in the vivarium were kept in cages at a temperature of 20°-22°C, without limitation of mobility, with free access to water with an adjustable light schedule (12 hours - light, 12 hours - darkness). Blood was taken after rapid decapitation of the animals under anesthesia. The work with animals was carried out in accordance with the principles of humanism laid down in the directives of the European Community (86/609/EEC) and the Declaration of Helsinki, in accordance with the "Animal experimentation legislations".

The data of 65 adolescents aged 13-17 years with AH (Group 1) were used for the clinical study (the age of adolescents is comparable to the biological age of the experimental animals used). The comparison group consisted of 65 normotensive adolescents of the same age and sex ("copy-

pair" type) (Group 2). The common inclusion criterion for all groups was either voluntary informed consent of teenagers 15 years of age or older or of the parents/legal representatives of the adolescents.

The main inclusion criterion for Group 1 was confirmed AH based on the measurement of BP in repeated office measurements ≥95 percentile for age, height and sex or ≥140/90 mmHg in adolescents older than 16 years. Exclusion criteria for Group 1 were secondary hypertension and the presence of severe somatic diseases.

The main inclusion criteria for Group 2 were comparability by age, sex, place of residence and other basic criteria, as well as the presence of a normal BP level when measured 3 times, and the absence of acute disease or exacerbation of chronic diseases at the time of examination.

Exclusion criterion for all groups was intake of antioxidant drugs within the last 6 months.

Blood samples (5 ml) were collected from the ulnar vein in standard vacuum tubes with EDTA. The erythrocyte population was separated from the other blood components by centrifugation at 1500 g for 5 min, at 4°C. The erythrocyte pellet was washed 3 times with a 0.9% (wt/vol) NaCl solution. Aliquots of ethylenediaminetetraacetic acid plasma and washed erythrocytes were used immediately or kept frozen at -40°C, not exceeding one month. We estimated the LPO-AOD parameters by plasma concentrations of primary/secondary products of LPO (DB, CDs, KD-CT, and TBARs and antioxidant indexes (TAA, SOD activity, α-tocoferol, and retinol).[18] The concentration of CDs and KD-CT was detected at 232 nm in plasma heptane extracts. For conversion of absorption units to μmol/L, we used the coefficient of molar absorption (K=$2.2 \cdot 10^5 M^{-1} C^{-1}$). TBARs levels were detected by fluorometry. Blood plasma total antioxidant activity (TAA) level was detected photometrically. α-tocopherol and retinol levels were detected in plasma by fluorometry. Fluorometry for SOD activity in hemolysate activity were determined. The measurements were conducted with a Shimadzu RF-1501 spectrophotometer (Japan) consisting of two blocks: a UV-1650PC spectrophotometer and a RF-1501 spectrofluorimeter.

Statistical analysis was performed using the Statistica 6.1 software package (Stat-Soft Inc., USA). The normality of distribution of continuous variables was tested by the Kolmogorov-Smirnov test with the Lilliefors correction and Shapiro-Wilk test. For descriptive analysis, results are presented as mean±standard deviation (SD), median (Me) and interquartile range (IQR; 25th to 75th percentiles). For data with normal distribution, inter-group comparisons were performed using Student's t-test. Differences of continuous variables departing from the normal distribution, even after transformation, were tested by the Mann-Whitney U-test. Spearman's rank correlation coefficient was calculated to measure the strength and direction of the relationship between two variables. A probability value of $P<0.05$ was considered statistically significant.

Results and Discussion

At the first stage, we analyzed the data of the LPO-AOD system in ISIAH rats in comparison with WAG animals (Table 1).

In hypertensive rats, there was an increase in the average values of the final TBA-active LPO products (by 1.77 times; $P<0.001$) and a decrease in the activity of a number of antioxidant factors: lower values of TAA (by 1.72 times; $P<0.0001$), α-tocopherol (by 2.23 times; $P<0.0001$), and retinol (by 1.32 times; $P=0.026$) compared with WAG rats (Table 1).

Table 1.

The content of LPO products and components of AOD system in rats of the ISIAH line (M±SD, Me, IQR [P_{25}; P_{75}])

Parameters	WAG rats	ISIAH rats
Compounds with conjugated DB, units	1.74±0.35 1.81 1.54 − 1.87	1.88±0.19 1.38 1.76 − 2.07
CDs, μmol/L	0.94±0.31 0.86 0.73 − 0.89	1.16±0.13 1.14 1.12 − 1.22
KD-CT, units	0.34±0.04 0.34 0.31 − 0.37	0.36±0.08 0.36 0.31 − 0.38
TBARs, μmol/L	0.84±0.13 0.79 0.78 − 0.94	1.49±0.40* 1.66 1.07 − 1.83
TAA, units	16.45±2.71 17.14 14.36 −18.90	9.57±2.65* 10.34 8.29 − 10.52
SOD activity, units	2.06±0.24 1.98 1.94 − 2.14	2.16±0.37 2.02 1.84 − 2.51
α-tocopherol, μmol/L	10.91±2.55 11.20 9.85 − 12.30	4.89±1.55* 5.14 3.48 − 6.24
Retinol, μmol/L	0.54±0.15 0.51 0.48 − 0.65	0.41±0.08* 0.39 0.37 − 0.41

* - P<0.05 between two groups

At the second stage, we analyzed the data of the LPO-AOD system in adolescents of Group 1 (Table 2) and discovered that in patients with AH, in relation to the data of Group 2, there was a statistically significant decrease in the value of primary LPO–CDs products by 1.34 times ($P=0.0028$) , as well as an increase in the content of intermediate LPO–KD-CT by 1.21 times ($P=0.0132$) and final TBA-active products by 1.43 times ($P<0.0001$). The state of the AOD system in Group 1 was characterized by a decrease in the α-tocopherol level by 1.38 times ($P<0.0001$), in the absence of changes in the values of TAA, SOD activity and retinol content (Table 2).

A high-brand line of rats with persistent ISIAH was determined to be the closest experimental model to humans for this pathological condition.[14,17] The ISIAH line is characterized by, among other things, an increase in BP at rest and a significant increase under mild emotional stress, the presence of specific morphological changes in organs and systems, an imbalance in the system of neuroendocrine regulation, and changes in behavioral reactions.[13] It is known that AH in humans is also associated with severe pathological conditions accompanied by numerous polysystem disorders, decreased immunity, early occurrence of atherogenic shifts,

a significant imbalance of neurovegetative and endocrine influences, and significant changes in central and regional hemodynamics.[3,6]

Table 2.

The content of LPO products and components of AOD system in adolescents with AH (M±SD, Me, IQR [P_{25}; P_{75}])

Parameters	Comparison group	Group of adolescents with AH
Compounds with conjugated DB, units	1.5±0.48 1.48 1.08 − 1.84	1.47±0.63 1.39 0.99 − 1.84
CDs, μmol/L	1.42±0.7 1.44 0.75 − 2.06	1.06±0.67* 0.98 0.52 − 1.56
KD-CT, units	0.33±0.17 0.28 0.2 − 0.4	0.4±0.17* 0.36 0.28 − 0.48
TBARs, μmol/L	0.73±0.29 0.73 0.49 − 0.87	1.04 ±0.5* 0.91 0.65 − 1.38
TAA, units	13.33±4.14 13.12 10.79 − 15.75	14.96±6.33 14.26 10.34 − 19.22
SOD activity, units	1.56±0.18 1.57 1.44 −1.72	1.56±0.17 1.58 1.43 − 1.67
α-tocopherol, μmol/L	7.66±3.33 7.32 5.25 − 9.00	5.56±2.3* 5.06 4.06 − 6.27
Retinol, μmol/L	0.8±0.33 0.73 0.56 − 0.99	0.9±0.41 0.86 0.62 − 1.12

* - P<0.05 between two groups

When analyzing the experimental data, we found that rats of the ISIAH line have a high activity of LPO reactions (an increase in the final TBA-active products). Our data are consistent with the results of a number of studies indicating the altered reactivity of various systems in animals of this line.[13] Thus, characteristic violations in the biochemical status of the animals, indicating an increase in the level of cholesterol-containing fractions of lipids, glucose, lactic acid, etc., were revealed earlier.[19] It can be assumed that these disorders can directly affect the state of nonspecific bodily reactivity systems, provoking the development of OS in hypertensive animals. Thus, it is possible to point to the presence of a significant shift toward prooxidant activity in rats of the ISIAH line in comparison with WAG rats.

In the analysis of the primary link of the LPO process in AH adolescents that includes the formation of compounds with unsaturated substrates with conjugated double bond and primary products – CDs. Previously, the presence of LPO activation had not been revealed, since the value of the CDs was lower than in Group 2. In this case, it is legitimate to note an increase in the rate of transition of the primary LPO products to subsequent metabolites, especially since changes in the secondary link showed a statistically significant increase in the content of intermediate LPO products - KD-CT. The activation of LPO processes can be judged by a significant

increase in the concentration of final TBA-active LPO products. The increase of secondary and final LPO metabolites in AH adolescents may be a sufficient criterion for the conclusion about the activation of free radical reactions, especially since a variety of LPO products, including TBA-active LPO products, have a multilateral damaging effect on most biopolymers and cell structures.[20,21] According to some data in the literature, a possible explanation for the growth of reactive LPO products in hypertension may be an imbalance in the neuroendocrine regulation system, resulting in systemic metabolic disorders with atherogenic changes in blood composition.[22-24] In addition, the development of this disease provoked changes in the fatty acid composition of blood plasma lipids: an increase in the total content of saturated and monounsaturated fatty acids with a reduction in the concentration of polyunsaturated components.[8] The consequence of such destabilization is the development of OS in patients with AH. With respect to the studied problem, it should also be noted that the generation of toxic LPO products is closely related to the synthesis of nitric oxide, the concentration of which positively correlates with the activity of an angiotensin converting enzyme, which is one of the most important factors in the regulation of BP.[6,11] Having a free radical structure, as well as functioning as a signaling molecule of the cardiovascular system, nitric oxide has a significant regulatory effect on the system's activity, supporting vasodilation at the required level and regulating regional hemodynamics. Thus, the activation of LPO reactions can lead to serious consequences, including at the level of the vascular bed.

The limiting factor of the LPO processes in the body is the high activity of antioxidant factors that make up the overall antioxidant status.[8,11] It was found that the increased values of toxic metabolites in hypertensive animals took place against the background of a significant decrease in antioxidant protection factors—TAA and the content of fat-soluble vitamins. It is known that α-tocopherol is a strong antioxidant of exogenous origin.[25] The mechanism of its action is due to the ability of the mobile hydroxyl of the vitamin molecule chromane nucleus to interact directly with free radicals: active radicals of oxygen, unsaturated fatty acids and their peroxides.[26] It is likely that the accumulation of toxic metabolites in ISIAH rats may be due to insufficient activity of antioxidant factors.

In AH, adolescents had an imbalance of the LPO-antioxidant system, with changes in the level of antioxidant components. Thus, in the AH group, there was a significant decrease in the α-tocopherol value in the absence of changes in the values of TAA, SOD activity, and the level of non-enzymatic antioxidants. It is known that due to the lipophilic properties, the α-tocopherol molecule has the ability to be embedded in the lipid bilayer of cell membranes and thus have a membrane-protective and membrane-stabilizing effect. In addition, this fat-soluble vitamin supports the functional stability of the external plasma membrane of cells and participates in the regulation of tissue respiration in mitochondria and the work of cell enzyme systems that interfere with the LPO activity.[27] The decrease in the concentration of α-tocopherol in the blood of patients is the evidence of stress in the AOD system, which is confirmed by a significant accumulation of intermediate and final LPO products.

Conclusion

Thus, changes in nonspecific mechanisms of bodily reactivity in animals and humans under conditions of AH indicate the development of a predominantly unidirectional disadaptive reaction, reflecting the prevalence of pathogenetic reactions (accumulation of prooxidant factors) over sanogenetic (antioxidant defense activation) ones. Species differences in this case consist in the difference in the intensity of reactions and the number of parameters involved in the development of the pathological process. We can talk about the formation of stable pathological reactions in animals (accumulation of final metabolites), while in humans there is a dynamics of the pathological process (growth of intermediate and final metabolites). Reactivity of the AOD system in animals was characterized by a more pronounced decrease in protective reserves, whereas in humans there were changes in a single antioxidant parameter. Thus, animals of the ISIAH line can be a genetic model for the study of new mechanisms of the body's adaptive reserves under the influence of AH; however, a number of differences have been identified that require consideration in the studies of AH in the experiment in order to develop methods of treatment and optimization of sanogenetic mechanisms aimed at leveling pathological effects in patients with AH.

Competing Interests

The authors declare that they have no competing interests.

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**INTERNATIONAL
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The Electrical Activity of the Heart during Ventricular Repolarization and Types of the Remodeling of the Athlete`s Heart

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Abstract

The aim of this work was to investigate the electrical activity of the athletes' hearts with different types of the left ventricular remodeling, as well as a control group of untrained people, by the body surface potential mapping before and immediately after exercising. The study of the heart's electric field in athletes of the same sex, age and sport qualification, but different sport disciplines, showed that the reaction of the heart to physical activity is reflected in different changes of the amplitude-temporal characteristics of the extremes on the body's surface in athletes with eccentric and concentric myocardial remodeling. (**International Journal of Biomedicine. 2019;9(4):297-299.**)

Key Words: ventricular repolarization • ventricular remodeling • electrical activity • body surface potential mapping

Abbreviations

ARD, aortic root diameter; **BSPM**, body surface potential mapping; **HR**, heart rate; **LVEDD**, left ventricular *(LV)* end-diastolic diameter; **LVM**, LV mass; **LVMI**, LV mass index; **PWT**, posterior wall thickness; **LVRWT**, LV relative wall thicknes*s*; **LVEF**, LV ejection fraction; **SWT**, septal wall thickness; **VR**, ventricular repolarization.

Introduction

The specificity of sports specialization[1] defines the type of "sports heart" remodeling.[2,3] In endurance athletes, an increase of myocardial preload leads to an increase in the size of the heart and ventricular cavities and an increase in the stroke volume, and forms the eccentric hypertrophy of the left ventricle. In strength athletes an increase of myocardial afterload leads to an increase in the mass of the LV myocardium without dilatation of the cavities, and forms the concentric myocardial hypertrophy.[3,4] Structural and functional cardiac remodeling leads to electrophysiological remodeling.[5] Using standard ECG on an athlete`s heart during VR, the general particularities are: J-point elevation, ST segment elevation/depression, tall and peaked T-wave, and isoelectric, biphasic or inverted T-waves.[6,7] When an athlete performs physical exercise, these ECG features disappear, which confirms their functional, rather than structural, origin.[8] BSPM method, known as a noninvasive, multichannel, synchronous recording of electrical potentials of the heart on the thoracic surface from multiple unipolar leads, is a more informative method for studying the functional state of the heart, which makes it possible to obtain more data on the electrical processes in the myocardium than with the standard electrocardiography.[9,10]

The aim of this work was to investigate the electrical activity of the athletes' hearts with different types of the LV remodeling, as well as a control group of untrained people, by the BSPM before and immediately after exercising.

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Materials and Methods

The 25 athletes were examined by the methods of echo- and electrocardiography. All athletes were male with the sports qualifications of candidates for Master of Sports of Russia and Master of Sports of Russia. Three sporting disciplines predominantly made up the study group: swimming (n=10; age of 19.5±0.5 years; weight of 76.0±9.5 kg; height of 179±6 cm), weightlifting (n=8; age of 19.5±1.5 years; weight of 77.6±8.3 kg; height of 174.3±6.0 cm) and cross-country skiing (n=7; age of 18.9±1.1 years; weight of 76±4.0 kg; height of 178.1±3.2 cm). The control group comprised 9 healthy adult volunteers (age of 19,7±0,8 years; weight of 70,8±6,4 kg; height of 176,9±4,9 cm).

Echocardiography

Two-dimensional EchoCG was perfumed with the subjects resting in a left lateral decubitus position using LOGIC Pro (GE, USA) with a 5MHz transducer. The heart images obtained in M- and B- modes in the standard parasternal long-axis and four chambers positions, according to guidelines of the American Society of Echocardiography,[11] were used to measure LVEDV, SWT, PWT, and ARD. The Devereux formula (1986) was used to calculate LVM (g). We calculated LVMI (g/m^2) as the ratio of LVM to body surface area, LVRWT as the ratio of double PWT to LVEDV, and LVEF using the Teichholz method.

Multichannel electrocardiography

The heart's electrical activity in the young men was studied using multichannel ECG during VR. BSPM with 64 unipolar leads covering the thorax was performed; Standard lead II was used as the reference.[9] The electrodes were located evenly on the ventral and dorsal surfaces of the torso with a 3-5cm distance between them. The electrodes were attached to 8 flexible strips, each containing 8 electrodes. BSPM was recorded at rest and during the recovery period after submaximal physical exercise. Using a bicycle ergometer (KETTLER, Germany), subjects performed two workloads, each lasting 5 minutes. The first (moderate) workload corresponded to 1.5 W/kg body weight. The second workload was submaximal and was calculated according HR at the fifth minute of the first workload. During the second workload, HR of the subjects reached 170 bpm. After each load, there was a 3-minute recovery period.

We analyzed the amplitude characteristics of the positive and negative extremes (the amplitude of the maximum and the minimum, respectively) and the time they reached the maximum amplitudes at the period of VR (the maximum time and the minimum time, respectively).

The study was conducted in accordance with ethical principles of the WMA Declaration of Helsinki (1964, ed. 2013) and approved by the KSC UB RAS Ethics Committee. Written informed consent was obtained from all participants.

Statistical analysis was performed using the statistical software SPSS version 22.0. The normality of distribution of continuous variables was tested by the Shapiro–Wilk test. Variables were presented as mean+SD. For data with normal distribution, inter-group comparisons were performed using Student's t-test.

Differences of continuous variables departing from the normal distribution were tested by the Mann-Whitney U-test. A probability value of $P<0.05$ was considered statistically significant.

Results

According to recommendations[11] as the result of an echocardiographic study, two types of LV remodeling were revealed in the subjects: eccentric (cross-country skiers and swimmers) and concentric (weightlifters).

Indicators of LVM, LVMI, and LVRWT were as follows: in skiers— 217.3±31.6 g, 112.7±15.0 g/m^2, and 0.35±0.01; in swimmers—192.6±1.3 g, 100.0±9.8 g/m^2; and 0.37±0.02; in weightlifters—153.4±40.7 g, 87.4±19.3 g/m^2, and 0.33±0.01, respectively.

In comparison with the untrained people, significant differences were revealed in the indicators of LVM and LVMI for skiers and swimmers, and in the indicator of LVRWT for weightlifters. A statistically significant difference was revealed between skiers and weightlifters in the indicators of LVM and LVMI.

The amplitude of positive extremum in weightlifters and untrained people increased during the recovery period (2, 3 min), in swimmers and skiers at the cessation of exercise (1 min recovery). The maximal increase in positive extremum was found in skiers.

The amplitude of negative extremum was increased (vs. baseline) in all subjects at the cessation of exercise, and decreased during 3 minutes of the recovery period until it reached the initial value (excluding untrained people).

In all subjects, the time required to reach the maximal values for positive and negative extremes decreased significantly in comparison with initial values after exercise. The maximal decrease was obtained in swimmers (by 44% for t_{min} and 40% for t_{max}), and minimal in weightlifters (by 39% for t_{min} and t_{max}) (Table 1).

According to anthropometric data, the compared groups were similar. Studies on humans and animals have shown that the chest size, fat tissue and size of the pectoral muscles have a slight effect on the heart's electrical activity on the body surface.[9,12] Therefore, the difference in the ratio of muscle, fat and bone tissue among athletes of different sports[13] can be neglected, and the revealed differences in the BSPM characteristics are due to the influence of the type of myocardial remodeling. During physical workload, the indicators of HR and stroke volume increase rapidly; as a result, the strength of myocardial contraction increases, and these changes are much greater in athletes than in untrained people.

In comparison with weightlifters, the cross-country skiers and the swimmers have a larger heart size and volume of the left ventricle, but have less thickness of the interventricular septum and posterior wall of the left ventricle. Earlier, we showed that in cross-country skiers and weightlifters the duration of the ventricular depolarization period of the heart was the same, but the duration of the different phases of depolarization was different. These differences indicate a difference in the heart's electrical activity in athletes with eccentric and concentric types of myocardial remodeling.

According to the BSPM results of this study, during the period of VR, the temporal characteristics of the extremes after exercise change maximally in endurance athletes (swimmers and skiers), and minimally in strength athletes (weightlifters). As a result of a static training in weightlifting, a ventricular pressure overload leads to a thickening of the heart wall,[15] and the diameter of the myofibrils in the cardiomyocytes increases without an increase in their number.[16] In dynamic training in cross-country skiing, overload of ventricles by volume leads to lengthening of cardiomyocytes. According to experimental data on animals with pathological hypertrophy of the left ventricular, the t_{max} extremes increase during VR.[17] In our study, there were no differences in the indicators of the maximum time and the minimum time between athletes with eccentric and concentric myocardial remodeling at baseline, which confirms the physiological origin of wall thickening in weightlifters.

Table 1.

The amplitude-temporal characteristics of the extremes in subjects during rest and recovery period after submaximal workload

Extremes	Subjects	Baseline	Recovery period after physical workload		
			1 min	2 min	3 min
max, mV	untrained people	0.86±0.05	0.86±0.25	1.05±0.35	0.98±0.36
	cross-country skiers	0.99±0.12#	1.07±0.41	0.82±0.38	0.88±0.28
	swimmers	0.76±0.17˟	0.94±0.10	0.93±0.10	0.74±0.09*
	weightlifters	0.91±0.16	0.87±0.31	0.87±0.40	0.97±0.30
min, mV	untrained people	0.34±0.04	0.40±0.09	0.39±0.12	0.42±0.03*
	cross-country skiers	0.35±0.08	0.50±0.09*	0.53±0.16*	0.30±0.06#
	swimmers	0.36±12	0.39±0.10	0.41±0.10	0.33±0.10
	weightlifters	0.33±0.11	0.44±0.24	0.39±0.13	0.32±0.14
t_{max}, ms	untrained people	220±16	136±14*	149±18*	161±20*
	cross-country skiers	256±54	166±5*#	197±17#	228±37#
	swimmers	250±32	150±14*˟	173±26*	197±35*
	weightlifters	225±37	156±27*	160±44*	175±57
t_{min}, ms	untrained people	234±14	141±14*	154±19*	156±18*
	cross-country skiers	272±39#	165±7*#	198±15*#	199±47*
	swimmers	263±20^	147±16*	160±20*	207±33*^
	weightlifters	226±38	156±17*	175±22*	190±39

$P<0.05$ in comparison to: *- baseline; #- between cross-country skiers and untrained people; ˟- between swimmers and cross-country skiers; ^- between swimmers and untrained people

Thus, the study of the heart's electric field in athletes of the same sex, age and sport qualification, but different sport disciplines, showed that the reaction of the heart to physical activity is reflected in different changes of the amplitude-temporal characteristics of the extremes on the body's surface in athletes with eccentric and concentric myocardial remodeling.

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Competing Interests

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Pulmonology

Evaluation of the Effectiveness of Immunomodulatory Therapy in Chronic Obstructive Pulmonary Disease

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Abstract

The purpose of our study was to evaluate the effectiveness of sodium deoxyribonucleate (Derinat, solution for intramuscular injection) in combination with standard therapy in the treatment of COPD Group C patients in outpatient settings.

Materials and Methods: The study included 80 patients (43 men and 37 women) with COPD (Group C), mean age of 51.7±1.4 years. Blood sampling for the study was carried out 3 times—before the start of therapy, and on days 5 and 15. Using monoclonal antibodies, we determined the number of lyphocytes carrying markers CD3+, CD+, CD8+, and CD72+. The quality of life (QL) indicators were evaluated using the St. George's Respiratory Questionnaire (SGRQ). For at least 15 days, patients received standard COPD therapy. All patients were divided into 2 groups: Group 1 included 41 patients who received Derinat 75 mg intramuscularly once daily for 5 days; then - 5 injections with an interval of 48 hours against the background of standard therapy. Group 2 included 39 patients who continued to receive standard COPD therapy.

Results: The inclusion of Derinat in the complex therapy of COPD contributed to the normalization of the T-cell to B-cell ratio, an increase in the number of T suppressors. Assessing the clinical effects of combination therapy with the inclusion of the studied drug, a marked decrease in shortness of breath, cough, and the amount of sputum excreted can be noted in comparison with standard therapy. The improvement of the immunological status and clinical indicators against the background of complex therapy was accompanied by an increase in QL. (**International Journal of Biomedicine. 2019;9(4):300-303.**)

Key Words: sodium deoxyribonucleate • pro-inflammatory mediators • immunomodulatory therapy

Introduction

Chronic obstructive pulmonary disease (COPD) is currently the fourth leading cause of death in the world [1] but is projected to be the third leading cause of death by 2020.[2] It should be noted that in most cases, COPD is diagnosed only in the late stages of the disease. A competent assessment of the dynamics of the COPD course is possible only with an in-depth study of all the links in the pathogenesis of the disease, namely chronic systemic inflammation and an imbalance in the system of local and systemic immune responses.

The trigger agent for the onset of COPD is abnormal inflammation, which occurs in the small bronchi and bronchioles under the influence of various factors. Secretion of pro-inflammatory cytokines in the mucous membrane of the bronchi leads to the activation of fibroblast structures of connective tissue and development of fibrosis. All these mechanisms trigger a cascade of important pathogenic reactions, which ultimately provoke an imbalance between the immune response of the bronchial mucosa and their reparative properties. Thus, systemic inflammation is associated with local inflammation accompanied by the production of biologically active substances.[3-5] Macrophages are a leading factor in the pathophysiology of COPD. It has been found that the number of them in the airways reliably correlates with the severity of the disease.[6]

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The initiation and prolongation of local inflammation is provided by neutrophils containing a complex of pro-inflammatory mediators, in particular, IL-6, IL-1β, TNF-α and CRP.[2,7] Systemic inflammation is interconnected with local inflammation and is the result of the release of biologically active substances into the systemic circulation, activation of leukocytes in the peripheral blood, as well as stimulation of hematopoietic organs with pro-inflammatory mediators. The biomarkers of the inflammatory process in COPD are IL-8, TNF-α.[8] The number of these cytokines correlates with the number of neutrophils.[9]

The relationship between the immunosuppressive effects of COPD risk factors and the inflammatory response to an infectious agent is not currently in doubt, and a number of studies confirm this.[6,10,11] In this regard, adequate immunomodulatory therapy can help increase the effectiveness of treatment. Immunomodulators are widely represented in the pharmaceutical market; however, it is extremely difficult to assess their declared effectiveness. Many studies have shown that the maximum clinical effect of immunomodulating drugs in the complex treatment of COPD can be obtained by combining the immunotropic and reparative effects of the drugs, which together provide a pronounced clinical efficacy.[7,12]

At present, sodium deoxyribonucleate (Derinat) related to nucleic acid derivatives is of particular interest as an immunomodulator in chronic diseases. According to the literature, sodium deoxyribonucleate is an agonist for Toll-like receptor 9 (TLR-9, CD289).[13,14] The immunomodulating effect of the drug is due to the interaction of the active substance (cytosine-guanine) with TLR9 on immunocompetent cells, which leads to the subsequent activation of a number of immune mechanisms. First, the stimulation of TLR in dendritic cells increases their ability to influence the differentiation of T helper cells in the direction of the formation of Type 2 T helper cells (Th2). Under the influence of Th2, B-lymphocytes differentiate into plasma cells secreting IgG_2, IgG_4, and IgM. Stimulated by TLR9, epithelial cells enhance the secretion of sIgA, which performs both the barrier function and the function of opsonin for interaction with the cellular element of the local immune response: macrophages and NK.[7,15] Thus, stimulation of macrophage TLR9 with an increase in IFNγ production leads to the activation of three levels of antiviral macrophage response.[3]

The purpose of our study was to evaluate the effectiveness of sodium deoxyribonucleate (solution for intramuscular injection, 15 mg/ml) in combination with standard therapy in the treatment of COPD Group C patients in outpatient settings.

Materials and Methods

We conducted a randomized controlled clinical trial. The study included 80 patients (43 men and 37 women) with COPD (Group C), mean age of 51.7±1.4 years.

The investigation was approved by local ethics committees, and written informed consent was obtained from all participants.

All patients had a smoking index of more than 18 pack-years and a history of no data on the presence of atopy and bronchial asthma. The diagnosis of COPD was based on a) clinical symptoms (cough, sputum production, shortness of breath), b) a history of exposure to risk factors, and c) signs of airflow limitation on spirometry: a post-bronchodilator FEV1/FVC ratio < 70%.[16]

Blood sampling for the study was carried out 3 times—before the start of therapy, and on days 5 and 15. Using monoclonal antibodies, we determined the number of lyphocytes carrying markers CD3+, CD4+, CD8+, and CD72+. Lymphocytes were isolated by sedimentation in the density gradient of ficoll verographin, according to the Böyum method. Immediately after isolation from the blood, their viability was assessed. The lymphocyte absolute number in peripheral blood was calculated according to Friemel's criteria.[17]

The quality of life (QL) indicators were evaluated according to scores on the St. George's Respiratory Questionnaire (SGRQ)[18] before the study, and on Day 15 of therapy. Scores are based on a scale of 0 to 100, with lower scores indicating better functioning. For the SGRQ, a decrease in the score reflects an improvement. The minimum important difference in the SGRQ total score has been reported to be a change of -4 points.[19,20]

Since the development of COPD by bronchitis type makes it possible to predict the deterioration of the functional state of the patient and the increased risk of exacerbation of the disease, special attention was paid to the evaluation of coughing using the Chung scale score.[21]

For at least 15 days, patients received standard COPD therapy: salmeterol 25 μg (one inhalation twice daily) and fluticasone 500 μg twice daily. All patients were randomly divided into 2 groups: Group 1 included 41 patients who received Derinat 75 mg intramuscularly once daily (slow injection for 1.5-2 minutes) for 5 days; then - 5 injections with an interval of 48 hours against the background of standard therapy. Group 2 included 39 patients who continued to receive standard COPD therapy. Patients of Groups 1 and 2 were comparable in their age, gender, clinical performance, and duration of observation

Statistical analysis was performed using StatSoft Statistica v10.0 Baseline characteristics were summarized as frequencies and percentages for categorical variables and as mean±SEM for continuous variables. Student's unpaired and paired t-tests were used to compare two groups for data with normal distribution. Mann-Whitney U test and Wilcoxon criterion were used to compare means of variables not normally distributed. A probability value of $P<0.05$ was considered statistically significant.

Results

The study results showed that in all patients before therapy, the levels of CD3+, CD4+ and CD8+ lymphocytes were decreased relative to the physiological norm by an average of 22.4±2.7%, and the values of CD72+ lymphocytes were higher than normal values by 11.4±2.7%, which indicated a prolonged sluggish chronic course of COPD in the observed patients.

Table 1.

The changes in blood lymphocyte populations and subpopulations during treatment

Day	CD3+			CD4+			CD8+			CD72+		
	Group 1	*P*	Group 2	Group 1	*P*	Group 2	Group 1	*P*	Group 2	Group 1	*P*	Group 2
Initial data	59.7±0.61	>0.05	58.1±0.59	26.4±0.51	>0.05	26.7±0.44	29.1±0.88	>0.05	28.8±0.62	16.4±0.37	>0.05	17.1±0.59
Day 5	63.1±0.39*	<0.05	59.8±0.41	31.4±0.34*	<0.05	27.8±0.57*	33.7±1.2*	<0.05	31.1±0.37*	12.8±1.4*	<0.05	15.7±0.11
Day 15	66.4±0.27*	<o.o5	61.4±0.22*	35.7±0.49*	<0.05	31.7±0.19*	41.7±0.31*	<0.05	36.3±1.1*	9.7±0.22*	<0.05	12.4±0.37*

*P<0.05 - compared to initial data

On Day 5 of therapy, in Group 1 there was a significant increase in the levels of CD3+, CD4+, and CD8+ lymphocytes and a decrease in the CD72+ level (Table 1). By this time, in Group 1 the severity of clinical symptoms (cough, sputum, shortness of breath) was significantly reduced, which was not observed in Group 2 (Table 2). On Day 15 of therapy, in Group 1 the marked dynamics for all cells increased significantly. In Group 2, there was also a dynamics similar to Group 1, but to a much lesser extent, which was expressed by the presence of significant differences between groups at all stages of treatment. On Day 15 of therapy, in Group 1 the severity of all clinical symptoms continued to significantly decrease, in contrast to Group 2, where only cough and shortness of breath significantly decreased.

Table 2.

The dynamics of clinical symptoms during treatment

Symptom	Before treatment		Group 1		Group 2	
	Group 1	Group 2	Day 5 after therapy	Day 15 after therapy	Day 5 after therapy	Day 15 after therapy
Cough	2.3±0.2	2.4±0.1	1.4±0.2*	0.8±0.1	2.2±0.2*	1.8±0.1*
Sputum	1.5±0.2	1.4±0.1	1.1±0.1*	0.6±0.1*	1.2±0.1	0.9±0.1
Shortness of breath	2.9±0.2	2.8±0.1	1.9±0.1*	1.1±0.1*	2.4±0.2*	1.6±0.1*

*P<0.05 - compared to initial data

Table 3.

Mean changes in SGRQ scores on Day 15 of therapy

Group	Symptoms score (points)	Activity score (points)	Impact score (points)
Group 1	-8.1±0.4	-4.6±0.3	-0.8±0.2
Group 2	-3.8±0.2	-2.1±0.2	1.4±0.1

On Day 15 of therapy, an analysis of QL using the SGRQ questionnaire indicated that more pronounced shifts on the domains of "Symptoms," "Activity" and "Impacts" (social functioning, psychological disturbances) were recorded in Group 1. In Group 2, a low QL was maintained (Table 3).

Conclusion

The inclusion of Derinat in the complex therapy of COPD contributed to the normalization of the T-cell to B-cell ratio, an increase in the number of T suppressors, which probably can increase the expression of receptors mediating the Fas-dependent mechanism of apoptosis induction, contributing to the normalization of the protective function of the bronchial mucosa and a pronounced reparative effect. However, more accurate conclusions regarding the immunotropic effects of Derinat can be obtained during a longer study with the participation of a larger group of COPD patients. Assessing the clinical effects of combination therapy with the inclusion of the studied drug, a marked decrease in shortness of breath, cough, and the amount of sputum excreted can be noted in comparison with standard therapy. The improvement of the immunological status and clinical indicators against the background of complex therapy was accompanied by an increase in QL.

Competing Interests

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Pulmonology

The Comparison of Inspiratory Muscle Training Effectiveness in COPD Patients with Obesity and Normal Weight

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Abstract

The objective of this study was to compare the effectiveness of inspiratory muscle training in COPD patients with obesity and patients with normal body weight.

Materials and Methods: The study involved 2 groups of patients with COPD (clinical Group D) similar in spirometric parameters with signs of airflow limitation on spirometry (FEV_1<50% of predicted, a post-bronchodilator FEV_1/FVC ratio<70% of predicted). Group 1 consisted of 45 obese COPD patients (BMI>30 kg/m^2). Group 2 included 44 COPD patients with normal body weight (BMI from 18.5 kg/m^2 to 24.9 kg/m^2). The groups were comparable in age, gender, severity of bronchial obstruction, concomitant pathology, and drug therapy. All patients underwent IMT for 8 weeks. Breathe-Link Live Feedback Software was used to evaluate such parameter as SI (Strength Index), PIF (Peak Inspiratory Flow), Average Pressure, Average Power, Average Flow, Average Volume for the entire sessions were estimated. At the baseline and after 8 weeks all patients underwent an assessment of the lung function using spirometry (including FVC, FEV_1), and the severity of dyspnea using the mMRC questionnaire (The Modified Medical Research Council).

Results: A comparative analysis of the estimated parameters of training sessions showed a significant increase in SI, PIF, Average Power, and Average Flow in patients of Group 1. In patients of Group 2, we found only a tendency to be increased in these parameters, with the exception of Average Flow. A statistically significant increase in the FEV_1 and FVC parameters was observed only in Group 1. Statistical analysis showed a close correlation between the SI and the severity of dyspnea on the mMRC scale (r=-0.52, *P*<0.05) in Group 1.

Conclusion: The results of our study indicate a positive effect of IMT in patients with COPD, and a greater effect in patients with obesity than in those with normal body weight. Clinically, an improvement in the IMT parameters was expressed in a decrease in dyspnea and an improvement in spirometric indicators. (**International Journal of Biomedicine. 2019;9(4):304-307.**)

Key Words: chronic obstructive pulmonary disease • obesity • inspiratory muscle training

Abbreviations

6MWT, the 6-minute walk test; **BMI**, body mass index; **COPD**, chronic obstructive pulmonary disease; **FVC**, forced vital capacity; **FEF**, forced expiratory flow; **IMT**, inspiratory muscle training; **PR**, pulmonary rehabilitation; **QL**, quality of life.

Introduction

COPD is a widespread disease that significantly affects QL of patients; exacerbations and worsening of the disease course are one of the main reasons for hospitalization, largely determining the prognosis.[1] Despite the success of medical treatment for COPD, many patients continue to notice symptoms of the disease, such as progressive dyspnea, cough, and poor exercise tolerance.[2] Muscle dysfunction in COPD, the most extensively studied systemic manifestation of the disease, can involve both respiratory and peripheral muscles.[3-6] It is considered to be of multifactorial origin, with local and systemic factors interacting to modify, in different ways, the phenotype and function of any specific muscle.[7,8] Both pulmonary hyperinflation and

increased airway resistance increase the work of breathing, which is mainly dependent on inspiratory muscles.[8] Like other striated muscles in the whole body, respiratory muscles can also be influenced by systemic factors, such as inflammation and oxidative stress, nutritional depletion and the effect of certain drugs used in COPD treatment.[7,9-14] According to GOLD (2018), much attention is paid to PR programs that include physical training, especially of the muscles of the upper shoulder girdle and respiratory muscles, which have been shown to reduce the severity of the clinical disease course, the frequency of exacerbations, and hospitalizations.[1,15] A recent meta-analysis showed that only the use of IMT in patients with moderate and severe COPD led to a significant reduction in the severity of dyspnea. In addition, these patients showed an improvement in exercise tolerance.[16] Thus, IMT can be considered as an addition to treatment in patients with COPD who cannot fully participate in general physical training due to the presence of a concomitant pathology. In the available literature, however, there is very little information about the effectiveness of IMT in patients with COPD and obesity. Nevertheless, this component of the PR program in patients with COPD and obesity may be the most effective and preferred, given the impossibility of providing adequate exercise regimes due to the presence of a concomitant pathology of the cardiovascular system, which is more common in COPD patients with obesity than in COPD patients with normal body weight.

The objective of this study was to compare the effectiveness of inspiratory muscle training (IMT) in COPD patients with obesity and patients with normal body weight.

Materials and Methods

The study involved 2 groups of patients with COPD (clinical Group D) (average age of 63.5±7.2 years), similar in spirometric parameters with signs of airflow limitation on spirometry (FEV_1<50% of predicted, a post-bronchodilator FEV_1/FVC ratio<70% of predicted). Group 1 consisted of 45 obese COPD patients (BMI>30 kg/m^2). Group 2 included 44 COPD patients with normal body weight (BMI from 18.5 kg/m^2 to 24.9 kg/m^2). The groups were comparable in age, gender, severity of bronchial obstruction, and concomitant pathology. Exclusion criteria were cardiovascular diseases with increased LVEDP, a need for oxygen support, COPD exacerbation within 1 month, a history of spontaneous pneumothorax, signs of bullous disease in X-ray examination, severe osteoporosis combined with a history of spontaneous fracture of the ribs, lung surgery, and damage of the tympanic membrane.

All patients were familiarized with the IMT program; no additional regular physical exercises were prescribed to anyone. None of the patients had contraindications or restrictions for IMT using a breathing trainer. All patients received long-acting bronchodilators and inhaled glucocorticosteroids on a regular basis.

Respiratory muscles were trained using the POWERbreathe K5 breathing simulator (POWERbreathe International Ltd., Southam, England, United Kingdom).[17] The patient was in a sitting position, the air outlet through the nose when performing a breathing maneuver was blocked

with a nasal clip. Training began with a warm-up for 1 minute at 50% of the expected full load. Then a training session was held, consisting of 30 breaths through the device with a certain resistance. Training was terminated upon completion of the program or upon the appearance of undesirable symptoms—cough, dyspnea, feeling of severe tiredness, and pain in the chest. In order to ensure safety during training sessions, oxygen saturation was monitored. Training sessions were performed 3 times a week for 8 weeks. Breathe-Link Live Feedback Software (POWERbreathe International Ltd., Southam, England, United Kingdom) was used to evaluate a number of parameters:

- SI (Strength Index) is an indicator of the strength of the respiratory muscles obtained from the peak inspiratory flow, namely the predicted value of maximum inspiratory pressure. In contrast to PImax (maximum inspiratory mouth pressure), dynamic assessment allows for the evaluation of inspiratory muscle output throughout the total lung volume. This is considered more appropriate for measuring inspiratory muscle performance than isometric assessments. The POWERbreathe has been validated, and its accuracy to measure dynamic inspiratory muscle pressure has been demonstrated. Pressure is plotted at every moment throughout each lung volume, which creates a line through IMT. The highest point in this line is called the SI. The use of the SI in clinical practice is preferable, especially with the integration of this parameter into automated IMT programs using modern electronic devices.

- PIF (Peak Inspiratory Flow) is an indicator that reflects the ability of the respiratory muscles to contract rapidly and overcome the natural resistance and elasticity of the respiratory system.

- Average Pressure for the entire session: represents the average pressure created in the airways due to the force of the inspiratory muscles during training.

- Average Flow for the entire session: represents the average flow generated in the airways due to the force of the respiratory muscles during training.

- Average Power for the entire session: represents the average power of muscle activity, which combines strength and speed (pressure×flow), averaged for individual breaths of the session.

- Average Volume for the entire session: this is a measure of the average amount of air that was inhaled during training.

In addition, at the baseline and after 8 weeks all patients underwent an assessment of the lung function using spirometry (including FVC, FEV_1), and the severity of dyspnea using the mMRC questionnaire (The Modified Medical Research Council). All tests were carried out by the same researcher without knowledge of a group affiliation (participants were blinded).

The study was carried out in compliance with Ethical Principles for Medical Research Involving Human Subjects, Adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964, and amended by the 52nd WMA General Assembly, Edinburgh, Scotland, October 2000. The study was

approved by the Ethics Committee of Voronezh State Medical University. Written informed consent was obtained from each patient.

All data was evaluated with STATGRAPHICS Plus 5.1. The normality of distribution of continuous variables was tested by the Kolmogorov-Smirnov test with the Lilliefors correction and Shapiro-Wilk test. Baseline characteristics were summarized as frequencies and percentages for categorical variables and as mean±SD for continuous variables. Student's unpaired and paired t-tests were used to compare two groups for data with normal distribution. Pearson's correlation coefficient (r) was used to determine the strength of the relationship between the two continuous variables. A probability value of $P<0.05$ was considered statistically significant.

Results and Discussion

A comparative analysis of the estimated parameters of training sessions showed a significant increase in SI, PIF, Average Power, and Average Flow in patients of Group 1. In patients of Group 2, we found only a tendency to be increased in these parameters, with the exception of Average Flow (Table 1).

Table 1.

IMT parameters in the studied groups before and after training sessions

Parameters	Group 1 (n=45)			Group 2 (n=44)			P_{1-2} after 8 weeks
	before	P	after 8 weeks	before	P	after 8 weeks	
SI, cmH_2O	19.7±4.58	<0.05	28.83±6.21	18.80±5.11	>0.05	21.33±7.92	<0.05
PIF, l/sec	0.76±0.21	<0.05	1.53±0.32	0.78±0.26	>0.05	0.87±0.34	<0.05
Average pressure, cmH_2O	6.99±2.31	>0.05	9.34±4.7	5.10±3.61	>0.05	6.59±2.58	>0.05
Average power, watt	0.31±0.11	<0.05	0.83±0.26	0.24±0.12	>0.05	0.36±0.21	<0.05
Average flow, l/sec	0.48±0.12	<0.05	0.80±0.17	0.46±0.16	<0.05	0.78±0.26	>0.05
Average volume, l	0.71±0.28	>0.05	0.74±0.51	0.91±0.35	>0.05	1.10±0.62	>0.05

According to the estimated spirometric parameters, after 8 weeks of IMT, statistically significant differences between the groups were observed. In Group 1, FEV_1 increased from 44.1±3.3% to 48±2.7%, and FVC from 62±3.6% to 68±4.1% ($P<0.05$). Group 2 also showed an improvement in these parameters, but no statistically significant changes were found.

According to the severity of dyspnea at the baseline, in accordance with the mMRC questionnaire, there were no significant differences between the groups. Statistically significant differences were noted after 8 weeks from the beginning of IMT and were manifested in a significant decrease

in mMRC scores in Group 1, and the lack of improvement in Group 2 (3.4±0.5 and 2.8±0.5, P=0.01; 3.5±0.6 and 3.4±0.2, P=0.12, respectively). Statistical analysis showed a close correlation between the SI and the severity of dyspnea on the mMRC scale (r=-0.52, $P<0.05$) in Group 1.

The data obtained do not contradict the results of a study by Villiot-Danger et al., in which 20 patients with morbid obesity (BMI=45±7 kg/m^2) took part.[19] Despite the fact that the authors did not show an increase in the strength of the respiratory muscles, a decrease in dyspnea was observed in COPD patients. In addition, IMT significantly improved the results of 6MWT and QL, while in the group without IMT changes were not observed. It was also suggested, that IMT could potentially prevent the early onset of respiratory muscle fatigue during exercise, and that in obese patients exercise tolerance may be increased due to a decrease in dyspnea, which, in turn, could be the basis of the improvement in QL observed in patients.

Thus, taking into account the results of our study, the IMT program was more effective in patients with COPD and obesity than in patients with normal body weight, especially in relation to such significant integral parameters as SI and PIF. A possible explanation for this may be that obese patients had worse muscle condition due to the peculiarities of their behavior with limited physical activity and lifestyle. By analogy with skeletal muscles, training and increasing the strength of the respiratory muscles is dose-dependent. Training with a constant ongoing load limits the load that a patient with COPD can reach, as fatigue develops quickly and rest is required.[20] IMT protocols, suggesting an interval approach with the inclusion of rest periods, during which there is a decrease in the severity of symptoms that appear in the exercise period, are more effective and allow optimizing the achievement of tolerance to large loads. This in turn contributes to the maximum increase in strength and endurance of the trained respiratory muscles.[21,22]

Thus, the results of our study indicate a positive effect of IMT in patients with COPD, and a greater effect in patients with obesity than in those with normal body weight. Clinically, an improvement in the IMT parameters was expressed in a decrease in dyspnea and an improvement in spirometric indicators. However, further studies are needed to finally assess the effect of various types and modes of respiratory muscle training in patients with COPD and obesity.

Competing Interests

The authors declare that they have no competing interests.

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INTERNATIONAL JOURNAL OF BIOMEDICINE

Assessment of Non-Motor Symptoms in Essential Tremor

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Abstract

In the clinical picture of essential tremor (ET), in addition to tremulous hyperkinesis, the importance of non-motor manifestations has recently been discussed. Despite their high occurrence, in most cases these manifestations remain unverified. The purpose of this study was to assess the incidence of non-motor symptoms (NMS) in patients with ET. The study included 3 groups comparable by sex and age. Group 1 (the main group) consisted of 53 patients with ET; Group 2 consisted of 57 patients with Parkinson's disease (PD); Group 3 consisted of 111 individuals without ET or PD, and without burdened heredity for extrapyramidal diseases. In study Groups the distribution by ethnicity was as follows: 22(41.5%) ethnic Yakuts and 31(58.5%) ethnic Russians in Group 1, 29(50.9%) ethnic Yakuts and 28(49.1%) ethnic Russians in Group 2, and 67(60.4%) ethnic Yakuts and 44(39.6%) ethnic Russians in Group 3. All subjects filled out the NMSQuest scale, which contains 30 questions for various groups of NMS. The analysis of NMS using the NMSQues scale in the three study groups showed a similarity between ET plus and PD in non-motor manifestations. The spectrum of NMS in patients with ET plus of both ethnic groups is heterogeneous and prevails in patients of the Russian ethnic group. Thus, Yakut patients with ET plus and PD showed a similarity in the frequency of hyposmia to Russian representatives with ET plus and PD in hyposmia, dysphagia, pain, sadness and restless legs syndrome. Excessive sweating was found in more than 64% of patients with ET plus of both ethnic groups. The results indicate a similarity in manifestations of ET plus and PD, which is possibly due to both the genetic and phenotypic affinity of these nosologies, and suggests that ET plus can be a transitional form of PD. (**International Journal of Biomedicine. 2019;9(4):308-312.**)

Key Words: essential tremor • Parkinson's disease • non-motor symptoms • NMSQuest

Introduction

ET is considered the most common disease of the extrapyramidal system, with a slowly progressing and disabling course.[1] A classic manifestation of the disease is a progressive kinetic-postural hand tremor, most often in combination with a tremor in a different location. ET is now considered to be a "syndrome" that can be associated with other symptoms. Based on a new classification of tremor,[2] ET can be classified as Essential Tremor and Essential Tremor plus. ET plus is a tremor with similar characteristics but may have additional neurological signs, such as impaired tandem gait or memory, dystonia or other mild neurological signs of unknown significance.[2] In the clinical picture of the disease, in addition to tremulous hyperkinesis, the importance of non-motor symptoms (NMS) has recently been discussed.[3] The spectrum of NMS in ET includes cognitive, psychiatric, sensory and other disorders (sleep disturbances, decreased body mass index, decreased quality of life).[3-11] NMS in ET, along with obligate symptoms, make up a common, rather complex phenotype of the disease.[12]

A number of authors do not exclude the probability of a manifestation of the disease with NMS.[9,10] Patients with ET are diagnosed with a variety of cognitive impairments: from mild/moderate impairment to dementia.[4] Psychiatric symptoms of ET include depression, increased anxiety, apathy, and changes in personality traits.[3] In general, studies show that patients with ET are observed with a high frequency of depression, apathy, situational anxiety, and social phobia,[13,15,16] often

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diagnosed with sensory impairment in the form of hearing loss and olfactory dysfunction.[17,18] The spectrum of NMS in ET is supplemented by sleep disturbances in this category of patients.[19]

Materials and Methods

The study included 3 groups comparable by sex and age. Group 1 (the main group) consisted of 53 patients (average age of 62.62±2.3 years) with ET: 19(35.8%) men and 34(64.2%) women. In Group 1, the distribution by ethnicity was as follows: 22(41.5%) ethnic Yakuts and 31(58.5%) ethnic Russians. Group 2 consisted of 57 patients (average age of 67.1±1.02 years) with Parkinson's disease (PD), including 23(40.4%) men and 34(59.7%) women. In Group 2, the distribution by ethnicity was as follows: 29(50.9%) ethnic Yakuts and 28(49.1%) ethnic Russians. Group 3 (the control group) consisted of 111 individuals (average age of 63.4±0.93 years) without ET or PD, and without burdened heredity for extrapyramidal diseases. In Group 3, the distribution by ethnicity was as follows: 67(60.4%) ethnic Yakuts and 44(39.6%) ethnic Russians.

All subjects filled out the NMSQuest scale, which contains 30 questions for various groups of NMS.[20] Each positive answer was scored as 1 point.

Statistical analysis was performed using statistical software package SPSS version 17.0 (SPSS Inc, Chicago, IL). Categorical variables were analyzed using the Chi-square test with the Yates' correction. The critical level of statistical significance for the three groups was determined at $P \leq 0.05/3 = 0.017$ (P_{1-2}, P_{2-3}, P_{1-3}).

The study was carried out in compliance with Ethical Principles for Medical Research Involving Human Subjects, Adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964, and amended by the 52nd WMA General Assembly, Edinburgh, Scotland, October 2000. The study was approved by our regional ethics committee. All patients gave their written informed consent.

Results and Discussion

According to the results of the survey, in Group 1 the most frequent NMSs were nightly urination (64.2%), excessive sweating (62.3%), insomnia (41.5%), dizziness (feeling light-headed) (41.5 %), sadness (39.6%), constipation (39.6%), subjective feeling of memory loss (35.8%), anxiety (34.0%), hyposmia (30.2%), false urge to urinate (26.4%), and incomplete defecation (26.4%). The analysis revealed statistically significant differences in symptoms, such as hypersalivation, constipation, sexual dysfunction, falls and daytime sleepiness due to their frequent occurrence in patients of Group 2 ($P<0.017$). In Group 1, falls were due to tremor of the lower extremities in one person and vertigo in two people. At the same time, statistically significant differences in dysphagia, hyposmia and apathy were found due to their rare frequency in the healthy individuals of Group 3. Patients of Group 1 were characterized by a high frequency of excessive sweating and insomnia, unlike patients of Group 2.

Table 1.
Distribution of NMS of in the study group

NMS	Group 1	Group 2	Group 3	P-value
Hypersalivation	5(9.4%)	19(38%)	1(2.6%)	P=0.000 P_{1-2}=0.001 P_{1-3}=0.234 P_{2-3}= 0.000
Hyposmia	16(30.2%)	24(48%)	3(7.7%)	P=0.000 P_{1-2}=0.064 P_{1-3}=0.008 P_{2-3}= 0.000
Dysphagia	14(26.4%)	12(24%)	2(5.1%)	P= 0.026 P_{1-2}=0.777 P_{1-3}=0.008 P_{2-3}=0.015
Nausea. vomiting	6(11.3%)	6(12%)	1(2.6%)	P=0.444
Constipation	21(39.6%)	40(80%)	15(38.5%)	P=0.000 P_{1-2}=0.000 P_{1-3}=0.909 P_{2-3}=0.000
Enuresis	1(1.9%)	2(4%)	0	P=0.798
Incomplete defecation	14(26.4%)	0	6(15.4%)	P=0.001 P_{1-2}=0.000 P_{1-3}= 0.205 P_{2-3}=0.014
False urge to urinate	14(26.4%)	7(14%)	2(5.1%)	P=0.020 P_{1-2}=0.118 P_{1-3}=0.008 P_{2-3}=0.306
Nocturia	34(64.2%)	22(44%)	18(46.2%)	P=0.084
Unexplained pain	13(24.5%)	15 30%)	6 (15.4%)	P=0.27
Weight fluctuations	2(3.8%)	7(14%)	2(5.1%)	P= 0.11
Memory loss	19(35.8%)	31(62%)	19(48.7%)	P=0.03 P_{1-2}=0.008 P_{1-3}=0.215 P_{2-3}=0.210
Apathy	14(26.4%)	13(26%)	3(7.7%)	P=0.054
Hallucinations	3(5.7%)	3(6%)	0	P=0.3
Difficulty concentrating	10(18.9%)	14(28%)	7(17.9%)	P=0.42
Sadness	21(39.6%)	22(44%)	6(15.4%)	P=0.000 P_{1-2}=0.652 P_{1-3}=0.012 P_{2-3}=0.004
Anxiety	18(34.0%)	19(38%)	13(33.3%)	P=0.88
Hypoactive sexual desire disorder	4(7.5%)	32(64%)	(20.5%)	P=0.000 P_{1-2}=0.000 P_{1-3}=0.068 P_{2-3}=0.000
Sexual dysfunction without sexual desire disorder	2(3.8%)	14(28%)	0	P=0.000 P_{1-2}=0.001 P_{1-3}=0.615 P_{2-3}=0.000

Table 1.

Distribution of NMS of in the study group (Continued)

NMS	Group 1	Group 2	Group 3	*P*-value
Feeling light-headed	22(41.5%)	28(56%)	15(38.5%)	P=0.189
Falls	3(5.7%)	11(22%)	2(5.1%)	P=0.012 P$_{1-2}$=0.016 P$_{1-3}$=0.724 P$_{2-3}$=0.025
Excessive daytime sleepiness	11(20.8%)	26(52%)	9(23.1%)	P=0.001 P$_{1-2}$=0.001 P$_{1-3}$=0.790 P$_{2-3}$=0.006
Imsomnia	22(41.5%)	10(20%)	16(41%)	P=0.037 P$_{1-2}$=0.018 P$_{1-3}$=0.964 P$_{2-3}$=0.030
Vivid dreaming	11(20.8%)	10(20%)	9(23.1%)	P=0.936
Sleep-talking	10(18.9%)	14(28%)	4(10.2%)	P=0.111
Restless legs syndrome	9(17.0%)	10(20%)	8(20.5%)	P=0.891
Swelling (edema)	12(22.6%)	6(12%)	5(12.8%)	P=0.273
Excessive sweating	33(62.3%)	8(16%)	4(10.2%)	P=0.000 P$_{1-2}$=0.000 P$_{1-3}$=0.000 P$_{2-3}$=0.431
Double vision	4 (7.5%)	1 (2%)	1 (2.6%)	P=0.313
Illusions	2 (3.8%)	0	0	P=0.658

Symptoms such as hyposmia, apathy, and dysphagia were equally common in Groups 1 and 2 (Table 1).

Considering the presence of other extrapyramidal phenomena in the clinical aspect of ET, we also evaluated NMS in patients with ET plus in the ethnic aspect. Patients with ET plus of the Yakut and Russian ethnic groups comprised 17/45(37.8%) and 28/45(62.2%) patients, respectively. Group 2 included 29/57(50.9%) ethnic Yakuts and 28/57(49.1%) ethnic Russians. Group 3 consisted of 35/57(61.4%) ethnic Yakuts and 22/57(38.6%) ethnic Russians.

Statistically significant differences were shown between the 3 study groups for such NMS as hypersalivation, hyposmia, constipation, sexual dysfunction, and daytime sleepiness, due to their frequent occurrence in ethnic Yakuts of Group 2 (Figure 1). We found no statistically significant difference in the incidence of hyposmia in patients with ET plus and patients with PD of the Yakut ethnic group.

In patients with ET plus of the Russian ethnic group, sweating was diagnosed statistically significantly more often compared to Groups 2 and 3 (*P*<0.017) (Table 2). Statistical differences were identified for apathy (35.7% in Group 1 and 39.3% in Group 2) compared to Group 3 (0%).

At the same time, constipation, difficulty concentrating, and sexual dysfunction were statistically significantly more common in patients of Group 2 compared to Group 1. Patients with ET and PD were similar in such NMS as hypersalivation, hyposmia, dysphagia, pain, sadness, and restless legs syndrome (*P*>0.017).
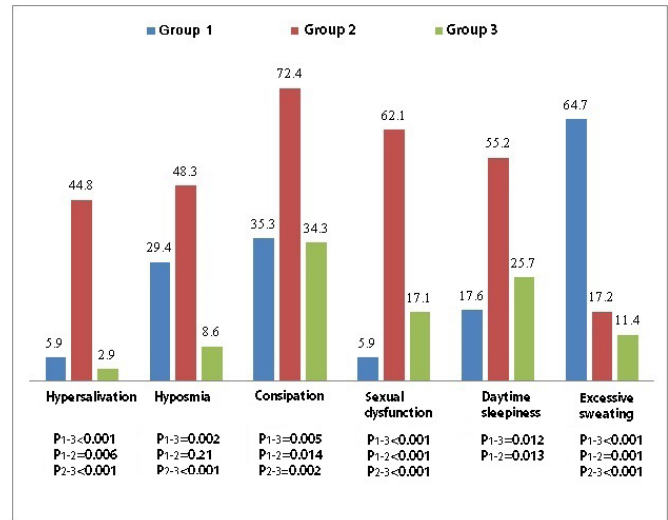
Fig. 1. *The spectrum of NMS in the Yakut representatives of the study groups*

Table 2.

The spectrum of NMS in the Russian representatives of the study groups

NMS	Group 1 (n=28)	Group 2 (n=28)	Group 3 (n=22)	*P*-value
Hypersalivation	2(7.1)	9(32.1)	1(4.5)	P=0.031 P$_{1-2}$=0.019 P$_{1-3}$=0.813 P$_{2-3}$=0.039
Hyposmia	9(32.1)	14(50)	2(9.1)	P=0.009 P$_{1-2}$=0.174 P$_{1-3}$=0.108 P$_{2-3}$=0.002
Dysphagia	10(35.7)	13(46.4)	2(9.1)	P=0.017 P$_{1-2}$=0.415 P$_{1-3}$=0.029 P$_{2-3}$=0.004
Nausea. vomiting	2(7.1)	8(28.6)	0	P=0.024 P$_{1-2}$=0.036 P$_{1-3}$=0.581 P$_{2-3}$=0.019
Constipation	13(46.4)	23(82.1)	7(31.8)	P=0.001 P$_{1-2}$=0.005 P$_{1-3}$=0.295 P$_{2-3}$=0.000
False urges to urinate	8(28.6)	3(10.7)	0	P=0.042 P$_{1-2}$=0.093 P$_{1-3}$=0.019 P$_{2-3}$=0.325

Table 2.

The spectrum of NMS in the Russian representatives of the study groups (Continued)

NMS	Group 1 (n=28)	Group 2 (n=28)	Group 3 (n=22)	*P*-value
Unexplained pain	7(25.0)	13(46.4)	2(9.1)	P=0.013 P_{1-2}=0.094 P_{1-3}=0.279 P_{2-3}=0.011
Apathy	10(35.7)	11(39.3)	0	P=0.005 P_{1-2}=0.783 P_{1-3}=0.008 P_{2-3}=0.004
Difficulty concentrating	5(17.8)	15(53.6)	2(9.1)	P=0.001 P_{1-2}=0.005 P_{1-3}=0.634 P_{2-3}=0.001
Sadness	14(50.0)	17(60.7)	4(18.2)	P=0.009 P_{1-2}=0.420 P_{1-3}=0.020 P_{2-3}=0.002
Sexual dysfunction without sexual desire disorder	2(7.1)	14(50.0)	8(36.4)	P=0.002 P_{1-2}=0.000 P_{1-3}=0.027 P_{2-3}=0.335
Falls	3(10.7)	8(28.6)	0	P=0.042 P_{1-2}=0.093 P_{1-3}=0.325 P_{2-3}=0.019
Restless legs syndrome	5(17.8)	11(39.3)	1(4.5)	P=0.01 P_{1-2}=0.076 P_{1-3}=0.318 P_{2-3}=0.004
Excessive sweating	18(64.3)	8(28.6)	2(9.1)	P=0.000 P_{1-2}=0.007 P_{1-3}=0.000 P_{2-3}=0.176

Conclusion

Thus, analysis of non-motor symptoms using the NMSQues scale in the three study groups showed a similarity between ET plus and Parkinson's disease in non-motor manifestations. The spectrum of non-motor symptoms in patients with ET plus of both ethnic groups is heterogeneous and prevails in patients of the Russian ethnic group. Thus, Yakut patients with ET plus and Parkinson's disease showed a similarity in the frequency of hyposmia to Russian representatives with ET plus and Parkinson's diseasein hyposmia, dysphagia, pain, sadness and restless legs syndrome. Excessive sweating was found in more than 64% of patients with ET plus of both ethnic groups. The results indicate a similarity in manifestations of ET plus and Parkinson's disease, which is possibly due to both the genetic and phenotypic affinity of these nosologies, and suggests that ET plus can be a transitional form of Parkinson's disease.

Competing Interests

Sources of Funding

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ORIGINAL ARTICLE

Obstetrics and Gynecology

The Relationship between Morphological and Functional Features of Preterm Placentas and the Results of Bacteriological Examination of the Discharge from the Cervical Canal of Women with Preterm Birth

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Abstract

The aim of this study was to determine the relationship between the morpho-functional features of placentas from preterm births (PBs) with the results of bacteriological examination of the discharge from the cervical canal in women with spontaneous preterm birth (SPB). The study included 227 pregnant women at gestational age between 28 and 36 weeks and 6 days, who underwent examination in the period from 2017 to 2018. Depending on the gestational age, they were divided into 3 groups. In general, a strong relationship was found between the presence of pathological changes in placentas and the presence of opportunistic flora in women (R=0.722, *P*<0.001). The constructed mathematical models make it possible to determine, with a high degree of certainty, the main bacteria for all the studied groups of pregnant women, thereby identifying the risk group of women at the stage of pregnancy planning, predicting complications and increasing the possibility of deliver a full term baby. (**International Journal of Biomedicine. 2019;9(4):313-319.**)

Key Words: preterm birth • placenta • opportunistic flora • placental insufficiency

Introduction

Preterm birth (PB) is a multifactorial syndrome with a variety of risk factors and long-term health consequences for the child.[1-3] Every year, an estimated 15 million babies are born preterm (before 37 completed weeks of gestation), and this number is rising.[3] Approximately 1 million children die each year due to complications of PB.[4] Common causes of PB include multiple pregnancies, infections and chronic conditions such as diabetes and high blood pressure.[3]

There is a large body of evidence that a cascade of activations of cellular components and mediators of inflammatory pathways results in onset of labor and membrane rupture.[5,6] Supporting the fetus through the preceding gestation, the placenta is a very critical organ in explaining the pathogenesis of spontaneous preterm birth (SPB). Placental pathology provides important diagnostic information to ascertain the cause of PB. Intra-amniotic infection, as is well known, is one risk factor of SPB.[6-8] Bacterial infection and the subsequent inflammatory response are recognized as an important cause of PB. It is hypothesized that these organisms ascend the cervical canal, colonize placental tissues, cause chorioamnionitis and in severe cases infect amniotic fluid and the fetus.[9] However, after 32 weeks' gestation, infection may be a less common cause of PB; instead, many cases of spontaneous preterm labor leading to PB appear to be caused by placental insufficiency (PI).[1] The pathoanatomical investigation of placentas from PBs is useful for assessing the etiology of SPB and the prognosis for the child.[10]

The aim of this study was to determine the relationship between the morpho-functional features of placentas from PBs with the results of bacteriological examination of the discharge from the cervical canal in women with SPB.

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Materials and Methods

Our study included 227 pregnant women at gestational age between 28 and 36 weeks and 6 days, who underwent examination in the period from 2017 to 2018. Depending on the gestational age, they were divided into 3 groups. Group 1 included 73 women at gestational age between 28 and 30 weeks and 6 days; Group 2 included 81 women at gestational age between 31 and 33 weeks and 6 days, Group 3 included 73 women at gestational age between 34 and 36 weeks and 6 days.

All women underwent an assessment of vaginal microcenosis and the quantitative and qualitative composition of the biotope of the cervical discharge, as we described earlier.[8]

The examination of placentas was carried out according to a standardized scheme, including macroscopic analysis, material sampling and histological analysis in 3 stages. At Stage 1 of the histological examination, we performed an assessment of placental maturity;[11] at Stage 2 - a semi-quantitative assessment of the severity of certain structural indicators of the placenta (from 1 to 3 points); and at Stage 3 - the determination of the degree of PI by the totality of the mass of the fetus and placenta, the degree of maturity or immaturity of the villi, the severity of compensatory reactions, and involutive changes.

The study was conducted in accordance with ethical principles of the WMA Declaration of Helsinki (1964, ed. 2013) and approved by the RUDN University Ethics Committee. Written informed consent was obtained from all participants.

Statistical analysis was performed using the Statistica 8.0 software package (StatSoft Inc, USA). Baseline characteristics were summarized as frequencies and percentages for categorical variables and as mean±SEM for continuous variables. Mann-Whitney U test and Kruskal-Wallis test were used, respectively, to compare means of 2 and 3 or more groups of variables not normally distributed. The frequencies of categorical variables were compared using Pearson $\chi2$ or Fisher's exact test, when appropriate. The Pearson's correlation coefficient and Spearman's rank correlation coefficient were was used to determine the strength and direction of the relationship between two variables. The odds ratio (OR), its standard error and 95% confidence interval (CI) were calculated. Logistic regression was used to model dichotomous outcome variables. We calculated the sensitivity, specificity, positive predictive values, negative predictive values, and accuracy of the test results. A value of $P<0.05$ was considered statistically significant.

Results

The average weight of placentas in Groups 1, 2 and 3 was 237.3±49.1g, 228.3±94.1g, and 318.1±90.5g, respectively (Kruskal Wallis test: H=18.30641, *P*=0.0001). Macroscopically, placentas had an ovoid or irregular shape, small size, with additional bulges, more thickened in the center, with thinning at the edges. The umbilical cord was attached at the edges (paraplacental), in single observations—in the central zone. The length of the umbilical cord was in the range from 40 cm to 68 cm (an average of 46±0.6 cm). It was noted that the vessels passing through the umbilical cord had a less than normal

winding and spiral course. The fetal surface of placentas was smooth, individual placentas had whitish areas. The maternal surface had smoothed or flattened cotyledons. In all studies, fine-grained calcifications were noted. Morphological changes in placentas are presented in Figures 1-8.
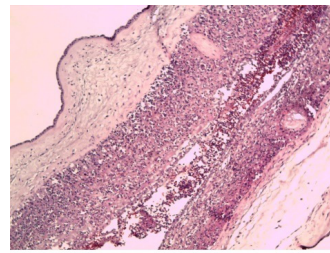
Fig.1. *Leukocyte infiltration in placentas (H&E staining, x400).*
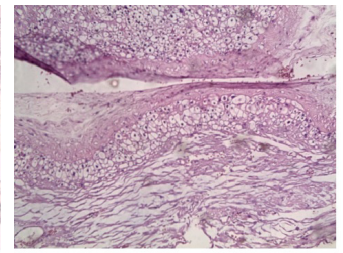
Fig.2. *Signs of productive inflammation (H&E staining, x400)*
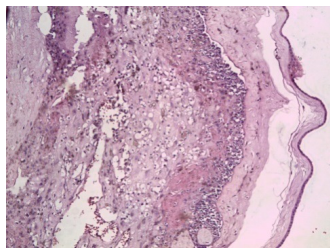
Fig.3. *Signs of inflammation in the amnion (H&E staining, x400).*
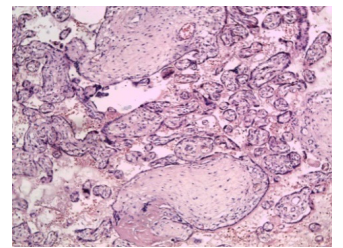
Fig.4. *Intermediate chorionic villi with a small number of capil-laries, sometimes surrounded by fibrinoid masses (H&E staining, x400).*
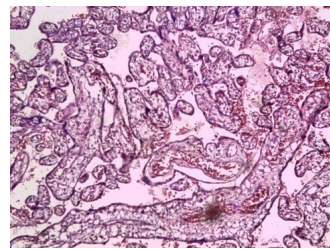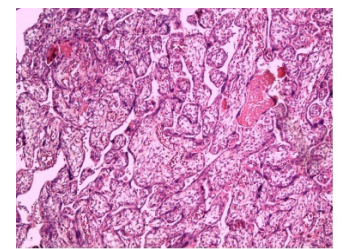
(A)　　　　(B)

Fig.5 (A,B). *Intermediate chorionic villi (the 1st and 2nd ramifi-cations). In many fields of view, the capillaries of the supporting and intermediate villi are "empty" (H&E staining; x400)*
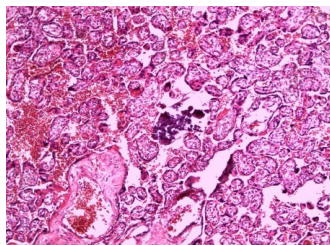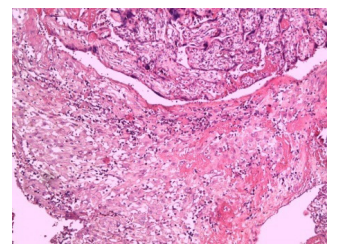
(A)　　　　(B)

Fig.6 (A,B). *Diffuse inflammation with foci of calcareous deposits in the form of calcifications in decidual tissue.*

In some places, the immature terminal chorionic villi are closely adjacent to each other, therefore, the intervillous space is weakly expressed. The chorionic villi are partially surrounded by a fibrinoid roller. There is plethora of immature villi and their "desolation" in some places. In the intervillosis space, erythrocyte "fields" are noted, as well as foci of calcareous deposits in the form of calcifications (H&E staining, x 400).
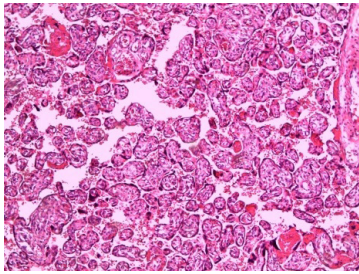
Fig.7. *Pathological immaturity of terminal chorionic villi (H&E staining, x 400).*

(A) (B)
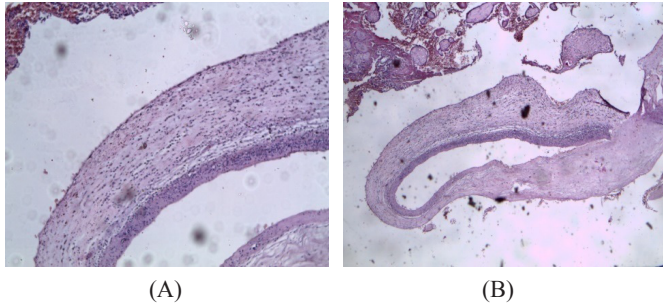
Fig.8. *Choriodeciduite. Severe leukocyte infiltration within the dicidal and trophoblastic layers. Moderate edema of spongy space. (H&E staining; A - x400, B - x200).*

The data from the correlation analysis of the relationship between the state of the placenta and the data from bacteriological examination of the cervical discharge are presented in Tables 1-9.

Table 1.

Placentas with histological signs of inflammation and necrotic changes in Group 1

Group 1	Signs + n=36	Signs - n=37	P	OR (95% CI)	R
E.coli	6(16.7%)	12(32.4%)	0.1153	0.51(0.22-1.22)	
Candida spp	4(11.1%)	6(16.2%)	0.5259	0.69(0.21-2.23)	
Enterococcus faecalis	15(41.7%)	12(32.4%)	0.4139	1.28(0.7-2.35)	
Str. agalactiae	10(27.8%)	3(8.1%)	0.0281	3.43(1.03-11.44)	0.472 P=0.003
Staph. epidermidis	10(27.8%)	4(10.8%)	0.0656	2.57(0.89-7.45)	0.307 P=0.042
Staph. aureus	3(8.3%)	4(10.8%)	0.7193	0.77(0.19-3.2)	
Staph. haemolyticus	8(22.2%)	2(5.4%)	0.0367	4.11(0.94-18.06)	0.328 P=0.029
Enterobacter aerogenes	9(25%)	2(5.4%)	0.0193	4.63(1.07-19.95)	0.472 P=0.003
Acinebacter	10(27.8%)	3(8.1%)	0.0281	3.43(1.03-11.44)	0.603 P<0.001
Str. viridans	3(8.3%)	6(16.2%)	0.3057	0.51(0.14-1.9)	
Staph. warneri	5(13.9%)	7(18.9%)	0.5621	0.73(0.26-2.1)	
Serratia marcescens	3(8.3%)	5(13.5%)	0.4787	0.62(0.16-2.39)	

Table 2.

Placentas with histological signs of pathological immaturity in Group 1

Group 1	Signs+ n=38	Signs- n=35	P	OR (95% CI)	R
E.coli	6(15.8%)	12(34.3%)	0.0670	0.46(0.19-1.09)	
Candida spp.	4(10.5%)	6(17.1%)	0.4114	0.61(0.19-2)	
Enterococcus faecalis	20(52.6%)	7(20%)	0.0039	2.63(1.27-5.45)	0.472 P=0.003
Str. agalactiae	10(23.7%)	3(8.6%)	0.0818	2.76(0.81-9.39)	0.307 P=0.042
Staph. epidermidis	11(28.9%)	3(8.6%)	0.0272	3.38(1.03-11.11)	0.328 P=0.029
Staph. aureus	3(7.9%)	4(11.4%)	0.6085	0.69(0.17-2.87)	
Staph. haemolyticus	3(7.9%)	7(20%)	0.1329	0.39(0.11-1.41)	
Enterobacter aerogenes	5(13.2%)	6(17.1%)	0.6345	0.77(0.26-2.29)	
Acinebacter	10(26.3%)	3(8.6%)	0.0477	3.07(0.92-10.25)	0.405 P=0.008
Str. viridans	8(21.1%)	1(2.9%)	0.0182	7.37(0.97-55.97)	0.419 P=0.005
Staph. warneri	4(10.5%)	8(22.9%)	0.1556	0.46(0.15-1.4)	
Serratia marcescens	3 (7.9%)	5 (14.3%)	0.3825	0.55 (0.14- .14)	

Table 3.

Placentas with histological signs of placental insufficiency and hypertension in Group 1

Group 1	Signs + n=28	Signs - n=45	P	OR (95% CI)	R
E.coli	12(42.9%)	6(13.3%)	0.0070	3.21(1.36-7.59)	0.378 P=0.008
Candida spp.	9(32.1%)	1(2.2%)	0.0003	14.46(1.94-108.11)	0.405 P=0.004
Enterococcus faecalis	15(53.6%)	12(26.7%)	0.0206	2.01(1.11-3.64)	0.326 P=0.031
Staph. haemolyticus	7(25%)	6(13.3%)	0.2295	1.88(0.70-5.01)	
Staph. epidermidis	9(32.1%)	5(11.1%)	0.0265	2.89(1.08-7.76)	0.339 P=0.027
Staph. aureus	5(17.9%)	2(4.4%)	0.0584	4.02(0.84-19.32)	0.386 P=0.008
Enterobacter aerogenes	9(32.1%)	2(4.4%)	0.0013	7.23(1.68-31.07)	0.563 P<0.001
Acinebacter	5(17.9%)	8(17.8%)	0.9931	1(0.36-2.77)	
Str. viridans	8(28.6%)	1(2.2%)	0.0009	12.86(1.7-97.37)	0.559 P<0.001
Staph. warneri	3(10.7%)	9(20%)	0.2979	0.54(0.16-1.81)	
Serratia marcescens	6(21.4%)	2(4.4%)	0.0239	4.82(1.04-22.25)	0.350 P=0.014

In Group 1, the number of placentas with signs of inflammation and necrotic changes was significantly higher in women with isolated Acinetobacter, Strep. agalactiae, Staph. haemolyticus, and Enterobacter aerogenes. The number of placentas with signs of pathological immaturity was higher in women with isolated Acinetobacter, Enterococcus faecalis, Staph. Epidermidis, and Str. Vridans. The number of placentas with signs of PI and hypertension was higher in women with isolated E. coli, Candida spp., Enterococcus faecalis, Staph. Epidermidis, Enterobacter aerogenes, Str. Viridans, and Serratia marcescens.

Table 4.

Placentas with histological signs of inflammation and necrotic changes in Group 2

Group 2	Signs + n=47	Signs - n=34	P	OR (95% CI)	R
E.coli	6(12.8%)	9(26.5%)	0.1171	0.48(0.19-1.23)	
Candida spp.	4(8.5%)	8(23.5%)	0.0604	0.36(0.12-1.1)	
Enterococcus faecalis	14(29.8%)	4(11.8%)	0.0542	2.53(0.91-7.02)	0.260 P=0.024
Str. agalactiae	6(12.8%)	4(11.8%)	0.8925	1.09(0.33-3.55)	
Staph. epidermidis	11(23.4%)	4(11.8%)	0.1832	1.99(0.69-5.72)	0.267 P=0.033
Staph. aureus	3(6.4%)	6(17.6%)	0.1114	0.36(0.1-1.35)	
Staph. haemolyticus	5(10.6%)	7(20.6%)	0.2135	0.52(0.18-1.49)	
Enterobacter aerogenes	8(17%)	2(5.9%)	0.1326	2.89(0.66-12.78)	0.257 P=0.036
Acinebacter	12(25.5%)	2(5.9%)	0.0210	4.34(1.04-18.15)	0.817 P<0.001
Str. viridans	8(17%)	1 (2.9%)	0.0466	5.79(0.76-44.13)	0.484 P<0.00
Staph. warneri	10(21.3%)	1 (2.9%)	0.0174	7.23(0.97-53.87)	0.491 P<0.001
Serratia marcescens	3(6.4%)	3 (8.8%)	0.6789	0.72(0.16-3.37)	

In Group 2, the number of placentas with signs of inflammation and necrotic changes was significantly higher in women with isolated Str. Vridans, Acinetobacter, and Staph. Warneri. The number of placentas with signs of pathological immaturity was higher in women with isolated E. coli, Candida spp., Enterococcus faecalis, Staph. Epidermidis, and Acinetobacter. The number of placentas with signs of PI and hypertension was higher in women with isolated E. coli, Staph. aureus, Staph. haemolyticus, Acinetobacter, and Str. Viridans.

In Group 3, the number of placentas with signs of inflammation and necrotic changes was significantly higher in women with isolated Enterococcus faecalis and Staph. Warneri. The number of placentas with signs of pathological immaturity was higher in women with isolated Enterococcus faecalis and Staph. aureus. The number of placentas with signs of PI and hypertension was higher in women with isolated E. coli, Staph. aureus, and Acinetobacter.

Table 5.

Placentas with histological signs of pathological immaturity in Group 2

Group 2	Signs + n=48	Signs - n=33	P	OP (95% CI)	R
E.coli	13(27.1%)	2(6.1%)	0.0167	4.47(1.08-18.51)	0.319 P=0.041
Candida spp.	1(2.1%)	11(33.3%)	0.0001	0.06(0.01-0.46)	
Enterococcus faecalis	15(31.3%)	3(9.1%)	0.0184	3.44(1.08-10.94)	0.335 P=0.001
Strep. agalactiae	4(8.3%)	6(18.2%)	0.1855	0.46(0.14-1.5)	
Staph. epidermidis	13(27.1%)	2(6.1%)	0.0167	4.47(1.08-18.51)	0.527 P<0.001
Staph. aureus	8(16.7%)	1(3%)	0.0550	5.5(0.72-41.92)	0.493 P<0.001
Staph. haemolyticus	6(12.5%)	6(18.2%)	0.4794	0.69(0.24- 1.95)	
Enterobacter aerogenes	7(14.6%)	3(9.1%)	0.4603	1.6(0.45-5.76)	
Acinebacter	12(25%)	2(6.1%)	0.0268	4.13(0.99-17.23)	0.607 P<0.001
Str. viridans	8(16.7%)	1(3%)	0.0550	5.5(0.72-41.92)	0.476 P<0.001
Staph. warneri	4(8.3%)	7(21.2%)	0.0964	0.39(0.12-1.24)	
Serratia marcescens	2 (4.2%)	4 (12.1%)	0.1792	0.34 (0.07-1.77)	

Table 6.

Placentas with histological signs of PI and hypertension in Group 2

Group 2	Signs + n=44	Signs - n=37	P	OP (95% CI)	R
E.coli	14(31.8%)	1(2.7%)	0.0008	11.77(1.62-85.36)	0.793 P<0.001
Candida spp.	9(20.5%)	3(8.1%)	0.1192	2.52(0.74-8.64)	0.283 P=0.051
Enterococcus faecalis	1(2.3%)	17(45.9%)	0.0000	0.05(0.01-0.35)	
Str. agalactiae	4(9.1%)	6(16.2%)	0.3315	0.56(0.17-1.84)	
Staph. epidermidis	6(13.6%)	9(24.3%)	0.2174	0.56(0.22-1.43)	
Staph. aureus	8(18.2%)	1(2.7%)	0.0272	6.73(0.88-51.35)	0.317 P=0.009
Staph. haemolyticus	3(6.8%)	9(24.3%)	0.0272	0.28(0.08-0.96)	
Enterobacter aerogenes	8(18.2%)	2(5.4%)	0.0816	3.36(0.76-14.87)	0.276 P=0.032
Acinebacter	12(27.3%)	2(5.4%)	0.0095	5.05(1.21-21.12)	0.727 P<0.001
Str. viridans	8(18.2%)	1(2.7%)	0.0272	6.73(0.88-51.35)	
Staph. warneri	3(6.8%)	8(21.6%)	0.0527	0.32(0.09-1.1)	
Serratia marcescens	2(4.5%)	4(10.8%)	0.2835	0.42(0.08-2.17)	

Table 7.

Placentas with histological signs of inflammation and necrotic changes in Group 3

Group 3	Signs + n=49	Signs - n=24	P	OR (95% CI)	R
E.coli	13(26.5%)	2(8.3%)	0.0707	3.18(0.78-12.99)	0.324 P<0.001
Candida spp.	4(8.2%)	6(25.0%)	0.0859	0.33(0.10-1.05)	
Enterococcus faecalis	25(51%)	6(25%)	0.0346	2.04(0.97-4.3)	0.6483 P<0.001
Str. agalactiae	6(12.2%)	3(12.5%)	0.9752	0.98(0.27-3.58)	
Staph. epidermidis	11(22.4%)	4(16.7%)	0.5657	1.35(0.48-3.79)	
Staph. aureus	30(61.2%)	9(37.5%)	0.0563	1.63(0.93-2.87)	0.358 P=0.009
Staph. haemolyticus	7(14.3%)	5(20.8%)	0.4783	0.69(0.24-1.94)	
Enterobacter aerogenes	8(16.3%)	7(29.2%)	0.2021	0.56(0.23-1.36)	
Acinebacter	12(24.5%)	7(29.2%)	0.6688	0.84(0.38-1.86)	
Str. viridans	8(16.3%)	1(4.2%)	0.1377	3.92(0.52-29.56)	
Staph. warneri	4(8.2%)	6(25%)	0.0494	0.33(0.1-1.05)	
Serratia marcescens	3(6.1%)	4(16.7%)	0.1506	0.37(0.09-1.51)	

Table 8.

Placentas with histological signs of pathological immaturity in Group 3

Group 3	Signs + n=48	Signs - n=25	P	OR (95% CI)	R
E.coli	13(27.1%)	2(8%)	0.0555	3.39(0.83-13.84)	0.324 P<0.001
Candida spp	5(10.4%)	5(20%)	0.2977	0.52(0.17-1.63)	
Enterococcus faecalis	15(31.3%)	16(64%)	0.0072	0.49(0.29-0.82)	0.577 P<0.001
Str. agalactiae	5(10.4%)	4(16%)	0.4911	0.65(0.19-2.21)	
Staph. epidermidis	9(18.8%)	6(24%)	0.5983	0.78(0.31-1.95)	
Staph. aureus	32(66.7%)	7(28%)	0.0017	2.38(1.23-4.61)	0.713 P<0.001
Staph. haemolyticus	7(14.6%)	5(20%)	0.5535	0.73(0.26-2.06)	
Enterobacter aerogenes	7(14.6%)	8(32%)	0.0805	0.46(0.19-1.11)	
Acinebacter	12(25%)	7 28%)	0.7816	0.89(0.4-1.98)	
Str. viridans	8(16.7%)	1(4%)	0.1183	4.17(0.55-31.47)	0.256 P=0.057
Staph. warneri	5(10.4%)	5(20%)	0.2585	0.52(0.17-1.63)	
Serratia marcescens	3(6.3%)	4(16%)	0.1794	0.39(0.09-1.61)	

Table 9.

Placentas with histological signs of PI and hypertension in Group 3

Group 3	Signs + n=48	Signs - n=25	P	OR (95% CI)	R
E.coli	14(29.2%)	1(4%)	0.0116	7.29(1.02-52.3)	0.493 P<0.001
Candida spp.	9(18.8%)	1(4%)	0.0820	4.69(0.63-34.94)	0.304 P=0.001
Enterococcus faecalis	24(50%)	7(28%)	0.0711	1.79(0.9-3.56)	0.277 P=0.012
Str. agalactiae	5(10.4%)	4(16%)	0.4911	0.65(0.19-2.21)	
Staph. epidermidis	9(18.8%)	6(24%)	0.5983	0.78(0.31-1.95)	
Staph. aureus	32(66.7%)	7(28%)	0.0017	2.38(1.23-4.61)	0.372 P=0.001
Staph. haemolyticus	7(14.6%)	5(20%)	0.5535	0.73(0.26-2.06)	
Enterobacter aerogenes	7(14.6%)	8(32%)	0.0805	0.46(0.19-1.11)	
Acinebacter	16(33.3%)	3(12%)	0.0487	2.78(0.89-8.64)	0.309 P=0.002
Str. viridans	8(16.7%)	1(4 %)	0.1183	4.17(0.55-31.47)	
Staph. warneri	6(12.5%)	4(16%)	0.6798	0.78(0.24-2.52)	
Serratia marcescens	5(10.4%)	2(8%)	0.7393	1.3(0.27-6.24)	

In general, a strong relationship was found between the presence of pathological changes in placentas and the presence of opportunistic flora in women (R=0.722, *P*<0.001). The risk of developing pathological changes in the placenta increased by more than 6 times (OR=6.14, 95% CI=2.32-9.48) with the presence of opportunistic flora. Binary logistic regression analysis made it possible to obtain a mathematical model that reliably identifies significant bacteria, the presence of which allows us to predict the revealed changes in the structures of placentas (Table 10-12).

Table 10.

Mathematical model for placentas with signs of inflammation and necrotic changes

Logistic regression (logit) Dep. Var: placentas with signs of inflammation and necrotic changes. Loss: Max likelihood (MS-err. scaled to 1) Final loss: 99.064595337 Chi-square (3)=69.181 *P*=.00000

	Const.B0	E.coli -	Staph. aureus	Acinebacter
Estimate	0.2001	-1.5361	-1.672	-2.1480
Standard Error	0.2144	0.4014	0.79112	0.771
t(228)	0.93308	-3.826	-2.1134	-2.783
P-value	0.3517	0.0001	0.03564	0.0058
-95%CL	-0.222	-2.3271	-3.2308	-3.668
+95%CL	0.6226	-0.7451	-0.11314	-0.627
Wald's Chi-square	0.870	14.643	4.46662	7.749
P-value	0.3507	0.00013	0.03457	0.0053
Odds ratio (unit ch)	1.2215	0.215	0.18787	0.116
-95%CL	0.8005	0.0975	0.0395	0.025
+95%CL	1.863	0.474	0.89301	0.533
Odds ratio (range)		0.215	0.18787	0.1167
-95%CL		0.097	0.039	0.025
+95%CL		0.474	0.893	0.533

Classification of Cases OR: 40.04 Perc. correct: 84.14%

	Pred. - 1	Pred. - 0	Percent - Correct
1	127	7	94.78
0	29	64	68.82

Mathematical model for placentas with signs of of inflammation and necrotic changes: sensitivity -77.27%, specificity-78.95%, and accuracy -77.97%

Table 11.

Mathematical model for placentas with signs of pathological immaturity

Logistic regression (logit) Dep. Var: placentas with signs of pathological immaturity. Loss: Max likelihood (MS-err. scaled to 1) Final loss: 110.43963104 Chi-square 3)=90.738 *P*=0.0000

	Const.B0	Staph. aureus	Candida spp.	Staph. epidermidis
Estimate	-1.2375	3.8453	2.8023	1.4181
Standard Error	0.18690	0.62581	1.104293	0.3792
t(228)	-6.6211	6.1445	2.5377	3.7395
P-value	<0.0001	<0.0001	0.01182	0.0002
-95%CI	-1.6058	2.61222	0.62646	0.670
+95%CL	-0.86927	5.0784	4.97832	2.165
Wald's Chi-square	43.84013	37.754	6.4400	13.98
P-value	<0.0001	<0.0001	0.01116	0.00018
OR (unit ch)	0.2900	46.77496	16.484	4.1295
-95%CL	0.2007176	13.629	1.8709	1.956
+95%CL	0.4192561	160.52	145.23	8.718
OR (range)		46.774	16.484	4.129
-95%CL		13.629	1.8709	1.956
+95%CL		160.52	145.2301	8.718

Classification of Cases OR: 40.04 Perc. correct: 84.14%

	Pred. - 1	Pred. - 0	Percent - Correct
1	127	7	94.78
0	29	64	68.82

Mathematical model for placentas with signs of pathological immaturity: sensitivity - 94.78% (95% CI: 89.61%-97.45%), specificity - 68.82% (95% CI: 58.81% - 77.33%), and accuracy -84.14% (95% CI: 78.29%-88.64%)

Table 11.

Mathematical model for placentas with signs of PI and hypertension

Model: Logistic regression (logit) Dep. var: placentas with signs of PI and hypertension. Loss: Max likelihood (MS-err. scaled to 1) Final loss: 106.99411981 Chi-square (3)=100.20 *P*=0.0000

	Const.B0	Enterobacter aerogenes	Candida spp.	E.coli -
Estimate	0.5749	1.606	4.4158	-2.9758
Standard Error	0.22071	0.618	1.10256	0.3818
t(240)	2.60481	2.5963	4.00511	-7.7929
p-value	0.00976	0.0100	<0.0001	<0.0001
-95%CL	0.14013	0.3875	2.2439	-3.7281
+95%CL	1.0097	2.825	6.5878	-2.2236
Wald's Chi-square	6.7850	6.7412	16.04094	60.729
P-value	0.00919	0.0094	<0.0001	<0.0001
OR (unit ch)	1.7769	4.9844	82.75615	0.05100
-95%CL	1.1504	1.473	9.4305	0.0240
+95%CL	2.744813	16.861	726.209	0.1082
OR (range)		4.984437	82.75615	0.0510
-95%CL		1.47343	9.430586	0.0240
+95%CL		16.86175	726.209	0.10821

Classification of Cases OR: 17.60 Perc. correct: 80.62%

	Pred. - 1	Pred. - 0	Percent - Correct
1	95	25	79.17
0	19	88	82.24

Mathematical model for placentas with signs of PI and hypertension: sensitivity -79.17% (95% CI: 71.05%-85.47%), specificity - 82.24% (95% CI: 73.92%-88.33%), and accuracy - 80.62% (95% CI: 74.28%-85.69%).

Conclusion

In pregnant women, the growth of opportunistic flora in diagnostically significant titers in the discharge from the cervical canal has a negative effect on the morphological and functional state of the placental complex in SPB. The constructed mathematical models make it possible to determine, with a high degree of certainty, the main bacteria for all the studied groups of pregnant women, thereby identifying the risk group of women at the stage of pregnancy planning, predicting complications and increasing the possibility of delivering a full-term baby.

Competing Interests

The authors declare that they have no competing interests.

Sources of Funding

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ORIGINAL ARTICLE

Women's Health

Somatotypological Characteristics of Adult Women with Type 2 Diabetes in Yakutia

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Abstract

The aim of the study was to present somatotypological characteristics of women (age 36-74 years) of Yakut ethnicity with type 2 diabetes (T2D).

Materials and Methods: The examined women with diagnosed T2D belonged to the age group of 36-55 years (n=24) and the group of 56-74 years (n=64). The comparison group (n=826) consisted of women of the same age periods who were not suffering from T2D. All examined women were born and permanently resided in Yakutia. Anthropometric examination, bioelectrical impedance analysis and the somatotypological method of examination were performed.

Results: The results indicate a significant predominance of overweight and obesity in Yakut women with T2D. Somatotypological analysis by the Rees-Eysenck body index, the Tanner scale and the Heath–Carter method also revealed a number of features. In the group of women with T2D, we found that individuals with andromorphic body type (according to sexual dimorphism index) and mesoectomorphic body type (according to the Heath–Carter index) were prevalent and that the proportion of individuals with asthenic somatotype, according to the Rees-Eysenck body index, was smaller. Identification of marked body types in the female population of Yakutia can serve as an additional prognostic criterion in a complex of studies aimed at the early detection of T2D.(**International Journal of Biomedicine. 2019;9(4):320-323.**)

Key Words: type 2 diabetes • women • somatotype • Yakutia

Abbreviations

AO, abdominal obesity; **BIA**, bioelectrical impedance analysis; **BShI**, body shape index; **BMI**, body mass index; **BW**, body weight; **BH**, body height; **BI**, the Brugsch index; **ChC**, chest circumference; **HC**, hip circumference; **HCI**, the Heath–Carter index; **ISD**, sexual dimorphism index; **IRPW**, the index of the relative pelvic width; **REBI**, the Rees-Eysenck body index; **T2D**, type 2 diabetes; **WC**, waist circumference.

Introduction

In the past three decades, the prevalence of type 2 diabetes (T2D) has risen dramatically in countries of all income levels. T2D comprises the majority of people with diabetes around the world, and is largely the result of excess body weight and physical inactivity.[1]

The average prevalence of T2D in Russia as of January 1, 2019, was 2,885.7/100 thousand of the population.[2] In Yakutia, there is a significant and constant increase in the number of people with T2D. By 2016, according to the Federal Register of Diabetes, the number of patients with T2D had risen to 20,508 people.[3]

Healthy diet, regular physical activity, maintaining a normal body weight and avoiding tobacco use are ways to prevent or delay the onset of T2D. Early diagnosis of diabetes can delay the occurrence of formidable complications, thereby prolonging the patient's working capacity and active

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life. Along with the known methods for the early diagnosis of T2D, such as BIA and anthropometric examination, there is also the somatotypological method, which is non-invasive and relatively non-laborious. It is known that the reactivity of the body, temperament characteristics, and endocrine and immunological status of a person are all related to the somatotype.[4,5] In this regard, somatotypological diagnosis is a promising method for a personalized approach to the prevention and early diagnosis of T2D.

The aim of the study was to present somatotypological characteristics of women (age 36-74 years) of Yakut ethnicity with T2D.

Materials and Methods

We examined 88 women who were hospitalized in the endocrinology department of the Center for Emergency Medicine in the period from January to March 2019 with diagnosed T2D. The examined women belonged to the age group of 36-55 years (n=24) and the group of 56-74 years (n=64). The comparison group (n=826) consisted of women of the same age periods who were not suffering from T2D. All examined women were born and permanently resided in Yakutia. Anthropometric examination, BIA and the somatotypological method of examination were performed. Exclusion criteria were the presence of an implanted *pacemaker and* pregnancy.

Anthropometric examination was carried out according to standard methods.[6] BH was measured using a Tanita digital anthropometer with an accuracy of 0.1 cm. BW was measured without clothing using medical scales with an accuracy of 50 g.

The girths of hip, chest and waist were determined using centimetric tape. The diameters of shoulders (ShD), intercristal diameter (ID) of the pelvis, and transverse and anteroposterior diameters of the chest were measured with a large caliber compass with an accuracy of 1 mm.

BMI was calculated using Quetelet's formula (in kg/cm^2). WC was measured using centimetric tape at the navel level on a horizontal line (in cm). BMI between 18.5 kg/m² and 25 kg/m² indicated a normal weight. BMI<18.5 kg/m² was considered underweight. BMI between 25 kg/m² and 29.9 kg/m² was considered overweight. BMI≥30 kg/m² indicated obesity.[7] The WC/HC ratio of ≥0.8 was considered elevated.[8] All women examined were identified by somatometric indices.[9]

IRPW was calculated by the formula:
$$IRPW=ID(cm)\times100/BH\ (cm)$$
The values of IRPW <16.0 characterized the narrow pelvis; IRPW 16.0-17.9 the intermediate pelvis; and IRPW ≥18.0 the wide pelvis.

The body shape was determined by BShI:
$$BShI=ID(cm)\times100/ShD(cm)$$
The values of BShI <70.0 characterized the trapezoid shape; BShI 70-74.9 the intermediate shape; and BShI ≥75.0 the rectangle shape.

The relative width of the chest was determined using BI:
$$BI=ChC(cm)\times100/BH(cm)$$
The values of BI <50.0 characterized the narrow chest; BI 50.0-55.0 the medium-wide chest; and BI >55.0 the wide chest.

Somatotypes were diagnosed using the following methods: the Rees-Eysenck body index (REBI), the Tanner scale and the Heath–Carter method.

The somatotype according to REBI[10] was determined by the formula:
$$REBI= BH\times100/(ChC\times6)$$
The values of REBI <96.0 characterized the picnic somatotype; REBI 96-106 the normosthenic somatotype; and REBI >106.0 the asthenic somatotype.

The body type was determined in accordance with the Tanner scale (sexual dimorphism index, ISD),[11] calculated by the formula:
$$ISD = 3xBAD-ID,$$
where BAD - bisacromial diameter (shoulder width), cm. The values of ISD <73.1 characterized the gynomorphic body type; ISD 73.1-82.1 the mesomorphic body type; and ISD >82.1 the andromorphic body type.

The body type, according to HCI, was assessed based on BIA using the ABC-01 MEDASS device.[12] To measure total body impedance, a pair of electrodes is placed at the extremity of the upper limbs and another pair at the extremity of the lower limbs.[13,14] The examination protocols automatically calculated scores of endomorphy, mesomorphy and ectomorphy. According to HCI, there are 13 somatotypes, which are determined by a combination of ecto-, meso-, and endomorphy scores.

The study was approved by our regional ethics committee. Written informed consent was obtained from all patients before inclusion in the study.

Statistical analysis was performed using statistical software package SPSS version 17.0 (SPSS Inc, Chicago, IL). Variables were presented as median (Me) and interquartile ranges (IQR; 25th to 75th percentiles). Mann-Whitney U test was used to compare means of 2 groups of variables not normally distributed. The frequencies of categorical variables were compared using Pearson χ^2 or Fisher's exact test, when appropriate. A probability value of $P<0.05$ was considered statistically significant.

Results and Discussion

According to study data, average BH and BW were 157.5 [153.0; 161.7] cm and 75.0 [70.0; 88.3] kg, respectively. BMI was equal to 31.45 [27.1; 33.6] kg/m². A BMI deficiency was not found among the women examined. Normal BMI was determined in 8(9.1%) women, overweight in 28(31.8%), and obesity in 52(59.1%) women. WC and HC were 95.5 [89.0; 103.7] cm and 103.5 [95.0; 109.5] cm, respectively. AO (WC ≥88 cm) was detected in 78(88.6%) women. The WC/HC ratio was 0.94 [0.86; 0.99]: within the normal range in 8(9.1%) women and increased in 80(90.9%) women.

Somatotyping with REBI in patients found the asthenic somatotype in 11.4% of cases, the normosthenic somatotype in 50.0%, and the picnic somatotype in 38.6% of cases. In the comparison group, we found the asthenic samotype in 31.4% of cases, the normosthenic samotype in 41.0%, and the picnic samotype in 27.6% of cases.

We found the gynomorphic body type, according to ISD, in 47.7% of patients, the mesomorphic body type in 43.2%, and the andromorphic body type in 9.1%. The distribution of somatotypes in the comparison group was as follows: the gynomorphic type - 33.2%, mesomorphic type - 64.9%, the andromorphic type - 1.9%.

According to HCI, 93.2% of patients had a mesoectomorphic body type. A balanced ectomorphic type was detected in 2.3% of cases, an endoectomorphic type in 4.5%. The scores of endomorphy, ectomorphy and mesomorphy are presented in Table 1. In the comparison group, somatotyping with HCI was not performed.

Table 1.

The scores of endomorphy, ectomorphy and mesomorphy in T2D women according to HCI

Somatotype	Scores of endomorphy	Scores of ectomorphy	Scores of mesomorphy
Mesoectomorphic type (n=82)	2.54 [1.95; 3.05]	6.54 [6.32; 6.93]	5.11 [4.86; 5.41]
Balanced ectomorphic type (n=2)	5.63	6.89	5.79
Endoectomorphic type (n=4)	5.16	7.39	3.51

The intermediate and wide pelvis, according to IRPW, was found in 10.2% and 89.8% of patients, respectively. The relative width of the chest, according to BI, was —as follows: the medium-wide chest in 6.8% of patients and the wide chest in 93.2%. Intermediate body shape, according to BShI, was found in 2.3% of patients and the rectangle shape in 97.7%.

In the age structure of the examined women with T2D, the age group of 56-74 years was predominant. BH in the examined T2D women did not significantly differ from the general population indicators of Yakut women. BW and BMI in T2D women were significantly higher than in women without diabetes. A. Guryeva et al.[15] found that the average BW of Yakut women in the age group of 36–55 years was 62.3 [55.0; 70.0] kg and in the age group of 56-74 years - 61.2 [55.2; 70.5] kg. BMI was 24.87 [22.23; 27.64] kg/m^2 and 26.62 [22.93; 29.43] kg/m^2, respectively. In accordance with BMI, in the examined group of T2D women, none were underweight. Most women (59.1%) were obese and 31.8% of them were overweight. In the comparison group, obesity was detected significantly less frequently (15.3% in the age group of 36-55 years and 20.1% in the age group of 56-74 years), and normal BW was recorded more often (in 48.6% and 39.8%, respectively).

It was found that WC in T2D women was significantly higher than in women without T2D (95.5cm versus 88.0cm). The WC/HC ratio was also higher in T2D women compared to comparison group (0.94 versus 0.89).

Analysis of the somatotypological characteristics of T2D women, according to REBI, revealed a predominance of individuals with the normosthenic somatotype (50.0%).

Among women with T2D, the asthenic somatotype was much less common, compared with the comparison group (χ^2=9.556, *P*<0.01). A comparative analysis of the distribution of body types according to the Tanner scale showed a statistically significant predominance of the andromorphic body type among T2D women (9.1% versus 1.9%) (χ^2=14.304, *P*<0.001). Elderly women with the normosthenic and picnic somatotypes, according to REBI, and andromorphic body type, according to the Tanner scale, can be assigned to the risk group for the development of T2D. In the group of T2D women, only 3 somatotypes were revealed, according to HCI: mesoectomorphic, balanced ectomorphic, and endoectomorphic types. There was a significant predominance of individuals with a mesoectomorphic body type (93.2%).

According to IRPW, BI and BShI, we can conclude that most of the T2D women had a wide pelvis, a wide chest, and a rectangular body shape.

Numerous studies in the field of clinical anthropology indicate the predisposition of representatives of different somatotypes to the occurrence of certain pathologies.[16] For the development of T2D, the results show a lesser predisposition of individuals with an asthenic body type. In women, the andromorphic body type is an extreme somatotype, which can be considered a risk factor for the development of T2D. Thus, the results indicate a significant predominance of overweight and obesity in Yakut women with T2D. Somatotypological analysis by the Rees-Eysenck body index, the Tanner scale and the Heath–Carter method also revealed a number of features. In the group of women with T2D, we found that individuals with andromorphic body type (according to ISD) and mesoectomorphic body type (according to HCI) were prevalent and that the proportion of individuals with asthenic somatotype, according to REBI, was smaller. Identification of marked body types in the female population of Yakutia can serve as an additional prognostic criterion in a complex of studies aimed at the early detection of T2D.

Competing Interests

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Women's Health

Age-related Features of the Cytological Diagnosis of Cervical Dysplasia of Different Degrees

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Abstract

The purpose of this study was to investigate the incidence of cervical intraepithelial neoplasia (CIN) and cervical cancer (CC) in women of different age groups who underwent cervical cytological testing from 2016 to 2018. According to our study, the frequency of CD incidence was inversely dependent on the degree of dysplasia in all age groups of women receiving survey from 2016 to 2018. The CIN1 incidence rate increased from 2016 to 2018, while the rate of CIN 2, CIN 3 and CC decreased. An analysis of the distribution patterns of CIN 1, CIN 2, CIN 3 and CC according to age revealed that the peak ages of the CIN1-3 incidence was 26-35 years. In the age group of 46-55 years, there was a sharp increase in CC—by 2.5 times compared with the age groups of 26-35 years and 36-45 years. Although the peak ages of CC incidence was ≥56 years. The peak ages of the indirect signs of HPV infection were 18-29 years and 30-44 years, characterizing the peculiarity of the immune status of these age groups. Thus, the peak age group in which women develop both CIN1 and CIN2+ is 26-35. Patient's age has a considerable influence on the natural history of CIN—independent of CIN grade and HPV high-risk infection. (**International Journal of Biomedicine. 2019;9(4):324-328.**)

Key Words: cervical intraepithelial neoplasia • cervical cancer • human papillomavirus

Abbreviations

CC, cervical cancer; **CIN**, cervical intraepithelial neoplasia; **CD**, cervical dysplasia; **LSIL**, low-grade squamous intraepithelial lesion; **HSIL**, high-grade squamous intraepithelial lesion; **HPV**, human papillomavirus.

Introduction

According to the Russian statistics, in 2016, 16,423 new cases of cervical cancer (CC) were recorded. In the structure of the morbidity of the female population with malignant neoplasms, CC occupies fifth place (5.3%).[1] Invasive CC is preceded by a long phase of pre-invasive disease called cervical intraepithelial neoplasia (CIN), also known as cervical dysplasia (CD). CINs ascertained by histological examination are classified in three grades based on increasing degrees of cellular change and disorganization. The strongest factor influencing the natural history of CIN is the presence of high-risk human papillomavirus (HPV) infection.[2,3] In particular HPV 16 and 18 increase the risk for persistent disease.[4] Low-grade squamous intraepithelial lesion (LSIL, also known as CIN1) is now recognized as a histological diagnosis of benign viral replication that should be managed conservatively. Despite evidence on differences in the clinical course of CIN2 and CIN3, the updated World Health Organization 2014 histopathological classification graded these lesions as a single entity: high-grade squamous intraepithelial lesion (HSIL).[5] If left untreated, CIN2 or CIN3 (collectively referred to as CIN2+) can progress to CC. It is estimated that approximately 1%–2% of women

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have CIN2+ each year, with higher rates reported for women of HIV-positive status, at 10%.[6-10] A recent 5-year longitudinal investigation from Northern Italy [11] of 310 patients with CIN2+ indicates that none of the 172 women with a negative HPV DNA test at 6 months post-treatment had residual or recurrent CIN2+ during the 2-year surveillance period. Thus, the authors conclude that that HPV DNA is highly predictive of disease eradication.

According to a study conducted in the United States, the annual incidence of CIN 1 was 1.2 per 1,000 with a rate of 1.5 per 1,000 for CIN 2/3. CIN 1 incidence peaked among women aged 20 to 24 years (5.1 per 1,000), with CIN 2/3 rates highest among those 25 to 29 years (8.1 per 1,000).[12] Another US study found that 412,000 women in the United States are diagnosed with CIN annually, with an associated cost of approximately $570 million. CIN incidence was highest among women aged 21 to 30 years (3.3 and 3.6 per 1,000) and women aged 31 to 40 years (2.9 and 2.7 per 1,000).[13] CC tends to occur in midlife and is most frequently diagnosed in women between the ages of 35 and 44.[14] According to the Russian statistics, the incidence of CC was the highest in the age group of 30-34 years (23.76%), compared with other age groups.[1]

The purpose of this study was to investigate the incidence of CIN and CC in women of different age groups who underwent cervical cytological testing from 2016 to 2018.

Materials and Methods

Cytological material with signs of CD of different degrees from 931 women aged between 18 and 88 years, who applied to medical institutions for preventive and diagnostic purposes from 2016 to 2018, was analyzed in the laboratory of pathomorphology, histology and cytology of the NEFU Medical Institute Clinic. The material of the cytological study consisted of smears of cervical mucosa and the cervical canal, stained according to the method of Romanovsky-Giemsa. Diagnosis of CIN (1-3) and CC was performed according to Bokhman's classification (1976). We conducted a comparative study for the incidence of CD in the dynamics of a 3-year examination period by year (2016, 2017 and 2018). The study was conducted with subjects grouped according to age: the age groups of 18-25 years (n=144/15.5%), 26-35 years (n=222/23.8%), 36-45 years (n=212/22.7%), 46-55 years (n=192/20.6%), and ≥56 years (n=161/17.3%).

Results and Discussion

In accordance with the years of the survey, 128(13.7%) women underwent a cytological study in 2016, 322(34.5%) women in 2017, and 481(51.6%) women in 2018. According to the data of cytological analysis, the highest rate falls on LSIL (CIN1), which was recorded in 578(62.1%) women. HCIL (CIN2 and CIN3) were found in 241(25.9%) and 97(10.4%) women, respectively (Fig. 1, 2). CC was detected in 15(1.6%) examined women (Fig. 3). The high frequency of CIN1 may reflect a high prevalence HPV in examined women. In addition, CIN1 may not be a true neoplastic lesion

and just reflects the changes due to HPV infection and be difficult to distinguish from reactive and other non-neoplastic histological changes that resolves spontaneously. Generally, CIN1, as acute infection, has a high regression rate with recommended management.
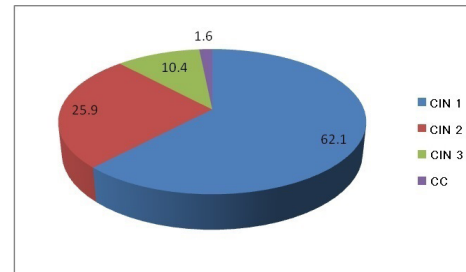
Fig.1. *The incidence of different types of CIN and CC in women who underwent cervical cytological testing from 2016 to 2018.*
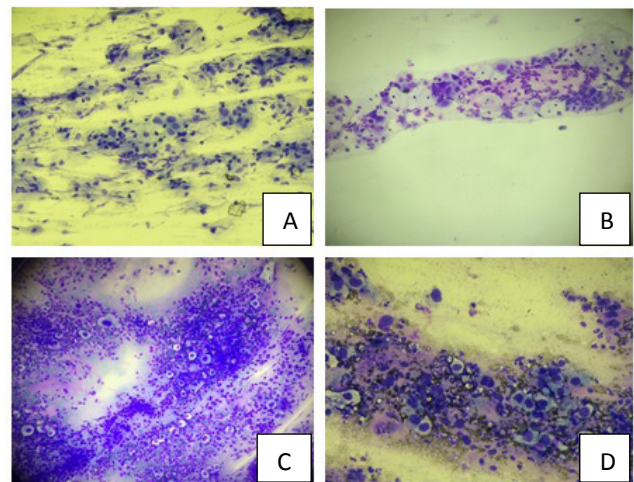
Fig. 2. *A – CIN 1, B – CIN 2, D – CIN 3; x200*
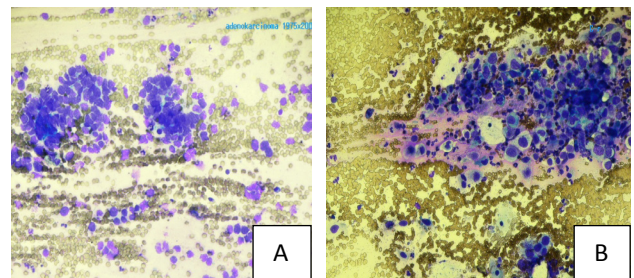
Fig. 3. *A – cervical adenocarcinoma, B – squamous cervical cancer; x200.*

Analysis of the frequency of different types of CD and CC over a 3-year period (from 2016 to 2018 inclusive) (Fig.4) showed an increase in the CIN1 rate from year to year with a simultaneous decrease in the rate of CIN2 and CIN3. The incidence of CIN1 in 2018 increased by 52.6% compared to 2016, while the incidence of CIN2 decreased by 24.3%, and the incidence of CIN3 decreased by 3.33 times. The CC incidence decreased by 2.2 times over a three-year period.

This indicates a positive trend in the development of high-grade dysplasia. The decrease in the rate of CIN2+ and CC can be associated with constant monitoring of the development of dysplasia in the dynamics and effective treatment.
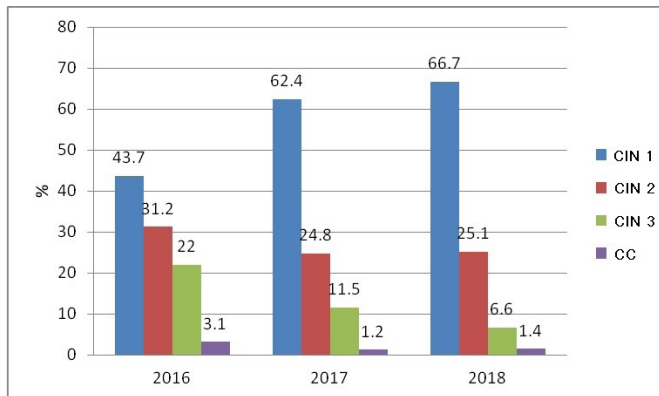
Fig.4. *The frequency dynamics of different types of CD and CC over a 3-year period (from 2016 to 2018 inclusive).*

A study of the incidence of CD depending on age showed that CIN1 was the most frequently diagnosed pathology in all age groups.(Fig.5) The highest frequency of CIN1 (14.2%) was observed in the age group of 26-35 years, the lowest frequency (10%) was observed in the age group of ≥56 years. The highest frequency of CIN2 (6.4%) was observed in the age groups of 26-35 years and 36-45 years. The lowest frequency of CIN2 (3.7%) was found in the age group of 46-55 years. The highest frequency of CIN3 (3%) was observed in the age group of 26-35 years, and the minimum frequency of CIN3 (0.8%) in the age group of 18-25 years. The incidence of CC increased depending on the age of the patients. In the age groups of 46-55 years and ≥56 years, this indicator was 2.5 and 3 times higher, respectively, than in the age group of 26-45 years (with no cases in the age group of 18-25 years). The maximum incidence of CC was observed in the age group of ≥56 years - 6(0.6%) cases. Thus, the progression of the severity of CD develops over decades, and as the severity of CD increases, the risk of further progression increases and the chance of regression decreases.

The link between HPV and CC has been extensively investigated over the past decade.[15] In the majority of HPV-infected individuals, the virus will clear out naturally. An immune-deficient woman will develop CC within 5 to 10 years, while for a woman with a normal immune system this time frame may be extended up to 15 to 20 years or even longer.[16] Persistent infection with high-risk HPV types and altered viral gene expression are the cornerstone of HPV-induced carcinogenesis.[17]

HPV infection has specific sites for localization. In order to complete the HPV infection generation cycle, the virus requires a stratified epithelium. The usual progression begins with initial virus infestation of the basal cell nucleus, overcoming the host defense mechanisms. A long-recognized, pathognomonic feature of HPV infection is the appearance of halo or koilocytotic cells in the differentiated layers of the squamous epithelium.[18]

The maximum incidence of indirect signs of HPV infection was observed in the age group of 26-35 years. (Fig.6, 7) It is estimated that in about 70% of young women, HPV infection spontaneously disappears after 12 months from the date of detection. Long-term preservation of HPV linked mainly to infection by high-risk HPV types (mainly HPV 16 and 18).[4] In menopausal women, indirect signs of viral infection were rare.
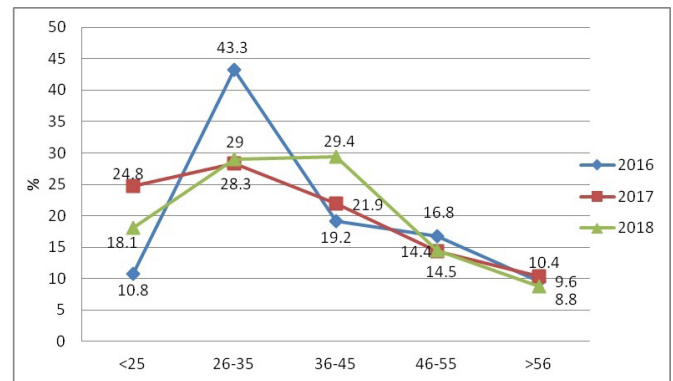
Fig.6. *The incidence of indirect signs of HPV infection in different age groups.*

Fig.5. *The incidence of CD in different age groups.*
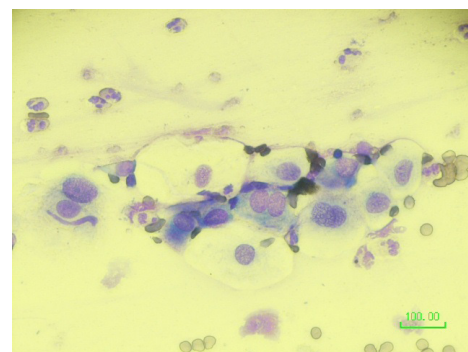
Fig.7. *Binucleated cells with their nuclei pressing against each other in a smear from the cervix in HPV infection. Romanowsky-Giemsa staining; x400.*

Conclusion

Thus, according to our study, the frequency of CD incidence was inversely dependent on the degree of dysplasia in all age groups of women receiving survey from 2016 to 2018. The CIN1 incidence rate increased from 2016 to 2018, while the rate of CIN 2, CIN 3 and CC decreased. An analysis of the distribution patterns of CIN 1, CIN 2, CIN 3 and CC according to age revealed that the peak ages of the CIN1-3 incidence was 26-35 years. In the age group of 46-55 years, there was a sharp increase in CC—by 2.5 times compared with the age groups of 26-35 years and 36-45 years. Although the peak ages of CC incidence was ≥56 years. The peak ages of the indirect signs of HPV infection were 18-29 years and 30-44 years, characterizing the peculiarity of the immune status of these age groups.

Thus, the peak age group in which women develop both CIN1 and CIN2+ is 26-35. The age group of 46-55 years and older presents a sharp increase in CC incidence. Patient's age has a considerable influence on the natural history of CIN—independent of CIN grade and HPV high-risk infection. Observational management should be considered for selected young patients with CIN.[19-22]

Prevention, early detection, and effective treatment of malignant tumors is one of the most important sections of modern medicine. The WHO guideline provides recommendations for screening and treatment of precancerous lesions to prevent CC. A new screen-and-treat strategy applies to all women regardless of HIV status, but specific recommendations for women living with HIV have been developed.[23]

Competing Interests

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Reproductive Health

Association of Lipid Peroxidation/Antioxidant System Activity with Glutathione S-Transferase P1 Polymorphisms in Infertile Men

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Abstract

The purpose of this study was to assess the association of two polymorphic loci *Ile105Val/Ala114Val* of the *GSTP1* gene with the parameters of oxidative stress (OS) in men with infertility.

We retrospectively analyzed the results of a survey of 222 men (mean age of 29.9±5.3 years) of infertile couples. A control group of 104 men (30.2±3.6 years) was formed, consisting of healthy men with realized reproductive function. An analysis of the frequency with which the *Ile105Val* polymorphism of the *GSTP1* gene occurred in men with infertility and in fertile men found statistically significant differences ($\chi2$=7.487; *P*=0.024). When comparing the frequency distribution of the genotypes of the *Ala114Val* polymorphism of the *GSTP1* gene in infertile men and fertile patients, no significant differences were found between the groups ($\chi2$=3.823; *P*=0.14). In men with infertility, carriers of the heterozygous genotype of the *GSTP1 Ile105Val* polymorphism presented an increase in reduced glutathione activity by 7% (*P*=0.0004), a decrease in glutathione reductase activity by 20% (*P*=0.03) in serum, and a decrease in SOD activity by 8% (*P*=0.01) in the ejaculate, unlike fertile men with heterozygous polymorphism, who had an increase in the total antioxidant activity of the blood serum by 20% (*P*=0.0001) and a decrease in glutathione peroxidase activity by 24% (*P*=0.03) in the ejaculate. In men with infertility, carriers of the heterozygous genotype of the *GSTP1 Ala114Val* polymorphism presented a decrease in α-tocopherol concentration by 15% (*P*=0.002), an increase in glutathione peroxidase activity by 25% (*P*=0.0004) in the blood and a decrease in SOD activity by 7% (*P*=0.01) in the ejaculate, unlike fertile men with heterozygous polymorphism, who had an increase in the concentration of serum conjugated dienes by 19% (*P*=0.0001) and a decrease in glutathione-S-transferase activity by 32% (*P*=0.03) in the ejaculate. Carrier identification of the *GSTP1 Ile105Val* and *Ala114Val* polymorphic loci, as well as the determination of the enzymes of the thiol-disulfide system, can be recommended for an additional assessment of the risk of developing reproductive disorders in men. (**International Journal of Biomedicine. 2019;9(4):329-333.**)

Key Words: male infertility • oxidative stress • glutathione-s-transferase • gene polymorphism

Abbreviations

AOA, antioxidant activity; **AOD**, antioxidant defense; **CDs**, conjugated dienes; **GPO**, glutathione peroxidase; **GR**, glutathione reductase, **GSSG**, oxidized glutathione; **GST**, glutathione-S-transferase; **GSTP1**, glutathione S-transferase pi 1 gene; **GSH**, reduced glutathione; **LPO**, lipid peroxidation; **OS**, oxidative stress; **ROS**, reactive oxygen species.

Introduction

In modern society, the newly formed family is often faced with a situation where the natural desire to reproduce cannot be realized. From 14% to 20% of couples of reproductive age suffer from infertility. Male infertility accounts for about 40% of infertile marriages. Genetic factors in 30%–50% of cases can be a reason for various forms of male infertility.[1-4] The process of spermatogenesis is influenced by a precisely controlled cascade of activation and deactivation of certain genes. Currently, much attention is paid to the study of polymorphic variants of predisposition genes, which, unlike mutations, appear less distinct in the phenotype, but are not always neutral and often lead to the appearance of metabolic products with altered physicochemical

properties and functional activity parameters.[5,6] The most suitable genetic markers for research are polymorphic variants of biotransformation genes of xenobiotics, the expression of which, unlike other classes of genes, is directly regulated by the effects of environmental factors of a chemical nature.[7,8] Genetic testing in the pre-symptomatic period makes it possible to identify the hereditary tendencies in the development of future diseases in the genome and, based on modern medical experience, to identify ways of their early prevention. In the pathogenesis of diseases of the reproductive organs, nonspecific processes occurring at the cellular level are essential.[9-11]

LPO processes play an important part in disrupting the vital activity of cells and molecular mechanisms, which is associated with the formation of free radicals, which damage the structure and function of membranes.[2,6,11] The processes of LPO and antioxidant protection are a single system that provides cellular homeostasis at the optimal level for the body and is one of the regulatory mechanisms of metabolism. The excess of free radicals and OS caused by them, on the one hand, can have a negative effect on spermatogenesis, but on the other hand, the normal functioning of spermatozoa requires the presence of physiological amounts of ROS.[12-14] In excess, ROS can initiate abnormalities in spermatozoa by inducing oxidative damage to cellular lipids, proteins and DNA, which are the mechanisms of the pathogenesis of male infertility.[5] ROS have a negative effect even within physiological concentrations, since they can stimulate premature capacitation and such irreversible events as the acrosome reaction.[13,15] An indispensable companion of spermatogenesis anomalies can also be a disruption of the activity of thiol-dependent ensembles.[16-18] Pathospermia is accompanied by a decrease in the activity of such antioxidant enzymes as GPO and GST and a decrease in the content of GSH in sperm and seminal plasma.[19-24] Based on the above description, the problem with this study is that the same risk factors, depending on the genetic characteristics of men, can either reduce fertility or not. It is the genetically determined features of the functioning of the xenobiotic biotransformation system that make each individual unique in relation to his adaptive abilities. Two genetic variants in glutathione S-transferase pi 1 gene (GSTP1)—Ile105Val (amino acid isoleucine 105 changed to a valine [rs1695]) and Ala114Val (amino acid alanine 114 changed to a valine [rs1138272])—have been shown to confer altered catalytic activity of GSTP1.[25-27]

The purpose of this study was to assess the association of two polymorphic loci *Ile105Val/Ala114Val* of the *GSTP1* gene with the parameters of OS in men with infertility.

Materials and Methods

We retrospectively analyzed the results of a survey of 222 men (mean age of 29.9±5.3 years) of infertile couples. A control group of 104 men (30.2±3.6 years) was formed, consisting of healthy men with realized reproductive function. All men had a laboratory and clinical examination by an andrologist, including an ultrasonic scan of scrotum and prostate. Macroscopic and microscopic examination of ejaculate was performed in accordance with the WHO recommendations (2010). Patients with the genetic causes

of infertility (AZF-deletions, CFTR-mutations, mutational changes of the number of CAG repeats controlled by androgen receptors) were excluded from the study. In serum and ejaculate of the examined men, the content of thiobarbituric acid (TBA)-active products (TBA-AP) was determined by the method of V.B. Gavrilova et al. (1984). The level of retinol and α-tocopherol was estimated by the method of R.Ch. Chernyauskene et al. (1984), total antioxidant activity (AOA) according to G.I. Klebanov et al. (1988). The content of reduced glutathione (GSH) and oxidized glutathione (GSSG) was determined by the method of P.Y. Hissin (1976). The activity of GST, GPO and GR was determined using Randox reagents. The concentration of conjugates during the reaction was registered spectrophotometrically with a wavelength of 340 nm using a BTS-350 spectrofluorophotometer.

DNA samples were genotyped for polymorphisms in the GSTP1 gene. DNA was isolated from venous blood samples using the sorbent method with the certified reagent kit DNA-Sorb-B (Central Research Institute of Epidemiology, Moscow, Russia).

Genotyping for two common variants in the *GSTP1* gene, c.313 A>G (Ile105Val, rs1695) and c.341 C>T (Ala114Val, rs1138272) was performed using methods described by Watson et al.[28] Amplification products were detected in 3% agarose gel; the electrophoresis results were registered and documented with the help of the system of computer gel documentation GelDoc.

The statistical analysis was performed using the software package Statistica 6.1 (StatSoft, USA) and Biostat. Deviation from Hardy-Weinberg equilibrium and differences in allele distributions between the two groups were assessed by $\chi2$- test and Yates' chi-square test. Two-tailed *P* values <0.05 were considered statistically significant.

Results and Discussion

It is known that the degree of expression of various isoenzymes of enzymes of the biotransformation system in different organs and systems varies. Unfortunately, data on the functioning of the glutathione system of spermatozoa as a target organ are rare and indicate the importance of this system in implementing such pathological states as patospermia (asthenozoospermia, oligozoospermia, teratozoospermia), as well as secretory and excretory-toxic types of infertility.[16,21,23] Xenobiotic detoxification system enzymes are involved in metabolic reactions aimed at reducing the activity of foreign substances; the deletion polymorphism of the GST gene family can contribute to the formation of reproductive disorders in men.[29,30]

An analysis of the frequency with which the *Ile105Val* polymorphism of the *GSTP1* gene occurred in men with infertility and in fertile men found statistically significant differences ($\chi2=7.487$; *P*=0.024) (Table 1) . Men in the control group with proven fertility have a homozygous *Ile105Ile* genotype in 54% of cases, while in men with infertility this genotype was observed in 67% of cases. The heterozygous genotype of the *Ile105Val* polymorphism was more common in the group of fertile patients than in patients with infertility

(33% and 28%, respectively). At the same time, fertile patients carry the mutant genotype *Val105Val* (14%) 2.8 times more often than men with infertility (5%). Probably the presence of this genotype is not associated with an increased risk of reproductive disorders in men.

Table 1.
The frequency distribution of the genotypes of the Ile105Val and Ala114Val polymorphisms of the GSTP1 gene in fertile and the infertile men

Polymorphism	Genotype	Control n=104	Infertility n=160	P	χ^2
GSTP1 Ile105Val	Ile/Ile	56 (53.8%)	107 (66.9%)	0.02	7.49
	Ile/Val	33 (31.7%)	44 (27.5%)		
	Val/Val	15 (14.4%)	9 (5.6%)		
GSTP1 Ala114Val	Ala/Ala	90 (86.5%)	123 (76.9%)	0.19	3.31
	Ala/Val	13 (12.5%)	35 (21.8%)		
	Val/Val	1 (0.96%)	2 (1.2%)		

When comparing the frequency distribution of the genotypes of the *Ala114Val* polymorphism of the *GSTP1* gene in infertile men and fertile patients, no significant differences were found between the groups (χ2=3.823; *P*=0.14). Heterozygous genotypes were found in 21.7% of the infertile men and 13% of the fertile men. The *Val114Val* genotype was found in 2 patients with infertility and in 1 fertile man. Carriers of the mutant allele *Val114* constituted 1% in both groups.

In men with infertility, carriers of the heterozygous genotype of the *GSTP1 Ile105Val* polymorphism presented an increase in GSH activity by 7% (*P*=0.0004), a decrease in GR activity by 20% (*P*=0.03) in serum, and a decrease in SOD activity by 8% (*P*=0.01) in the ejaculate, unlike fertile men with heterozygous polymorphism, who had an increase in the total AOA of the blood serum by 20% (*P*=0.0001) and a decrease in GPO activity by 24% (*P*=0.03) in the ejaculate (Figure 1).
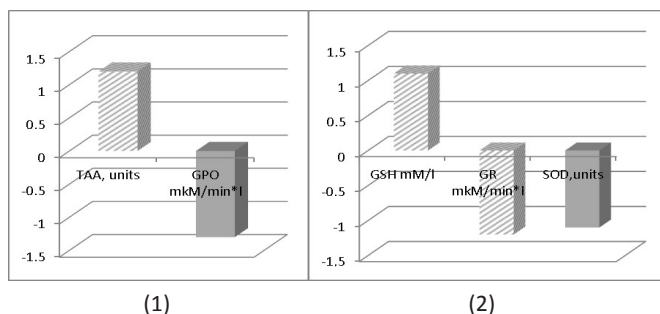
Fig.1. *Statistically significant indicators of the LPO/AOD system in the blood and ejaculate of fertile (1) and infertile (2) men, carriers of the heterozygous genotype of the GSTP1 Ile105Val polymorphism. Here and further, the blood indicators are cross-hatched.*

The increased level of the blood total AOA and reduced GPO activity in the ejaculate indicate that antioxidant protection in the group of fertile men is implemented already

in the first stages of blocking peroxidation in response to the activation of LPO processes. In men with infertility, the main effect of GSH is realized through participation in the work of antioxidant enzymes. As a substrate for them, GSH acts as a donor of hydrogen atoms for peroxides. SOD performs not only a protective, but also a regulatory function, being a key element in the regulation of constant oxygen concentration. A decrease in SOD activity reduces the inactivation of the superoxide radical, which leads to an increase in the degree of OS. A decrease in the activity of GSH is probably due to its active participation in the GSSG bioregeneration process. A decrease in the power of the antioxidant protection enzyme, in particular one of the components of the thiol-disulfide system, which may not cope with the LPO processes, thereby enhancing them, indicates the development of OS.

In men with infertility, carriers of the heterozygous genotype of the *GSTP1 Ala114Val* polymorphism presented a decrease in α-tocopherol concentration by 15% (*P*=0.002), an increase in GPO activity by 25% (*P*=0.0004) in the blood and a decrease in SOD activity by 7% (*P*=0.01) in the ejaculate, unlike fertile men with heterozygous polymorphism, who had an increase in the concentration of serum CDs by 19% (*P*=0.0001) and a decrease in GST activity by 32% (*P*=0.03) in the ejaculate (Figure 2).
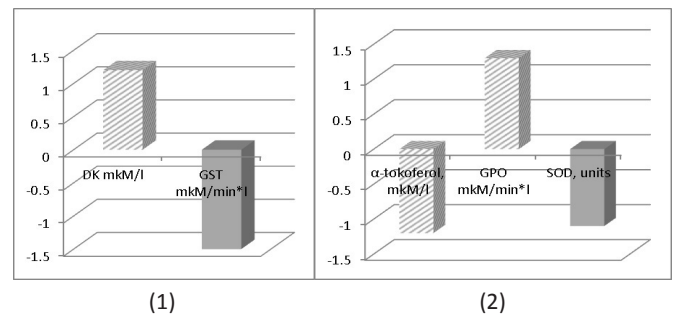
Fig.2. *Statistically significant indicators of the LPO/AOD system in the blood and ejaculate of fertile (1) and infertile (2) men, carriers of the heterozygous genotype of the the GSTP1 Ala114Val polymorphism.*

Normally, CDs are involved in regulating membrane permeability, growth rate of organisms, and cell proliferation. GST is the most important multifunctional protein of the ejaculate, since it not only protects against xenobiotics and their metabolites, but also localizes on the surface of spermatozoa and plays the role of a trigger that initiates their interaction with the zonapillucida ligands at the stage of initiation of the acrosomal reaction. Therefore, the determination of GST activity in the ejaculate can be used to determine the fertilizing ability of spermatozoa in men.

Alpha-tocopherol performs several functions, giving a combined antioxidant effect. So interacting with the hydroxyl radical OH, it has a suppressive effect on singlet oxygen. Being a radical trap, α-tocopherol is actively involved in blocking the LPO processes and increasing its concentration, possibly due to excessive formation of free radicals in the LPO process. The decrease in the concentration of α-tocopherol in the blood of men with infertility, carriers of the heterozygous

GSTP1 polymorphism (*AIa114Val*), occurs due to its active participation in metabolic reactions. GPO efficiently interacts with phosphotidylcholine, cholesterol and cholesterol ester hydroperoxides, and is also capable of reducing phospholipid hydroperoxides. It is known that, together with tocopherol, GPO almost completely suppresses LPO in biomembranes. The increased activity of GPO in the blood of men with infertility is likely to be compensatory. SOD activity in the ejaculate of men with infertility is not enough to inactivate reactive oxygen species at the site of their formation, which can lead to diffusion in the medium of tissue macromolecules.

Conclusion

Thus, it has been found that the glutathione disulfide system is an important component of antioxidant protection, especially against endo- and exogenous metabolites formed during OS. A genetically determined imbalance in the system of glutathione-dependent antioxidant protection determines the LPO activation and contributes to a significant weakening of the metabolic and detoxifying functions of the body. As a result, the susceptibility of cells to the damaging effects of xenobiotics increases significantly; xenobiotics negatively affect spermatogenesis and cause "breakdowns," so subtle and responsive to any change in the external and internal constants of the germ cell formation process. Genetically determined features of the functioning of the xenobiotic biotransformation system make each individual unique in relation to his adaptive capacity—stability or sensitivity to damaging exogenous and endogenous factors. Carrier identification of the *GSTP1 Ile105Val* and *Ala114Val* polymorphic loci, as well as the determination of the enzymes of the thiol-disulfide system, can be recommended for an additional assessment of the risk of developing reproductive disorders in men. Evaluation of the real state of reproductive health of the male population and the forecast of its changes in the future is not only an important scientific problem, but also essential for monitoring the reproductive health of the population, as well as for creating new medical programs for its preservation and improvement.

Competing Interests

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Epidemiology & Population Health

Surgical Service Key Performance Indicators for the Arctic Regions of Russia

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Abstract

The article represents surgical service key performance indicators (KPIs) in the Republic of Sakha (Yakutia) (RS(Y)) for the period between 2014 and 2016. According to the official statistics, the Arctic regions of RS(Y) are surgically understaffed, though formally there is high demand for medical staff and beds in surgery. The understaffing is due to peculiarities of RS(Y) its vast territory, low occupancy rate, the presence of sparsely populated territories and seasonal isolation of the population.(**International Journal of Biomedicine. 2019;9(4):334-337.**)

Key Words: surgical service • key performance indicators • staffing • air medical service • Yakutia

Introduction

The regions of Russia differ in the availability of medical services and medical staffing, the level of life, and development of transport and communication. A considerable part of normative legal documentation in the field of healthcare does not take into account specific characteristics of vast territories, the regions of the Far North in particular. RS(Y) is not an exception. Implementing the principles of healthcare organization and improving the availability of surgical services, including high tech medical services, are some of the priority tasks for the modern healthcare system. It should also be noted that federal and regional health care institutions have incomparably different capabilities to render surgical aid.[1-4]

The historical background of the lifestyle in RS(Y) has been defined by the presence of a great number of sparsely populated villages far from administrative centers and medical healthcare centers. These conditions have led to the creation of a very specific life-support system for the local population.

There are available, but incomplete, medical and preventive healthcare institutions for the local population, a high demand for specialized and air medical emergency service, as well as specialized exit medical aid.[1,5,6] These peculiarities demand development and implementation of differentiated regional mechanisms in the state healthcare policy in the regions of the Far North of the Russian Federation (RF).

Materials and Methods

The evaluation of surgical service KPIs in RS(Y) is represented for the period of 2014-2016. We analyzed the provision of surgical service and staffing, availability of hospital beds, and air medical service calls in cases of surgical pathology.

Results

Yakutia (the Sakha Republic) is the largest subject (3103.2 km²) of the Russian Federation. It is one of the coldest regions in the world. In 2010, Yakutia had a population of 958,500 people; the population density of the Republic was 0.31 persons per 1 km², while in a number of the Arctic regions, it ranged from 0.1 to 0.01 persons per 1 km². About 40% of

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the territory lies above the Arctic Circle, where only 7% of the population lives, including the indigenous peoples of the North who lead a traditional nomadic way of life. All these factors have a certain impact on the medical aid organization for the population.

Ethnically, it is represented by the Yakuts (49.9%), the Russians (37.8%), the Ukrainians (2.2%), the Evenks (2.2%), the Evens (1.6%), and other ethnicities (6.3%). Currently, Yakutia consists of 35 administrative regions located in different climate zones, having different social and economic backgrounds, with an uncommon and differing network of medical and preventive healthcare institutions.

More than ninety percent of the Republic's territory is in the area of seasonal transport service, where communication is mainly by air, water and road (seasonal). About 76% of 34 districts do not have reliable transport links with the center of the Republic and surrounding regions. The most remote village is situated at a distance of 3,189km from Yakutsk, and in the interior, the distance from the medical centers to the Central District Hospital averages about 400km and year-around travel is not possible. Difficulties in healthcare service organization, determined by the low density of population and underdevelopment of transport infrastructure in the Far North, cause high demands for all kinds of resources.

The level of surgical service provision tended to decrease from 2014 to 2016, from 74% to 73.2% (Table 1). The level of paramedical personnel also tended to decrease, from 83.1% to 80.7%. However, the medical staffing in RS(Y) is higher in comparison to the situation in the RF as a whole: 48 per 10,000 population in 2014, 48.5 per 10,000 population in 2015, and 48.1 per 10,000 population in 2016. The surgical service provision also tended to increase: 2.0 per 10,000 population in 2014 and 2.2 per 10 000 population in 2016. Provision of paramedical personnel is also higher than in the RF (115.1 per 10 000 population in 2014, 113.3 in 2015, and 112.7 in 2016) (Table 1).

Table 1.

The level of medical and paramedical provision in RS(Y) from 2014 to 2016 (per 10,000 population)

Indicators	2014	2015	2016	The RF
Medical staffing	6186.25	6259	6310.5	
Individuals	4580	4637	4617	543.6 thousand
Staffing, %	74.0	74.1	73.2	
physician staffing	48.0	48.5	48.1	37.2
Surgical staffing	2.0	2.0	2.2	
Paramedical staffing	13234.25	13300.25	13409.5	
Individuals	10994	10844	10816	1309.8 thousand
Staffing, %	83.1	81.5	80.7	
Nurse staffing	115.1	113.3	112.7	89.6

The provision of hospital beds per 10,000 individuals is decreasing considerably; thus, it was 107.0 in 2014 and

dropped to 98.2 in 2016. Nevertheless, the indicator is higher than in the RF in whole (Table 2).

Table 2.

The provision of hospital beds in RS(Y) from 2014 to 2016 (per 10,000 population)

Indicators	2014	2015	2016	The RF
Average number of beds occupied per day (including beds for fee)	10214	10186	9425	1097.1 thousand
Hospital bed provision per 10,000 patients	107.0	106.4	98.2	75.0
Bed occupancy	314	322	323	319
Bed turnover	25	27	28	27.9
Hospital bed per 1 person	3.332	3.290	3.104	
Admission rate per 100 patients	26,8	27,3	26,4	
Average hospital stay	12.5	12.0	11.7	11.4
Inpatient mortality rate	0.7	0.7	0.8	1.71

By the end of 2016, there were 1,314 surgical beds; more of them (636 beds) were surgical beds for adults: 251 were traumatology beds for adults, 72 were neurosurgical ones (Table 3). Historically, the structure of hospital bed supply was established according to the needs of the local population. On the whole, inpatient mortality rate in surgical beds of RS(Y) was 0.9 by the end of 2016. There was the highest hospital mortality rate in neurosurgical and proctological beds (2.4 and 2.3 respectively).

Thus, the statistics confirm the common data that the regions of the Far North are better provided with hospital beds and medical staff per 10,000 patients than in the RF as a whole.[7] It is certainly well grounded that these are the regions of the Far North that have difficult transport infrastructure, seasonal isolation of the population and severe natural and climate conditions for living. All these conditions predispose the region toward upkeep of networks of medical and healthcare institutions in each populated area.

The surgical staff requirement confirms the indicator that the regions of the Republic are well provided. The most understaffed regions are Abyysky, Allaikhovsky, Bulunsky, Verkhoyansky, Kobiaysky, Ust-Maysky, Ust-Yansky, and Verkhne-Kolymsky. The surgeons are required each year. It should be noted that the most problematic regions are the Arctic regions of the Republic, i.e. Abyyskiy, Allaikhovsky and Bulunsky districts. The indicator there reaches up to 50% of staff provision (Table 4).

As RS(Y) is a vast territory, in emergency cases the air medical service is required. Annual calls for air medical service vary; however, the data per 1000 patients are rather stable, 1.6 in 2014 and 1.6 in 2016 (Table 5).

As described in Table 6, the air medical service calls for surgical treatment were almost two times more in the period of 1993-1995. The number of planned and emergency operations was also two times more. The dynamics of 2013-2015 is rather stable in the number of air service calls and planned/emergency operations.

Table 3.

Surgical beds profile in RS(Y)

Hospital bed profile	Number of beds by 31.12.16	Admission rate per 1000 individuals	AHS	Hospital bed per 1 person	AHS per year	Turn-over	IMR
Surgical profile, total	1314	41.8	10.5	0.440	324	31	0.9
Among them: adult surgery	636	30	10	0.299	330	33	1.3
pediatric surgery	21	3.2	8.1	0.026	322	40	0.7
adult neurosurgery	72	2.4	13.4	0.033	318	24	2.4
pediatric neurosurgery	15	1.3	13.1	0.017	295	22	0.3
cardiac surgery	33	1.2	10.5	0.013	306	29	1.2
vascular surgery	32	1.8	7.5	0.013	318	42	0.1
adult traumatology	251	8.9	12.5	0.112	315	25	0.6
pediatric traumatology	30	4.4	10.6	0.047	407	38	0
burn	50	0.9	18	0.016	304	17	1.2
adult urological	43	1.8	9.6	0.017	309	32	0.2
pediatric urological	20	3.2	8	0.025	331	41	0
proctological	25	0.9	10.2	0.009	363	35	2.3
pediatric contaminated surgery	20	2.4	10.6	0.025	330	46	0
maxillofacial surgery	30	2	7.1	0.014	330	46	0
pediatric maxillofacial surgery	10	0.9	6.6	0.006	147	22	0
pediatric dental surgery							
adult orthopedic	26	1.2	10.3	0.012	329	32	0

AHS - Average hospitalstay; IMR - Inpatient mortality rate

Table 5.

The air medical service calls in RS(Y) from 2014 to 2016

Indicators	2014	2015	2016
Numbers of calls	1497	1460	1580
Per 1,000 patients	1,6	1,5	1,6

Table 6.

The air medical service calls for surgical treatment in RS(Y)

Indicators	1993	1994	1995		2013	2014	2015
Number of calls	3953	3331	2986		1594	1547	1632
Operations	…	1011	787		418	389	368

Table 4.

The surgical staff provision in RS(Y) from 2014 to 2016 (per 10,000 population)

Districts	Surgical staff provision		
	2014	2015	2016
Abyysky	50.0	50.0	50.0
Adlansky	85.7	69.6	69.6
Allaikhovsky	50.0	50.0	100.0
Amginsky	100.0	66.7	66.7
Anabarsky	200.0	100.0	100.0
Bulunsky	44.4	44.4	47.1
Verkhne-Viluysky	133.3	133.3	133.3
Verkhne-Kolymsky	100.0	100.0	50.0
Verkhoyansky	85.7	85.7	33.3
Viluysky	88.9	94.1	100.0
Gorniy	171.4	240.0	80.0
Zhigansky	50.0	100.0	100.0
Kobiaysky	94.1	94.1	57.1
Lensky	52.6	55.6	64.9
Megino-Kangalassky	83.3	96.0	96.0
Mirninsky	83.9	90.3	82.5
Momsky	100.0	100.0	100.0
Namsky	120.0	120.0	44.4
Nerungrinsky	58.0	65.7	67.7
Nizhnekolymsky	100.0	80.0	66.7
Nyurbinsky	75.0	100.0	100.0
Oimiakonsky	42.9	28.6	100.0
Olyokminsky	75.0	75.0	75.0
Oleneksky	100.0	100.0	100.0
Srednekolimsky	100.0	66.7	100.0
Suntarsky	100.0	100.0	100.0
Tattinsky	100.0	100.0	50.0
Tomponsky	88.9	88.9	88.9
Ust-Adansky	133.3	100.0	100.0
Ust-Maysky	70.6	70.6	53.3
Ust-Yansky	50.0	33.3	66.7
Khangalassky	85.7	83.3	83.3
Churapchinsky	142.9	100.0	100.0
Eveno-Bytantaicky	100.0	100.0	100.0
Healthcare Committee, Yakutsk	82.9	81.0	66.7
Republican institutions	96.2	64.3	66.1
RS(Y), total	85.3	75.4	73.2

Conclusion

The analysis of surgical service KPIs for RS(Y) has definitely described high demand for surgical specialists in the regions of the Far North. The situation will increase

prospectively more as surgical technologies are developed. Besides that, the building of a new oncologic dispensary is planned. RS(Y) has preserved the existing medical staff of surgeons, thus it has increased the number of staff to develop and implement new high-tech methods of treatment. Moreover, it has preserved the medical staff of surgeons in all central regional hospitals, even in the Arctic regions where the density of population is extremely low.

Competing Interests

The authors declare that they have no competing interests.

Sources of Funding

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ORIGINAL ARTICLE

Polar Medicine

Polyunsaturated Fatty Acids of Blood Serum and the Assessment of their Ratios in Clinically Healthy Adults Living in the Arctic Territories of Russia

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Abstract

Background: The study of the content of polyunsaturated fatty acids (PUFAs) in permanent residents of the Arctic territories, characterized by high activity of lipid metabolism, is of undoubted interest. The aim of this study was to assess the composition of omega-3 and omega-6 PUFAs in blood serum by gas-liquid chromatography and individual PUFA ratios in clinically healthy adults living in the Russian Arctic and Sub-Arctic regions.

Materials and Methods: A total of 1,556 healthy adult residents (the age groups of 22-35, 36-45 and 46-60 years) of the northern territories were examined. Of these, 661 people were living in the Sub-Arctic region (SAR) and 895 people in the Arctic region (AR). Analysis of PUFA composition in blood serum was determined by gas-liquid chromatography after transesterification to volatile fatty acid methyl esters (FAME). We determined the content of ω-3 PUIFAs: α-linolenic acid (ALA), eicosatrienoic acid (ETE), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), docosapentaenoic acid (DPA); and ω-6 PUFAs: dihomo-γ-linolenic acid (DGLA), linoleic acid (LA), arachidonic acid (AA), docosatetraenoic acid with the common name adrenic acid (AdA).

Results: In the clinically healthy individuals born and permanently living in SAR and AR, there was an age-related increase in LA level combined with an increase in the levels of AA, AdA and DGLA, as well as an increase in ALA level combined with an increase in the levels of EPA, DPA and DHA, the content of which was higher in AR individuals. In all age groups of adult residents of SAR, an increase in the AA/EPA ratio and a decrease in the EPA/DPA and (DGLA EPA)/DHA values were observed relative to similar age groups of AR, which indicates disorders in the PUFA metabolism and intensification of pro-inflammatory eicosanoid synthesis. In AR, a statistically significant increase in both ω-3 and ω-6 PUFAs can be a compensatory-adaptive reaction aimed at preserving the lipid component of cell membranes and reducing the risk of their destruction. An increase in the AA/DGLA ratio can be considered as a hidden risk criterion for the synthesis of pro-inflammatory eicosanoids.(**International Journal of Biomedicine. 2019;9(4):338-344.**)

Key Words: ω-3 and ω-3 polyunsaturated fatty acids • adults • Arctic and Sub-Arctic regions

Introduction

The lifestyle modification in recent years associated with the restriction of physical activity, an increase in the calorie content of food products and a steady increase in emotional stress provokes adaptive changes in the content of serum lipids and in the redistribution of the composition of fatty acids in lipids of cell membranes. All this potentiates the main risk factors for dyslipidemia, diabetes and obesity.[1-3]

A chain of consecutive disorders, starting with the pathology of the fatty acid transporter protein, leads to a cell deficiency of essential fatty acids. Depletion of essential PUFAs in the cell membrane pool is a negative factor for the synthesis of oxylipins (eicosanoids)—bioactive lipid

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metabolites of PUFAs.[4-6] It has been shown that an imbalance of oxylipins in the body causes chronic inflammation, arterial hypertension, coronary heart disease, atherosclerosis, and diabetes mellitus.[7-9] Disturbances in the synthesis of eicosanoids lead to metabolic syndrome. At the same time, the transformation of the FA profile in membrane lipids not only changes the synthesis of oxylipins, but also causes a violation of their physicochemical properties with a decrease in fluidity, receptor sensitivity and membrane permeability.[10,11] At the same time, the composition of essential blood serum PUFAs, reflecting the structural and functional changes in cells and the organism as a whole, significantly depends on the nature of nutrition.[12-14]

Although early studies have shown associations between PUFA imbalances and a number of diseases,[3,15,16] there is insufficient information on the content of PUFAs in clinically healthy individuals living in the Arctic and Arctic regions.[17,18] Polar human physiology merits intensified study because such a study would increase our knowledge of basic principles of biomedicine.

The aim of this study was to assess the composition of omega-3 and omega-6 PUFAs in blood serum by gas-liquid chromatography and individual PUFA ratios in clinically healthy adults living in the Russian Arctic and Sub-Arctic regions.

Materials and Methods

A total of 1,556 healthy (I and II health groups) adult residents (the age groups of 22-35, 36-45 and 46-60 years) of the northern territories were examined. Of these, 661 people (206 people in the age group of 22-35 years, 144 people – in the age group of 36-45 years, and 311 people in the age group of 46-60 years old) were living in the Sub-Arctic region (SAR) (Primorsky, Konoshsky and Pinezhsky districts of the Arkhangelsk oblast) and 895 people (259 people – the age group of 22-35 years, 244 people in the age group of 36-45 years and 392 people in the age group of 46-60 years) in the Arctic region (AR) (Nenets and Yamalo-Nenets okrugs, Mezensky district of the Arkhangelsk oblast). As you know, the territories of AR are less comfortable for living than SAR due to climatic factors. In particular, AR is characterized by widespread permafrost, severe and prolonged winters with low temperatures, intense wind conditions and sharp changes in atmospheric pressure. Among the people surveyed in AR, 78% were indigenous northern peoples (Nenets, Komi, etc.) and 22% were the local Caucasoid population. Exclusion criteria were the presence of cardiovascular diseases and their complications, diabetes mellitus, thyroid diseases, acute pathological conditions and exacerbations of chronic diseases.

The study was conducted in accordance with ethical principles of the WMA Declaration of Helsinki (1964, ed. 2013) and approved by the FCIARctic Ethics Committee. Written informed consent was obtained from all participants.

Blood samples (5 ml) were obtained from the ulnar vein on an empty stomach between 8:00 and 10.00 a.m. in IMPROVACUTER' Evacuated Blood Collection Tubes. The composition of PUFAs in the blood serum was determined by gas-liquid chromatography on an Agilent 7890A chromatographic system (Agilent Technologies, Santa Clara, California, USA) equipped with a flame ionization detector. SGE Analytical Science™ BPX90 GC Capillary Columns were used for fast separation of isomers of Fatty Acid Methyl Esters.[19] We determined the content of ω-3 PUIFAs: α-linolenic acid (C18:3 n-3, ALA), eicosatrienoic acid (20:3 n-3, ETE), eicosapentaenoic acid (C20:5 n-3, EPA), docosahexaenoic acid (C22:6 n-3, DHA), docosapentaenoic acid (C22:5 n-3, DPA); and ω-6 PUFAs: dihomo-γ-linolenic acid (C20:3 n-6, DGLA), linoleic acid (C18:2 n-6, LA), arachidonic acid (C20:4 n-6, AA), docosatetraenoic acid (C22:4 n-6), with the common name adrenic acid (AdA).

Chromatograms were integrated manually using ChemStation software (version B.03.01, Agilent Technologies). Fatty acid concentrations (μg/ml) were calculated by comparing the peak area of a fatty acid of interest with the internal standard (decanoic acid). The ratios of individual PUFAs were also calculated: AA/DHA, AA/EPA, AA/DGLA, AA/AdA, EPA/DPA, DHA/DPA, LA/AdA, ETE+EPA/DHA.

Statistical analysis was performed using the statistical software SPSS version 22.0 (SPSS Inc, Chicago, IL). The normality of distribution of continuous variables was tested by one-sample Kolmogorov-Smirnov test. Variables were presented as median (Me) and interquartile ranges (IQR; 25th to 75th percentiles). Mann-Whitney U test was used to compare means of 2 groups of variables not normally distributed. A probability value of $P<0.05$ was considered statistically significant.

Results

Comparison of the median values (Me) of LA, which is a substrate for the formation of other ω-6 PUFAs, showed that in both regions its content increased with age. Moreover, the LA content in the age group of 46-60 years in SAR was significantly higher than in the same age group in AR. The marked age-related increase in the LA level was accompanied by a decrease in the proportion of people with an LA deficit (in SAR from 11.2 to 6.1%, $P=0.038$; in AR from 12.0 to 4.8%, $P=0.001$) and an increase in the proportion of people with an excess (in SAR from 10.2 to 22.2%, $P<0.001$; in AR from 9.7% to 10.5%, $P=0.42$).

The proportion of AA, the main substrate for the synthesis of pro-inflammatory eicosanoids, also increased with age in both regions. Moreover, in the age groups of 22-35, 36-45 and 46-60 years in AR, its content was significantly higher compared with similar age groups in SAR. It should be noted that the AA level in the age group of 22-35 years in SAR was shifted toward lower normative levels (below the norm - 21.4% and above the norm - 9.7%; $P=0.22$); in AR, we noted equal percentages: below the norm (15.5%) and above the norm (15.9%). In the age groups of 36-45 and 46-60, there were fewer AA deficient conditions, and more excess AA values (13.9% and 12.5%; 16.2% and 19.5% in SAR; 9.1% and 25.1%; 7.9% and 25.8 in AR). However, in all age groups in AR, compared with SAR, AdA levels were significantly higher, which, together with high AA levels, is an unfavorable factor in enhancing the synthesis of pro-inflammatory eicosanoids (Table 1).

Table 1.

Levels of omega-3 and omega-6 PUFAs in blood serum in clinically healthy adults living in the Russian Arctic and Sub-Arctic regions

PUFA (Norm) µg/ml	Region	Age group			P-value	
		22-35 years (1)	36-45 years (2)	46-60 years (3)	P (between the age groups)	P (between the same age groups for SAR and AR)
		Me (25; 75)	Me (25; 75)	Me (25; 75)		
n	SAR	206	144	311		
	AR	259	244	392		
LA (C18:2 n-6) 371.97-1041.0	SAR	642.57 (453.5; 806.7)	672.44 (444.7; 936.8)	766.21 (555.1; 960.7)	$P_{1-2}=0.428$ $P_{1-3}<0.001$ $P_{2-3}=0.013$	$P_{1-1}=0.777$ $P_{2-2}=0.577$ $P_{3-3}=0.002$
	AR	643.42 (470.3; 824.4)	692.98 (521.9; 887.7)	686.68 (524.7; 858.4)	$P_{1-2}=0.031$ $P_{1-3}=0.010$ $P_{2-3}=0.976$	
AA (C20:4 n-6) 26.33-139.53	SAR	59.07 (27.71; 104.01)	62.93 (35.44; 110.9)	78.46 (32.8; 123.6)	$P_{1-2}=0.241$ $P_{1-3}=0.005$ $P_{2-3}=0.182$	$P_{1-1}=0.002$ $P_{2-2}<0.001$ $P_{3-3}<0.001$
	AR	81.56 (41.5;118.2)	96.22 (56.56;139.5)	91.26 (58.95;141.3)	$P_{1-2}=0.001$ $P_{1-3}<0.001$ $P_{2-3}=0.880$	
AdA (C22:4 n-6) 1.09-6.15	SAR	1.94 (1.35; 2.95)	1.32 (1.02;2.11)	1.56 (1.06; 2.27)	$P_{1-2}=0.066$ $P_{1-3}=0.203$ $P_{2-3}=0.461$	$P_{1-1}=0.012$ $P_{2-2}<0.001$ $P_{3-3}<0.001$
	AR	3.15 (1.77; 5.41)	3.52 (1.95; 5.01)	3.19 (2.08; 5.01)	$P_{1-2}=0.866$ $P_{1-3}=0.947$ $P_{2-3}=0.651$	
ALA (C18:3 n-3) 1.51-9.57	SAR	3.54 (1.86; 5.72)	4.258 (2.50; 6.36)	4.68 (3.08; 6.92)	$P_{1-2}=0.011$ $P_{1-3}<0.001$ $P_{2-3}=0.084$	$P_{1-1}=0.000$ $P_{2-2}=0.001$ $P_{3-3}<0.001$
	AR	4.64 (2.98; 6.62)	5.38 (3.53; 7.17)	5.80 (4.056; 8.72)	$P_{1-2}=0.009$ $P_{1-3}<0.001$ $P_{2-3}=0.014$	
EPA(C20:5 n-3) 3.36-71.38	SAR	7.11 (3.26; 12.31)	8.91 (3.97; 16.11)	9.98 (4.67; 19.39)	$P_{1-2}=0.019$ $P_{1-3}<0.001$ $P_{2-3}=0.224$	$P_{1-1}<0.001$ $P_{2-2}<0.001$ $P_{3-3}<0.001$
	AR	13.80 (6.48; 27.12)	20.24 (10.69; 38.42)	23.07 (10.4; 45.99)	$P_{1-2}<0.001$ $P_{1-3}<0.001$ $P_{2-3}=0.350$	
DHA (C22:6 n-3) 8.84-138.9	SAR	20.56 (9.39; 50.81)	27.38 (12.33; 59.93)	37.01 (12.49; 71.57)	$P_{1-2}=0.095$ $P_{1-3}<0.001$ $P_{2-3}=0.122$	$P_{1-1}=0.048$ $P_{2-2}<0.001$ $P_{3-3}<0.001$
	AR	29.297 (13.89; 53.05)	44.23 (22.10; 74.75)	45.64 (21.11; 85.08)	$P_{1-2}<0.001$ $P_{1-3}<0.001$ $P_{2-3}=0.247$	
ETE (20:3 n-3) 3.72-44.32	SAR	15.04 (6.01; 24.23)	15.74 (7.92; 27.72)	19.45 (7.62; 32.11)	$P_{1-2}=0.126$ $P_{1-3}=0.001$ $P_{2-3}=0.166$	$P_{1-1}=0.245$ $P_{2-2}=0.075$ $P_{3-3}=0.258$
	AR	15.13 (7.89; 25.79)	19.314 (10.61; 30.21)	20.52 (11.0; 31.1)	$P_{1-2}=0.009$ $P_{1-3}<0.001$ $P_{2-3}=0.409$	
DPA (C22:5 n-3) 3.23-19.93	SAR	4.03 (2.11; 6.56)	3.15 (2.42; 5.78)	3.34 (2.31; 4.85)	$P_{1-2}=0.505$ $P_{1-3}=0.407$ $P_{2-3}=0.809$	$P_{1-1}<0.001$ $P_{2-2}<0.001$ $P_{3-3}<0.001$
	AR	8.26 (5.16; 13.46)	8.64 (5.05; 14.61)	9.43 (6.02; 13.37)	$P_{1-2}=0.522$ $P_{1-3}=0.120$ $P_{2-3}=0.563$	

In both regions, the content of EPA, the main antagonist of AA and AdA, was significantly higher in the age groups of 36-45 and 46-60 compared with the age group of 22-35 years. However, among the inhabitants of SAR, the EPA level in all age groups was significantly lower compared to similar age groups in AR. In addition, in SAR we found an age-related, statistically significant decrease in the percentage of people with EPA deficiency from 23.2% to 15.6% (*P*=0.031) (in AR from 11.2% to 9.5%; *P*=0.83). In AR, on the contrary, we noted a statistically significant increase in the percentage of people with EPA excess from 5.0% to 12.3% (*P*=0.002) (in SAR from 1.5% to 2.9%; *P*=0.86). A decrease in the proportion of EPA in the blood was associated with a decrease in the proportion of DPA, especially in SAR.

With regard to DHA, a dynamics similar to EPA was found. Moreover, the level of DHA was significantly higher in AR compared to SAR. In both regions with age, we found a decrease in the percentage of people with DHA deficiency from 20.3% to 15.8% (*P*=0.062) in SAR and from 18.1% to 9.2% (*P*=0.001) in AR, and an increase in the percentage of people with DHA excess from 0.5% to 8.1% (*P*<0.001) in SAR and from 3.1% to 13.0% (*P*<0.001) in AR. That is, in SAR, the proportion of people with DHA deficiency was greater but the proportion with excess was less. Consequently, DHA deficiency in residents of both regions increases the likelihood of synthesis of pro-inflammatory eicosanoids, especially in SAR.

The content of the essential ALA, which is the precursor of EPA and DHA, was significantly increased in the age groups of 36-45 and 46-60 in both regions relative to the age group of 22-35 years. Moreover, in the age group of 22-35 years, the limits of its fluctuations were multidirectional. In SAR, they were shifted mainly toward lower than normative values (below the norm - 17.5% and above the norm - 6.6%, *P*<0.001), and in AR, on the contrary, higher than normative values (above the norm - 7.3% and above the norm -14.3%, *P*=0.017). In the age groups of 36-45 and 46-60, in both regions, ALA content was shifted only toward higher than normative values, but the proportion of people with an excess of it in SAR was less than in AR (10.40% and 15.80% versus 14, 80% and 26.40%; *P*=0.216 and *P*<0.001).

Thus, in the clinically healthy individuals born and permanently living in SAR and AR, there was an age-related increase in LA level combined with an increase in the levels of AA, AdA and DGLA, as well as an increase in ALA level combined with an increase in the levels of EPA, DPA and DHA, the content of which was higher in AR individuals.

Thus, in clinically healthy residents of SAR and AR, we revealed a statistically significant, age-related increase in the level of ω-3 and ω-6 PUFAs, which may be a compensatory-adaptive reaction of the body to constantly acting the extreme factors of the North. At the same time, a statistically significant increase in the level of AA and AdA among residents of AR of all age groups, relative to similar age groups of SAR, occurred against the background of a statistically significant increase in the main inhibitors and competitors for cyclooxygenase and lipoxygenase metabolic pathways of EPA and DHA. All of the above, in our opinion, may indicate an increase in the synthesis of anti-inflammatory eicosanoids and an improvement in the fluidity of the phospholipid liquid crystal structure of cell membranes, and may indirectly indicate a slight decrease in the risk of developing metabolically caused diseases in people of AR. One of the reasons for the age-related changes in the content of PUFAs found in the blood of northerners (especially in SAR) that we have identified may be both disorders in PUFA active transport and an unbalanced diet, in particular, the consumption of easily digestible carbohydrates and trans fats. [12,14] Since PUFAs are the main substrate of lipid peroxidation, their low content in the blood of residents of SAR indicates an increase in oxidative processes and the formation of atherogenic lipid fractions.[20,21] At the same time, the revealed changes in the level of AA, namely in residents of AR, were combined with a higher frequency of occurrence of excess states of LA. This, in our opinion, happens because the set of climatic factors of the North stimulates the release of stress hormones and enhances lipolysis. In addition, an increase in the level of AA and its age-related excess in residents of AR occurred with a significant deficiency of its main inhibitor and competitor for cyclooxygenase and lipoxygenase metabolic pathways, EPA, the level of which also increased, but the deficit remained high. This, in our opinion, can lead to a change in the physicochemical properties of plasma membranes and activation of the synthesis of eicosanoids with pro-inflammatory activity.

During the analysis, we also studied the ratio of PUFAs, taking into account the age and region of residence (Table 2). Traditionally, the availability of PUFAs comes down to their qualitative and quantitative determination, analysis of the ratios of ω-3 and ω-6 acids between themselves and their total content ($\sum$ω-6/$\sum$ω-3). However, the results of studies in recent years indicate that these coefficients do not always correctly reflect the functional properties of ω-3 and ω-6 PUFAs.[22-25] Considering that one of the real reasons for the transformation of PUFA composition is the change in their role in metabolism, we analyzed some indicators of this process: the AA/ETE ratio indirectly reflects the activity of D5-desaturase; the DHA/DPA ratio shows the activity of enzymes in the last stage of FA biosynthesis; the AA/AdA ratio and EPA/DPA ratio are indicators of the activity of FA elongases. In addition, the ratios of AA/DHA and (ETE+EPA)/DHA reflected the metabolic state in the eicosanoid cycle, and the AA/EPA ratio allowed us to assess the level of cellular inflammation. The informative significance of the presented criteria has been proved for patients with cardiovascular pathology,[1,3] but there are no data on clinically healthy individuals.

The analysis of the values we obtained did not reveal significant changes in the value of AA/DGLA in people of both regions with increasing age, but when comparing similar age groups, the value of this ratio in people of AR was significantly higher compared to SAR. This indirectly indicates an increase in the activity of D5-desaturase catalyzing the reaction of substrate synthesis for eicosanoids of the second and fourth series. At the same time, in both regions, we found an age-related decrease in the AA/EPA ratio and an increase in the AA/AdA ratio, which characterize the relationship between the precursor of eicosanoid synthesis and the inhibitor of their formation. A comparison of

similar age groups of SAR and AR showed that the inhabitants of SAR, on the one hand, had a significantly higher AA/EPA ratio, but on the other hand, the values of EPA/DPA and (DGLA+EPA)/DHA were significantly lower, which indicates disorders in the metabolism of PUFAs and intensification of pro-inflammatory eicosanoid synthesis (Table 2).

Table 2.

The ratio of PUFAs in blood serum in clinically healthy adults living in the Russian Arctic and Sub-Arctic regions

The ratio of PUFAs, conv. units	Region	Age group			P-value	
		22-35 years (1)	36-45 years (2)	46-60 years (3)	P (between the age groups)	P (between the same age groups for SAR and AR)
		Me (25; 75)	Me (25; 75)	Me (25; 75)		
n	SAR	206	144	311		
	AR	259	244	392		
AA/DHA	SAR	2.55 (1.78; 3.86)	2.27 (1.58; 3.54)	2.19 (1.52; 3.24)	P_{1-2}=0.068 P_{1-3}=0.001 P_{2-3}=0.286	P_{1-1}=0.623 P_{2-2}=0.519 P_{3-3}=0.050
	AR	2.63 (1.90; 3.79)	2.30 (1.67; 3.0)	2.06 (1.38; 2.96)	P_{1-2}<0.001 P_{1-3}<0.001 P_{2-3}=0.014	
AA/EPA	SAR	8.97 (4.79; 15.40)	6.43 (3.8; 13.27)	6.80 (3.9; 13.06)	P_{1-2}=0.896 P_{1-3}=0.006 P_{2-3}=0.985	P_{1-1}<0.001 P_{2-2}<0.001 P_{3-3}<0.001
	AR	5.45 (2.81; 9.33)	4.05 (2.36; 7.59)	3.85 (2.25; 7.85)	P_{1-2}=0.013 P_{1-3}=0.001 P_{2-3}=0.484	
AA/ DGLA	SAR	4.34 (3.35; 5.93)	4.28 (3.14; 5.89)	4.22 (3.24; 5.66)	P_{1-2}=0.280 P_{1-3}=0.198 P_{2-3}=0.981	P_{1-1}=0.014 P_{2-2}=0.001 P_{3-3}=0.009
	AR	4.81 (3.52; 7.71)	5.10 (3.69; 6.98)	4.52 (3.36; 6.69)	P_{1-2}=0.925 P_{1-3}=0.100 P_{2-3}=0.073	
DHA/DPA	SAR	4.82 (3.86; 7.08)	4.15 (3.0; 4.99)	4.61 (3.82; 5.23)	P_{1-2}=0.050 P_{1-3}=0.235 P_{2-3}=0.270	P_{1-1}=0.404 P_{2-2}=0.042 P_{3-3}=0.178
	AR	4.60 (3.58; 6.01)	4.78 (3.53; 6.33)	4.88 (3.66; 6.54)	P_{1-2}=0.468 P_{1-3}=0.223 P_{2-3}=0.697	
AA/AdA	SAR	24.19 (20.68; 33.53)	27.83 (23.14; 33.72)	23.33 (18.62; 30.38)	P_{1-2}=0.352 P_{1-3}=0.354 P_{2-3}=0.044	P_{1-1}=0.408 P_{2-2}=0.259 P_{3-3}<0.001
	AR	26.19 (20.30; 41.31)	28.88 (23.07; 43.0)	29.63 (22.64; 46.28)	P_{1-2}=0.085 P_{1-3}=0.020 P_{2-3}=0.511	
EPA/DPA	SAR	1.997 (1.56; 2.45)	1.97 (1.24; 2.68)	1.89 (1.19; 2.41)	P_{1-2}=0.747 P_{1-3}=0.500 P_{2-3}=0.726	P_{1-1}=0.05 P_{2-2}=0.011 P_{3-3}<0.001
	AR	2.38 (1.39; 3.93)	2.698 (1.60; 4.68)	2.84 (1.65; 4.22)	P_{1-2}=0.104 P_{1-3}=0.031 P_{2-3}=0.761	
DGLA+EPA/DHA	SAR	0.92 (0.68; 1.43)	0.94 (0.65; 1.51)	0.832 (0.65; 1.27)	P_{1-2}=0.760 P_{1-3}=0.328 P_{2-3}=0.250	P_{1-1}<0.001 P_{2-2}=0.134 P_{3-3}=0.005
	AR	1.121 (0.87; 1.61)	1.03 (0.78; 1.43)	0.96 (0.73; 1.33)	P_{1-2}=0.021 P_{1-3}<0.001 P_{2-3}=0.150	

Thus, in clinically healthy northerners, especially those living in SAR, pronounced changes were found in PUFA metabolism and eicosanoid biosynthesis due to inhibition of the activity of enzymes in the initial and last stages of the essential PUFA metabolism with reciprocal inhibition of synthesis of the cyclooxygenase and lipoxygenase metabolites of ω-3 PUFAs. Obviously, an increase in the AA/EPA ratio in residents of SAR and in the AA/DGLA ratio in residents of AR may be a significant factor in the increased risk for the development of metabolic syndrome in the examined adults.

The results obtained indicate a transformation of the PUFA composition in the blood serum of clinically healthy adults, due to a change in their metabolism, competitive inhibition of the biosynthesis of ω-3 PUFAs, and the marked increase in the formation of ω-6 PUFAs and pro-inflammatory eicosanoids. It can be concluded that residents of the Arctic, especially living in SAR, starting from 22-35 years old, develop changes in lipid metabolism at the cellular level.

Conclusions

In all age groups of adult residents of the Sub-Arctic region, an increase in the AA/EPA ratio and a decrease in the EPA/DPA and (DGLA+EPA)/DHA values were observed relative to similar age groups of the Arctic region, which indicates disorders in the PUFA metabolism and intensification of pro-inflammatory eicosanoid synthesis.

In the Arctic region, a statistically significant increase in both ω-3 and ω-6 PUFAs can be a compensatory-adaptive reaction aimed at preserving the lipid component of cell membranes and reducing the risk of their destruction. An increase in the AA/DGLA ratio can be considered as a hidden risk criterion for the synthesis of pro-inflammatory eicosanoids.

Competing Interests

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Experimental Medicine

Comparison of the Pathological Picture of Experimental Diabetic Nephropathy in Rats at Early (1 month) and Late (8 months) Stages

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Abstract

The research objective was to investigate the pathological picture of diabetic nephropathy in the 8-month streptozotocin (STZ)-induced diabetes mellitus and compare it with the previously obtained picture of diabetic nephropathy in the 1-month STZ-induced diabetes mellitus (DM).

Materials and methods: Experiments were conducted on 15 male Wistar rats aged 2-3 months and weighing 250–280 g. To induce DM, the animals were injected intraperitoneally 1ml of STZ solution in the citrate buffer at a dose of 65 mg/kg. In accordance with modern ideas about the peculiarities of DM modeling using STZ for more selective modeling of type 2 DM, the rats were previously injected with an intraperitoneal solution of cytoflavin based on a nicotinamide dose of 115 mg/kg. After 8 months of the experiment, the concentration of glucose, protein, and creatinine was determined in urine. The animals were euthanized under ethereal anesthesia and both kidneys were extracted, cleaned and washed with a physiological solution. In the renal tissues of animals, we determined the concentration of thiobarbituric acid reactive products, total pro-oxidant activity, total antioxidant activity, activity of antioxidant enzymes (catalase, superoxide dismutase, and glutathione peroxidase). With the help of a morphometric method of research, the area of renal glomeruli and the area of capillary lumens were measured, and after a special computer processing of digital photos, the total area of the vascular bed in the glomerulus and the area of mesangium in the renal glomerulus were assessed.

Results: Diabetic nephropathy in the 8-month STZ-induced DM was more pronounced than with the 1-month STZ-induced DM. The revealed biochemical and morphological signs of the 8-month STZ-induced DM indicate the irreversible nature of its course and the impossibility of its pharmacological correction.**(International Journal of Biomedicine. 2019;9(4):345-349.)**

Key Words: diabetes mellitus • streptozotocin • diabetic nephropathy

Introduction

The problem of finding ways to effectively treat diabetes mellitus (DM) and its complications remains one of the main issues of modern medicine.[1] Among the most frequent and dangerous complications of this disease, diabetic nephropathy (DN) takes one of the leading positions.[2]

According to modern ideas, DN is a disease caused by a set of pathological processes initiated by hyperglycemia and hyperglucosuria.[3] It has been established that aldosterone-associated mechanisms, free-radical oxidation (FRO) processes, and lipid metabolism disorders can make a significant contribution to kidney damage.[3] In addition, advanced views on the pathogenesis of DN increasingly focus on possible pathological modification of proteomic nephroprotection mechanisms in DM, operating in the norm.[4] For example, endogenous dipeptide carnosine (β-alanyl-L-histidine) is known to play a protective role in renal pathologies, including DN.[5,6] It is possible that a certain modification of its structure or function against the background of diabetes deprives it of nephroprotective properties, which contributes to the development of pathology.

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All of the above allows researchers to identify targets in the pathogenesis of DN for the development of new approaches to targeted correction of the disease. At the same time, an important question remains unresolved: At what stages of DN are effective medicinal corrections possible? Earlier, having conducted a study, we described the biochemical and pathological picture of DN on the background of experimental streptozotocin *(STZ)*-induced DM in rats for a period of 1 month.[7,8] The outcome of the 1-month STZ-induced DM was that hyperglucosuria and proteinuria developed, that FRO process was activated and that a number of characteristic morphological signs of glomerulus damage had emerged.[7,8] However, it is widely believed that persistent nephropathy develops only after 8 months of DM modeling. In this regard, the research objective was to investigate the pathological picture of DN in the 8-month STZ-induced DM and compare it with the previously obtained picture of DN in the 1-month STZ-induced DM.

Materials and Methods

Experiments were conducted on 15 male Wistar rats aged 2-3 months and weighing 250–280 g. Animals were grown in the Department of Animal and Human Genetics of the Federal Research Center "Institute of Cytology and Genetics" of the Siberian branch of the Russian Academy of Sciences (Novosibirsk). The work with animals was carried out in accordance with the principles of humanism laid down in the directives of the European Community (86/609/EEC) and the Declaration of Helsinki, in accordance with the "Animal experimentation legislations".

To induce DM, the animals were injected intraperitoneally 1ml of STZ solution in the citrate buffer at a dose of 65 mg/kg. In accordance with modern ideas about the peculiarities of DM modeling using STZ for more selective modeling of type 2 DM, the rats were previously injected with an intraperitoneal solution of cytoflavin based on a nicotinamide dose of 115 mg/kg.[9] After STZ administration, the animals were in a common metabolic cell on a standard laboratory diet, in conditions of free access to fluids. Two weeks before the end of the experiment, the rats were distributed to individual cells adapted for urine collection in order to assess the biochemical markers of kidney function on the last day of the experiment.

After 8 months of the experiment, the animals were euthanized under ethereal anesthesia and both kidneys were extracted, cleaned and washed with a physiological solution, one of which was used to determine the activity of FRO process in the renal tissue, and the other one for morphological research.

In urine, the concentration of glucose, protein, and creatinine was determined on the automatic biochemical analyzer DIRUICS-T240. Glucose concentration was determined by the method of enzymatic oxidation of glucose in the presence of glucose oxidase, which is based on the measurement of the optical density of the colored quinone imine compound. To determine protein concentration, a biuretic method was used, which is based on the formation of a blue-violet color complex with copper ions, the optical density

of which is directly proportional to the protein concentration. Creatinine concentration was determined by a kinetic method without deproteinization based on the Jaffe reaction with formation of a red-orange colored complex.

The activity of the FRO processes was estimated by a combination of pro-oxidant and antioxidant indicators.[10] Indicators of oxidant status were determined in the homogenate of the cortical substance of the kidneys. The total concentration ratio of all pro-oxidants and free-radical metabolites, and total pro-oxidant activity (TPA), were assessed by the intensity of coloring of the fluorescent complex formed by the interaction of Tween-80 peroxidation products and thiobarbituric acid. Additionally, the concentration of malondialdehyde (MDA) and other thiobarbituric fatty acid reactive products (TBARP) was determined in tissues.

The activity of the antioxidant system was investigated in the homogenate of the cortical substance of the kidneys. Total antioxidant activity (TAA) was assessed as an integrative activity indicator of all enzymatic and nonenzymatic factors of neutralization of free radicals by degree of oppression of Fe^{2+}/Tween-80 ascorbate-dependent oxidation by tissue homogenate. To assess the antioxidant status of cells, the activity of antioxidant enzymes was determined: catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx). The CAT activity was determined by suppressing sodium molybdate with the oxidation enzyme of hydrogen peroxide: hydrogen peroxide oxidizes sodium molybdate to form colored products; the decomposition of hydrogen peroxide under the action of catalase reduces the degree of coloring of the samples. The calculation was carried out relative to the control sample. The SOD activity was assessed at the content of nitroformazan in the sample, which is a colored product of nitrotetrazolium oxidation by superoxide radicals formed by interaction of phenazinmetasulfate and nicotinamidinucleotide (NADN). To determine the glutathione peroxidase (GPx) activity, the concentration of reduced glutathione was measured in a colored reaction with Ellman's reagent.

For morphological studies, the material was fixed in 10% neutral formalin solution. The material was dehydrated in isopropyl alcohol using a carousel-type dehydration machine TISSUE-TEK VIPTM6 (Sakkura, Japan), and then poured into paraffin using the paraffin filling station TISSUE-TEK TEC 5(Sakkura, Japan). Histological sections 2–4 μm thick were obtained using a semi-automatic rotary microtome Accu-Cut SRM (Sakkura, Japan). In each preparation, 20–25 glomeruli were evaluated. For testing, 3-4 preparations of each kidney were taken from each animal. All preparations were photographed with a digital camera with various zoom, which allowed fully visualizing at what level the glomeruli were located. We investigated glomeruli, which were located on one and the same level and approached the shape of a circle; elongated or deformed glomeruli were not included. We also did not investigate glomeruli that were cut superficially and were too small to avoid the impact of the cut level. The sections were stained with H&E in a TISSUE-TEK Prisma machine for micro-section automatic staining (Sakkura, Japan). Histochemical staining on neutral glycosaminoglycans was also carried out by Schiff-reagent, according to McManus,

and on acidic glycosaminoglycans with a 1% alcian blue solution on 3% acetic acid, according to Steedman (pH 2.5). Preparations were placed under a coating film, TISSUE-TEK Film, in a device for the automatic enclosure of micro-sections (Sakkura, Japan). Morphometric studies were performed using a specially created computer image analysis system consisting of a Leica DME microscope(Germany), a Leica EC3 digital camera (Leica Microsystems AG, Germany), a personal computer, and Video Test Morphology 5.2 software. With the help of a morphometric method of research, the area of renal glomeruli and the area of capillary lumens were measured, and after a special computer processing of digital photos, the total area of the vascular bed in the glomerulus and the area of mesangium in the renal glomerulus were assessed.

Statistical analysis was performed using StatSoft Statistica v12.0. The results of biochemical studies are presented as the median (Me) and interquartile range (IQR; 25th to 75th percentiles). The results of morphometric studies are presented as the mean (M) and standard error of the mean (SEM). Statistical comparisons between groups were performed using the Mann–Whitney U-test. A probability value of $P<0.05$ was considered statistically significant.

Results

Experiments showed that after 8 months of *STZ*-induced DM, there was a pronounced glycosuria (Table 1). Glucose concentration in urine exceeded the level of 1 month by 2.7 times ($P=0.0000$), and glucose excretion was 1.7 times higher ($P=0.0000$). The levels of diuresis, creatinine excretion and protein concentration in urine after 1 month and 8 months of *STZ*-induced DM did not differ significantly. At the same time, the level of protein excretion at 8 months was significantly lower, by 2.1 times, compared to the indicator of 1 month ($P=0.002$).

Against this background, the study of the activity of FRO process in the kidneys of rats with 8-month DM showed that levels of TPA decreased by 2.5 times ($P<0.0001$), TAA by 4.1 times ($P<0.0001$), and SOD activity by 2 times ($P=0.014$), in comparison with 1-month DM. TBARP concentration, GPx and CAT activities did not differ significantly between time groups (Table 2).

The results of a morphological study, presented in Table 3, showed that after 8 months of STZ-induced DM, the kidney glomeruli of the experimental animals were enlarged, surpassing the indicator of healthy animals by 1.3 times ($P=0.0003$), and were almost no different from the indicator in the group of the 1-month *STZ*-induced DM.

In addition, there was a significant expansion of the intercapillary space due to the accumulation of Schiff-positive mesangium and connective tissue (Fig. 1a). The mesangium area in glomeruli exceeded the level of healthy rats by 1.5 times ($P=0.001$) on average, which also generally corresponded to the indicator recorded after 1 month of *STZ*-induced DM. At the same time, the total area of vessels in the glomerulus (Fig. 1b) was 1.7 times less than the level in the 1-month STZ-induced diabetic rats ($P=0.0000$) and 2.7 times less than the level of healthy rats ($P=0.0000$). The area of capillary lumens in the glomerulus of the 8-month STZ-induced diabetic rats (Fig. 1c) was inferior to the level of the 1-month STZ-induced diabetic rats by 1.9 times ($P=0.0000$), which, in turn, did not differ from intact rats. The basal membranes of the glomeruli capillaries were significantly thickened, the capillary lumen was narrowed. The capsule of the kidney glomeruli looked thickened. There was no capsule cavity in most tubules. Glomeruli capillaries were full-blooded. In the kidney interstitium, there were foci of nephrosclerosis and lymphoplasmacytic infiltration.
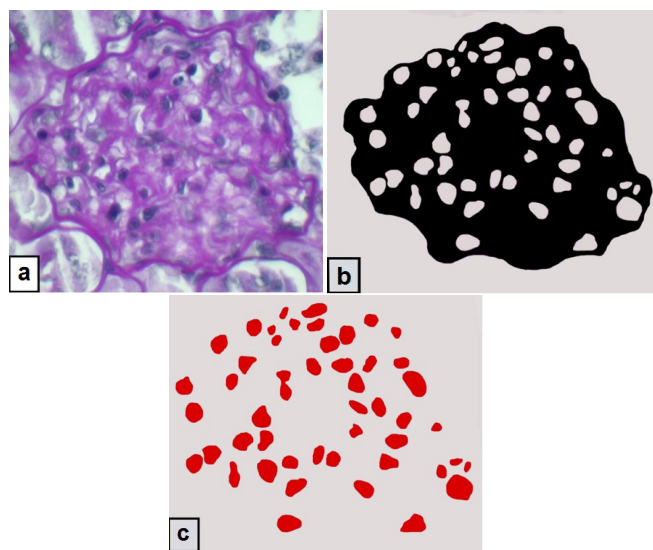
Fig. 1. Computer analysis of the mesangium area and glomerular capillaries after 8 months of STZ-induced DM: 1a – kidney glomerulus; 1b – mesangium area is significantly enlarged; 1c – reduction of the glomerular capillary lumen area. Staining with Schiff reagent according to McManus; x1200.

The canal lumens were expanded; the basal membranes of the canals were thickened. Nephrocytes were in a state of hyaline-drop dystrophy. The blood vessels were full-blooded. The walls of the arteries were thickened; the elastic membranes were hyperplastic. The veins were full-blooded. Finally, among the massive expansion of connective tissue, it was almost impossible to differentiate podocytes.

Table 1.

Indicators of renal function in STZ-induced diabetic rats

Group	Diuresis (ml/day)	Urine glucose concentration (mmol/L)	Urinary glucose excretion (μmol/day)	Urine protein concentration (mg/ml)	Urinary protein excretion (mg/day)	Urinary creatinine excretion (μmol/day)
After 1 month of STZ-induced DM	8.0 (7.0; 16.4)	0.7 (0.5; 9.5)	8.7 (4.7; 20.9)	2.0 (1.5; 2.8)	18.0 (13.3; 31.2)	64.0 (45.9; 113.1)
P-value	$P>0.05$	$P=0.0000$	$P=0.0000$	-	$P=0.002$	$P=0.0000$
After 8 months of STZ-induced DM	5.7 (3.6; 9.0)	1.9 (1.8; 2.6)	14.8 (9.1; 20.0)	2.0 (0.9; 2.8)	8.7 (6.6; 12.6)	59.9 (42.6; 78.3)

Table 2.

Indicators of FRO process activity in the kidneys of STZ-induced diabetic rats

Group	TBARP (μmol/mg)	TPA (%)	TAA (%)	GPx (%)	CAT (%)	SOD (%)
After 1 month of STZ-induced DM	8.5 (8.5; 8.8)	70.7 (68.6; 71.2)	46.3 (43.6; 46.9)	45.3 (42.1; 47.8)	10.3 (6.8; 13.0)	23.1 (18.4; 26.0)
P-value	*P*>0.05	*P*=0.0001	*P*=0.0001	*P*=0.0000	*P*>0.05	*P*=0.014
After 8 months of STZ-induced DM	6.6 (6.0; 7.0)	28.4 (24.1; 32.6)	11.3 (8.9; 14.8)	55.1 (50.7; 63.1)	12.9 (8.7; 16.9	11.3 (9.2; 15.6)

Table 3.

Morphological characteristics of rat kidneys in STZ-induced DM and in normal conditions

Parameter	Norm (1)	After 1 month of STZ-induced DM (2)	After 8 months of STZ-induced DM (3)	*P*-value (<0.05)
Area of kidney glomeruli (μm^2)	6174.7±257.5	7918.9±367.5	8174.9±310.6	P_{1-2}=0.002 P_{1-3}=0.0003
Total area of vessels in glomerulus (μm^2)	2900±27.4	1825.4±25.3	1083.0±57.8	P_{1-2}=0.0000 P_{1-3}=0.0000 P_{2-3}=0.0000
Glomerular capillary lumen area (μm^2)	47.5±3.7	42.8±4.3	22.2±1.5	P_{2-3}=0.0000 P_{1-3}=0.0000
Mesangium area of glomeruli (μm^2)	4738.7±43.3	6849.2±45.5	7090.3±577.3	P_{1-2}=0.0000 P_{1-3}=0.0000
Podocytes (number)	10.2±0.20	4.9±0.40	Not detected	P_{1-2}=0.0000

Discussion

Summarizing the above-mentioned data, we found a clear picture of the DN progression in the 8-month STZ-induced diabetic rats in comparison with the 1-month STZ-induced diabetic rats. Analysis of biochemical markers of DN showed that with 8-month DM, an increase in glucose concentration in urine and renal excretion level occurred as expected in comparison with 1 month of the disease.

In addition, the results of determining the parameters of oxidative damage to the renal tissue of the 8-month STZ-induced diabetic rats were noted. There was a pronounced decrease in the value of integrative indicators: TPA and TAA, as well as a twofold decrease in the SOD activity. In our opinion, this testified to the depletion of protective biochemical resources of the kidneys against the background of prolonged oxidative stress caused by large glycosuria.

Finally, there was clear morphological evidence of DN progression. First, this is shown by an almost double decrease in the total area of vessels in the glomerulus and the area of glomerulus capillary lumen relative to 1 month of DM. At the same time, it should be noted that the area of glomerulus capillary lumen did not differ from the norm after 1 month of STZ-induced DM. Attention is also drawn to the fact that the histological methods used failed to verify podocytes in the kidney glomerulus. According to modern ideas, the violation of the structure and function of these cells plays a decisive role in the DN development.[11,12] It is possible that the recorded phenomenon most clearly indicates a severe course of chronic 8-month DN, which seems to be already irreversible during this period and is not subject to drug therapy.

Conclusion

Thus, DN in the 8-month STZ-induced DM is more pronounced than with the 1-month STZ-induced DM. The revealed biochemical and morphological signs of the 8-month STZ-induced DM indicate the irreversible nature of its course and the impossibility of its pharmacological correction.

Competing Interests

The authors declare that they have no competing interests.

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Changes in Oxidative Phosphorylation Activity in Fibroblasts at p38 MAPK Pathway Inhibition

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Abstract

Background: Mitochondrial oxidative phosphorylation (OxPhos) accounts for more than 90% of the cellular ATP production, plays the role in reactive oxygen species (ROS) generation and programmed cell death. In addition, it contributes to such cellular processes as proliferation, differentiation and cell aging. Currently, several signaling systems are known to participate in regulation of OxPhos and activity of cytochrome c-oxidase (CcO), the terminal enzyme of the mitochondrial electron transport chain. However, data on mechanisms and key units involved in the signal transduction are still being supplemented.

Methods: Peritoneal fibroblasts were isolated from the omentum of Wistar rats by fragmenting the dissected tissue and disaggregating the fragments in collagenase solution (200 U/ml). The primary culture of fibroblasts was cultured in Dulbecco's Modified Eagle's Medium (DMEM) containing 10% Fetal Bovine Serum (FBS), 1% antibiotic/antimycotic at 37°C, 80% RH and 5% CO_2 in Biostation CT, Nikon. To obtain a culture, the fibroblasts were subcultured every 7 days. After the third passage the culture was treated with SB203580 at the concentration of 10 μM or with SB203580 in combination with bFGF at the dose of 133 pg/ml. Cells for immunofluorescent studies were fixed with 70% ethanol and stained with antibodies to CcO subunit I.

Results: When exposed to SB203580 or the combination of SB203580 and bFGF, marked changes were observed in the fibroblast culture: in both cases there was intensive collagen destruction; attached fibroblasts rounded and detached from the substrate. When exposed to SB203580, non-rounded cells started to vacuolate actively while vacuoles occupied the entire cytoplasm. Introduction of the p38 inhibitor into the culture of activated fibroblasts caused a more intensive cell detachment and collagen destruction. Moreover, the formation of large conglomerates containing several dozens of cells connected with collagen fibers was observed in the areas characterized by the highest cell density. Immunofluorescent staining made it possible to reveal a certain increase in the cell area occupied by CcO after fibroblasts exposure to SB203580 and a significant increase in the area of the enzyme distribution in the studied cells (more than 5-fold in comparison with the control group) when simultaneously adding SB203580 and bFGF. In addition, one-way ANOVA test demonstrated a statistically significant increase in the CcO fluorescent staining intensity in both cases.

Conclusion: The analysis of the results indicates that SB203580, a p38 MAPK inhibitor, influences both peritoneal fibroblast morphology and the energy status of the cells under study increasing the amount of CcO, the terminal enzyme of the mitochondrial electron transport chain, in the cells, although no cell change to active proliferation or apoptosis was observed. Fibroblast culture stimulation by bFGF significantly enhances the effect of SB203580, which implies a greater OxPhos complex expression in case of impaired p38 MAPK signaling pathway activation. (**International Journal of Biomedicine. 2019;9(4):350-355.**)

Key Words: peritoneal fibroblast • oxidative phosphorylation • p38 MAPK • SB203580 • bFGF • OxPhos

Abbreviations

ATP, adenosine triphosphoric acid; **bFGF**, basic fibroblast growth factor; **cAMP**, 3',5'-cyclic adenosine monophosphate; **CcO**, cytochrome c-oxidase; **CHO cell line**, Chinese hamster ovary cell line; **DMEM**, Dulbecco's Modified Eagle's Medium; **EGFR**, Epidermal Growth Factor Receptor; **ERK**, extracellular signal-regulated kinase; **FBS**, Fetal Bovine Serum; **IL-1**, Interleukin-1; **MAPK**, mitogen-activated protein kinases; **MMP**, matrix metalloproteinase; **OxPhos**, oxidative phosphorylation; **ROS**, reactive oxygen species, **TGF-β**, Transforming growth factor beta, **TNFα**, tumor necrosis factor alpha.

Introduction

Under normal physiological conditions mitochondrial OxPhos accounts for more than 90% of the cellular ATP production in most cells and tissues. Mitochondria are also involved in the maintenance of calcium homeostasis, carry out critical reaction steps of steroid hormone metabolism, pyrimidine synthesis and elimination of ammonia through the urea cycle. Moreover, they are considered one of the major programmed cell death modulators and the source of reactive oxygen species.[1-3]

That is why mitochondrial dysfunctions caused by defective electron transport complexes or by dysfunction of separate chain elements are linked to a variety of diseases and pathological conditions of the humans, including so-called mitochondrial diseases (Alzheimer's disease and Parkinson's disease), cancer, diabetes, cardiovascular diseases, as well as myocardial ischemia/reperfusion.[2] Normally, OxPhos regulation also has a great impact on such key processes as cell differentiation and cell aging. So, it was shown that formation of myofibroblasts is closely linked to metabolism switching from OxPhos to glycolysis.[4] Studies conducted on skin-derived fibroblasts revealed age-related decline in efficiency of OxPhos.[5] The role of OxPhos in cell proliferation still remains a subject of debate. Along with the data saying that OxPhos in actively proliferating cells, both tumor cells and non-oncogenic actively proliferating cells (non-transformed fibroblasts, lymphocytes, macrophages, thymocytes, endothelial cells and embryonic stem cells)[6,7] is inhibited and energy metabolism is carried out by means of glycolysis and pentose phosphate pathway even in the presence of oxygen (the Warburg effect),[2,8] there are studies demonstrating an increase in OxPhos in the cells of certain cancer types (including leukaemias, lymphomas, pancreatic ductal adeno-carcinoma, melanoma and endometrial carcinoma),[9] as well as an increase in OxPhos during active proliferation of fibroblasts.[7]

Mitochondrial complex IV or CcO is the terminal enzyme of the electron transport chain. It catalyzes the electron and proton transfer across the membrane to molecular oxygen to make H_2O. CcO is also one of the three H^+ pumps (along with complexes I and III) that generate the proton gradient across the inner mitochondrial membrane, which powers the ATP synthesis.[2, 3] Besides energy function, CcO also contributes to ROS generation during oxidative stress, although the data about the potential role of this enzyme in the process are contradictory. On the one hand, it is known that CcO dysfunction or enzyme deficit increases reactive oxygen species generation (for example, an increased ROS production was observed in the cells with enzyme knock-out mRNA). This is explained by the fact that even though CcO does not participate directly in ROS generation it can affect their generation by consuming the electrons that could have been involved in oxygen activation.[10, 11] On the other hand, there was a certain volume of evidence published suggesting that CcO subunit I can exhibit pro-oxidant properties and the hydrogen peroxide sensitivity of cells increases with overexpression of CcO subunit I.[12] In addition, under hypoxic conditions complex IV can participate in NO production thus mediating the impact on NO-dependent signaling pathways.[13]

It is known that both under normal and pathological conditions several signaling pathways are involved in OxPhos regulation and CcO activity. However, the data about the mechanisms and various molecules participating in the signal transduction are still being supplemented. The molecules affecting CcO activity are the thyroid hormone, 5-diiodothyronine (T2) that activates the enzyme even in the presence of an allosteric ATP inhibitor;[2,3] glucagon that has a suppressive effect;[14,15] EGFR, which is, when activated, translocated to cytoplasm and localized in the mitochondrial membrane where it phosphorylates CcO subunit II, which leads to a partial inhibition of its activity and cell transition from the aerobic to glycolytic and pentose phosphate pathways;[16] Smad4, a negative regulation mediator in TGF-β signaling that binds with CcO during apoptosis.[17] CcO is competitively inhibited by nitrogen oxide (NO), carbon monoxide (CO), non-competitively inhibited by hydrogen sulphide (H_2S), hydrogen cyanide (HCN) and sodium azide (NaN_3).[2,3] Mitochondrial complex IV is also a target for cAMP-dependent regulation, in addition, increasing cAMP levels in CHO cells led to inhibition of CcO activity under hypoxic conditions and in case of ischemia/reperfusion;[2,14,15] the enzyme activity is also inhibited by TNFα, a pro-inflammatory cytokine, and cytochrome P450.[3,18]

The goal of this study was to investigate the impact of the p38 MAPK signaling pathway on OxPhos activity of peritoneal fibroblasts in culture.

Materials and Methods

Primary fibroblast culture isolation from the rat omentum

The experiment for isolation of primary fibroblast culture from the omentum of mature Wistar rats (200 g) was carried out. Animals were housed in accordance with the Good Laboratory Practice (GLP) rules. The experiments were performed in accordance with the norms for the humane treatment of animals regulated by the International Guidelines of the Association for the Assessment and Accreditation of Laboratory Animal Care in accordance with the protocol approved by the Institutional Animal Care and Use Committee of the Irkutsk Scientific Center of Surgery and Traumatology. All the operative interventions were conducted aseptically. Animals were anesthetized with an intramuscular injection of 2% rometar at the dose of 0.2 mL/kg.

The primary culture was obtained by fragmenting the dissected omentum and disaggregating the tissue fragments at 37°C in the solution containing 200 U/ml of collagenase, 2% antibiotic/antimycotic (10, 000 U/ml of penicillin, 10,000 μg/ml of streptomycin and 25 μg/ml of amphotericin B, Gibco) in DMEM. Collagenase activity in the suspension was inhibited by equal amount of DMEM containing 15% FBS (Sigma-Aldrich) and 1% antibiotic/antimycotic. Then the cells were twice washed in DMEM spiked with 10% FBS, 1% antibiotic/antimycotic by centrifuging the suspension at 500 G for 5 minutes. Isolated fibroblasts were cultured in DMEM containing 10% FBS, 1% antibiotic/antimycotic at 37°C, 80% RH and 5%

CO_2 in the Biostation CT, Nikon. To obtain a culture, fibroblasts were subcultured every 7 days.

After the third passage the culture was treated with SB203580 (4-[5-(4-fluorophenyl)-2-[4-(methylsulfonyl)phenyl]-1H-imidazol-4-yl]pyridine) at the concentration of 10 μM, bFGF at the dose of 133 pg/ml or their combination. The cells that were not exposed to the active substances served as controls (an appropriate amount of DMEM was added to the culture).

Immunofluorescence staining

Cells for immunofluorescence studies were fixed with 70% ethanol and stained with antibodies to CcO (anti-OxPhos Complex IV subunit I monoclonal antibody, Invitrogen, Cat. ND 0589, Lot 459600) in a dilution of 1:200. Alexa fluor 568 goat anti-mouse IgG (H+L) (Invitrogen, Cat. NA-11031, Lot 822389) in a dilution of 1:300 was used as secondary antibody. Nuclei were stained with Hoechst (Invitrogen, Cat. NH-3570, Lot822389), 1:300.[19,20]

Results

Morphological changes in peritoneal fibroblast culture

At the first stage of the study, we derived primary fibroblast culture from the rat omentum. After 7 days, the cells were subcultured to obtain a pure culture, and after three passages, the fibroblasts were introduced in the experiment.

To activate the cells, bFGF at the dose of 133 pg/ml was added. 30 minutes after exposure of fibroblast culture to bFGF, marked changes were observed (intensive collagen destruction, collagen fibers started to grow thinner). At the same time, some rounding of the attached fibroblasts was observed, some of them detached from the matrix and moved freely in the culture medium. A formation of conglomerates containing several dozens of fibroblasts was observed in the areas characterized by the highest cell density (Fig. 1 A, 1B). During the next day, the cell conglomerates thickened, producing more compact formations, which, however, disintegrated upon mechanical action (when moving the culture bottle or adding the medium).

To block the signaling of the p38 MAPK pathway, cells were exposed to SB203580, the mitogen-activated protein kinase inhibitor, at the concentration of 10 μM. Both the intact culture and the one activated by fibroblast growth factor (133 pg/ml) were subjected to the exposure. 30 minutes later, marked changes were observed in the fibroblast culture: in both cases there was intensive collagen destruction, collagen fibers started to grow thinner; attached fibroblasts rounded, detached from the substrate and moved freely in the culture medium. When exposed to SB203580, 2 hours after the substance adding, non- rounded cells started to vacuolate actively with vacuoles occupying the entire cytoplasm (Figure 1C). Introduction of two active substances into the fibroblast culture caused more intensive cell detachment and collagen destruction. Moreover, the formation of large conglomerates containing several dozens of cells connected with collagen fibers was observed in the areas characterized by the highest cell density (Figure 1D). During the next day, the cell conglomerates thickened, producing more compact formations, which disintegrated upon mechanical action (when moving the culture bottle or

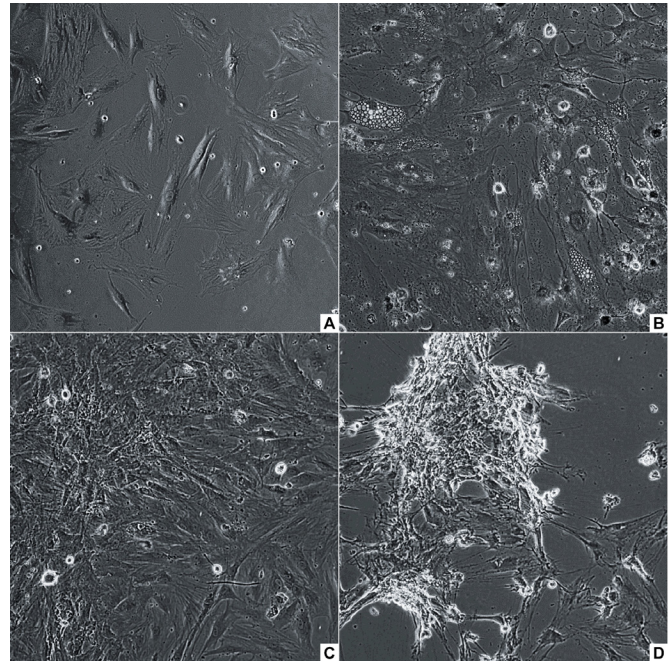adding the medium) and broke up into separate cells or smaller cell clusters.

Fig. 1. *Fibroblasts with dense intercellular junction and high content of collagen fibers before exposure to bFGF (A); Fibroblast conglomerate 30 minutes after exposure to bFGF(B); Fibroblast conglomerates 30 minutes after exposure to SB203580 (C) and SB203580 in combination with bFGF (D).*

Immunofluorescence studies

To study the OxPhos activity, cultured cells were fixed and stained with antibodies to mitochondrial CcO subunit I on Day 3 after exposure to active substances. Nuclei were stained with Hoechst.

Specific color staining of distinctly structured oval-shaped nuclei was observed in the controls. Mitochondrial complex IV was localized pointwise in the cells in direct proximity to the nuclei (Figure 2A).

After immunofluorescence staining of fibroblasts cultured with bFGF, irregular-shaped nucleus content with different Hoechst staining intensity was visualized. At the same time, the color of the mitochondrial complex IV in fibroblasts was uneven and was absent in most of the cells under study (Figure 2B).

After fibroblast cultivation with p38 MAPK inhibitor SB203580, the color of the mitochondrial complex IV in certain cells became more intense, being localized in the entire cell and represented by numerous pointwise formations. Nucleus content was characterized by irregular shape and indistinct contours and the Hoechst staining intensity was different (Figure 2C).

Fibroblasts exposed to p38 MAPK inhibitor in combination with bFGF were characterized by even more intense CcO staining. In this case, OxPhos fluorescence occupied a larger part of the cell volume, partially overlapping the color of the nuclei. At the same time, the cell nuclei were characterized by a more pale color and less structured content as well as by indistinct contours (Figure 2D).
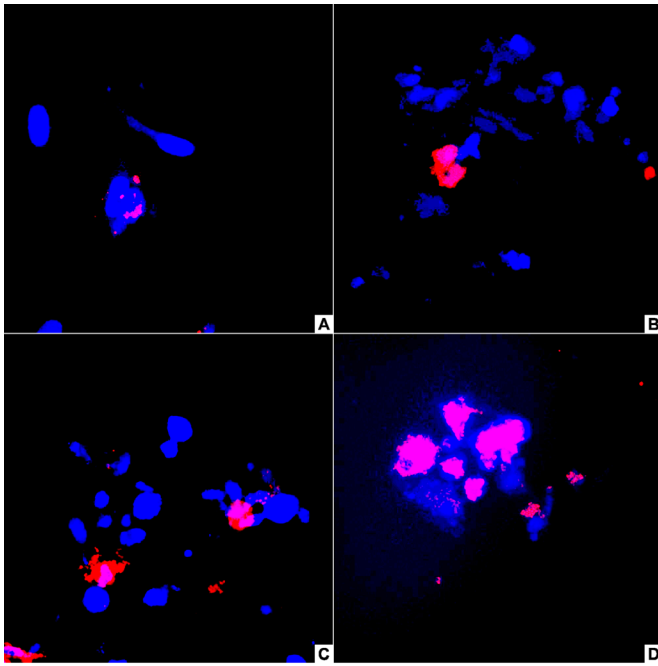
Fig. 2. *OxPhos fluorescence staining (red color). Fibroblast, control group (A), Fibroblasts with bFGF (B), SB203580 effect on fibroblasts (C), SB203580 and bFGF effect on fibroblasts (D). Hoechst (blue color).*

Statistical analysis of the data obtained also confirmed a significant increase (more than 5-fold) in the area of the cell stained with antibodies to mitochondrial complex IV when fibroblasts were exposed to the combination of SB203580 and bFGF, whereas introducing only the p38 MAPK inhibitor into the culture did not significantly increase the area of the fluorescently stained enzyme in the cultured cells (Figure 3). Uneven staining observed in the cells under study and, accordingly, a wide interquartile range in the analyzed cell groups are associated with heterogeneity of the resulting culture (cultured fibroblasts are at different stages of the cell cycle).
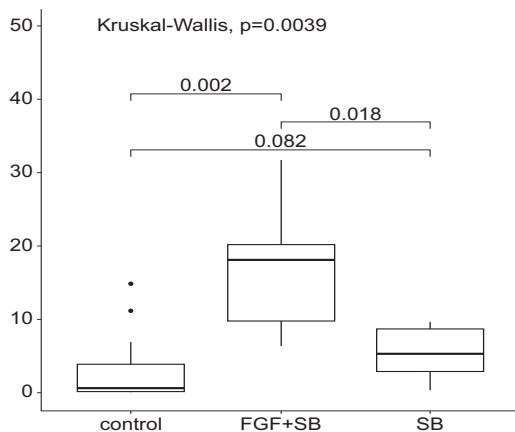

Fig. 3. *One-way analysis of variance (Kruskal-Wallis test) of the changes in the area of the cell occupied by CcO in the controls and the samples treated with SB203580 as well as with the combination of SB203580 and bFGF. A pairwise comparison was made using the Wilcoxon test. Median values, the 1st and the 3d quartiles are presented. Differences are considered statistically significant at P<0.05.*

At the same time one-way analysis of variance showed that total fluorescence staining intensity of complex IV is significantly greater through exposure to SB203580, both in intact cells and in bFGF-activated fibroblasts: 6 and 4 times more, respectively. This may suggest that the concentration of CcO in mitochondria increases in p38 MAPK inhibitor-incubated fibroblasts. However, the intracellular distribution of these organelles in the cell is quite tight, whereas after incubation of bFGF-activated fibroblasts with SB203580, there is less increase in the concentration of the OxPhos complex in mitochondria but its intracellular localization becomes far more distributed.

Discussion

MAPKs constitute a protein family relaying signal transduction, amplification and integration and thus providing for appropriate physiological responses of mammalian cells, such as proliferation, differentiation, inflammation and apoptosis.[21-24] One of the MAP kinase subfamilies, p38 MAPK, is known to be activated under stress conditions caused by ultraviolet radiation, heat shock, osmotic stress, lipopolysaccharide, protein synthesis inhibitors, pro-inflammatory cytokines (IL-1, TNF-α, etc.), and also by certain mitogens realizing the effect through tyrosine kinase receptors in particular. In response to the stimulus, p38 MAPK system can interrupt the cell cycle in the G1/S phase during mitotic spindle formation, thus launching one of the physiological response scenarios.[25] For example, p38 MAPK is known to be involved in the differentiation of adipocytes, cardiomyocytes, chondroblasts, erythroblasts, myoblasts and neurons.[26]

The association of p38 MAPK with OxPhos level and CcO activity in particular, is underexplored, however, there is some evidence that genetic knockout of p38 in Purkinje neurons suppressed the mitochondrial respiration in male mice while increasing CcO expression in female mice.[27] Ronda et al. (2010) report on p38 MAPK-mediated effects of estradiol on the muscle cells treated with hydrogen peroxide indicating that MAPK inhibition leads to inability of the hormone to prevent cell damage exerted by oxidative stress, while mitochondrial membrane disintegration was determined by measuring CcO activity in the cytosol.[28] In addition, the study conducted on hepatocarcinoma cells demonstrated a positive dependence of CcO subunit IV expression from p38 MAPK activity.[29]

Our results demonstrate an increase in CcO levels during p38 MAPK inhibition, which may indicate that p38 MAPK negatively regulates the CcO expression in peritoneal fibroblasts of adult animals (Figure 2C).

bFGF performs numerous functions in the body, it participates in wound repair, stimulates angiogenesis both under normal and pathological conditions (carcinogenesis), influences vascular tone and blood pressure, participates in inflammatory responses, is essential to normal fetal development (since it induces embryonic and extra-germ cell division and is of great importance for limbs development), plays a role in the brain cortex development as well. In addition, FGF is one of the cell proliferation and cell death suppression

mediators during carcinogenesis.[30,31] At the cellular level, FGF stimulates fibroblast and endothelial cells proliferation, promotes migration of endotheliocytes regulating proteolysis and adhesion molecule expression.[31,32]

FGF and p38 MAPK interaction has not been well-defined, but there is some evidence of these molecules interaction in the regulation of the processes associated with cell proliferation and differentiation. Thus, Matsumoto et al.[33] investigated the role of p38 activation in FGF-2-stimulated angiogenesis: in collagen gel cultures, bovine capillary endothelial cells formed tubular growth-arrested structures in response to FGF-2, while FGF induced more potent MAPK activation. Treatment with the p38 inhibitor, however, enhanced cell morphogenesis and increased cell proliferation.[33] There is also data indicating that fibroblast growth factor (at the concentration of 100 ng/ml) affects chondrocyte proliferation and differentiation inhibiting cell multiplication both *in vitro* and *in vivo*, whereas regulation was mediated by ERK1/2 and p38 MAPK signaling pathways. In this case, the use of MAPK inhibitors prevented FGF-induced growth of chondrocytes.[34] In addition, p38 MAPK is essential to FGF-induced proliferation of fibroblast-like 3T3 mouse cells.[35] At the same time, the sustained p38 MAPK activation is crucial to FGF-induced cell death in cells of Ewing's sarcoma as compared to transient p38 MAPK activation, which mediates FGF-induced cell proliferation.[36]

In the study under discussion, SB203580, a p38 MAPK inhibitor, enhanced the effect of the fibroblast growth factor by increasing the area occupied by CcO in the cultured fibroblasts.

In addition to participating in the transduction of signals that cause cell proliferation and differentiation, p38 MAPK is also a modulator of metalloproteinase synthesis regulation, which provide for remodeling of collagen extracellular matrix. For example, synthesis and secretion of collagenase-1 (MMP-1) expressed by several cell types including fibroblasts, MMP-3, expressed in lamellar epithelial cells and MMP-9 of sarcoma cells depend on the p38 MAPK activity.[37]

In our paper, p38 MAPK inhibition in combination with fibroblast growth factor introduction could have caused impaired elimination of extracellular collagen, which resulted in incomplete collagen utilization by enzymes during cell rounding and detachment and the remaining fibrils contributed to cell conglomerate formation.

Thus, the analysis result indicates that p38 MAPK activity inhibition influences both fibroblast morphology and the energy status of the cells under study increasing the amount of CcO, the terminal enzyme of the mitochondrial electron transport chain, in the cells, although no cell change to active proliferation or apoptosis was observed.

Competing Interests

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Experimental Medicine

Cold Stress and Endogenic System Ethanol – Acetaldehyde

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Abstract

The article presents data on the effects of cold stress on changes in the concentrations of endogenous ethanol (EE) and acetaldehyde (EA), and the activities of the key enzymes involved in their metabolism—alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (AHD) in laboratory animals. Mature male Wistar rats were used in the experiment. Animals in the vivarium were kept in cages at a temperature of 20°-22°C, without limitation of mobility, with free access to water with an adjustable light schedule (12 hours - light, 12 hours - darkness). Simulation of cold stressing was carried out for 7 weeks at a temperature of 1-2°C, starting from 0.5 hour to 6 hours per day by the fourth week of the experiment. The concentration of EE and EA was determined by gas chromatography-mass spectrometry (GC-MS). The analysis of the activity of the ADH and AHD enzymes was performed by standard spectrophotometric methods. The experiment showed that during cold stresses, the survival rate in rats strongly correlates with the EE content in the blood (r=0.757÷0.923). This fact suggests that when adapting cold-adapted animals to the effects of low temperatures, it is important to increase the EE concentration in the body, and the consumption of EE compensates for the increased need of the body in cold conditions for this metabolite. Based on the data obtained, a probable mechanism of the participation of EE in the processes of adaptation of higher vertebrates to cold is presented, which includes specific adaptive changes in the activities of the ADH and AHD enzymes.(**International Journal of Biomedicine. 2019;9(4):356-360.**)

Key Words: adaptation • endogenous ethanol • acetaldehyde • dehydrogenase • laboratory rats • cold stresses
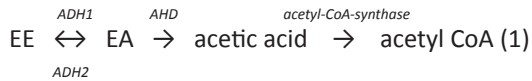
Introduction

The most stressing factor for higher vertebrate organisms in the North is cold. Under these conditions, dehydrogenase enzyme systems, which provide bioenergetic processes even with a lack of oxygen, are very important. One such system includes ADH and AHD metabolizing EE and EA.[1-4] It has been established that maintaining an optimal state of homeostasis when adapting to cold in the series "cold-adapted mammals (including humans) → small cold-adapted animals → large aboriginal cold-adapted animals of the North → hibernating animals," regardless of species specificity and living conditions, is largely due to the ratio of concentrations of EE and EA in the blood, depending on the liver activity of ADH and AHD.[5] It has also been shown that the blood concentration of EE and EA of indigenous people in the conditions of the North is 30%–40% higher than the

values of similar parameters in the conditions of the central zone of Russia.[6,7] Moreover, if these homeostatic parameters are stable in humans and animals under conditions of small amplitude of seasonal temperature fluctuations, in the North, people find seasonal dynamics of EE in the blood: in the summer, it is 1.7 times higher than in winter. This, along with the all-season increase in the EA content in the blood, indicates an increase in the importance of these metabolites for life support in extreme climatic conditions.

It is known that EE can be formed when EA is reduced, which occurs in the process of oxidative decarboxylation of pyruvate when the intermediate product is cleaved in the pyruvate decaboxylase complex. It has also been shown that EE can be formed during the decarboxylation of lactate, which accumulates in red skeletal muscles during glycolysis under conditions of oxygen deficiency.[8] In turn, the metabolism of EE and EA is associated with the main metabolism through the formation of acetyl-CoA. The concentrations of EE and EA and their ratio are provided by the enzyme system ADH and AHD. The ADH-dependent reaction is reversible; the AHD-dependent is irreversible (Equation 1). ADH-dependent

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conversion of EE occurs by first oxidizing it to EA, then to acetic acid, followed by the formation of its conjugate with coenzyme A, acetyl-CoA, which further enters the most diverse catabolic and anabolic transformations.

$$EE \underset{ADH2}{\overset{ADH1}{\longleftrightarrow}} EA \overset{AHD}{\longrightarrow} \text{acetic acid} \overset{\text{acetyl-CoA-synthase}}{\longrightarrow} \text{acetyl CoA} \quad (1)$$

There are 2 groups of AHD isoforms: with high affinity for aldehyde—AHD_1 (Michaelis constant $K_M = 0.1$–1.0 μM) and with low affinity—AHD_2 ($K_M = 0.1$-1.0 mM).[2,9,10] Due to the heterogeneity of the isoenzyme composition of both enzymes, the levels of EE, EA and their ratios can vary within rather wide limits.

EE and EA are considered to be metabolites that control a large part of the mechanisms of homeostasis, which ensure the optimal vital activity of living organisms, especially under extreme living conditions.[1,5] The normal level of homeostasis of laboratory animals and humans is maintained at blood concentrations of EE of about 0.05-0.2 mM, and EA is about 0.3-0.8 μM. The biological role of EE is diverse: (1) is a high-energy compound and under normal conditions can provide up to 10% of the body's energy needs; (2) participates in the maintenance of the liquid-crystalline, fluid state of the lipid layer of membranes, "liquefying" (fluidizing) them;[11-13] (3) is a regulator of lipid peroxidation in cell membranes, showing properties of a free radical trap and activating cholesterol synthesis.[11]

EA is chemically very active, does not penetrate cell membranes, but can change their permeability to other substances. The biological functions of EA are: (1) regulation of bioenergetic reactions at the stage of terminal oxidation of electron transfer from NADH to FAD by flavin enzymes; (2) regulatory modification (through the formation of Schiff bases) of opioid peptides; (3) with the participation of EA, endogenous morphine and morphine-like compounds are synthesized; (4) regulates the exchange of the most important neurochemical mediators of the amine nature: dopamine, norepinephrine, serotonin; hormone adrenaline.[12-14]

Everything discussed above allows us to consider the EE-EA system as an important element of non-specific regulatory systems of the body, providing the body's adaptive capabilities to the effects of stress, including cold.

Emotional stress, through the above mechanisms, leads to a decrease in the blood levels of EE, then EA, and can provoke their replenishment due to exogenous administration of alcohol.[7,15] Therefore, it has been found that in animals and people with a reduced level of EE in the blood, especially with prolonged exposure of the body to intense-stress environmental factors, an increased tendency to use exogenous ethanol to stabilize homeostasis.

The purpose of this study is to study changes in the state of the system of endogenous ethanol, acetaldehyde and the enzymes that metabolize them during cold stresses in a model experiment, using laboratory animals as an example.

Materials and Methods

Mature male Wistar rats were used in the experiment. Animals in the vivarium were kept in cages at a temperature of 20°-22°C, without limitation of mobility, with free access to water with an adjustable light schedule (12 hours - light, 12 hours - darkness). Simulation of cold stressing was carried out for 7 weeks at a temperature of 1°-2°C, starting from 0.5 hour to 6 hours per day by the fourth week of the experiment. Every day, the animals at the same time were placed in a specially equipped basement room, in which a constant cooling regime of 1°-2°C and a light mode similar to the vivarium were maintained. To maintain the natural rhythm of functioning, the rats were distributed at 4 p.m. At the same time, 2 groups of animals were studied: Group 1 - control (CG) animals were kept in stationary conditions of the vivarium (n=50); Group 2 - experimental (EG) rats were subjected to chronic cold stresses (n=150). After 4 weeks, depending on the nature of the change in the level of EE, the animals of EG were divided into 2 groups. EG1 included animals in which the EE level after the initial decline increased (n=58). EG2 included animals (n=92), in which the EE level decreased, compared with the control. In turn, the animals of EG2 were divided into 2 subgroups: EG2a (n=20) and E2b (n=72). Animals of EG2b, unlike rats of EG2a, were given the opportunity to consume a 10% ethanol solution under conditions of free choice. On Day 50 after the start of the experiment, part of the animals of EG2b (EG2b1; n=20)) were deprived of the possibility of alcohol consumption while maintaining all other conditions. By the end of the 10th week, the survival of the animals in each group was recorded. All blood sampling and decapitations were carried out in the morning when the animals were in a calm state. Blood to determine the concentration of EE and EA was taken from the tail, by cutting the tip of the tail with a sharp razor obliquely spiral. The decapitation of EG1 animals in order to study the ADH and AHD in the liver was carried out on Days 14, 28, and 49 of the experiment.

The experiments were performed in accordance with the norms for the humane treatment of animals, which are regulated by the International Guidelines of the Association for the Assessment and Accreditation of Laboratory Animal Care.

When studying the exchange of EE and EA, the following methods were used:

1. Concentrations of EE and EA in whole blood of animals were determined by GC-MS using a MAESTRO MSD chromatomass-mass spectrometer based on an Agilent 7820 gas chromatograph and an Agilent 5975 mass spectrometer.[17]

2. For kinetic studies, the purification of enzymes from rat liver was performed according to the following procedure. Frozen liver was washed from the blood, repeatedly perfusing it in a chilled saline solution (t=0°C) to a pale-yellow color. The liver tissue was homogenized in the cold. The final dilution of the homogenate was 2:1 (2 ml of a 0.05 M solution of glycine per 1g of the homogenate). To remove incompletely destroyed cells and nuclei, the homogenate was centrifuged for 30 min at 7000g (t=2°C). Chilled ethanol (t=0°C) was added to the supernatant to 20% final concentration. The mixture was shaken vigorously, and then centrifuged again. The supernatant was passed through a column with Sephadex G-100 (1.5×90cm) equilibrated with a 0.5 M glycine solution (t=2°C). In the selected fractions, the activities of ADH and AHD were determined.

3. The activities of ADH and AHD were determined on a Shimazu two-beam spectrophotometer and expressed in μmol/min per 1g of the liver. To obtain the kinetic parameters (Michaelis ADH and AHD constants by NAD, ethanol, NADH, and acetaldehyde), the dependence of the initial velocity on the concentration of one of the substrates (coenzymes) was measured at saturating concentrations of coenzyme. Differentiation of AHD isoforms into 2 groups was performed by the kinetic method.[18]

Statistical analysis was performed using the statistical program Stat Plus 2007. The normality of distribution of continuous variables was tested by one-sample Kolmogorov-Smirnov test. Continuous variables with normal distribution were presented as mean+SEM. Means of 2 continuous normally distributed variables were compared by independent samples Student's t test. Mann-Whitney U test test was used to compare means of 2 groups of variables not normally distributed. The frequencies of categorical variables were compared using Pearson χ^2 test. A value of $P<0.05$ was considered significant.

Results and Discussion

Changes in EE concentration detected in the blood of laboratory rats under conditions of low-temperature exposure are presented in Table 1. On Day 14 of cold stress, the EE level decreased by 3.2 times in animals of EG. On Day 28 of the experiment, rats exposed to cold stresses (EG) were divided into 2 groups depending on the level of EE. EG1 included animals whose blood EE level, after the initial decrease, increased by 3.4 times compared to the control, and 5 times on Day 49 of the experiment. EG2 included rats in which the EE level after the initial decrease practically did not increase by Day 28.

Table 1.

The concentration of EE (mmol/l) in the blood of rats during cold stresses

Day	Concentration of EE (mmol/l) in rat blood			P-value
	CG (n=50)	EG (n=150)		
1	0.16±0.03	0.16±0.03		
14	0.17±0.04	0,05±0.01		P=0.0000
		EG1 (n=58)	EG2 (n=92)	
28	0.16±0.04	0.55±0.12	0.08±0.02	$P_{EG1,CG} = 0.0046$ $P_{EG2,CG} = 0.037$ $P_{EG1,EG2} = 0.0059$
		EG2a (n=20)	EG2b (n=72)	
49	0.16±0.03	0.8±0.14	0.07±0.02 / 1.1±0.21	$P_{EG2a,CG} = 0.0205$ $P_{EG2b,CG} = 0.000$ $P_{EG2a,EG1} = 0.000$ $P_{EG2a,EG1} = 0.1296$ $P_{EG2b,EG1} = 0.000$ $P_{EG2a, EG2b} = 0.000$

From Day 28 of the experiment, 70 animals of EG2 were given the opportunity to consume a 10% ethanol solution under the conditions of free choice (EG2b), unlike animals of EG2a. On Day 49 after the experiment, the EG2b1 animals were deprived of the possibility of alcohol consumption while maintaining all other conditions. As a result, after 10 weeks, 87% of the animals in this group died (Fig.1). The survival rate of the rats in other groups was as follows: 92% - in EG1, 27% - in EG2a, and 81% - in EG2b.
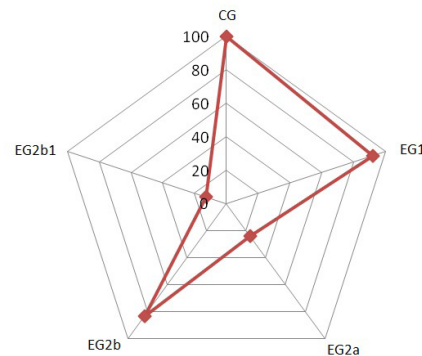

***Fig. 1.** The survival rate of animals after 10 weeks of the experiment (%).*

During cold stressing, the survival rate in rats of all groups strongly correlates with the EE content in the blood (r=0.757÷0.923). This fact suggests that when adapting cold-adapted animals to the effects of low temperatures, it is important to increase the concentration of EE in the body, and the consumption of exogenous ethanol makes up for the increased body need in cold conditions for this metabolite. To identify the mechanisms for increasing the level of EE in the blood during adaptation to cryotherapy, a study was made of the state of the dehydrogenase EE metabolism system in the group of EG1 rats, which have the highest percentage of survival (Table 2).

The kinetic calculation of Scheme (1), carried out considering the obtained concentration values and catalytic parameters of ADH, the activity of AHD_1 and AHD_2, showed that at the first stage of cold stresses (Day 14), a sharp decrease in the concentration of EE is associated with a change in enzyme activities. ADH_1 activity decreases by 1.75 times and ADH_2 activity by 2.1 times, primarily due to a 1.8 times decrease in enzyme concentration. AHD activity also decreases by 1.4 times, first, due to a 1.6 times decrease in the share of AHD_1 (Table 2). Therefore, the EA level is reduced to a lesser extent than EE. By Day 28 of the experiment, there was some restoration of the levels of concentration and activity of ADH. This led to an increase in the EE concentration, in relation to the control, by 3.4 times, and to EA - by 2.0 times. By Day 49 of the experiment, the levels of ADH and AHD completely recovered, which resulted from increasing the concentration of EE by 5 times on the background of increasing the EA level by 5.5 times. This contributed to the smooth flow of cold adaptation.

Table 2.

Characteristics of the metabolic chain of EE in the body of laboratory rats of the EG1 group during cold stressing

Parameters	Control (n = 15)	Cold stress period (days)			P-value
		14 (n=10)	28 (n=10)	49 (n=10)	
$ADH_1^{(1)}$ activity (μmol/min $\times$g)	0.35±0.04	0.20±0.03	0.25±0.03	0.40±0.04	P_{c-14}=0.0065 P_{14-9}=0.0009 P_{28-49}=0.0080
$ADH_2^{(2)}$ activity (μmol/min $\times$g)	0.79±0.08	0.37±0.07	0.50±0.08	0.82±0.06	P_{c-14}=0.0006 P_{c-28}=0.0177 P_{c-28}=0.0001 P_{14-49}=0.0052 P_{28-49}=
ADH concentration (nmol/g)	3.1±0.5	1.7±0.4	2.5±0.4	3.3±0.5	P_{c-14}=0.0397 P_{14-49}=0.0230
k_{1ADH} (min^{-1}) $^{(3)}$	113±12	120±12	100±12	120±15	
k_{2ADH} (min^{-2}) $^{(4)}$	255±20	220±20	200±25	248±20	
AHD activity (μmol/min$\times$g), including (%) AHD_1 AHD_2	1.10±0.12 40±5 60±5	0.80±0.05 25±6 75±6	1.20±0.15 42±5 48±5	1.00±0.09 40±5 60±5	P_{c-14}=0.0308 P_{14-28}=0.0215
[EE], mM	0.16±0.03	0.05±0.01	0.55±0.12	0.8±0.14	P_{c-14}=0.0021 P_{c-28}=0.0046 P_{c-49}=0.0001 P_{14-49}=0.0000 P_{28-49}=0.1928
[EA], μM	1.0±0.2	0.5±0.2	2.0±0.3	5.5±0.3	P_{c-14}=0.0909 P_{c-28}=0.0110 P_{c-49}=0.0000 P_{14-49}=0.0000 P_{28-49}=0.0000
[EE]/[EA]	160±16	100±10	275±28	145±15	

(1) ADH_1 activity in the ethanol oxidation reaction; (2) ADH_2 activity in the acetaldehyde reduction reaction;
(3) The catalytic constant of ADH in the ethanol oxidation reaction; (4) The catalytic constant of ADH in the acetaldehyde reduction reaction.

The mechanism of the cold-adaptive action of EE apparently consists, first, in its ability to easily penetrate into all cells of the body, reduce the viscosity of the lipid layer of cell membranes and optimize functioning at lower temperatures of membrane tissue-specific, transport and receptor complexes. [6,8,9,13] It consists second in the easy and fast mobilization of EE, primarily as a catabolic substrate; therefore, its concentration in the blood at the first stage of adaptation to cold decreases. However, on Day 28 of cold stress, the EE level is not only restored, but increases in overcompensation mode by 3.4-5.0 times. This happens due to specific adaptive changes in the activity of ADH and AHD, as well as to ADH- and AHD-dependent increases in the EA level, which reduces the level of aerobic metabolism and energy losses of the body in cold conditions. [3-5] At the same time, those animals whose EE level remains low on the background of cold stresses (EG2b) begin to feel the need for EE replenishment due to exogenous intake. Realization of this need allows the body to survive during cold stresses. A deprivation of the possibility of exogenous use of ethanol in doses that contribute to cold adaptation leads, with a high probability, to the death of animals (EG2b1). The results of the experiment confirm the significance of ethanol in the processes by which homoiothermal animals adapt to cold.

Competing Interests

Sources of Funding

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Cytokine Profile and Its Correction by Immunomodulators in Experimental Bronchopulmonary Inflammation in Rats

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Abstract

The aim of this study was to assess the changes in the levels of the blood serum pro- and anti-inflammatory cytokines in rats with experimental bronchopneumonia and after administration of immunomodulators.

Materials and Methods: Research was carried out on mature male Wistar rats (n=111), weighing 220-250g. The bronchopulmonary inflammation in the rats was modeled by administration of 0.1 ml of gum turpentine in the rats' trachea under etherization. All rats were divided into four groups: Group 1 included intact animals (n=18); Group 2 included animals having bronchopneumonia (n=19); Group 3 included animals having bronchopneumonia treated with Krezacin (n=15); Group 4 included animals having bronchopneumonia treated with Metaprot (n=16). Right after the operation and for the next 5 days, a solution of one of the test immunomodulators was injected (once a day) abdominally in the experimental animals: 25mg/kg of Krezacin or 25mg/kg of Metaprot. On Day 5 of the experiment, the animals were decapitated under anesthesia and blood samples were collected. The concentration of cytokines in the blood was determined by the flow immunofluorometry method with the device Bio-RadLaboratories (USA).

Results: The administration of Krezacin in animals with bronchopneumonia, in comparison with untreated animals, was accompanied by a statistically significant increase in the levels of IL-1β (by 3.43 times), IL-2 (by 1.95 times), INF-γ (by 3.29 times), and MCP-1 (by 2.07 times) and a decrease in the levels of TNFα (by 1.85 times) and IL-6 (by 2.20 times). Administration of Krezacin increased anti-inflammatory cytokines considerably: IL-4 by 5.22 times and IL-10 by 2.41 times. Analysis of results after administration of Metaprot demonstrates an increase in the levels of IL-1β (by 2.57 times), IL-2 (by 2 times), INF-γ (by 3.21 times), and MCP-1 (by 1.98 times) and a decrease in the levels of TNFα (by 2.17 times) and IL-6 (by 2.5 times), compared to data on untreated animals. Anti-inflammatory cytokines also increased greatly: IL-4 by 5.3 times and IL-10 by 3.71 times.

Conclusion: The results obtained show that investigated preparations reduce the severity and intensity of bronchopneumonia in rats. Krezacin and Metaprot demonstrate the properties of true immunomodulators, increasing protective properties of cytokines and providing the adaptive response of the body in severe bronchopulmonary pathology. (**International Journal of Biomedicine. 2019;9(4):361-365.**)

Key Words: experimental bronchopneumonia • rats • cytokines • immunomodulators

Abbreviations

IL, interleukin; **INF**, interferone; **MCP-1,** monocyte chemotactic protein 1; **TNF**, tumor necrosis factor; **SB RAS** - Siberian branch of the Academy of Sciences.

Introduction

Upper respiratory tract infections are considered a serious health problem due to high prevalence, complex clinical course and severe complications of disease.[1] As a rule, the treatment efficacy of acute bronchopulmonary diseases, particularly pneumonia, depends on adequate antibacterial treatment as well as the presence of secondary immunodeficiency status.[2,3]

It should be noted that diverse antibacterial therapies are becoming less effective against a wide range of pneumonia pathogens, resulting in long-term morbidity and chronicity.[4] As noted, acute bronchopulmonary diseases are accompanied by a progression of chronic hypoxia, alterations in immune response, and the development of oxidative stress.[5,6] The severity of this kind disease necessitates the development and research of new types of antihypoxic and immunotropic medicines.[7-11] Krezacin (hydroxyethylammonium methylphenoxyacetate) and Metaprot (ethylthiobenzimidazole hydrobromide monohydrate) were created in Russia in the middle of the last century and proved to have antihypoxic, energy-stabilizing (ergotropic) and immunomodulating properties.[12-16] However, studies of these medicines regarding bronchopulmonary diseases have not been carried out.

In fact, inflammatory and immune reactions are the result of interactions among numerous body systems, where cytokines (interleukin, colony-stimulating and growth factors, interferons, chemokines, etc.) are an connecting link between them.[17-22] Different cytokines possessing pro-inflammatory or anti-inflammatory effects not only perform a regulatory function within the immune system, but also provide multicomponent connections with the nervous and endocrine systems.[23]

Thus, it is rather important to investigate the presently unknown pathogenetic mechanisms of the inflammatory process in the bronchopulmonary tissue and the protective effect of immunomodulators, focusing on changes in the cytokine blood levels of the experimental animals. In regard to the above, the aim of this study was to assess the changes in the levels of the blood serum pro- and anti-inflammatory cytokines in rats with experimental bronchopneumonia and after administration of immunomodulators.

Material and Methods

Research was carried out on mature male Wistar rats (n=111), weighing 220-250 g. The bronchopulmonary inflammation in the rats was modeled by administration of 0.1 ml of gum turpentine in the rats' trachea under etherization. Animals in the vivarium were kept in cages at a temperature of 20°-22°C, without limitation of mobility, with free access to water with an adjustable light schedule (12 hours - light, 12 hours - darkness).

All rats were divided into four groups: Group 1 included intact animals (n=18); Group 2 included animals having bronchopneumonia (n=19); Group 3 included animals having bronchopneumonia treated with Krezacin (n=15); Group 4 included animals having bronchopneumonia treated with Metaprot (n=16).

Right after the operation and for the next 5 days, a solution of one of the test immunomodulators was injected (once a day) abdominally in the experimental animals: 25 mg/kg of Krezacin or 25 mg/kg of Metaprot. The choice of dose level was determined by previous studies and evidence of the effectiveness of the medicines in these doses.[8,11] On Day 5 of the experiment, the animals were decapitated under anesthesia and blood samples were collected. Blood serum prepared by centrifugation at 2500 rpm for 20 minutes at +4°C was frozen and stored at -20°C until testing.

The concentration of cytokines in the blood was determined by the flow immunofluorometry method with the device Bio-RadLaboratories (USA). MilliplexMapRatCitokine/Chemokine sets were used in accordance with the instructions of the producing firm. The method is based on the specific binding of the studied cytokines to the solid phase in suspension of polystyrene granules with a fluorescent label and is conjugated to the appropriate anticytokine monoclonal antibodies. The results were evaluated by the flow fluorimeter, where borders are automatically divided by the specific fluorescence of their own labels. Using standard calibration dilutions, the concentration of the studied cytokines in the tested samples was calculated automatically by the Bio-plex Manager computer program and was expressed in pcg/ml.

For the treatment of bronchopneumonia in rats, Krezacin and Metaprot were used. Krezacin was invented at the A.E. Favorsky Irkutsk Institute of Chemistry SB RAS in the 1990s; after that it was actively studied at the S.N. Kirov Military Medical Academy (Saint Petersburg). Metaprot was created as an adaptogen for military medicine exclusively in the 1970s. Both preparations are classified as phytohormones and are highly effective adaptogens; they act as stress protectors and have an anti-toxic effect.[13]

The work with animals was carried out in accordance with the principles of humanism laid down in the directives of the European Community (86/609/EEC) and the Declaration of Helsinki, in accordance with the "Animal experimentation legislations". The study was approved by the Local Ethic Committee of the S.M. Kirov Military Medical Academy.

Statistical analysis was performed using the Statistica 6.1 software package (Stat-Soft Inc., USA). The normality of distribution of continuous variables was tested by the Kolmogorov-Smirnov test with the Lilliefors correction and Shapiro-Wilk test. An F-test was used to test if the variances of two populations are equal. Differences of continuous variables departing from the normal distribution, even after transformation, were tested by the Mann-Whitney U-test. A probability value of $P \leq 0.05$ was considered statistically significant.

Results and Discussion

The modeling of experimental bronchopulmonary inflammation in the rats was accompanied by considerable changes in blood serum pro- and anti-inflammatory cytokines (Fig.1). Therefore, a statistically significant decrease in the levels of IL-2 (by 1.85 times), IFN-γ (by 1.43 times) and MCP-1 (by 2.07 times). In addition, levels of TNFα (by 3.16

times) and IL-6 (by 4.68 times) were greatly increased. The concentration of anti-inflammatory cytokines IL-4 and IL-10 was notable decreased – by 1.78 times and by 1.71 times, respectively (Fig.2).
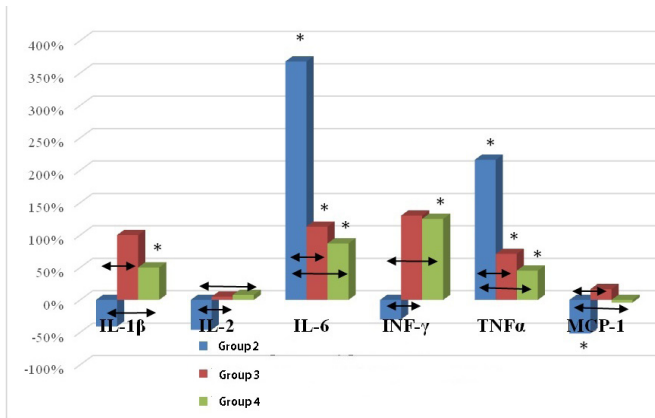

***Fig. 1.** Changes in the pro-inflammatory cytokine levels in rats with bronchopneumonia treated by different immunomodulators.* ** - statistically significant differences compared to intact animals (values are taken as 0%);* ◄► *- statistically significant differences between groups*

***Fig. 2.** Changes in the anti-inflammatory cytokine levels in rats with bronchopneumonia treated by different immunomodulators.* ** - statistically significant differences compared to intact animals (values are taken as 0%);* ◄► *- statistically significant differences between groups*
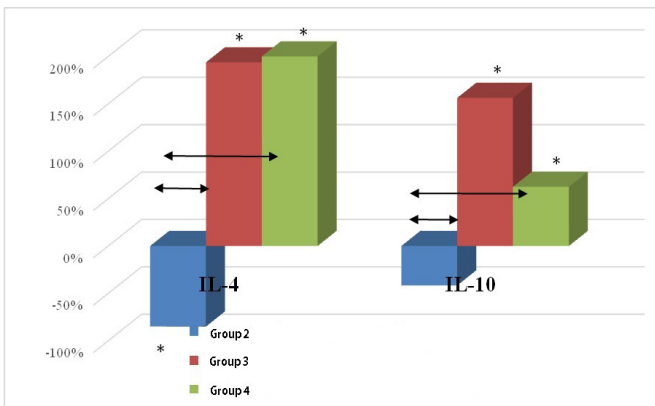
The decreased concentration of both pro-inflammatory and anti-inflammatory (IL-4, IL-10) factors in blood in rats with inflammation stands out. At the same time, the levels of TNFα and IL-6 counts were increased.

Findings indicate that experimental bronchopneumonia in rats can be the cause of secondary immunodeficiency development and acute depression of immunoregulatory functions of cytokines, which are responsible both for the development of inflammation and for limiting its severity. Such disarrangement of the cascade process in regulating properties in cytokines that act upon integrating the nervous, endocrine and immunological mechanisms of inflammation certainly points to the intensity and severity of bronchopulmonary inflammation, as well its destructive nature.

This finding is also supported by the fact that TNFα blood content increased more than 3 times and TNFα was considered as the strong factor of overproliferation and apoptosis.[1,12] TNFα is an inducer for progression of both local and systemic inflammation and promotes as well the synthesis of a wide range of pro-inflammatory cytokines that worsen tissue damage. In addition to pro-inflammatory cytokines, TNFα also increases lipid signal transduction mediators such as prostaglandins and platelet activating factor. TNFα governs cell survival signaling pathways, proliferation and regulates metabolic processes.[25] Moreover, almost a 5-fold increase of IL-6 level evidences the high severity and intensity of the systemic inflammatory process, because IL-6 is known as the "cytokine of damage," produced not only by monocytes, but also by T cells, macrophages, fibroblasts, and endothelial cells.[17,26] Affected by inflammatory mediators, the synthesis of this cytokine begins even at the early stages of the inflammation. During the later stages, it induces the differentiation of lymphocytes, as well as T-killer precursors into mature effector cells, which ensure target cells lysis, while stimulating the production of other interleukins.[27] In addition, IL-6 also activates T lymphocytes with the formation of plasma cells and antibodies.[28,29] In high doses it modulates the expression of hepatocyte-specific genes, which encode proteins of the acute phase of inflammation and, thereby, provide the transformation of inflammation into a chronic form.[30,31]

The administration of Kezacin in animals with bronchopneumonia, in comparison with untreated animals, was accompanied by a statistically significant increase in the levels of IL-1β (by 3.43 times), IL-2 (by 1.95 times), INF-γ (by 3.29 times), and MCP-1 (by 2.07 times) and a decrease in the levels of TNFα (by 1.85 times) and IL-6 (by 2.20 times) (Fig.1). Administration of Krezacin increased anti-inflammatory cytokines considerably: IL-4 by 5.22 times and IL-10 by 2.41 times (Fig.2).

Analysis of results after administration of Metaprot demonstrates an increase in the levels of IL-1β (by 2.57 times), IL-2 (by 2 times), INF-γ (by 3.21 times), and MCP-1 (by 1.98 times) and a decrease in the levels of TNFα (by 2.17 times) and IL-6 (by 2.5 times), compared to data on untreated animals (Fig.1). Anti-inflammatory cytokines also increased greatly: IL-4 by 5.3 times and IL-10 by 3.71 times (Fig.2).

The administration of used immunomodulators considerably limits the impact of the pathological process influencing the cytokines levels in rats' blood. Thus, the levels of pro-inflammatory cytokines IL-1β, IL-2, chemokine MCP-1, and INF-α, as well as anti-inflammatory cytokines IL-4, IL-10 increased significantly. That indicated not only a partial recovery of immunoregulatory function, but also cytokine-producing properties of B cells, T helper 1 cells and T helper 2 cells.

It should be noted that immunomodulators used in this study limited the cytotoxic action of IL-6, and therefore can be considered as perspective anti-inflammatory mediators. Apparently, the recovery of the production and escalated levels of anti-inflammatory cytokines IL-4 and IL-10, after administration of immunomodulators, can be considered as a sanogenetic action of Krezacin and Metaprot.

The administration of immunomodulators also decreased animal mortality rate. The survival rate in untreated bronchopneumonia was 48%, but after administration of Krezacin and Metaprot it was 65% and 60%, respectively.

Conclusion

The results obtained show that investigated preparations reduce the severity and intensity of bronchopneumonia in rats. They demonstrate the properties of true immunomodulators, increasing protective properties of cytokines and providing the adaptive response of the body in severe bronchopulmonary pathology.

Competing Interests

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Biotechnology

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The Study of the Growth of *Escherichia coli* on Pectins

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Abstract

The growth of *E. coli* on pectins isolated from various plant sources was studied. We used commercial apple pectin AU701 and low-methyl esterified pectins (6-22%) from callus cultures of tansy *Tanacetum vulgare* L. (TV, tanacetan), duckweed *Lemna minor* L. (LM, lemnan), and campion *Silene vulgaris* (M.) G. (SV, silenan). Bacterial growth was also tested on the enzymatic degradation products of tanacetan pectin. For comparison, bacterial growth was studied on easily metabolizable carbon sources – glucose and lactose. *E. coli* was cultivated on solid media in Petri dishes and in liquid nutrient media in Erlenmeyer flasks at a temperature of +37°C and at room temperature. It was found that *E. coli* colonies do not form with growth on gels of tanacetan, lemnan and silenan. When growing on a gel of apple pectin, a weak bacterial growth is detected. However, *E. coli* is capable of growth on soluble products of enzymatic hydrolysis of tanacetan pectin – oligogalacturonides. (**International Journal of Biomedicine. 2019;9(4):366-369.**)

Key Words: *Escherichia coli* • liquid and solid nutrient media • pectin • pectin hydrolysis products • glucose • lactose

Abbreviations

AP, apple pectin; **CaPGPs**, calcium-pectic gel particles; **CFU**, colony forming units; **PG-TV**, products of hydrolysis of tanacetan; **LM**, pectin lemnan; **SV**, pectin silenan; **MPA**, meat-peptone agar; **MPB**, meat-peptone broth; **MA**, must agar; **MM**, mineral medium; **SGF**, simulated gastric fluid; **SIF**, simulated intestinal fluid; **SCF**, simulated colonic fluid.

Introduction

Escherichia coli, a gram-negative, non-sporulating facultative anaerobe, is a typical inhabitant of the lower intestines of endothermic animals and humans.[1] Intestinal microbiota consists of more than 500 species of bacteria that make up 10^{10}-10^{11} cells per gram of colon contents. Although anaerobic bacteria in the intestines exceed the number of *E. coli* from 100:1 to 10000:1, *E. coli* is the dominant aerobic organism in the gastrointestinal tract of mammals.[2]

Most strains of *E. coli* are harmless mammalian commensals; however, some strains can cause intestinal or extra-intestinal diseases.[3] The main function of the commensal flora in the intestine, especially in the colon, is the fermentation of indigestible food residues and endogenous mucus produced by the epithelium.[4] In the digestive tract, commensal strains of *E. coli* are localized in the mucus layer covering the epithelial cells of the colon, adapting their metabolism in this ecological niche.[5]

The mucus gel layer of the gastrointestinal tract (mucin), synthesized and secreted by the goblet cells of the host, lubricates and protects the intestinal epithelium from damage caused by food and digestive secrets. The mucus gel layer also acts as a trap for microorganisms, including pathogens, preventing their access to the epithelium.[6] Mucins of the intestinal mucosa are high molecular weight glycoproteins consisting of 80% carbohydrates and 20% protein.[7]

Although the intestines are usually considered anaerobic, the tissues surrounding the lumen are quite rich in oxygen and oxygen diffuses into the intestines and, in addition, oxygen is present in the swallowed air. That is, the intestines are not strictly anaerobic; therefore, the ability to have aerobic respiration provides *E. coli* with a great competitive advantage.[8]

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Most carbohydrates in the colon are in the form of mucosal polysaccharides, which degrade with resident anaerobes that dominate the intestinal biota.[9] Monosaccharides or disaccharides released from mucin and other mucosal glycoproteins support the growth of many intestinal bacteria such as *E. coli*, which do not form polysaccharide-degrading enzymes.[10]

In the literature, information on the growth characteristics of indigenous bacteria of the gastrointestinal tract of humans and animals, including *E. coli*, on pectic polysaccharides is quite limited and there are only few reports.[11,12]

The present work is devoted to the study of the growth of the gram-negative bacterium *E. coli* ARCIM B-8208 on pectins and products of their enzymatic hydrolysis.

Materials and Methods

Object of study

The object of the study was the gram-negative bacterium *E. coli* ARCIM B-8208. The culture was maintained on mowed MPA at a temperature of +4°C.

Conditions of cultivation of E. coli and conditions of cultivation of callus cultures

The seed was obtained by deep cultivation of *E. coli* for 2 days in Erlenmeyer flasks with a working volume of MPB of 200ml with stirring (220 rpm) and a temperature of +25°C.

To study the growth characteristics, we studied deep cultivation of *E. coli* under static conditions in Erlenmeyer flasks with a working volume of 50ml of liquid nutrient medium both at a temperature of +37°C and at room temperature.

The ability of *E. coli* to grow on solid media was studied in Petri dishes. The surface of the solid media was seeded with a suitably diluted bacterial inoculum and incubated at a temperature of +37°C.

Solid and liquid nutrient media were used in the experiments. Solid media: MPA, must agar (MA), solid media with different pectins. Liquid media: MPB, mineral medium (MM). The composition of the MPA: meat broth - 1 l, NaCl - 5 g/l, peptone - 10 g/l, agar - 30 g/l. The composition of the MPB: meat broth - 1 l, NaCl - 5 g/l, peptone - 10 g/l. MM composition (g/l): KH_2PO_4 – 0.25, NH_4Cl – 0.5, $MgSO_4 \cdot 7H_2O$ – 0.1, $CaCl_2$ – 0.005, $FeSO_4 \cdot 7H_2O$ – 0.005.

The following pectins were used as difficult metabolizable carbon sources in solid or liquid nutrient media: low-methyl esterified commercial AP AU701 with a degree of methoxylation of 36%-44% and with the molecular weight of 406 kDa (AP, Herbstreith & Fox KG, Germany), low-methyl esterified pectins (6-22%) from callus cultures of tansy *Tanacetum vulgare* L. (TV),[13] duckweed *Lemna minor* L. (LM),[14] and campion *Silene vulgaris* (M.) G. (SV)[15] with a molecular mass of >300 kDa. For comparison, *E. coli* was also cultivated in media with easily metabolizable carbon sources – glucose and lactose.

Callus cultures of tansy, duckweed and campion were grown on modified Murashige and Skoog agar medium.[16] The campion and duckweed callus cultures were cultivated with 1.0 mg/l of 2,4-dichlorophenoxyacetic acid (2,4-D) and 0.5 mg/l of 6-benzylaminopurine (BAP) added to the medium. The tansy callus was cultivated with the addition of 2,4-D(1.5mg/l)+BAP(0.5mg/l). The calluses were subcultured with an interval of 21 days (campion) and 28 days (tansy and duckweed) at the temperature of 26±1°C in the dark. The callus tissue was frozen at the end of the cultivation.[17,18] Pectins from callus cultures were isolated in the group of biotechnology of the Department of Molecular Immunology and Biotechnology of the Institute of Physiology.

Formation of pectic gels and CaPGPs

Pectic gels and spherical CaPGPs were obtained from AP and pectins of callus cultures in the presence of calcium ions by the method of ionotropic gelling.[19]

To obtain CaPGPs, pectins (30 mg) were dissolved in distilled water (1 ml) by slow stirring with an MM-5 magnetic stirrer (Russia) for 2-5 hours at room temperature until complete dissolution.

Gel particles of spherical shape were prepared by drop-by-drop injection of the pectin solution (3%) from a syringe through a needle with a hole diameter of 0.6 mm on a distance of 4-5 cm in the slowly stirred solution of calcium chloride (0.34 M) and further stirring for 20 minutes at room temperature. The resulting gel particles were then washed three times in distilled water with stirring for 5 minutes and dried for 10-14 hours at + 37°C.

Obtaining products of enzymatic degradation of CaPGPs

An artificial gastrointestinal medium was used to obtain degradation products of CaPGPs.

The swelling and degradation of CaPGPs were studied under conditions simulating the gastrointestinal fluid, namely, SGF solution (pH 1.25), SIF solution (pH 7.0) and SCF solution (pH 7.0). The SGF medium was prepared with NaCl (2.0 g/l), KCl (1.12 g/l), KH_2PO_4 (0.4 g/l) and $CaCl_2$ (0.11 g/l). The pH of the solution was adjusted to 1.25 by addition of 0.1N HCl solution. The SIF medium was prepared by addition of 1N $NaHCO_3$ solution to the SGF solution to the pH value of 7.0. The SCF medium was prepared by addition of pectinase (Sigma, 1.18 U/mg, USA) to the SIF solution.[20] The concentration of pectinase in SCF medium was 1.7 mg/ml (2.0 U/ml).

10 mg of dry gel particles of tanacetan pectin were placed in Petri dishes (diameter 3.5 cm) and subsequently incubated in 3 ml of the SGF (2 h), SIF (4 h) and SCF (18 h) solutions with shaking on a shaker (Titramax 1000, Heidolph, Germany) at 100 rpm and at +37 °C. The enzymatic hydrolysis products of tanacetan pectin (PH-TV) obtained in SCF medium were used as a carbon source to study the growth of *E. coli* on them.

Determination of growth parameters of E. coli

The optical density of *E. coli* suspensions during growth in liquid nutrient media was measured on an SF-103 spectrophotometer (Russia) at a wavelength of 660 nm.

The ability to grow *E. coli* in solid media was evaluated by the number of CFU (colony forming unit) by the Koch method. Samples (10 ml) were taken aseptically from bacterial suspensions and a series of 10-fold dilutions were prepared. From the obtained dilutions, 50 µl of aliquots of bacteria were sown on the surface of solid nutrient media. Petri dishes with crops were incubated at a temperature of +37°C. The grown colonies were counted on Days 1, 2, 3, 4, and 5 of incubation.

The number of CFU/ml was determined as the average of three replicates. Each experiment was carried out in three independent experiments.

When bacteria grew in liquid media with soluble carbon sources, the number of CFU in 1 ml of bacterial suspensions was calculated using a calibration graph of the dependence of CFU/ml on the optical density of the bacterial suspension (OD_{660}) (Fig. 1).
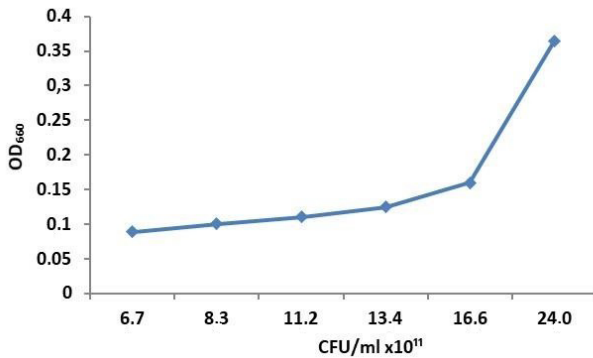

Fig. 1. *Calibration graph of the dependence of CFU/ml on OD_{660} of bacterial suspension.*

The statistical analysis was performed using the statistical software BioStat (version 4.03) and Microsoft Office Excell 2007. The mean (M) and standard deviation (SD) were calculated. A probability value of $P<0.05$ was considered statistically significant.

Results and Discussion

We studied the ability of the gram-negative bacterium *E. coli* ARCIM B-8208 to grow on the surface of solid pectin-containing media. Sources of pectins were commercial AP and the pectins tanacetan, lemnan, and silenan, isolated from callus cultures of tansy *Tanacetum vulgare* L., duckweed *Lemna minor* L. and campion *Silene vulgaris* (M.) G., respectively. Low methoxylated pectins, such as AP, tanacetan, lemnan, and silenan, form gels in the presence of calcium ions, and pectin molecules are cross-linked by calcium ions.[21] For comparison, MPA and MA, the standard media for the cultivation of bacteria, were used as solid media. The ability of solid media to modulate the formation of *E. coli* biofilms was evaluated by the number of CFU.

The most active colony formation occurs with the growth of *E. coli* on MA and MPA (Fig.2).

After 5 days of growth on MA and MPA, the number of *E. coli* colonies was $(14.3\pm1.8)\times10^{12}$ and $(13.0\pm2.6)\times10^{12}$ CFU/ml, respectively. With growth on gel of 3% AP, the formation of *E. coli* colonies was observed, like on MPA and MA, also after 2 days of growth, but in a smaller amount, and after 5 days of cultivation on AP, the number of CFU/ml was $(4.6\pm0.9)\times10^{12}$. On the contrary, the formation of *E. coli* colonies did not occur with growth on 3% gels of tanacetan, lemnan and silenan. Thus, gels obtained from callus pectins did not modulate the formation of *E. coli* biofilms.

We studied the effect on the growth of *E. coli* of enzymatic hydrolysis products of CaPGPs obtained under artificial gastrointestinal conditions. CaPGPs were obtained from the pectin tanacetan polysaccharide isolated from callus of tansy *Tanacetum vulgare* L.
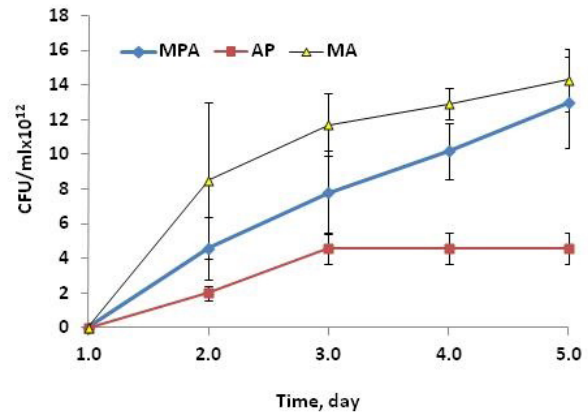

Fig. 2. *Growth of E. coli on solid media.*

The experiments were carried out under conditions of deep periodic cultivation in flasks, with stirring (220 rpm) and without stirring (under static conditions) *E. coli* culture broths at room temperature. It was found that more active bacterial growth occurs under static conditions, without stirring, and the amount of *E. coli* after 6 days of growth in a liquid MM with 1% glucose was $(18.5\pm0.2)\times10^{11}$ CFU/ml against $(13.2\pm0.1)\times10^{11}$ CFU/ml with stirring (Fig.3).
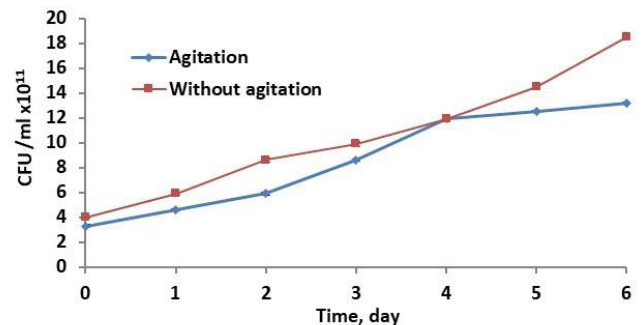

Fig. 3. *Growth of E. coli in liquid mineral medium (MM) with 1% glucose.*

We estimated the growth of *E. coli* in a liquid MM with both easily and hardly metabolizable carbon sources under static conditions at $+37°C$ (Fig.4). The most active growth of bacterium occurred in a mineral medium with easily metabolized carbon sources – lactose and glucose. The products of enzymatic hydrolysis of CaPGPs – oligogalacturonides obtained under conditions of an artificial gastrointestinal environment – contributed more to the growth of *E. coli* than did pectin tanacetan. The number of bacteria after 4 days of growth was $(43.5\pm0.6)\times10^{11}$ and $(25.5\pm0.2)\times10^{11}$ CFU/ml, respectively.

Thus, the gels of tanacetan, lemnan, and silenan do not contribute to the growth of *E. coli* ARCIM B-8208. When growing on a gel of AP, a weak bacterial growth was

detected. However, *E. coli* was capable of growth on soluble products of enzymatic hydrolysis of tanacetan pectin – oligogalacturonides.
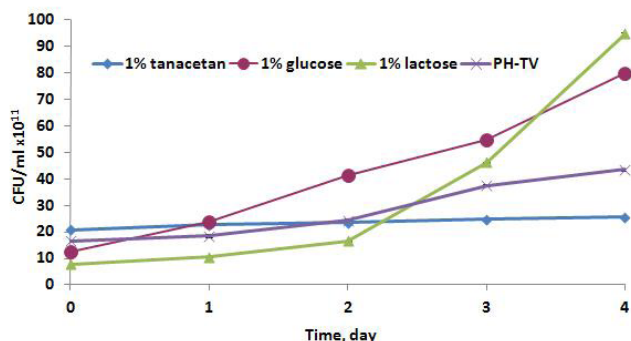

Fig. 4. *Growth of E. coli in a liquid mineral medium with different carbon sources.*

Apparently, in the colon, *E. coli* was also capable of growth on the products of enzymatic hydrolysis of pectins formed under the action of pectinases synthesized by the corresponding symbiotic microflora.

Competing interests

The authors declare that they have no competing interests.

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A Method for Performing a Gastrostomy Using a Polypropylene Mesh

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Abstract

Gastrostomy is one of the main palliative surgical methods for restoring enteral nutrition. The aim of the study was to develop a new method of gastrostomy that reduces the frequency of complications. The prototype for the described method is Depage-Janeway gastrostomy with use of the GIA stapler. The proposed method is characterized by the use of polypropylene mesh. This provides a tight attachment of the wall of the stomach to the anterior abdominal wall, which reduces the risk of complications. (**International Journal of Biomedicine. 2019;9(4):370-372.**)

Key Words: gastrostomy • palliative surgical methods • polypropylene mesh

Method Description

Gastrostomy is one of the most common palliative operations, the main indication for which is the need to restore enteral nutrition [1] in patients with severe neurological diseases associated with impaired swallowing, as well as in patients with a tumor obstruction of the upper digestive tract. However, indications for gastrostomy are constantly expanding and, in addition to solving palliative tasks, it is often used in a complex of rehabilitation [2] and therapeutic measures.[3] To date, about 100 different modifications of gastrostomy have been published in the literature. According to the method of application, they can be divided into 3 categories: open "traditional" methods (Witzel, Stamm – Kader, Toprover gastrostomy), laparoscopic gastrostomy, and percutaneous gastrostomy under endoscopic or radiological control. The traditional open methods for applying gastrostomy include Witzel gastrostomy,[4] which is still one of the most common operations performed in general surgical hospitals.[5]

Accordingly, with the expansion of indications for gastrostomy, this operation is still relevant to the improvement of existing gastrostomy techniques and the development of new ones;[6] it reduces the risk of gastrostomy insolvency, gastrostomy tube migration, and wound complications due to leakage of the structure, and it eliminates the need for the use of additional devices that fix the gastrostomy tube.

The purpose of the work was the development of a new method of gastrostomy to improve the treatment results in patients who have indications for applying gastrostomy.

Closest to the proposed method is Depage-Janeway gastrostomy using the GIA stapler.[7] This method was taken as a prototype method. The method of gastrostomy using a polypropylene mesh (Patent RU No. 2691924; priority of 06.18.2019; Bulletin No. 17) is implemented as follows. An upper median laparotomy is performed. The anterior wall of the stomach (Fig.1) is pulled up with two Babcock clamps to form a gastric tube 8-10 cm long. In addition, the diameter of the tube is calculated so that it allows insertion and removal of a Foley (18-22 F) catheter for feeding the patient. A GIA type stapler is placed perpendicular to the greater curvature of the stomach. The apparatus suture should end 2.5 cm from the greater curvature of the stomach. The device leaves 4 rows of sutures, 2 on each side, in the form of a double variable line of brackets. At the same time, the knife of the GIA apparatus cuts the stomach between both double rows of sutures, forming a closed gastric tube in the form of a diverticulum with a

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base at the greater curvature of the stomach. The apparatus suture is placed as the knotted cotton or silk seams. From a polypropylene mesh, 2 polypropylene mesh implants are modeled (Fig.1). The first of them is cut out in the form of an oval plate with a diameter of 6-7 cm, with a central hole (7 mm in diameter) through which the gastric tube is drawn. The second implant is cut out in a rectangular shape along the length of the gastric tube. The stomach tube is passed through the first implant, which is fixed to the gastric wall along the perimeter by separate sutures with 3/0 polypropylene thread. The second implant, in the form of a clutch, wraps the gastric tube and is fixed on the gastric tube with individual polypropylene sutures. Two implants are sutured together by separate sutures with 2/0 polypropylene thread. To the left of the midline incision, a hole of 1.5-2 cm is made in the projection of the left rectus abdominis muscle, where a Foley catheter (18-22F) is inserted into the abdominal cavity. The gastric tube is crossed along the diameter of the catheter, the catheter is inserted into the lumen of the stomach, and the catheter balloon is inflated through a special cannula. The gastric tube is fixed with separate sutures of polypropylene 2/0 thread to the parietal peritoneum and the muscular aponeurotic layer.
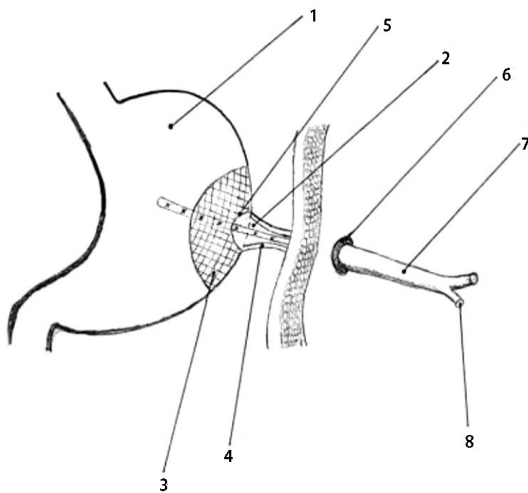
postoperative period with daily wound treatment, changing dressings after 3 weeks, the stomach-fixing sutures are gradually removed as the implants germinate with connective tissue, which provides an increasingly tight attachment of the stomach wall to the anterior abdominal wall.
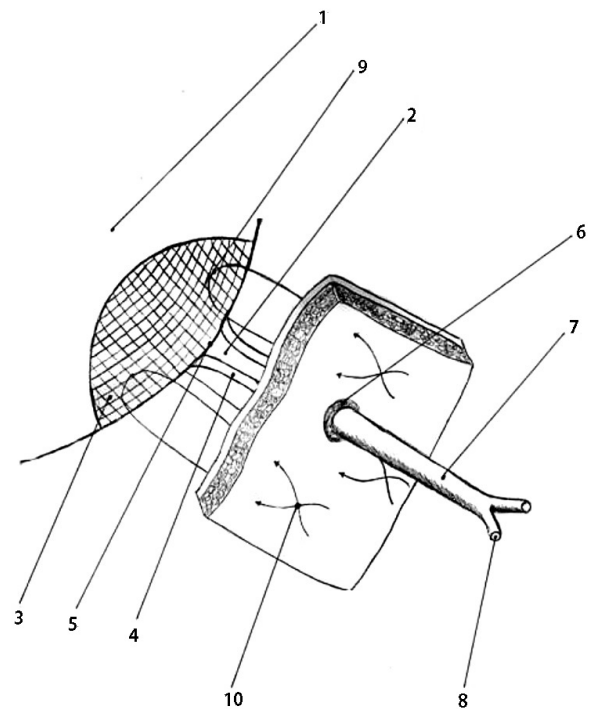

Fig. 2. *The final stages of the gastrostomy using a polypropylene mesh. In three places in the projection of the first implant, the anterior abdominal wall is stitched, as well as the wall of the stomach, with a mesh fixed by a serous-muscular suture with polypropylene thread. The wall of the stomach is firmly pressed to the anterior abdominal wall. On the skin, 3 knots are tied.*

1. The anterior wall of the stomach; 2. Gastric tube; 3. The first polypropylene mesh implant (oval shape); 4. The second polypropylene mesh implant (rectangular shape); 5. A surgical hole of 1.5-2 cm in the greater curvature of the stomach; 6. Aperture in the anterior abdominal wall through which the gastric tube is drawn; 7. Foley catheter; 8. Cannula for inflating a Foley catheter; 9. A mesh fixed by a serous-muscular suture with polypropylene 1/0 thread; 10. 3 knots on the skin

Fig. 1. *The first stages of gastrostomy using a polypropylene mesh. General view of the stomach with a formed gastric stalk with two mesh implants.*

1. The anterior wall of the stomach; 2. Gastric tube; 3. The first polypropylene mesh implant (oval shape); 4. The second polypropylene mesh implant (rectangular shape); 5. A surgical hole of 1.5-2 cm in the greater curvature of the stomach; 6. Aperture in the anterior abdominal wall through which the gastric tube is drawn; 7. Foley catheter; 8. Cannula for inflating a Foley catheter

In three places in the projection of the first implant, the anterior abdominal wall is stitched (Fig.2), as well as the wall of the stomach, with a mesh fixed by a serous-muscular suture with polypropylene 1/0 thread. The wall of the stomach is firmly pressed to the anterior abdominal wall. On the skin, 3 knots are tied. Hemostasis is performed. Laparotomic wound is sutured in layers. Further, during the course of the

The proposed method for performing gastrostomy using a polypropylene mesh is industrially applicable, because for its implementation in modern medical institutions with surgical hospitals, all the necessary materials and tools are available.

Discussion and Conclusions

When installing mesh endoprostheses, which is accompanied by violating the integrity of the anterior abdominal wall, there are a number of issues related to the development of postoperative complications: the formation of seroma or hematoma, vascular erosion, migration of the implant into

the abdominal cavity with the formation of pressure sores and intestinal fistulas,[8] a decrease in the physiological mobility of the abdominal wall, the formation of an adhesive intestinal obstruction in connection with the adhesion of the serous membrane of the small intestine.[9] The specific advantages of the proposed gastrostomy method are: 1) achieving tight attachment of the stomach wall to the anterior abdominal wall, which is absolutely necessary for a certain category of patients with a reduced level of reparative processes; 2) reducing the risk of postoperative ventral hernias; 3) eliminating the need for additional devices to fix the gastrostomy tube; 4) easing the maintenance of the gastrostomy tube, in particular, the procedure for replacing gastrostomy tubes—all of which taken together lead to a decrease in the number of complications, including in the long term.

At the present stage of development of palliative medicine, requirements are imposed to bear in mind the quality of life of the patient in the postoperative period.[9] Moreover, the quality of life is considered as a criterion for the effectiveness of rehabilitation measures.[10] The formation of a controlled, localized adhesive process between the wall of the stomach and the anterior abdominal wall avoids a decrease in the quality of life associated with the sensation of an implant in the area of the operation and intense pain in the early and late postoperative periods; it also facilitates the care of the gastrostomy and increases the mobility of patients in need of a long-lasting gastrostomy.

The risk of gastrostomy insolvency, migration of the gastrostomy tube, is reduced when using the developed method of gastrostomy because the implants perform a strengthening function in relation to the anterior abdominal wall. The need to use additional devices that fix the gastrostomy tube is eliminated due to the tight attachment of the stomach to the anterior abdominal wall. The number of wound complications is also reduced because this gastrostomy is closed and gastric contents do not get on the skin.

Competing interests

The authors declare that they have no competing interests.

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The Results of Surgical Treatment of Placenta Accreta in Women with a Previous Cesarean Delivery

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Abstract

This article presents the results of surgical treatment of placenta accreta in 18 women with a previous cesarean delivery according to the data of the Departments of gynecology at the Yakutsk Republican Clinical Hospital and Republican Hospital #1 (National health center) of the Republic of Sakha (Yakutia) for the period of 2018-2019. The diagnosis of placenta accreta spectrum (PAS) was based on ultrasound findings and MRI data. Seventeen patients underwent metroplasty. In PAS disorders, metroplasty is fertility-preserving surgery with minimal blood loss both by the laparotomic and laparoscopic approaches. (**International Journal of Biomedicine. 2019;9(4):373-375.**)

Key Words: placenta accreta spectrum • cesarean delivery scars • metroplasty • laparotomy • laparoscopy

Introduction

Placenta accreta is now the main cause of postpartum hemorrhage resulting in maternal and neonatal morbidity. Placenta accreta is often used as a general term but is defined by the levels of invasion of chorionic villi into maternal myometrium. In regard to terminology, recent guidelines suggested that placenta accreta spectrum (PAS) be used going forward. PAS includes (1) superficial placenta accreta (also called placenta creta, vera, or adherenta), where the villi attach directly to the surface of the myometrium without invading it; (2) placenta increta, where the villi penetrate deeply into the myometrium up to the external layer; and (3) placenta percreta, where the invasive villous tissue reaches and penetrates through the uterine serosa.[1] Abnormal adherence of the placenta to the myometrium is established in very early pregnancy. The diagnosis of a PAS is made on the basis of histopathologic examination and characterized by an absence of decidua, and chorionic villi are seen directly adjacent to myometrial fibers. Placenta accreta is the most common component of PAS and accounts for 75% of cases. Traditionally, PAS is thought to occur as a consequence of a localized uterine injury (e.g., previous cesarean section [PCS]), which can result in locally defective decidualization/scarring and abnormal placental adherence in a subsequent pregnancy.[2,3] In the presence of a low-lying placenta (placenta previa) and 3 PCSs, a woman would have a 61% risk of PAS.[4]

The development of PAS is a complex multifactorial process. Normal placentae do not proceed beyond the inner third of the myometrium. The underlying molecular mechanisms of invasive placentation are poorly understood; proposed hypotheses include a combination of primary absence of the decidua or basal plate, abnormal maternal vascular remodeling, and excessive extravillous trophoblastic invasion.[5] Extensive neovascularization is clearly evident in the majority of PAS cases. The current prevailing hypothesis for explaining this pathology is that a defect of the endometrium–myometrial interface, typically at the site of a prior hysterotomy, leads to a failure of normal decidualization in the corresponding uterine area. This allows extravillous

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trophoblastic infiltration and villous tissue to develop deeply within the myometrium, including its circulation, and to sometimes reach the surrounding pelvic organs.[6,7]

Prenatal diagnosis of RAS is a key element to improving maternal and perinatal outcome. Standard transabdominal ultrasonography is a reliable tool for diagnosing invasive placentation and is the primary tool for the antenatal diagnosis of the morbidly adherent placenta.[8,9,10] Warshak et al suggest a two stage protocol in evaluating a patient at risk for abnormal placentation using ultrasonography first, then MRI for cases that are inconclusive.[11] Riteau et al showed that ultrasound remains the more sensitive imaging modality for diagnosing accreta and suggest that MRI be complementary to ultrasound, especially in cases where there are few ultrasound signs.[12] A number of potential serum biomarkers have been investigated in PAS. At present, a sensitive serum biomarker for invasive placentation remains elusive.[13,14]
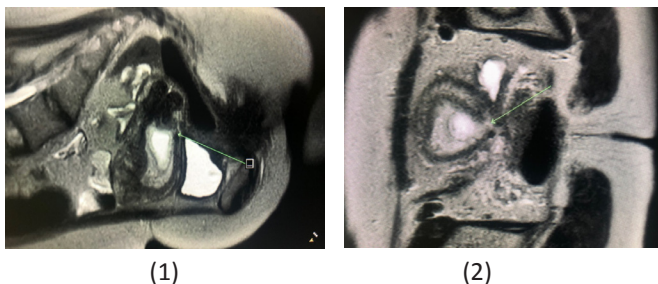
The objective of this study was to identify a risk group of women with a previous cesarean delivery for PAS and find the optimum surgical way to preserve a reproductive function.
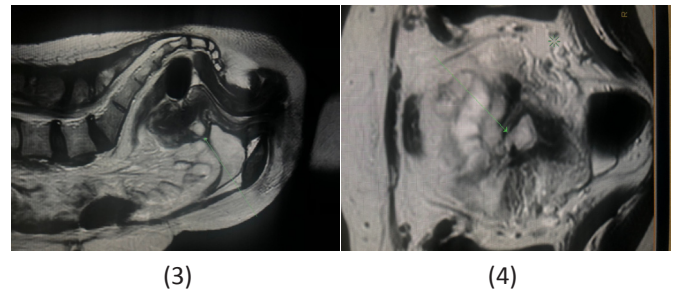
Materials and Methods

Based on the data of the Departments of gynecology at the Yakutsk Republican Clinical Hospital and Republican Hospital #1 (National health center) of the Republic of Sakha (Yakutia) for the period of 2018-2019, a total of 18 PAS cases with the PCS were analyzed and the results of surgical treatment with different approaches were compared.

Group 1 included 10 women aged from 28 to 41 years (mean age of 34.5 years) with placenta accreta into the scar from the PCS who underwent laparotomic surgery. Group 2 included 8 women aged from 20 to 39 years (mean age of 29.5years) with placenta accreta into the scar from the PCS *who* underwent laparoscopic surgery.

The diagnosis of PAS was based on ultrasound findings and MRI data. Pictures 1 and 2 show a thinned (up to 2 mm) postoperative scar with a niche of 12×10 mm in size. In the uterine cavity there is an embryo (48×26×47 mm in size); the chorionic villi are penetrating the postoperative scar. Pictures 3 and 4 show a thinned (up to 1.8 mm) postoperative scar with a niche of 15×12 mm. The MRI revealed an embryo (23×14×12 mm in size) lying close to the scar; the boundaries of the scar are blurred.

(1)   (2)

Pictures 1 and 2 show a thinned (up to 2 mm) postoperative scar with a niche of 12×10 mm in size. In the uterine cavity, there is an embryo (48×26×47mm in size); the chorionic villi are penetrating the postoperative scar.

(3)   (4)

Pictures 3 and 4 show a thinned (up to 1.8 mm) postoperative scar with a niche of 15×12 mm. The MRI revealed an embryo (23×14×12 mm in size) lying close to the scar; the boundaries of the scar are blurred.

In Group 1, at the moment of hospitalization, the gestation age was from 6 to 10 weeks. Two patients had planned pregnancies while the others were preparing for medical abortion (5 women), or were hospitalized with uterine bleeding after taking pills for abortion (3 women). Eight (80%) patients of Group 1 had 2 uterine scars. Eight patients underwent the emergency caesarian section in the first pregnancy: 1 of them had premature separation of the normally localized placenta, and 7 had inadequate uterine contraction. One of the patients underwent caesarian section at the 33rd week of gestation due to the premature separation of normally localized placenta. The secondary operation was performed routinely for other patients, taking into account the uterine scar from the previous pregnancy. Two (20%) patients of Group 1 had one uterine scar: one surgical intervention was caused by severe preeclampsia at the 32nd week of gestation and one surgical intervention was due to poor labor activity at the 39th week of gestation. The interpregnancy interval was from 6 months to 10 years, with an average of 5 years. In their medical history, 7 (70%) patients had from 1 to 3 medical abortions.

Nine (90%) patients of Group 1 underwent metroplasty. In 5 patients, the average blood loss was 100 ml. In 4 patients, blood loss was from 1L to 1.5 L: in three women bleeding was associated with taking abortion pills and in one case as the result of early miscarriage caused by uterine infection. All patients underwent vacuum aspiration of the uterus; as the hemorrhage continued, a laparotomy was performed, which confirmed the diagnosis of the PAS disorders. Three of the patients underwent metroplasty; one patient had subtotal hysterectomy as there was endomyometritis caused by uterine infection.

In Group 2, the medical history was as follows: 1 PCS in 1 patient, 2 PCSs in 6 patients (75%) and 3 PCSs in 1 patient. Five (62.5%) patients had from 1 to 5 previous medical abortions. Two patients applied for medical assistance after an unsuccessful medical abortion induced by taking pills at the period of 5-7 weeks of gestation. An early incomplete abortion was found in 3 patients, and 3 patients had progressive pregnancy after taking abortion pills (6, 7, and 8 weeks of gestation). All patients underwent laparoscopic metroplasty combined with vacuum aspiration. Total hemorrhage was 100 ml in 7 patients and 1000 ml in 1 patient at the 8th week of gestation.

Previous cesarean delivery scars excised during metroplasty represented thin and wide (1-3 cm) plates of fibrous tissue, granulation tissue, smooth muscle tissue, heterogeneous vessels, and trophoblast. The histological examinations confirmed the presence of trophoblast villi in the uterine scar in all cases; additionally, there were 3 cases of invasion into the myometrium. All patients were discharged in satisfactory condition.

In conclusion, early diagnosis of PAS disorders in patients with previous cesarean delivery scars contributes to adequate operative treatment with minimal blood loss. The risk factors for PAS are the presence of 2 or more cesarean sections, and location of the chorion on the front wall of the uterus, in the scar area or in the area of the internal orifice. In PAS disorders, metroplasty is fertility-preserving surgery with minimal blood loss both by the laparotomic and laparoscopic approaches.

Competing interests

The authors declare that they have no competing interests.

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CASE REPORT

Large Fungal Ball of the Paranasal Sinuses and Nasal Cavity: Two Case Reports

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Abstract

Two cases of a large fungal ball of the paranasal sinuses and nasal cavity are presented and its removal via an endoscopic approach is detailed. The clinical symptoms of the fungal body of the paranasal sinuses are not specific. With one-sided localization and large sizes of fungal bodies, they must be differentiated from neoplasms. The most informative non-invasive diagnostic method is computed tomography. We are presenting two cases of giant fungal bodies of the paranasal sinuses and identified specific CT signs. (**International Journal of Biomedicine. 2019;9(4):376-378.**)

Key Words: fungal ball • nose neoplasms • endoscopic sinus surgery

Introduction

Fungal balls of the paranasal sinuses are a non-invasive form of fungal rhinosinusitis, characterized mainly by a unilateral lesion, without involving the underlying mucosa in the inflammatory process.[1] The main clinical manifestations, such as nasal congestion, mucopurulent discharge or bleeding, and headache, are not specific and are found in many diseases of paranasal sinuses.[2]

The most informative non-invasive diagnostic methods are computed tomography (CT) and magnetic resonance imaging (MRI). According to the results of CT of the paranasal sinuses, it is possible to suspect the presence of a fungal ball in cases of total or subtotal darkening of the paranasal sinus with calcinates. In cases of long-term current processes, due to compression of the walls of the paranasal sinus on CT images, erosion of the paranasal sinus wall can be detected in about 3.6%-17% of cases.[3,4] An MRI is indicated for pregnant patients in cases of long-term, unilateral sinusitis.[5] On an MRI of paranasal sinuses, FBs are visualized on T1-weighted images in the form of areas with low signal intensity, and on T2-VI in the form of areas with a hypointense signal or parts of the "empty" signal.

Case Report 1

A 59-year-old white male presented to the otorhinolaryngology department. During the previous four years, manifestations of chronic right-sided rhinosinusitis had arisen. Two months after the patient first presented to the otorhinolaryngology department, the condition worsened, and the patient sought medical help in the outpatient clinic with complaints of painful pressure in the right half of the face, difficulty with nasal breathing on the same side, mucopurulent discharge on the right, and intoxication syndrome. According to the endoscopic examination of the nasal cavity after mucous membrane anemization, we revealed: in the nasal cavity on the right, the polyposis, a mucopurulent discharge, and the structures of the nasal cavity were not differentiated.

A CT scan was obtained showing a soft tissue density in the nasal cavity on both sides, the right maxillary sinus and cells of the ethmoid bone on the right, the bone structures of the right orbit were preserved and the nasal septum was displaced to the left (Fig.1). With the patient under general anesthesia, we

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performed infiltration with articaine+1:200000 adrenaline of the anterior end of the inferior nasal concha and lateralization of the inferior nasal concha, and we visualized the caseous mass (Fig.2) occupying the nasal cavity, right maxillary sinus and the right ethmoid labyrinth. After removing the mass, a single cavity of the right maxillary sinus and the right ethmoid labyrinth was determined. This cavity was washed with 0.9% saline solution. According to histological data, the mass was determined to be fungal flora of *Candida albicans*. On the control CT scan one week after the surgical intervention, we revealed a single cavity of the maxillary sinus and an ethmoid labyrinth on the right (Fig.3).
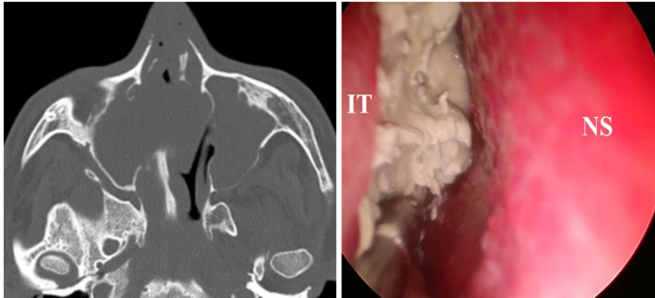

Fig. 1. *A 59-year-old white male. Axial CT scan at the level of the lesion. The isodensive shading on the right side, involving maxillary sinus and the ethmoid cells. The nasal septum is displaced to the left.*

Fig. 2. *Intraoperative photo: caseous masses occupying the right nasal cavity. Fungal flora: Candida albicans. The IT – inferior turbinate, NS – nasal septum.*

Fig, 3. *The control CT scan one week after the surgical intervention. A single cavity of the maxillary sinus and an ethmoid labyrinth on the right.*

Case Report 2

A 40-year-old white male presented to the otorhinolaryngology department of Tyumen Railway hospital. When admitted, he complained about the absence of nasal breathing through the right half of the nose, nasal discharge, a putrid odor from the nose, and a high-grade body temperature for 6 months. According to his medical history, since June 2018 there had been a difficulty with nasal breathing on the right, anosmia, and subfebrile body temperature. He was treated in the outpatient clinic, and underwent several courses of antibacterial therapy; the patient regularly used vasoconstrictive drops without positive dynamics. About 3-4 years ago, he had treatment for the teeth in the upper jaw on the right; there were no complications after the treatment. Patient had viral hepatitis C. A CT scan of the paranasal sinuses was performed and the patient was referred to the otorhinolaryngologic department with a diagnosis of paranasal sinus neoplasm.

According to the endoscopic examination of the nasal cavity after mucous membrane anemization, in the nasal cavity on the right the polyposis and a mucopurulent discharge was presented, and the structures of the nasal cavity were not differentiated. The nasal septum was sharply displaced to the left. The CT scan (Fig.4) showed destruction of the medial wall of the right maxillary sinus and of the structures of the ethmoid labyrinth on the right, and displacement of the median structures sharply to the left. Bone structures of the right orbit were preserved. Under general anesthesia, the right-sided FESS was performed. The fungal ball was fragmented and removed (Fig.5).

Fig. 4. *A 40-year-old white male. Coronal CT scan at the level of the lesion. Destruction of the medial wall of the right maxillary sinus and of the structures of the ethmoid labyrinth on the right, and displacement of the median structures sharply to the left.*
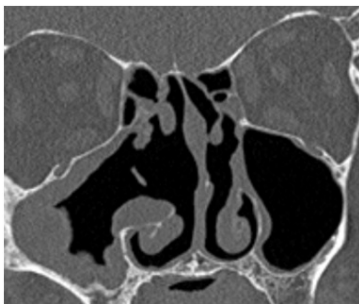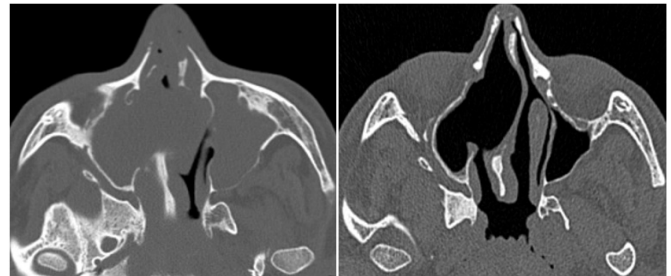
Fig. 5. *The control CT scan one week after the surgical intervention. A single cavity of the maxillary sinus and an ethmoid labyrinth on the right.*

Discussion

In recent years, the incidence of fungal rhinosinusitis has grown. Owing to imaging and diagnostic endoscopy, it is possible to define the classification, diagnosis and management of these diseases more precisely. Fungal rhinosinusitis is generally classified into invasive and non-invasive.

The non-invasive form, in its turn, is subdivided into allergic fungal sinusitis and fungal ball. The first to describe a case of a non-invasive fungal sinusitis was probably Mackenzie in 1893.[6]

The fungal ball (mycetoma) can be described as an accumulation of non-invasive dense fungal concretions at the level of the paranasal cavities. It is more often unilateral although rarely it can affect more sinus cavities. The disease generally occurs in adults, with a mean age of 64 years, predominantly among women. However, we have now presented two cases of a large fungal ball of the paranasal sinuses and nasal cavity in two male patients aged 40 years old and 59 years old.

The fungal ball differs from allergic fungal sinusitis, which generally affects more sinuses. The fungal ball is most often localized at the level of the maxillary sinus. The opacification of the sphenoid sinus is very rare, found in 5% of patients with diseases of the paranasal sinuses, of which only 5% are suffering from fungal ball.[7]

Patients with fungal ball are almost all immuno-competent, without significant alterations in the levels of immunoglobulin or IgG subclasses. Because of its slow evolution, few symptoms, and non-invasiveness, the diagnosis of paranasal sinus fungal ball is often late. Generally, the symptoms include headache, nasal breathing discharge, and facial algia and are often similar to symptoms of bacterial chronic rhinosinusitis.

CT findings associated with the presence of paranasal sinus fungal ball are characterized by the presence of inhomogeneous material at the level of the paranasal sinus involved, which often appears obliterated, with the presence of some material hyperdense spot or a single focus hyperdense. The sensitivity and specificity of CT, in the presence of these findings, was calculated respectively in 62% and 99%. Although the CT has high sensitivity and specificity, it is always necessary to rule out other sinus disorders, benign or malignant.

We have identified distinctive CT signs for the large paranasal sinus fungal ball: the destruction of the medial wall of the paranasal (maxillary) sinus by the fungal mass, intact orbital wall, and displacement of the nasal septum.

The treatment of fungal ball can be performed through the classic surgery of Caldwell-Luc, both using endoscopic techniques as it was in our case. The result is excellent with both techniques and rarely requires a systemic antifungal therapy.

Competing Interests

The authors declare that they have no competing interests.

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CASE REPORT

Aplasia and Hypoplasia of the Maxillary Sinus:
Three Case Reports

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Abstract

Aplasia and hypoplasia of the maxillary sinus (MS) are developmental anomalies, the incidence ranging from 1.5% to 10%. Usually patients with aplasia and hypoplasia of MS are asymptomatic, but in some cases they complain of a runny nose, facial pain and pressure, and headache. These symptoms are very similar to maxillary sinusitis. Thus, it is important to distinguish anomalies from pathology. In the present study, three case reports illustrate the importance of correct diagnostics. (**International Journal of Biomedicine. 2019;9(4):379-381.**)

Key Words: maxillary sinus • developmental anomalies • aplasia • hypoplasia

Introduction

The maxillary sinus (MS) starts to develop in the 10th week of embryonic life. The volume of the sinus is about 3.3 mm x 8.2 mm x 2.8 mm at the time of birth. Then, up to 8 years, the MS increases annually by 2mm in vertical and lateral dimensions and by 3mm in the anteroposterior direction. At the age of 10-12 years, the inferior border of the sinus reaches the level of the nasal cavity floor. The growth of the MS lasts until age 12-20 years.[1] The incidence of MS hypoplasia (MSH) ranges between 1.5% and 10%.[2] Usually, MSH is asymptomatic and is revealed by X-ray studies as an accidental find.[3] But in some cases, ignorance of radiological signs of the MSH or use of conventional radiographs can lead to diagnostic problems, so MSH is identified as an infectious disease or cancer.[4] Also, it is necessary to differentiate MSH from silent sinus syndrome. Thus, CT imaging and endoscopic examination are important diagnostic tools in cases of MSH. Hereinafter, cases of MSH in real clinical practice are illustrated.

Case Report 1

A 26-year-old female was referred to the department of otorhinolaryngology at the medical-sanitary unit 'Neftyanik'

with complaints of difficulty in nasal breathing, mucous discharge from the nasal cavity, and facial pain on the left side. The symptoms have bothered her for 3 years. X-ray of the nasal cavity and paranasal sinuses was repeatedly performed during exacerbations. Based on clinical and radiological findings, chronic maxillary sinusitis on the left side was diagnosed. During the exacerbation in 2018, a puncture of the left MS was performed, but no content was received. Medical therapy proved to be ineffective. Half a year after symptoms reappeared, a CT examination was performed and revealed MSH type 2 according to Bolger's classification, lateral position of the uncinate process, and type 3 right-side nasal septal deviation according to the classification of R. Mladina (Figure 1).

***Fig. 1.** Coronal CT scan. A decreased volume of the left MS, lateral position of the uncinate process – MSH type 2.*

The symptoms were due to the deviated nasal septum. Submucosal resection of nasal septum and correction of intranasal structures were performed. There were no symptoms after surgery, and no additional treatment was required. The patient was informed about the MSH.

Case Report 2

A 71-year-old female was admitted to the otorhinolaryngologist with complaints of facial pain on the left side, with a feeling of pressure in the left eye. Four years ago, the patient visited the otorhinolaryngologist with complaints of difficulty in nasal breathing, nasal discharge from the left half of the nasal cavity, and facial pain on the left side. CT was performed, the granuloma of root 6 of the upper tooth on the left was visualized. The patient was referred to the dentist-surgeon: the tooth was removed; the symptoms have disappeared. One year passed, the symptoms arose again and were not stopped by medical treatment. CT study was performed and evaluation of the coronal view revealed MSH type 2 according to Bolger's classification (Figure 2). The patient was referred to the department of otorhinolaryngology at the medical-sanitary unit 'Neftyanik' for surgery. An infundibulotomy was performed to access the MS; in the cavity of the MS a retention cyst was visualized and removed by using the shaver system. The postoperative course was uneventful, the patient was discharged in satisfactory health, and the symptoms were stopped.

Fig. 2. *Coronal CT scan.*
Complete opacification and a smaller size of the left MS, lateral position of the uncinate process - MSH type 2.

Case Report 3

A 36-year-old man was referred to the department of otorhinolaryngology at the medical-sanitary unit 'Neftyanik' with complaints of nasal discharges from the right half of the nasal cavity, lasting for 4 months. Before that, a puncture of the right MS had been performed twice, without giving relief. The patient was referred to CT examination, which showed a decreased volume of both MSs, opacification of the right MS, lateralized uncinate process, and fontanelle, which corresponds to type 2 hypoplasia according to Bolger's classification (Figure 3).

Fig. 3. *Coronal CT scan.*
A decreased volume of both MSs, the bilateral uncinate process – MSH type 2.

The right uncinate process was resectioned, the ostium of the right MS was enlarged, and sinus contents were removed. The postoperative course was uneventful; the patient was discharged in satisfactory health. During a follow-up examination after 6 months there were no complaints.

Discussion

The clinical cases given above demonstrate the need for high caution regarding the abnormalities of the MS. MSH is a rare condition and it is usually asymptotic. However, in some cases, there exist some nasal symptoms, which are associated with a deviated nasal septum or lateralized uncinate process. In this state of health, the MS drainage is impaired and surgery is required. MSH may lead to diagnostic problems. Usually it is disguised as chronic rhinosinusitis.[5] In these cases, medical therapy has no effect, and an attempt to puncture of the MS can lead to injury of the orbit.[6,7] Thus, CT imaging is the necessary tool to diagnose MSH. CT images characteristic of MSH are the impression of anterior and lateral walls into the sinus cavity and a decrease in horizontal and vertical dimensions, the maximum values of which are less than 50% of the diameter of the orbit.[8] It is very important to distinguish MSH from silent sinus syndrome, which always leads to enophthalmos.[9] The abnormal type of the MS is associated with abnormalities of the uncinate process and infundibulum. According to Bolger's classification, in MSH type 1 a mild hypoplasia is combined with normally developed uncinate process and with well-developed infundibulum. In MSH type 2, significant hypoplasia is associated with hypoplastic/absent uncinate process and poorly defined or absent infundibulum. The MSH type 3 is characterized as profound hypoplasia with absent uncinate process.[10] Thus, it is necessary to identity abnormalities of the MS. When MSH is diagnosed in time, it can save the patient from long-term medical treatment with no effect, performing unnecessary puncture of the MS, injury to the orbit, and just wasting of time. The main treatment of MSH in cases of the given symptoms is an endoscopic surgery.

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Competing Interests

The authors declare that they have no competing interests.

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Lymphocyte Recirculation: A Brief Review

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Abstract

This brief review provides up-to-date information on the study of lymphocyte recirculation, in particular, the main mechanisms and factors of an exogenous and endogenous nature, affecting the activity of lymphocyte recirculation and migration. (**International Journal of Biomedicine. 2019;9(4):382-385.**)

Key Words: lymphocyte recirculation • lymph node • immunocompetent cells

Abbreviations

APCs, antigen-presenting cells; **HEVs**, high endothelial venules; **LN**, lymph node; **LR**, lymphocyte recirculation

Recirculation is an essential process of continuous transport of immunocompetent cells between the lymphoid system and the blood, providing proper immunological control. The ability to recirculate is characteristic only of lymphocytes. Naive cells, using the circulatory and lymphoid systems as transport routes, continuously migrate between lymphoid and non-lymphoid organs and tissues.[1,2] During recirculation, the remaining naive lymphocytes are found in the secondary lymphoid tissues for about 10-20 hours. If there is no interaction with the antigen on the corresponding antigen-presenting cells (APCs), the cells exit through the efferent lymph ducts, eventually drain into the lymphatic system of the thoracic duct and reunite with the blood, and continue to recirculate throughout life. However, if the immune response is initiated in the CD4+ T-cell population by presenting with APC a peptide antigen associated with MHC class II, a change in the characteristic pattern of recycling occurs. Almost immediately after infection with an antigen, lymphocytes remain at this site for a day or more,[3] which is called a shutdown of the CD4 T-cell response. Recycling of lymphocytes into the secondary lymphoid organs is an important process in immunological surveillance and significantly increases the likelihood that antigen-specific T and B cells will encounter related antigens. The exception is the brain: due to the presence of the blood-brain barrier, it is considered that the brain is subject to limited immunological surveillance.[4,5] However, for example, under the influence of a strong stress factor, endothelial cells of the brain vessels are transformed, resulting in increased expression of cell adhesion molecules that facilitate the adhesion and extravasation of immunocompetent cells. The cells penetrating the brain change their phenotype and begin to produce predominantly pro-inflammatory cytokines, which leads to the initiation of an inflammatory response in the structures of the brain—neuroinflammation, accompanied by an increase in the manifestations of the anxiety-depressive state.[6-11]

Lymphocyte recirculation (LR) depends on the anatomical origin of the cells, the presence of inflammation and pro-inflammatory cytokines, adhesion molecules and the presence of receptors on the cells to these adhesion molecules. In LR, both continuously expressed and, in some cases, tissue-specific adhesion receptors are used. This allows resting lymphocytes to continuously move through tissue components, optimizing the effect on isolated antigens. Naive T lymphocytes recycle most efficiently through the peripheral lymph nodes (LNs), where there is an effective processing, concentration and presentation of foreign antigens. Having various surface receptors, various subpopulations of lymphocytes differ

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significantly in their adhesive characteristics, so T cells with a phenotype of memory cells penetrate into inflammatory foci more easily than naive cells. Thus, the most efficiently responding cell population is directed to areas of potentially active disease. Specific LR occurs back into the tissue in which they were activated. Thus, memory lymphocytes activated in the mesenteric LNs are located mainly in the intestine, while lymphocytes activated in the axillary LNs return to the skin, and lymphocytes stimulated in Peyer's patches pass through the regional LNs into the bloodstream, and then return "home"—in *lamina propria* of the mucous membrane. This specific recirculation is ensured by the presence of specific homing molecules on the surface of lymphocytes that bind to adhesion molecules (addressins) on the surface of the endothelial cells of venules of lymphoid tissue of the mucous membranes. The endothelium serves as a critical separation between the tissues and adjacent circulatory lymphatic sections. Activated immunoblasts (memory cells or effectors) recycle to areas of the mucous membrane, while small lymphocytes (naive cells) return to the lymphoid organs.[12] Moreover, the LR activity, for example, in the thymus, depends on age: it is higher in children than in adults and increases again in the elderly.[13] In children, 5%-15% of CD4+ thymocytes are of peripheral origin. It is believed that the recycling of mature CD4+ cells from the periphery to the thymus increases the differentiation of thymic epithelial cells (TEC) precursors to mTEC,[14] which leads to depletion of TEC precursors and promotes the involution of the thymus. Regulatory T(Treg) cells expressing the transcription factor Foxp3 are also actively recycled to the thymus. Thus, activated lymphocytes migrating into the thymus and APCs from the periphery can influence the development and differentiation of T lymphocytes and participate in the process of thymus involution.[15] However, the molecular mechanisms of the process of penetration of cells into the thymus, as well as their exact functions, have not yet been fully determined. Penetration of lymphocytes into LNs or Peyer's patches is achieved by transmigration through HEVs. HEVs carry many unique adhesion molecules that capture lymphocytes. They also have special intercellular connections that facilitate the penetration of the walls of blood vessels by these emigrating lymphocytes. The endothelium plays an essential, locally tuned role in regulating T cell migration and information exchange. Intimate cell-cell interaction between lymphocytes and endothelial cells provides instruction to T cells that influences their states of activation and differentiation. In addition, the endothelium can act as a non-hematopoietic, "semiprofessional," antigen-presenting cell. Close contacts between circulating T cells and antigen-presenting endothelium may play unique non-redundant roles in shaping adaptive immune responses within the periphery.[16] The main factors regulating the dynamic transfer of lymphocytes are members of the chemokine family, as well as lipid mediator sphingosine-1-phosphate (SlP). They perform this control by activating specific receptors, which contribute to the adhesion of lymphocytes inside specific microvessels of the secondary lymphatic organs, and subsequent migration within the lymphoid tissue, and exit into the blood.[17] Lymphocyte egress from LNs is strongly dependent on sphingosine-1-phosphate receptor type

1 (S1PR1), by which lymphocytes sense high concentration of S1P in lymph (~100 nmol/L) compared with LN parenchyma (~1 nmol/L) to exit LN. S1PR1 acts to overcome retention signals mediated by CCR7, CXCR4 and possibly additional chemoattractant receptors.[18,19]

The migration of lymphocytes from HEVs into lymphoid organs is ensured by the sequence of interactions between the cell adhesion molecules on the lymphocytes and the vascular endothelial cell molecules that line the vessels. At the same time, lymphocyte homing receptors and vascular adresants regulate the first stages of this process—lymphocyte binding. Subsequent movement of lymphocytes along the surface of endothelial cells and migration through the vessel wall (diapedesis) are regulated regardless of the initial binding. It is assumed that these last stages are mediated by the functional activation of integrins on the lymphocyte by chemoattractants located in the vessel wall.[20]

Brezinschek et al.[21] showed that CD4+ T cells acquire the capacity for transendothelial migration at a specific phase of maturation that is only minimally altered by the activation of either the T cell or the endothelial cells, or by the presence of specific chemokines in the subendothelial matrix.

Immunocompetent cells differ significantly in their adhesive properties, so analysis of T helper-cell subsets revealed that memory T cells bound severalfold stronger to ICAM-1 expressing transfectants compared to the CD4+ 45RA+ naive T cells, whereas adhesion to B7, LFA-3- and B7/LFA-3-expressing CHO cells was similar in both T-cell subsets.[22] Authors suggest that resting naive CD4+ T cells utilize preferentially the CD2/LFA-3 or CD28/B7 adhesion pathways upon adhesion to APCs, while memory CD4+ T cells utilize the CD2/LFA-3, CD28/B7 and LFA-1/ICAM-1 adhesion pathways.[22]

Stress hormones, cytokines, prostaglandins, and heavy metals capable of changing the adhesion properties of cells influence lymphocyte recycling. C. Spry showed that the administration of corticotropin reduces the egress of lymphocytes from the thoracic duct. In particular, their recirculation to the lymph decreased, and a smaller amount was restored.[28] Adrenergic regulation of the level of lymphocytes causes a decrease in the number of lymphocytes in the blood (which is associated with inhibition of the egress from LNs of antigen-primed memory T cells, but not effector cells), which is associated with the predominant expression of β2AR memory by T cells.[29-31] High adrenergic nerve activity can promote the priming of lymphocytes in the LN, but inhibit the inflammation caused by lymphocytes in the peripheral tissues.[32-34] A decrease in the activity of adrenergic nerves allows lymphocytes to leave LNs and gain access to peripheral tissues, which will facilitate the recognition and elimination of pathogens at the sites of infection. Consequently, adrenergic nerves can coordinate adaptive immune responses in LNs and peripheral tissues in order to maximize the host's defense effectiveness by generating a daily LR rhythm.[35]

Zalavina et al.[36] studied the structural features of the zonal and cellular organization of thymus in 20 pregnant Wistar rats with the introduction of cadmium. The revealed morphological restructuring of the thymus indicated the

accidental involution of the thymus, increased processes of death of thymocytes, and violation of the permeability of the components of the hematological barrier. The lymphocyte migration coefficient through individual postcapillary venules was reduced; however, the number of post-capillary venules involved in the process of effective migration increased, and this ensures the maintenance of recirculation processes of lymphoid cells at a sufficient level.

Lymphocyte egress from LNs is an important therapeutic target in T-cell-mediated autoimmune disease.[37] The presence of a viral antigen in LNs and spleen leads to a change in the rate of LR due to an increase in the average rate of T-cell binding (adhesion) to dendritic cells, and to a decrease in the rate of dissociation. This mechanism of the extreme retention of lymphocytes in lymphoid tissue through changes in the adhesive properties of APCs is probably also responsible for the lymphocyte shutdown phenomenon, in which the egress of lymphocytes from the antigen-stimulated LN can be temporarily stopped.[38] In addition, in some chronic inflammatory conditions, the formation of HEV-like vessels is induced, for example, with chronic Helicobacter pylorigastritis, inflammatory bowel disease and autoimmune pancreatitis, as well as with lymphoid stroma formed in some types of tumors. In these cases, activating factors include bacterial products (e.g., lipopolysaccharides) and pro-inflammatory cytokines (IL-1, TNFα, IFNγ). This allows for LR (homing), similar through HEVs to the secondary lymphoid organs. In the diffuse sclerosing variant of papillary thyroid cancer, the dominant population of cells attached to the surface of HEV-like vessels was CD8+ lymphocytes. With Hashimoto thyroiditis, there were no significant differences in the number of T and B cells. Such differences in the binding of lymphocytes to HEV-like vessels in various pathological conditions can be associated with various chemokines acting on these vessels.[39] It has been shown that FTY720 inhibits the egress of autoreactive T cells from the LN and, therefore, their migration to the target organ.[40] Animal studies have shown that treatment with β2AR agonists suppresses experimental autoimmune encephalomyelitis.[41-43] In sepsis, the number of lymphocytes in the peripheral blood decreases, mainly due to increased recycling of CD4+ cells.[44]

Thus, the study of LR is important for understanding the course of the normal immune response, as well as the formation of pathology. However, there are many unanswered questions regarding the recirculation of various subpopulations of lymphocytes and the role of humoral factors, such as cytokines, chemokines and hormones, in regulating the recycling of subpopulations of cells, and little is known about the signals that control cell retention in tissue, which also include adhesive interactions. The molecular mechanisms of the process of penetration of activated cells into the thymus, as well as their exact functions, are also not completely determined. Very little is known about the potential function of recirculating T cells in dampening acute immune responses, possibly via tissue exit of Tregs or of T cells with irrelevant antigenic specificities.[45] Clarification of the molecular basis of the specific interactions of lymphocytes with endothelium will improve our understanding of the regulatory roles of

adhesion molecules in other biological processes, such as the formation of immunodeficiency and metastasis. More insight into both the molecular mechanisms and the relevance of these processes will contribute to identifying new targets for immunomodulatory therapies.

Competing Interests

The author has no competing interests to declare.

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