ISSN 1813-1905 (print)
ISSN 2312-1017 (online)

1(81) `2023
YAKUT MEDICAL JOURNAL

YAKUT MEDICAL JOURNAL

SCIENTIFIC - PRACTICAL JOURNAL
OF THE YAKUT SCIENCE CENTRE OF COMPLEX
MEDICAL PROBLEMS

Published since 2003

Quarterly

Registered by the Office of the Federal Service on supervision in the field of communications, information technologies and mass communications in the Republic Sakha (Yakutia) December 13/2016

Registration number PI No.TU 14-00475

Subscription index: 78781
Free price

«Yakut Medical Journal» is included in:

the approved by the Higher Attestation Commission of the Russian Federation List of leading peer-reviewed scientific journals and publications, in which the main scientific results of dissertations for the acquisition of scientific degrees of Doctor and Candidate of science on biological sciences and medicine should be published

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RELATIVE LEUKOCYTE TELOMERE LENGTH IN PATIENTS WITH CHRONIC ALCOHOL ADDICTION DEPENDING ON CLINICAL AND ANAMNESTIC CHARACTERISTICS

DOI 10.25789/YMJ.2023.81.01

УДК 57.049:575.21:616.89:576.313.3

The studies conducted on human cell lines demonstrated that even moderate alcohol consumption caused the shortening of telomeres – structures that play a key role in of cellular aging. Nevertheless, published data on the effect of chronic alcohol intake on the changes in the relative leukocyte telomere length (LTL) in humans remain ambiguous. Such ambiguity may be attributed to the differences in the clinical symptoms of individuals with alcohol dependence. In this regard, the present study aimed to test for the hypothesis, which suggests the association of shorter telomeres with manifesting chronic alcoholism and to identify clinical and anamnestic characteristics associated with individual variance in relative telomere length in subjects with alcohol dependence. LTL assessment has been carried out via real-time PCR in patients diagnosed with alcohol dependence syndrome (ICD-10) (N = 272) and in control group (N = 254). Linear regression analysis demonstrated statistically significant effect of age in the total sample (βstand = -0.153, P = 0.009) and in men (βstand = -0.143, P = 0.026) on variance in LTL. Moreover, age-dependent telomeres shortening was characteristic only for patients’ group (βstand = -0.217, P = 0.017). The inclusion of clinical and anamnestic characteristics in the model resulted in a significant negative effect of age at onset of withdrawal syndrome on LTL (βstand = -0.343, P=0.001). The findings obtained are congruent with the data on the toxic effect of acetaldehyde and increased allostatic load accompanied by prolonged alcohol consumption, and confirm the presence of a compensatory effect in the cells, which is associated with regulated expression of genes responsible for maintaining telomere length.

Keywords: alcohol addiction, telomeres, cell aging, allostatic load, biomarkers, clinical and anamnestic characteristics

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Introduction. Telomeres are heterochromatin structures located at the ends of the chromosomes and consisting of 5’-TTAGGG-3’tandem repeats. These structures protect chromosomal ends from fusion and degradation, therefore, maintaining their integrity and stability, and play a key role in cellular aging [6]. Recent studies have demonstrated the influence of several environmental factors, including stress of various nature, on the modified telomeres length in humans in dynamics. However, the causal relationships between the effect of adverse environment and changes in telomere length are still incompletely understood. Together with individual variations in the number of telomeric repeats caused by age [11], sex and ethnic differences [18], unfavorable environment [10], chronic alcohol intake results in severe stress in biological systems, causing excessive allostatic load [19].

During the last decades a candidate gene approach [1, 2, 5, 9], genome-wide association studies (GWAS) [8], assessment of epigenetic changes [3, 13] have been used for the study of alcohol dependence and antisocial behavior in order to identify genetic predictors. Together with mentioned approaches, in recent years the assessment of the relative telomere length in peripheral tissues of individuals with various mental disorders became of high relevance [15, 17, 19]. The studies conducted on human cell lines demonstrated that even moderate alcohol treatment for one week resulted in telomeres shortening in different cell types [6]. A similar effect was observed in the case of cells’ exposure to acetaldehyde (an intermediate metabolite of ethanol) at the same concentration, which confirmed a toxic effect of this metabolite on cells, thus causing a premature “cell aging”. Other authors also indicated the changes in the expression of genes responsible for maintained telomere length (including shelterin complex genes), depending on the duration of ethanol exposure to human embryonic stem cells and its concentration [12]. Nevertheless, published data on the effect of chronic alcohol intake on changes in the relative leukocyte telomere length (LTL) remain ambiguous. In particular, both telomeres shortening in addicted individuals [14, 17] and the absence of such association have been reported [15, 20]. Such ambiguity may be attributed to the differences in the severity of clinical symptoms (in particular, the presence of acute alcoholic psychosis, delirium, etc.) in individuals with alcohol dependence. Despite the LTL studies, which assessed the effect of both alcohol addiction and comorbid addiction with other psychoactive substances [19], no studies evaluating the role of clinical and

anamnestic parameters on LTL in subjects with alcohol dependence have been carried out to date.

Considering the abovementioned data, the present study aimed to test for the hypothesis, which suggests the association of shorter telomeres with manifesting chronic alcoholism, form the one hand. On the other hand, it was suggested to identify clinical and anamnestic characteristics associated with individual variance in relative telomere length in subjects with alcohol dependence.

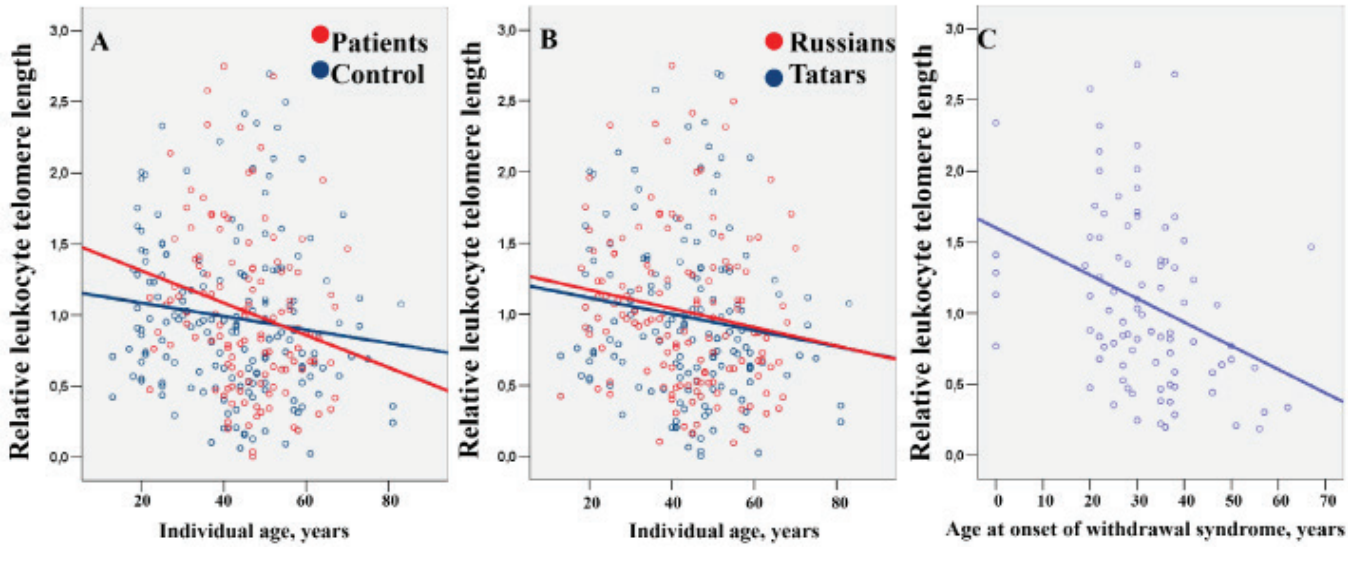
Materials and methods. The study sample included patients diagnosed with “alcohol dependence syndrome” (ICD-10) (N = 272, 12% women) of different ethnicity (134 Russians, 112 Tatars, 26 subjects of mixed ethnicity). Mean age of patients was 45.54 ± 11.08 years. Control group (N = 254, 12% women) consisted of mentally healthy individuals without individual or family history of psychiatric disorders. Control group corresponded to the sample of patients by age (mean age 42.08 ± 15.68 years), sex and ethnic content (150 Russians, 107 Tatars and 15 individuals of mixed ethnicity). All the enrolled subjects signed an informed consent after they were acquainted with all the procedures. This study was approved by the local Bioethical Committee at the Institute of Biochemistry and Genetics (UFRC RAS).

A collection of biological material (peripheral blood) was conducted in 2009-2010 followed by DNA extraction via phenol-chloroform technique. LTL quantitative assessment was carried out via real-time PCR on “CFX96” Analyzer (BioRad, USA) using fluorescent intercalating dye IQ SYBR Green Supermix (BioRad, USA). PCR mix contained a pair of primers designed to telomeric region (T) and to single-copy beta-globin gene (*HGB*) as a conservative one (S) [7]. For each sample, which has been analyzed in triplicate, a mean value of cycle threshold (Ct) has been calculated for the conservative gene and for the telomeric region. The samples, which demonstrated differences in Ct values between the triplicates for more than 30%, have been excluded from the analysis. Pool DNA was used as a control sample in each PCR run (reproducibility was above 98%).

LTL for each individual was calculated based on the method based on the formula $2^{-\Delta\Delta Ct}$ described previously [10]. For this purpose a difference in the cycle thresholds for the telomeric and control PCR and a relative telomere length in a genome (T/S) was assessed according to the formula $T/S=2^{-\Delta\Delta Ct}$, where $\Delta\Delta Ct = (Ct_T(sample) – Ct_T(poolDNA)) - (Ct_S(sample) – Ct_S(poolDNA))$. A relative leukocyte telomere length in a genome (T/S) is proportional to $2^{-\Delta\Delta Ct}$ and a telomere length in the analyzed sample.

Statistical analysis included a series of multiple regression analyses, which included LTL as dependent variable, while status (being patient or mentally healthy subject), individual age, sex and ethnicity, age at onset of withdrawal syndrome and age at onset of first alcohol probe, family history of psychopathologies, number of hospitalizations in anamnesis and the number of premorbide traumatic brain injuries were included as independent predictors. In the case of statistically significant effect of a predictor we reported both regression coefficient (β) and standardized regression coefficient (β_{stand}). A correlation analysis was conducted via Spearman's rank correlation coefficient. Statistical analysis and data visualization was carried out with R v.4.1.2.

Results and discussion. Within the framework of the present study in order to test for the hypothesis on the association of LTL with alcohol dependence we conducted a series of linear regression analyses with sex and age inclusion as covariates. Statistically significant effect of age was determined in the total sample (β = -0.006, β_{stand} = -0.153, P = 0.009) and in men (β = -0.005, β_{stand} = -0.143, P = 0.026) on LTL variance (Table 1). Due to a small sample size of women, statistical analysis in women separately has not been conducted. A correlation analysis also demonstrated a negative correlation between LTL and age of individual (r= -0.178, P < 0.01). As a result of similar analyses performed in patients and control groups separately we revealed a statistically significant effect of age on telomeres shortening only in the group of addicted individuals (β = -0.011, β_{stand} = -0.217, P = 0.017), whereas a trend for a negative link between these two parameters was obtained for the control group (β = -0.005, β_{stand} = -0.133, P = 0.086). While dividing sample based on ethnic origin, we observed a trend for age-related telomeres shortening both in Russians (β = -0.007, P = 0.056) and in Tatars (β = -0.006, P = 0.084), which indicates the absence of significant effect of ethnicity on LTL decrease. Therefore, more rapid and statistically significant age-related decline in LTL is characteristic for patients with alcohol dependence compared to mentally healthy subjects regardless on ethnic origin (Fig., *a*). The



Dependence of relative leukocyte telomere length on age in a group of patients with alcohol dependence and control group (a), in individuals of different ethnic origin (b), on age at onset of withdrawal syndrome in patients with alcohol addiction (c).

data obtained are congruent with previously published findings on LTL drop in both mentally impaired individuals [11] and control ones [10], thus indicating an increased allostatic load.

At the same time, suggested hypothesis on a relation of shortened telomeres with a negative systematic organism exposure to ethanol has not been confirmed, since no statistically significant changes in LTL were obtained between the patients with alcohol addiction and the control group in the total sample (β = 0.024, P = 0.703) and in men (β = 0.010, P = 0.888) (Table 1). Since a relative decrease in telomere length was reported in Europeans compared to individuals of other ethnic groups [18], we conducted the analysis in Russians and Tatars separately. Nevertheless, a stratified analysis also failed to demonstrate a link between LTL and the presence or absence of alcohol addiction both in Russians (β = 0.045, P = 0.634) and Tatars (β = 0.032, P = 0.739) (Fig., *b*).

To date, no systematic meta-analysis has been published that makes it possible to make unambiguous conclusion on the link between telomere length and the presence of alcohol dependence. Nevertheless, there is evidence of the association of excessive alcohol consumption in mid-life with telomere shortening in elderly [17]. However, in the present study we failed to demonstrate differences in LTL between individuals with alcohol dependence and healthy controls. One of the possible causes may be attributed to individual differences in the activity of acetaldehyde dehydrogenase (ALDH2) – enzyme, which is responsible for acetaldehyde catalysis, and, consequently, for the accumulation of toxic products of ethanol degradation and their effects on cells. In particular, one of the studies demonstrated a negative association between LTL and enhanced alcohol consumption, which was prominent only in the case of a low-active form of ALDH2 related to the presence of a mutant allele (rs2074356 C/T or T/T genotypes) in the encoded gene [16]. Moreover, the highest effect of such association was more evident with age. At the same time, the average level of alcohol consumption, on the contrary, was associated with increased LTL; however, such association was characteristic only for carriers of high-active rs2074356 C/C genotype in the *ALDH2* gene [16]. In addition, telomere shortening was observed in patients with alcohol dependence only in the case of genetically determined high-active form of alcohol dehydrogenase (ADH), an enzyme involved in the ethanol conversion to acetaldehyde [14]. Thus, published data indicate a negative relationship between telomere length and the exposure to high doses of alcohol only in the case of toxic effects of ethanol degradation products (i.e., a low-active form of ALDH2 and a high-active form of ADH). Similarly to our findings on the association of LTL with alcohol consumption, several foreign researchers also demonstrated the absence of such link [20]. It should be noted that recent large-scale study, which evaluated the effect of the summarized

Table 1

Multiple regression analysis demonstrating the effect of several predictors and clinical and anamnestic characteristics on individual variance in LTL in the total sample, in men and patients with alcohol dependence

Group	Predictor	Reference group	Model 1		Model 2		Model 3		Model 4	
			β_{stand}	p-value	β_{stand}	p-value	β_{stand}	p-value	β_{stand}	p-value
Total sample	Intercept	-	1.266	<0.001	0.976	<0.001	1.202	<0.001	1.408	<0.001
	Status	control	-	-	0.022	0.703	-	-	0.054	0.353
	Sex	men	-	-	-	-	-0.120	**0.033**	-0.103	0.080
	Age	-	-0.153	**0.009**	-	-	-	-	-0.144	**0.016**
	P-value of model		**0.009**		0.703		**0.033**		**0.013**	
	Corrected r^2		0.023		<0.001		0.014		0.037	
Men	Intercept	-	1.263	<0.001	1.010	<0.001	1.202	<0.001		
	Status	control	-	-	0.009	0.888	0.042	0.512		
	Age	-	-0.143	**0.026**	-	-	-0.149	**0.022**		
	P-value of model		**0.026**		0.888		0.068			
	Corrected r^2		0.020		<0.001		0.022			
Patients with alcohol addiction	Intercept	-	1.601	<0.001	1.082	<0.001	1.713	<0.001	1.603	<0.001
	Sex	men	-	-	-	-	-	-	-0.052	0.659
	Age	-	-	-	-	-	-	-	0.060	0.747
	Ethnicity	Tatars	-	-	-	-	-	-	0.022	0.849
	Family history	no	-	-	-	-	-	-	0.051	0.659
	Age alcohol start	-	-	-	-	-	-	-	0.065	0.729
	Age withdrawal	-	-0.343	**0.001**	-	-	-0.361	**0.001**	-0.408	**0.039**
	Hospitalization	0	-	-	-0.151	0.129	-0.211	**0.047**	-0.275	**0.046**
	TBI	0	-	-	-	-	-	-	-0.008	0.947
	P-value of model		**0.001**		0.129		**0.001**		0.172	
	Corrected r^2		0.117		0.023		0.150		0.156	

Note. Corrected r^2 – corrected coefficient of determination; βstand – standardized regression coefficient; family history – family history of psychopathologies; age alcohol start – age at the first alcohol probe; age withdrawal – age at onset of withdrawal syndrome; hospitalization – number of hospitalizations in anamnesis; TBI – number of premorbide traumatic brain injuries. Statistically significant differences are marked in bold.

parameter of a healthy lifestyle (including moderate alcohol consumption) in more than 420 thousand individuals from the UK Biobank demonstrated only its insignificant effect (less than 0.2%) on individual variance in LTL [4]. Moreover, the authors failed to determine association of LTL with any of examined diseases. At the cellular level, the absence of significant long-term negative effects of ethanol on human cells has been also shown, which is explained by regulated expression of the genes associated with maintaining telomeres length in cells. In particular, in the case of short-term (3 days) exposure to ethanol of human embryonic stem cells, a decrease in the expression of six genes encoding shelterin complex subunits was observed, while longer exposure (7-14 days) was related to restored expression of these genes and the absence of ethanol-dependent telomere shortening [12].

In order to test the second hypothesis we performed the assessment of relation of clinical and anamnestic characteristics (age at onset of withdrawal syndrome and first probe of alcohol, family history of psychopathologies, number of hospitalizations in anamnesis and premorbide traumatic brain injuries) to individual differences in LTL in addicted patients. The data on the examined clinical and anamnestic characteristics in individuals with alcohol dependence is shown in Table 2. While including all characteristics in the regression model, a statistically significant effect o of age at onset of withdrawal syndrome (β = -0.017, β_{stand} = -0.343, P=0.001) n LTL was observed (Table 1, Fig., *c*). At the same time, sex (β = -0.083, P = 0.659), ethnicity (β = 0.027, P = 0.849), positive family history of psychopathologies (β = 0.065, P = 0.659), age at first probe of alcohol (β = 0.004, P = 0.729), presence of premorbide traumatic brain injuries (β = -0.002, P=0.947), number of hospitalizations in anamnesis (β = -0.027, P=0.129) demonstrated insignificant effect of LTL among patients (Table 1). A correlation analysis revealed a positive relation between patient age and age at onset of withdrawal syndrome (r = 0.603, P < 0.001) and the number of hospitalizations (r = 0.274, P = 0.006), and between age at first probe of alcohol and age at onset of withdrawal syndrome (r = 0.749, P < 0.001). At the same time, no correlation was observed between age at onset of withdrawal syndrome and the number of hospitalizations (r = -0.160, P = 0.148).

Our results on rapid decrease in LTL in patients with alcohol addiction may be related to with the toxic effect of acetaldehyde [16], which causes accelerated cellular aging of the body. In turn, revealed differentiation in telomere length depending on the age at onset of acute alcoholic psychosis (within the withdrawal syndrome) is probably caused by a correlation between the age of manifestation of withdrawal syndrome and individual age. This dependence is logically consistent with the duration of a negative effect of ethanol exposure on the organism. Despite the suggested hypothesis on the relationship of clinical and anamnestic characteristics with variations in telomere length between individuals with alcohol dependence, we failed to identify statistically significant relations with respect to the number of premorbide traumatic brain injuries and the number of hospitalizations, family burden with psychopathologies, and earlier age of the first probe of alcohol. To date, there are no published data on the association of telomere length with the severity of clinical symptoms in alcohol dependence. However, one of the studies reporting a trend for diminished telomere length individuals with chronic alcohol addiction with comorbid cocaine abuse has to be mentioned [19]. Moreover, as part of the assessment of the enhanced allostatic load, the authors demonstrated that chronic alcohol intake, together with the duration of cocaine addiction, older age and reduced LTL predicted decreased cognitive functioning. Another study, similar to our negative results, reported no association of LTL with even prolonged exposure to severe psychoactive substances (methamphetamine), following psychosis and withdrawal syndrome [15]. Accordingly, the data obtained by our group and published findings do not confirm that individual changes in LTL are attributed to comorbid use of other psychoactive substances, as well as to clinical and anamnestic characteristics.

Table 2

Mean LTL level depending on examined clinical and anamnestic characteristics in patients with alcohol addiction

Parameter	Mean±SD*	Parameter	Mean±SD*
Sex		Number of traumatic brain injuries	
Men (N = 239)	1.02±0.60	0	1.01±0.59
Women (N = 33)	0.89±0.48	1	0.99±0.57
		≥2	1.03±0.69
Ethnicity			
Russians (N = 150)	1.04±0.53	Age	45.54±11.08
Tatars (N = 107)	0.99±0.62		
Family history of psychopathology		Age of first alcohol probe	30.09±11.45
yes (N = 106)	1.16±0.63		
no (N = 166)	0.96±0.53		
Number of hospitalizations		Age at onset of withdrawal syndrome	31.40±12.68
1	1.28±0.48		
2	0.83±0.51		
≥3	0.91±0.66		

*Mean ± standard deviation. For age-related quantitative variables (individual age, age of first alcohol probe, age at onset of withdrawal syndrome) the values are reported for the variable instead of LTL mean

Discussion. Within the framework of the present study an attempt to associate individual variance in the relative leukocyte telomere length to clinical and anamnestic characteristics of patients diagnosed with alcohol dependence syndrome has been carried out for the first time. As a result of the study of various characteristics we detected statistically significant negative effect of age of onset of withdrawal syndrome on LTL, which is attributed to a positive correlation of this parameter with individual age. Moreover, congruent with published data on age-dependent telomere shortening, we demonstrated a rapid and statistically significant decline in LTL with age in subjects with alcohol addiction regardless ethnic origin. Determined relation is consistent with the suggestions on a toxic effect of acetaldehyde and enhanced allostatic load related to the duration of alcohol consumption. It should be mentioned that the findings obtained failed to demonstrate telomeres shortening in addicted individuals compared to control group, which has been previously reported in other ethnic groups, and is congruent to the presence of a compensatory effect in the cells related to regulated expression of the genes involved in maintaining telo-

meres length. Despite the association of the age at onset of withdrawal syndrome with telomere shortening in patients, we failed to confirm suggested hypothesis on a relation of other clinical and anamnestic characteristics with LTL variance. Our findings evidence in insignificant effect of such clinical and anamnestic parameters as enhanced number of premorbide traumatic brain injuries, family history of psychopathologies, and reduced age at first probe of alcohol on accelerated cellular aging of the organism.

The study was supported by the State Contract of the Ministry of Science and Education of RF (№AAAA-A16-116020350032-1) with partial support of the Ministry of Science and Higher Education of Russian Federation (№075-15-2021-595) (in the part of statistical analysis) and the Ministry of Science and Higher Education of the Republic of Bashkortostan (agreement no. 1, 2 December 2022) (in the part of molecular-genetic analysis). DNA samples for the study were provided by the IBG UFRC RAS collection “Collection of human biological materials” developed within the project № 007-030164/2.

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ASSOCIATION ANALYSIS OF POLYMORPHIC VARIANTS IN *ALDH7A1*, *AOC1*, *PSAP*, *ADCYAP1* GENES INVOLVED IN THE HISTAMINE METABOLISM WITH ASTHMA DEVELOPMENT IN INDIVIDUALS FROM THE REPUBLIC OF BASHKORTOSTAN

DOI 10.25789/YMJ.2023.81.02

УДК 616.248 (Астма)

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Many environmental and genetic factors are involved in asthma development, among which an important role in the disease formation and response to antihistamine therapy belongs to genes involved in the histamine metabolism (*HRH1*, *HRH2*, *HRH3*, *HRH4*, *HDC*, *HNMT, AOC1*, *MAOB*, *ALDH7A1*, etc.). Histamine is a central mediator of allergic inflammation the release of which leads to contraction of the bronchial smooth muscles, bronchial secretion and edema of the respiratory mucosa. The use of antihistamines performing competitive blockade of histamine receptors leads to inhibition of histamine effects. The aim of this study was to assess the role of polymorphic variants of aldehyde dehydrogenase 7 family member A1 *ALDH7A1* (rs13182402), amine oxidase copper containing 1 *AOC1* (rs1049793), prosaposine *PSAP* (rs11000016), adenylate cyclase activating polypeptide 1 *ADCYAP1* (rs2231187) genes involved in the histamine metabolism in asthma development in individuals from the Republic of Bashkortostan. DNA samples of 846 unrelated individuals of different ethnicity living in the Republic of Bashkortostan were used as the study material. Genotyping of polymorphic variants was performed by real-time PCR and RFLP analysis. The statistically significant association of the rs2231187**AA* genotype and the rs2231187**A* allele of the *ADCYAP1* gene with asthma, the rs2231187**A* allele of the *ADCYAP1* gene with disease manifestation in childhood, the rs2231187**AA* genotype of the *ADCYAP1* gene and the rs13182402**G* allele of the *ALDH7A1* gene with severe and moderate asthma was established in Bashkirs. The rs1049793**CC* genotype and the rs1049793**C* allele of the *AOC1* gene were associated with asthma in Russians. The results of this study are complementary to the previously published data regarding genetic aspects of asthma pathogenesis that suggest the involvement of *ALDH7A1*, *ADCYAP1* and *AOC1* gene polymorphisms in asthma development.

Keywords: bronchial asthma, histamine, pharmacogenetics, association.

Introduction. Asthma is one of the most common chronic diseases in children and adults. The prevalence of asthma in different countries varies from 1 to 18% [7; 9]. The results of a number extensive studies of asthma inheritance based on modern approaches are published nowadays. A number of asthma molecular genetic studies using the candidate gene approach as well as whole-genome analysis of associations are also performed in the Republic of Bashkortostan (RB) [2; 5]. Insufficient control of asthma symptoms observed with even regular taking the recommended antiinflammatory drugs and bronchodilators is a serious problem of modern medicine. The lack of effective asthma control is noted in 20-30% of patients and leads to an increased risk of airway remodeling both disease progression [3]. The modern definition of asthma severity is based on assessing the degree of asthma control [9]. According to the published data, 60-80% of the variability in patients' susceptibility to treatment is due to genetics [11].

Histamine is one of the main inflammatory mediators synthesized and stored in the vesicles of mast cells and basophils. Histamine is assumed to be involved in the allergic reaction immunomodulation through activation of cytokine production, changes in Th1- and Th2-lymphocyte function and regulation of dendritic cells. The inhalation of histamine into the lungs causes the direct bronchoconstriction [7; 14]. Histamine released from storage vesicles into the extracellular space upon immunological stimulation of mast cells and basophils activates the histamine receptors (H1-H4) [14]. The interaction of histamine with H1-receptors stimulates excessive secretion of nasal mucosa and contraction of bronchial smooth muscle, whereas the interaction of histamine with H2-receptors produces the secretion of

gastric acid. H3-receptors are expressed primarily in the central nervous system and operate as autoreceptors in presynaptic histaminergic neurons, suppressing histamine release and modulating other neurotransmitters. H4-receptors are found in cells of the immune system. The action of histamine to H4-receptors activates the secretion of cytokines [10]. The diamine oxidase DAO (AOC1) and histamine-N-methyltransferase HNMT enzymes are actively involved in the initial steps of extracellular and intracellular histamine degradation [4]. Genome-wide association studies found a number of polymorphic variants of genes involved in the histamine metabolism (*PSAP*, *SCG3*, *ADCYAP1*, etc.) associated with asthma (www.genome.gwas.org). Antihistamines are used to treat the symptoms of allergic diseases depending on the histamine release, particularly allergic asthma, and to reduce the frequency of asthma exacerbations [7; 10]. Currently, the first and second generation antihistamines with similar pharmacological effects are used. The main difference between antihistamines is that the second-generation drugs have less adverse effects because they are more selective against peripheral H1 receptors [10].

The frequencies of polymorphic variants of genes involved in the disease pathogenesis varies among geographic regions and populations, which makes a relevance of a molecular genetic study of asthma in groups of people with different origins. The aim of this study is to evaluate the role of polymorphic variants of aldehyde dehydrogenase 7 family member A1 *ALDH7A1* (rs13182402), amine oxidase copper containing 1 *AOC1* (rs1049793), prosaposine *PSAP* (rs11000016) and adenylate cyclase activating polypeptide 1 *ADCYAP1* (rs2231187) genes involved in the histamine metabolism in asthma development in individuals of different ethnicity from the RB.

Materials and methods. The study included 846 unrelated individuals aged 3-67 years from the RB. The group of patients consisted of 421 individuals with asthma of different ethnicities (Russians - 174, Tatars - 142, Bashkirs - 105) from the children's clinic of Bashkir State Medical University of the Ministry of Health of the Russian Federation, the allergology and pulmonology departments of Municipal Clinical Hospital № 21 and Republican Children's Clinical Hospital (Ufa) (239 males, 182 females). The subgroup of individuals with childhood onset asthma consisted of 258 cases with the manifestation of disease before the age of 18 (Russians - 94, Tatars - 111, Bashkirs - 53). The control group included 425 practically healthy individuals (181 males, 244 females) with low levels of total IgE (0-150 IU/ml), without bronchopulmonary and allergic diseases (Russians - 194, Tatars - 145, Bashkirs - 86). The participants or their parents signed an informed consent form. The study was approved by the Bioethics Committee of the IBG UFRS RAS (Protocol № 7 from 10.02.2011).

Genomic DNA was obtained by phenol-chloroform extraction. Genotyping of *ALDH7A1* rs13182402 (c. 517+395T>C), *PSAP* rs11000016 (g. 71819460C>T), *ADCYAP1* rs2231187 (c. 456 A>G, p. Lys152=) genes polymorphisms was performed according to the recommended protocol by using the CFX96 real-time PCR detection system (DNA-Synthesis, Moscow) (Bio-Rad, USA). Genotyping of the *AOC1* rs1049793 (c. 1990C>G, p. His664Asp) polymorphism was conducted by PCR-RFLP analysis. The comparison of allele and genotype frequencies in patient and control groups was based on the chi-square criterion for 2x2 contigency tables, the odds ratio (OR) and 95% confidence interval (CI 95%) were estimated. The statistical analysis of data was performed using the Plink 1.9 and WinPepi v.11.32 programs.

Results and discussion. Insufficient control of asthma is one of the widespread problems of modern medicine. The solution of this problem involves the importance of an extensive and comprehensive analysis of factors involved in the disease pathogenesis, as well as the patients' susceptibility to treatment, which is largely determined by heredity [11]. In the present work the study of polymorphic variants of *ALDH7A1* (rs13182402), *AOC1* (rs1049793), *PSAP* (rs11000016), *ADCYAP1 (*rs2231187) genes involved in the histamine metabolism in asthma patients and control groups from the RB was performed (Table 1). The distribution of genotype frequencies was shown to be consistent with the expected under Hardy-Weinberg equilibrium (p>0,05).

ALDH7A1 plays an important role in the detoxification of aldehydes, catabolism of lysine in the mitochondrial matrix (https://www.ncbi.nlm.nih.gov/gene/501) and degradation of histamine metabolic products [4]. The frequency of the rs13182402**G* allele in patients with severe and moderate asthma of Bashkir ethnicity (18,49%) was significantly higher than in the respective control group (9,52%, p=0,02; OR=2,16; 95%CI 1,11-4,18). Increased frequency of the rs13182402**G* allele was revealed in Bashkirs with severe and moderate-to-severe asthma (18,49%) than in the controls (9,52%, p=0,02; OR=2,16; 95%CI 1,11-4,18). According to the literature, the rs13182402**G* allele was associated with development of other multifactorial diseases, such as esophageal squamous cell cancer and osteoporosis [13]. Genome-wide association analysis revealed that the rs13182402**G* allele with a high level of significance was associated with asthma in individuals of European origin (www.genome.gwas.org)

The association analysis of the *AOC1* rs1049793 polymorphism with asthma development in Russians revealed that the *CC* genotype and the *C* allele were significantly more common in patients (54,07% and 70,35%) than in controls (40,53%; p=0,01; OR=1,73; 95%CI 1,14-2,62 and 62,89%, p=0,03; OR=1,4; 95%CI 1,03-1,91) (Table 1). The obtained data consistent with our previous results, which showed that the rs1049793**CC* genotype and the rs1049793**C* allele were associated with asthma development and low spirometry values in Russians [1]. According to the published data, the rs1049793 polymorphism causes a missense mutation resulting in decreased activity of the AOC1 enzyme involved in histamine degradation. Disorders in the metabolism of histamine may cause to its excessive accumulation in the body both an extensive or prolonged response of receptors to histamine [4; 7]. It was revealed that a haplotype including rare alleles of the *AOC1* rs1049793 and *HNMT* rs11558538 polymorphisms was associated with a more severe course of allergic rhinitis and higher histamine levels in blood serum in children with allergic diseases [6].

The study of the rs2231187 polymorphism of the *ADCYAP1* gene (PACAP, 18p11.32) encoding the PACAP protein involved in the histamine secretion (www.ebi.ac.uk/) in groups from the RB was carried out. The association of the rs2231187**AA* genotype and the rs2231187**A* allele with asthma development in Bashkirs was found (p=0,04; OR=1,83; 95%CI 1,02-3,26 and p=0,03; OR=1,66; 95%CI 1,05-2,63) (Table 1). A higher frequency of the rs2231187**A* allele was observed in Bashkirs with childhood onset asthma (81,13%) compared to controls (68,24%; p=0,02; OR=2,0; 95%CI 1,12-3,59). The rs2231187**AA* genotype was significantly more frequent in Bashkirs with severe and moderate asthma (63,01%) than in controls (47,06%, p=0,05; OR=1,92; 95%CI 1,01-3,63) and in patients with a mild course of asthma (59,38%). According to the literature, a meta-analysis of GWAS studies of

The distribution of allele and genotype frequencies of *ALDH7A1* rs1318240, *AOC1* rs1049793, *ADCYAP1* rs2231187, *PSAP* rs11000016 polymorphisms in case/control groups

Group		Genotypes, n (%)			Alleles, n (%)		N
rs13182402 (*ALDH7A1*)		***AA***	***AG***	***GG***	***A***	***G***	
Cases	Russians	144 (84.21)	25 (14.62)	2 (1.17)	313 (91.52)	29 (8.48)	171
	Tatars	109 (78.42)	28 (20.14)	2 (1.44)	246 (88.49)	32 (11.51)	139
	Bashkirs	77 (73.33)	25 (23.81)	3 (2.86)	179 (85.24)	31 (14.76)	105
Controls	Russians	154 (80.21)	35 (18.23)	3 (1.56)	343 (89.32)	41 (10.68)	192
	Tatars	117 (81.25)	26 (18.06)	1 (0.69)	260 (90.28)	28 (9.72)	144
		68 (80.95)	16 (19.05)	-	152 (90.48)	16 (9.52)	84
rs1049793 (*AOC1*)		***CC***	***CG***	***GG***	***C***	***G***	
Cases	Russians	**93 (54.07) p=0.01 OR=1.73 (1.14-2.62)**	**56 (32.56) p=0.02 OR=0.6 (0.39-0.92)**	23 (13.37)	**242 (70.35) p=0.03 OR=1.4 (1.03-1.91)**	**102 (29.65) p=0.03 OR=0.71 (0.52-0.98)**	172
	Tatars	66 (46.81)	60 (42.55)	15 (10.64)	192 (68.09)	90 (31.91)	141
	Bashkirs	34 (32.69)	54 (51.92)	16 (15.38)	122 (58.65)	86 (41.35)	104
Controls	Russians	77 (40.53)	85 (44.74)	28 (14.74)	239 (62.89)	141 (37.11)	190
	Tatars	59 (41.26)	71 (49.65)	13 (9.09)	189 (66.08)	97 (33.92)	143
	Bashkirs	35 (41.18)	40 (47.06)	10 (11.76)	110 (64.71)	60 (35.29)	85
rs2231187 (*ADCYAP1*)		***AA***	***AG***	***GG***	***A***	***G***	
Cases	Russians	84 (48.84)	61 (35.47)	27 (15.7)	229 (66.57)	115 (33.43)	172
	Tatars	80 (57.14)	50 (35.71)	10 (7.14)	210 (75.0)	70 (25.0)	140
	Bashkirs	**65 (61.9) p=0.04 OR=1.83 (1.02-3.26)**	34 (32.38)	6 (5.71)	**164 (78.1) p=0.03 OR=1.66 (1.05-2.63)**	**46 (21.9) p=0.03 OR=0.6 (0.38-0.95)**	105
Controls	Russians	94 (48.96)	82 (42.71)	16 (8.33)	270 (70.31)	114 (29.69)	192
	Tatars	75 (52.08)	55 (38.19)	14 (9.72)	205 (71.18)	83 (28.82)	144
	Bashkirs	40 (47.06)	36 (42.35)	9 (10.59)	116 (68.24)	54 (31.76)	85
rs11000016 (*PSAP*)		***CC***	***CT***	***TT***	***C***	***T***	
Cases	Russians	128 (74.85)	41 (23.98)	2 (1.17)	297 (86.84)	45 (13.16)	171
	Tatars	97 (69.29)	42 (30.0)	1 (0.71)	236 (84.29)	44 (15.71)	140
	Bashkirs	78 (74.29)	25 (23.81)	2 (1.9)	181 (86.19)	29 (13.81)	105
Controls	Russians	138 (72.25)	49 (25.65)	4 (2.09)	325 (85.08)	57 (14.92)	191
	Tatars	96 (67.13)	44 (30.77)	3 (2.1)	236 (82.52)	50 (17.48)	143
	Bashkirs	59 (70.24)	21 (25.0)	4 (4.76)	139 (82.74)	29 (17.26)	84

Note. N - number of individuals; n – number of the group, the frequency of alleles and genotypes is given in brackets; p – level of significance, indicated only for statistically significant differences ($p < 0,05$); OR – the odds ratio, in brackets – 95% confidence interval

asthma in individuals of European origin found that the *T* allele of the rs1291183 polymorphism localized near the *ADCYAP1* gene was highly significant associated ($4x10^{-6}$) with low percent predicted FEV1 values [8].

The analysis of the genotype distribution and allele frequencies of the *PSAP* rs11000016 polymorphism between asthma patients and controls revealed no statistically significant differences (Table 1). The *PSAP* gene is located in 10q22.1 chromosomal region and encodes a protein fragmented into four homologous sphingolipid activator proteins (saposins A - D) which are involved in the activation of certain lysosomal hydrolases. The mutations of saposin proteins cause a deficiency of lysosomal hydrolase and subsequent lysosomal accumulation disorders [12]. GWAS of individuals of European origin showed that the *PSAP* rs11000019 polymorphism was associated with childhood onset asthma (www.genome.gwas.org).

Meta-analysis of the associations of *ALDH7A1* (rs13182402), *AOC1* (rs1049793), *PSAP* (rs11000016) and *ADCYAP1* (rs2231187) genes polymorphisms with asthma development and clinical severity in Russians, Tatars and Bashkirs revealed no statistically significant differences ($p>0,05$).

Conclusion. In summary, we analyzed the associations of polymorphic variants of the aldehyde dehydrogenase 7 family member A1 *ALDH7A1* (rs13182402), amine oxidase copper containing 1 *AOC1* (rs1049793), prosaposine *PSAP* (rs11000016) and adenylate cyclase activating polypeptide 1 *ADCYAP1* (rs2231187) genes with risk of asthma development and clinical course severity. The association of the rs1049793**CC* genotype and the rs1049793**C* allele of the *AOC1* gene with asthma development in Russians was established. The associations of the rs2231187**AA* genotype and the rs2231187**A* allele of the *ADCYAP1* gene with asthma, the association of the rs2231187**A* allele of the *ADCYAP1* gene with childhood onset asthma, the associations of the 2231187**AA* genotype of the *ADCYAP1* gene and the rs13182402**G* allele of the *ALDH7A1* gene with severe and moderate asthma in Bashkirs were revealed. The results

are important for further understanding the influence of the polymorphic variants of genes involved in the histamine metabolism in the pathophysiology and clinical course of asthma.

This work was carried out within the framework of the state assignment of the Ministry of Science and Higher Education of the Russian Federation (no. 122041400169-2 and no. 075-03-2021-193/5), with partial support of the Megagrant of the Ministry of Education and Science of the Russian Federation (agreement no. 075-15-2021-595), RFBR grant (project no. 19-315-90055) and grant of the Saint-Petersburg State University (project no. 93025749). DNA samples were taken from the Collection of Human Biological Materials at the IBG UFRS RAS, supported by the Bioresource Collections Program of the FANO of Russia (agreement no. 007-030164/2) using the equipment of the Technological Platform of the Ufa Federal Research Center of the Russian Academy of Sciences on the basis of the Regional Analytical Center for collective use “Agidel”.

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ASSESSMENT OF VITAMIN D LEVEL AS A FACTOR OF BRONCHIAL ASTHMA CONTROL IN CHILDREN OF THE AMUR REGION

DOI 10.25789/YMJ.2023.81.03

УДК 616.248-053.2-07:577.16

Nowadays, control of bronchial asthma (BA) can be achieved only in 2/3 of cases, even in a controlled population with direct daily supervision of a doctor ensuring full compliance with standard treatment regimens. In this regard, factors that can affect the course of asthma, but those which are not taken into account in standard treatment regimens, are of particular importance. Interest in vitamin D as a modifier of atopic inflammation in BA is due to its noncalcemic effects realized through the VDR receptors of immune cells. 78 patients suffering from asthma were examined. The diagnosis of the disease and the degree of control over it were established based on the GINA 2020 criteria. The levels of vitamin D and interleukins in the blood serum were determined by ELISA. The level of immunocompetent cells was determined by flow cytometry. The presence of persistent infection was determined by PCR method. It was found that children with uncontrolled AD have more than two times (29.8 vs 64.5 ng/ml) lower level of vitamin D than patients who control the disease. Children with vitamin D deficiency have three times higher levels of one of the inducers of atopic inflammation – IL5 (0.62 vs 0.22 pg/ml) and require a higher dose of topical glucocorticosteroids (TGC) (347.3 vs 285.5 mcg) to control the disease. Thus, vitamin D has a significant impact on the level of asthma control in children, the immune status and the likelihood of persistence of the herpes virus type 6. Determination and correction of vitamin D deficiency should be recommended, since it influences on pathogenetically significant parameters of atopic inflammation in uncontrolled bronchial asthma in children.

Keywords: bronchial asthma, children, vitamin D, herpes virus type 6.

Introduction. In recent decades, there has been an increase in the prevalence of BA and its incidence has reached 15% among the child population of the Earth [14]. Russia as a whole [6] and the Khabarovsk Territory [8] in particular are not an exception. Twofold increase in the prevalence of BA among children in the Khabarovsk Territory was detected in the period from 2005 to 2020 (teenagers – from 12% to 25%, children under 14 years – from 11% to 20%). At the same time, the relatively lower official rates of the disease (about 2% in Russia and 7-15% in industrialized countries) are due to the method of record keeping of medical aid appealability. According to ISAAC studies asthma like symptoms are detected in 4-15% of children in various regions of our country, which corresponds to the indicators of other industrialized countries [4]. Such BA extension is due to, first of all, a change in the total genotype of the population of these countries, caused by a radical change in lifestyle in the last century, which leads to an ever wider spread of atopy in general, and BA in particular, thus making the disease practically not treatable. Therefore, the efforts of the medical community are aimed at improving the effectiveness of BA therapy, but not at its primary prevention. However, the proportion of patients who have managed to control BA does not exceed 30%, and complete control is achieved only in 5% of cases. Even when basic therapy is carried out under the direct supervision of a specialist according to accepted treatment regimens, with free provision of drugs and among patients without significant comorbidity of BA, complete control can be achieved only in 34% of cases, good results show 38% of patients and 28% of patients remain with uncontrolled course of the pathological process [18]. In Russia, there are similar ratios of the degrees of disease control [3]. Bronchial asthma is a multifactorial disease, so the causes of an uncontrolled course are very diverse, but in children it is almost always based on atopic inflammation, which is primarily due to congenital factors, but the likelihood and duration of their implementation, as well as the course of the disease, can be significantly modified under the influence of various exogenous factors [11, 13, 16], including regional ones. One of them is the level of insolation and the ability to perceive it by a person in connection with other climatic features. The Amur region is one of the leaders among the regions of Russia in terms of formal indicators of insolation, the number of sunny days for our region exceeds 300 per year. However, a rather harsh climate with large temperature fluctuations, up to extreme ones, does not allow to take full advantage of this benefit, so the level of vitamin D in the population is quite low. According to studies conducted in 2020, vitamin D deficiency was detected in almost half of the child population of our region [2, 7]. Meanwhile, the role of this vitamin in the pathogenesis of BA is known. Recent Iranian studies have shown that asthma patients have lower levels of vitamin D which decrease even more with disease aggravation [21]. Similar results were obtained in the same year by Indian researchers [19]. Several earlier epidemiological and in vivo studies have also found a connection between low vitamin D levels in serum and increased inflammation, decreased lung function, increased exacerbations, and general deterioration in patients with AD [12, 15, 20]. Later, a meta-analysis confirmed a significant reduction in objective indicators of obstruction, such as FEV1, with low vitamin D levels in both children and adults with BA [17]. In addition, a number of scientific works using meta-analysis methods show the effectiveness of vitamin D in the complex therapy of bronchial asthma. Vitamin D contributes to positive changes in the cytokine network during treatment, which is associated with its

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ability to regulate Th2 functions and, as a result, reduce the synthesis of interleukins -13 and -17, which are involved in the allergy pathogenesis [1]. Thus, the detection of vitamin D levels in children with bronchial asthma in our region and confirmation of its effect on the course of asthma is of great scientific interest and may be of high clinical significance.

Aim: To evaluate the effect of vitamin D on the controllability of the course of AD in children of the Amur region and its role in the pathogenesis of atopic inflammation.

Materials and methods. We examined 167 patients with asthma. The diagnosis of BA, the severity and degree of control over the disease were established according to the criteria of the current editions of GINA [14], the National Program "Bronchial asthma in children: a strategy for treatment and prevention" [6], and Clinical guidelines for the diagnosis and treatment of BA [5]. The study of lymphoid populations was carried out on a FACSCalibur Becton Dickinson cytometer. The panel of monoclonal antibodies ("BD") consisted of 7 parameters: CD3+/CD45+ (mature T-lymphocytes), CD19+/CD45+ (mature B-lymphocytes), CD3+/CD4+/CD45+ (T-helpers/inducers), CD3+/CD8+/CD45+ (T-killers/cytotoxic), CD3+/CD25+ (lymphocytic activation marker), CD(16+56)/CD45+ (natural killers). Neutrophil activity indicators were studied in spontaneous and stimulated tests of phagocytic activity with latex particles and in tests of NBT reduction to formazan ("FAN-test", "NBT-test", "Reacomplex", Chita city). To determine the levels of IgA, IgM, IgG, IgE and interlekins 4, 5, 6, 7, 8, 9, 10, 18 and TNFα, as well as the level of vitamin D in blood serum, an immunoenzyme method with the "Vector-Best" test systems was used. The determination was carried out by an automatic spectrophotometer Lazurite "Vector-Best". DNA of viral infections pathogens in swabs from the oropharynx was determined by PCR using the test system of OOO InterLabService (Moscow) AmlpSens®EBN/CMV/HHV6 – screen-FI. Detection was performed in real time using a C 1000 Touch CFX96 thermal cycler (BIO-RAD the USA). The DNA of Human herpes virus 4 type (EBN) - Epstein-Bar virus, and Human herpes virus 6 type (HHV6) - herpes 6 type, were detected. The research data were entered into the Excel-2013 electronic database. The statistical analysis of the results of the study used standard methods of variant statistics using the statistical software package: "STATISTICA" for "Windows" (version 10.0). In this paper, all parameters are presented as – M±m – mean ± "standard error of the mean" (SD/sqrt(n) = SEM (Standard Error Means), - where n is the sample size). The statistical hypothesis of equality of group means was tested using Student's t-test (two-sample t-test). When describing the reliability of the results of statistical analysis, the formula "$p<0.05$" was used. In addition, a significant difference in the proportions in the groups was assessed. The studies were carried out in accordance with the principles of the ongoing revision of the Declaration of Helsinki (64th WMA General Assembly, Fortaleza, Brazil, October, 2013). All data were collected with the personal consent of the subjects and their legal representatives. In all tables showing statistical processing, except for the primary one, the interviewees are presented under serial numbers. The Excel spreadsheet was password-protected and was only accessible to study participants. The study design was approved by the institution's ethics committee.

Results. The studies revealed that children with controlled BA have significantly ($p=0.006$) higher level of vitamin D (64.5±12.8 ng/ml) than patients with uncontrolled disease (29.8±5.4 ng/ml) (Figure 1).

Patients with vitamin D deficiency required a significantly higher dose of glucocorticosteroids (GCS) to achieve control over the disease (347.3 mg versus 285.5 mg) (Figure 1).

Discussion. The climatic features of the region affect the course of bronchial asthma in various ways. In particular, Khabarovsk has been repeatedly recognized as the sunniest city in Russia (2449 hours of sunshine per year). However, such climatic characteristics as the extreme temperature fluctuations and humidity do not allow us to take full advantage of the sun, since Khabarovsk residents are forced to wear clothing that covers almost entire body and spend a lot of time indoors. In this regard, residents of the Far East are more likely to have vitamin D deficiency rather than its excess. This study confirmed that despite equal insolation, the level of vitamin D in patients with BA is lower than in the comparison group, and decreases with disease aggravation. It was found that children with uncontrolled asthma have significantly lower levels of this vitamin as compared to patients who control the disease. Interest in vitamin D as a modifier of atopic inflammation in BA is due to its noncalcemic effects

Immunological parameters of patients with BA depending on the level of vitamin D

Indicator	Level of vitamin D		Reliability
	<30,0 ng/ml	≥30,0 ng\ml	Accuracy
IL4, pg/ml	0.2±0.09	0.94±0.21	0.03
IL5, pg/ml	0.62±0.18	0.22±0.13	0.04
CD19, absolute	366.0±31.7	427.0±24.2	0.05
CD19, %	13.7±1.05	16.1±1.2	0.05

The average levels of vitamin D in patients with a controlled course were within the normal range (more than 30.0 ng/ml), in children with an uncontrolled course, they were below the reference values.

When analyzing the effect of vitamin D on some clinical and pathogenetic parameters in BA, certain results were obtained (Table).

When studying some indicators of the immune status, it was revealed (Figure 6.6) that children suffering from BA with vitamin D deficiency have truly 3 times higher levels of interleukin-5 (0.62 pg/ml versus 0.22 pg/ml), which directly activates atopic inflammation, but at the same time significantly (4.5 times) lower levels of interleukin-4 (0.2 pg/ml versus 0.94 pg/ml). A significantly lower proportion of active B-lymphocytes (13.7% vs 16.1%) and their absolute number (366 vs 427) were also detected.

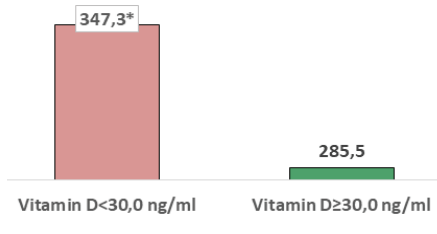

Fig. 1. The dose of TGC required to achieve control over the disease in vitamin D deficiency among children with BA

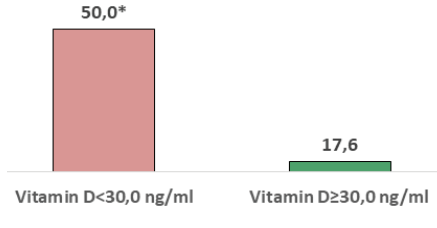

Fig. 2. Proportion of DNA of herpes virus 6 type shed from sputum in vitamin D deficiency in children with BA

realized through the VDR receptors of immune cells. Though insolation is the same for all residents of a given region, it has individual impact in each specific case. The significance of VDR receptor polymorphisms for the pathogenesis of bronchial asthma is known [9, 10]. Therefore, the connection between the level of vitamin D and the controllability of the course of asthma, as well as the mechanisms of its implementation, are of great interest. The study showed that the level of vitamin D affects precisely those immune parameters that play a major role in local atopic inflammation. Interleukin-4, which regulates the differentiation of the second type T-helpers and is more of a cytokine of lymphonodus, is reduced in the patients with vitamin D deficiency. Interleukin-5 directly regulating atopic inflammation is many times higher in situ. Moreover, such patients also have a sharply reduced barrier function of the epithelium, which is signified by more frequent emission of Epstein-Barr virus DNA.

Thus, the content of vitamin D has a significant effect on the level of control of asthma in children, affects the pathogenetically significant indicators of the immune status for atopic inflammation and the likelihood of persistence of the herpes virus type 6. Determination and correction of vitamin D deficiency should be recommended for children with uncontrolled bronchial asthma.

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THE EFFECT OF LIGHT ON THE FORMATION OF OSMIOPHILIC BODIES IN PINEALOCYTES AND CALCIFICATION OF THE PINEAL GLAND

DOI 10.25789/YMJ.2023.81.04

УДК 611.814.53:612.4.09:616-008.61/.63

In order to verify the assumption that changes in the content of osmiophilic bodies (OB) in pinealocytes in rats are a morphological marker not only of shifts in secretory activity and cal-cification of the pineal gland responding to round-the-clock lighting, but also reflect violations of pineal biorhythm, by methods of light and transmission electron microscopy, microrentgenospec-tral analysis in an experiment on rats, exposed to 48-hour exposure to bright light, the numerical density of single and grouped OB, their chemical composition was estimated. It is concluded that the grouped OB with the material of disposed mitochondria and calcifications are a residual manifestation of desynchronosis. Their content increases at an earlier time after exposure than the total content of various types of OB.

Keywords: pineal gland, exposure to light, rats, ultrastructure, chemical composition.

Introduction. In the northern latitudes beyond the Arctic Circle, such a phenomenon as a polar day is observed. In connection with the active development of the Arctic, the northern sea route, the study of the effects of round-the-clock lighting on the body is becoming increasingly relevant. The pineal gland plays an importance role in the regulation of daily biorhythms. Modeling the effect of 24-hour illumination with 48-hour exposure to bright light on white rats, we showed that hyperilluminated animals develop temporary desynchronosis of the daily activity of the pineal gland, which resulted in an increase in the content of calcified cells in pinealocytes [3, 4]. During the study, a complex of morphological markers of functional activity of the gland was considered [7], which made it possible to assess, among other things, the state of the mitochondrial apparatus, which is the exclusive site of synthesis of the main pineal hormone melatonin [10]. The specific volume of mitochondria in the cytoplasm of most large light pinealocytes (type IB) significantly exceeds the value of the indicator characteristic of all other types of cells of a neural nature, including hypothalamic neuroendocrine [1]. Mitochondria in pinealocytes, in addition, exhibit a diurnal fusion-division rhythm, and are subject to mitophagy [11]. Disposal is accompanied by the formation of single and grouped into aggregates from [2]. It is assumed that the utilization of damaged mitochondria is associated with the formation of OB accumulating in small light pinealocytes (type IA), dark pinealocytes (type II) and degenerating pinealocytes (type III), and that the formation of grouped OB is a consequence of a violation of the daily rhythm of mitochondrial fusion.

The aim of the study was to analyze the formation, chemical composition and changes in the numerical density of single and grouped OB in pinealocytes of the pineal gland in rats after the cessation of round-the-clock illumination with bright light.

Materials and methods of research. The work was performed on 80 mongrel white male rats weighing 180-200 g in accordance with the rules of laboratory practice (Order of the Ministry of Health of the Russian Federation No. 267 dated 06/19/2003). The illumination of animals in the daytime (from 8 to 20 hours) was 200 lux. The effect of round-the-clock lighting was modeled by placing rats in hyperilluminated cells for 48 hours (6 lamps LB-40, illumination 3500 lux). Experimental and control animals were withdrawn from the experiment at 11-12 hours of the day after 24 hours, 10, 30 and 180 days after the cessation of exposure to bright light. The gland was taken after decapitation of rats under ether anesthesia, fixed in 2.5% glutaraldehyde at 0.2 M cacodilate buffer (pH 7.4), post-fixed in 1% osmium tetraoxide solution, dehydrated in ethanol, poured into epon. The sections were made using LKB-III ultratome (Sweden), contrasted with uranyl acetate and lead citrate, and examined in a JEM-100 CX II transmission microscope (JEOL, Japan). The electron microscope JEM-210 (JEOL, Japan) and the energy dispersive spectrometer Oxford Instruments X-Max (Great Britain) were used for the microrentgen spectral analysis. In sections of the gland with an area of 0.06 mm2, the numerical density FROM was calculated using a 368-point test system. The data was processed using the software package "Statistica for Windows", version 7 (StatSoft Inc., USA).

Results and discussion. In rats, in type IA pinealocytes, along with OB, mitochondria with inclusions of osmiophilic material and grains of calcifications are detected, which are isolated by cisterns of the endoplasmic reticulum with the formation of autophagolysosomes. Autophagolysosomes filled with amorphous osmiophilic material contain lamellar structures, calcified grains, lipids. It is not so common in autophagolysomes to observe complete digestion of the recycled material, as well as the presence of calcified lamellar material in the intercellular space. In the daytime, when mainly small mitochondria are detected in the zone of the inactive Golgi complex of light pinealocytes in control animals, some of them, thanks to the folds of the outer mitochondrial membrane, can interact with each

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other and with OB, turning into osmiophilic clear-cut structures (Fig. 1).

In larger numbers, grouped and single OB (up to 7.5 microns in size) are found in control and experimental animals in pinealocytes of type II and III, as well as in apoptotic corpuscles. The OB is involved in the genesis of concretions, which is consistent with the data of other authors and is considered as a physiological process, and calcification of the contained OB (its amorphous type) itself is a reflection of aging and/or degenerative condition of the pineal gland [5, 6, 9]. Only sometimes at the site of pinealocyte death in rats, crystals with a size of 1.2 × 0.2 microns and rosettes of crystals are organized (Fig. 2).

In terms of the ratio of calcium and phosphorus, the crystals are close to hydroxyapatite [8]. Calcium, phosphorus, and sulfur are rich not only OB wich utilize mitochondria, but also in organelles capable of melatonin synthesis themselves, depositing, in addition, Ca^{2+}, involved in oxidation and phosphorylation, containing substances that include cysteine, for example, glutathione (Fig. 3).

After the cessation of round-the-clock illumination, the numerical density of grouped pinealocytes in the cytoplasm increases and exceeds the control by 10-180 days. The increase in the total numerical density is measured by 30-180 days (Fig. 4). Since hyperilluminated rats developed desynchronosis with suppression and then inversion of the exact rhythm of pineal gland activity [3], it can be assumed that osmophilic material and calcifications accumulated in small mitochondria with inhibited activity of IB pinealocytes activate utilization organelles with similar changes. The OB are formed, type IB cells acquire morphological features of type IA cells, the fusion of small mitochondria into large ones synthesizing melatonin is complicated. Small mitochondria without signs of osmiophilia and with osmiophilic material are more often combined after the cessation of exposure into clear-cut structures, often in combination with OB, therefore, after 24 hours in the phase of inversion of the circadian rhythm, there is no significant increase in the specific volume in the cytoplasm of pinealocytes of mitochondria in hyperilluminated rats [4]. In large mitochondria, not only the enlightenment of the matrix is noted, but also the deposition of osmiophilic material. The specific volume of mitochondria in pinealocytes in rats increases 10 days after the cessation of light exposure at the peak of the adaptive response to

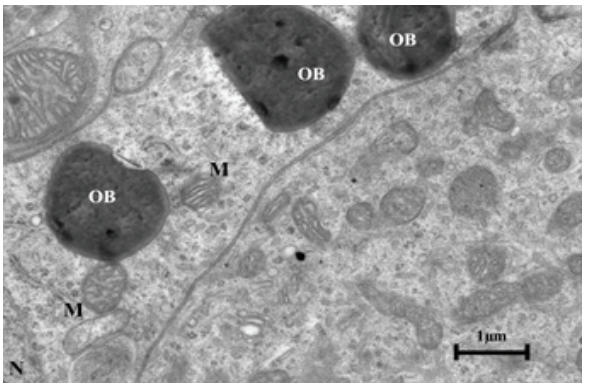

Fig. 1. Grouping of small mitochondria (M) and OB (N – pinealocyte nucleus)

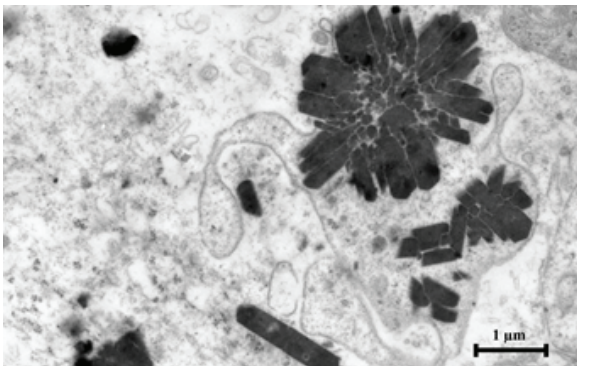

Fig. 2. Crystals at the site of pinealocyte death

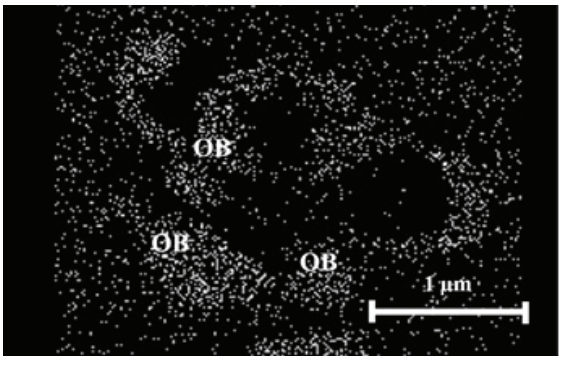

Fig. 3. Phosphorus distribution in the pinealocyte cytoplasm according to the data of dispersion microrentgen-ospectral analysis

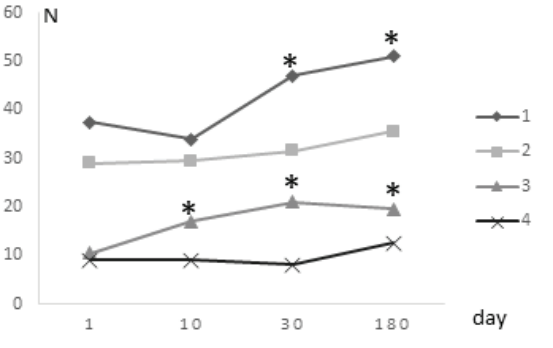

Fig. 4. Changes in the numerical density (N) of in the cytoplasm of pinealocytes in rats after exposure to light: 1 – total N in the experiment, 2 – total N in the control, 3 – grouped N in the experiment, 4 – grouped N in the control, asterisk – significant differences with the control

stress [4], which obviously activates mitophagy, the utilization of large mitochondria with signs of damage of the "dark" type with the formation of single OB. This may explain the earlier increase in the numerical density of grouped OB compared to the increase in the total numerical density of OB.

Conclusion. Thus, the peculiarity of the morphofunctional organization of the pineal gland, associated with the site of synthesis of the main pineal hormone melatonin in the mitochondria of pinealocytes, causes their frequent fusion with daily frequency, a significant specific volume in the cytoplasm, widespread calcification and osmiophilia of the mitochondrial matrix, utilization in the composition of OT, in place of which hydroxyapatite crystals or concretions of amorphous type. Violation of the circadian rhythm of mitochondrial fusion increases the content of grouped OT in pinealocytes, which can be considered as a residual manifestation of desynchronosis when adapting to round-the-clock lighting.

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STRUCTURAL CHARACTERISTICS OF DENTAL ANOMALIES IN SCHOOLCHILDREN DUE TO THE VAULT HEIGHT OF THE HARD PALATE WITH DIFFERENT DEGREES OF SEVERITY OF CONNECTIVE TISSUE DYSPLASIA

DOI 10.25789/YMJ.2023.81.05

УДК 616.315-007.24

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Nowadays a high level of CTD is prevailed, which has local and general phenotypic signs. At the same time, despite its wide study, the problems of diagnosis, treatment, prevention and comprehensive rehabilitation of its manifestations in the oral cavity and maxillofacial region remain unresolved. In this connection the research aim is to solve these problems, which have an extremely important practical, scientific and theoretical significance in dentistry, and also in medicine. The **research** o**bjective** is to study the structure of dentoalveolar anomalies of schoolchildren due to the hard palate vault height at different degrees of connective tissue dysplasia severity. **Materials and methods**. A clinical and craniometric study was performed in 964 children and adolescents aged from 12 to 15 years old diagnosed with CTD. The CTD severity was determined by the method of T. Milkovska-Dmitrova and A. Karkashev (1985). In this case, anomalies of occlusion, dental deformities and anomalies in the teeth position were determined taking into account the height of the hard palate vault at different CTD severity. The vault height of the hard palate was determined by the method of Ushnitsky I.D. et al. (2018). **Results**. The examined patients most frequently revealed a medium degree of severity, then a mild degree and less often a severe degree in the CTD structure severity. At the same time, in the structure of occlusal anomalies associated with distal occlusion, deep traumatic incisal overlap and underdevelopment of the upper jaw, dental arch anomalies including occlusion of the maxillary and mandibular dental arches, shortening of the maxillary and mandibular dental arches, displacement of the upper and lower central tremes, dental position anomalies such as close position of incisors, vestibular position of upper canines, protrusion of upper incisors, retrusion of upper and lower incisors, primary adentia, dystopia of upper canines are determined by increasing their prevalence in school children due to the height of the hard palate vault depending on CTD severity.

It should be noted that an opposite pattern to the previous pathologies was detected in some dentoalveolar anomalies, which is characterized by their decrease in the examined schoolchildren depending on the CTD severity. Thus, the occlusion anomalies trend was determined in reverse incisal occlusion, crossbite, mesial occlusion, retroposition of the mandible, as well as in some anomalies of tooth position, including palatine positioning of upper second incisors, tortoanomaly of the upper and lower jaws, inverse occlusion of upper incisors, macro dentition, disorders of the premolars eruption, lateropositioning of upper second incisors. **Discussion**. The comprehensive study revealed the main structural characteristics of anomalies of occlusion and position of teeth and dental deformities in schoolchildren due to the hard palate vault height at different degrees of CTD severity. **Conclusion.** The increase and decrease pattern in the prevalence of dentoalveolar anomalies in schoolchildren due to the height of the hard palate vault at different degrees of CTD severity has been established for the first time, which will allow taking timely measures to improve complex medical and social rehabilitation.

Keywords: connective tissue dysplasia, height of the hard palate vault, dentition deformities, occlusal anomalies, dental position anomalies.

Introduction. Nowadays, the problems of general and local phenotypic manifestations of CTD are widely studied [1, 5, 9, 19, 22, 24, 30, 32]. In spite of this, the problems of improving medical and social rehabilitation of patients with CTD have not been completely solved [3, 7, 10, 20, 31, 33]. In the structure of local phenotypic signs of CTD manifested in the organs and tissues of the oral cavity, congenital anatomical deformities of the hard palate and dental rows, abnormal occlusion and position of the upper and lower jaw teeth are most frequently identified [6, 13, 21, 23, 27].

It is important to emphasize that constitutional and morphological dysgenesis has a direct impact on the frequency of functional disorders of the maxillary system [11, 12, 26, 28]. Thus, the most frequent lesions of oral organs and tissues are TMJ dysfunction, dental anomalies, occlusion, distal occlusion, high gothic palate, deep incisal overlap, vestibular inclination of the anterior maxilla teeth, tooth dystopia and crowding, curvature of the nasal septum, etc. [4, 14, 15, 25, 29].

It should be noted that the dental problems of this congenital pathology have been insufficiently studied and reported in the literature [2, 8, 16, 34]. It dictates the need for further research improving diagnosis, treatment, prevention and comprehensive medical and social rehabilitation of general and local manifestations of CTD, which is an urgent problem in dentistry and medicine.

The purpose of the research is to determine the structural characteristics of occlusal anomalies and dental position abnormalities, dental r deformities in school-age children taking into account the height of the hard palate vault at different degrees of connective tissue dysplasia severity.

Materials and methods. The clinical and craniometric study was carried out in 964 children and adolescents aged from 12 to 15 years old with the CTD diagnosis. The examination was conducted in the urban district "Yakutsk" (Secondary school №5, №9, №26 and №35, Secondary school №2, Yakutsk City lyceum, Yakutsk City gymnasium, Gymnasium №8, Khangalassky ulus (district) of the Sakha Republic (Yakutia).

The CTD severity was interpreted by the method of T. Milkovska-Dmitrova and A. Karkashev (1985). The CTD severity in the examined children and adolescents was calculated according to the sum of points: 12 points in the mild degree, 23 - in a moderate degree, and in a severe degree - 24 or more points. The depth of the hard palate in case of deformities was determined using a well-known device for measuring the height of the hard palate vault [17].

A biometric study of the hard palate vault height with different CTD degrees was carried out: 633 at dental consultation and 331 jaw models by the method of Ushnitsky I.D. et al. (2018) [18]. Cast impressions of the jaws were obtained at the dental consultation, then biometric measurements were performed at the Department of Therapeutic, Surgical, Orthopedic Dentistry and Pediatric Dentistry of the Medical Institute of M.K. Ammosov North-Eastern Federal University and Yakutsk Specialized Dental Center of the Republic of Sakha (Yakutia). Morphometric studies of anatomical deformities of the upper and lower jaws were performed in the dental clinic and on diagnostic models by a special device including fixed and movable sponges for measuring external dimensions in the form of a truncated plate. These plates have two through holes of oval (rounded) shape each, designed for fixation of removable intraoral pads for fixed and movable measuring jaws, where there is a thread for connection with the screw of the pad in the hole. The measurement results are projected on the display in the digital caliper display.

We used the standard Pon-Linder-Hart method determining the width of the dentitions in children and adolescents, where we determined the relationship between the obtained total values of the mesiodistal incisors and the width of the dentition in the area of the first premolars and molars to obtain objective data. The measuring points were located in the middle of the longitudinal fissures of the first premolars and the anterior point of the intersection of the longitudinal and transverse fissures of the first molars of the maxilla. Mandible constrictions were studied on the measuring points, which are located on the distal point of the first premolar in contact with the second premolar (point between the premolars), and the median point on the vestibular surface or distal-cheeked cusp of the first molar. These measuring points, according to Pon, are used when the bite is permanent. There are anomalies of occlusion, dental deformities and dental position anomalies in various degrees of connective tissue dysplasia severity by standard methods.

The research was done by regulatory documents based on Russia's basic documents on the organization of scientific research.

The studies were conducted in compliance with the ethical principles of scientific medical research involving human subjects, as defined by the Declaration of Helsinki of the World Medical Association (1964, 2000), and the requirements set forth in the main regulatory documents of the Russian Federation on clinical trials. Prior voluntary parental consent was obtained in all examined children.

Statistical analysis of the results was performed with the software package "SPSS" version 22, license "IBM SPSS 22", as well as Pearson correlation analysis (r) and factor analysis by "Varimax" method.

Results and discussion. The most frequently revealed CTD structure data was medium severity degree (55,12±1,05%), then mild degree - 32,05±1,59%, and less frequently severe degree - 12,83±2,04% in the examined children. The results of the hard palate vault height using our device characterize that the hard palate vault height was up to 18.2±0.02 mm in children with mild CTD degree. At the same time, school-age children with medium (19.3±0.04 mm) and severe (32.4±0.02 mm) CTD show pronounced biometric changes in the height of the hard palate vault.

Table 1

Structural characteristics of occlusion anomalies at different degrees of connective tissue dysplasia severity, (%)

CTD severit degree based on the height of the hard palate vault	Distal occlusion	Reverse incisal occlusion	Intersecting occlusion	Deep trauma-traumatic incisal overlap	Vertical incisal overlap dysocclusion	Undeveloped upper jaw	Mesial occlusion	Open bite	Retroposition of lower jaw (sagittal gap)	Direct occlusion
Mild	10.69±0.44[2]	8.36±0.45[2]	5.97±0.47[2]	5.62±0.46[2]	5.38±0.46[2]	0.36±0.28	6.78±0.42[2]	0.44±0.31	31.57 ± 0.33	5.51±0.41[2]
Medium	28.93±0.36[1.2]	2.77±0.56[1.2]	5.35±0.46[1.2]	16.52±0.42[1.2]	12.97±0.44[1.2]	0.71±0.50[2]	5.85±0.47[1.2]	7.71±0.46[2]	18.98±0.41[1.2]	1.29±0.49[1.2]
Severe	37.58±0.38[1.2]	2.61±0.49[2]	3.64±0.55[1.2]	38.88±0.38[1.2]	4.69±0.55[1.2]	5.12±0.55[1.2]	2.77±0.56[1.2]	5.14±0.55[1.2]	11.86±0.51[1.2]	2.95±0.56[1]
Average values	25.73±0.25	4.58±0.04	4.98±0.02	20.34±0.11	7.68±0.07	2.91±0.01	5.13±0.05	6.42±0.03	20.80±0.06	3.25±0.03

Notes: 1 - degree of reliability calculated between CTD degrees of severity; 2 - degree of reliability calculated between mean values and indices of different CTD degrees of severity.

Table 2

Structural characteristics of dental anomalies in different degrees of connective tissue dysplasia severity, (%)

CTD severity degree due to the hard palate vault height	Dento-alveolar arches constriction of the upper and lower jaw	Shortening of dentoalveolar arches of upper and lower jaws	Displacement of upper and lower center line	Diastema	Tremes
Mild	27.51± 0.11[2]	17.54±0.16[2]	0.18±0.22	2.52±0.23	2.29±0.23
Medium	28.49±0.10[1.2]	21.18±0.14[1.2]	0.26±0.24[2]	4.22±0.22	2.55±0.23
Severe	31.48±0.09[1.2]	27.37±0.11[1.2]	2.67±0.23[1.2]	1.29±0.24	3.07±0.23
Average values	29.16±0.29	22.03±0.22	1.46±0.01	2.67±0.02	2.63±0.02

Distal occlusion was the most frequent in the occlusal anomalies data structure at different CTD degrees severity taking into account the hard palate vault height (Table 1), where the average rate was 25.73+0.25%, lower jaw retrosession (sagittal gap) - 20.80+0.06% and deep traumatic incisal overlap - 20.34+0.11%. Next in frequency were vertical incisional dysocclusion, which was 7.68+0.07%, open bite - 6.42+0.03%, mesial occlusion - 5.13+0.05%, cross bite - 4.98+0.02%, and reverse incisional occlusion - 4.58+0.04%. Straight occlusion 3.25+0.03% and underdevelopment of the maxilla 2.91+0.01% were less common.

The research revealed a pattern of increased frequency of occlusion anomalies depending on the CTD severity. Thus, distal occlusion in the moderate degree of congenital dysgenesis increased by 2.71 times compared with the mild degree and by 1.30 times compared with the moderate degree of CTD in the severe degree. A similar trend is determined in deep traumatic incisor overlap, where the rates were 2.94 and 2.35 times, and 1.97 and 7.21 times, respectively in maxillary underdevelopment data ($p \leq 0,05$).

It should be noted that an opposite pattern to the previous pathologies of the maxillary system has been revealed in some occlusal anomalies, which is characterized by their reduction depending on the CTD severity degree. Thus, reverse incisal occlusion in the moderate degree decreased by 3.01 times compared with the mild degree, and the severe degree by 1.06 times compared with the moderate degree of CTD. A similar situation is determined in the crossbite, where the values were 1.11 and 1.47 times, and in the mesial occlusion 1.17 and 2.11 times and the retrolateralization of the mandible (sagittal gap) 1.66 and 1.60 times, respectively ($p<0.05$). At the same time, in the indices of vertical incisal dysocclusion, open bite and straight occlusion, a variety of their frequency at different degrees of CTD severity was determined.

The indicators of the maxillary and mandibular arches occlusion prevailed, where their average value was at 29,16+0,29% in the structure of dental anomalies, due to the hard palate vault height (Table 2). Then there were shortening of maxillary and mandibular arches - 22,03+0,22%, less often we detected diastems - 2,67+0,02% and tremors - 2,63+0,02%, where the lowest value was noted in the data of upper central line displacement - 1,46+0,01%.

It should be noted that the narrowing of the maxillary and mandibular arches jaws at the average degree of congenital dysgenesis increased by 1.03 times compared to the mild degree, and at the severe degree by 1.10 times compared to the average degree of CTD. This trend is determined in deep traumatic incisor overlap, where the rates were 2.94 and 2.35 times, and in data of maxillary underdevelopment 1.97 and 7.21 times, three times 1.11 and 1.20 times, respectively. At the same time, no upward dynamics was detected in the rates of diastema detection, where the maximum value was detected in CTD of moderate severity. In general, the data characterize the presence of a trend increasing frequency of dentition anomalies depending on the severity of CTD.

In the general structure of tooth position anomalies due to the hard palate

Table 3

Structural characteristics of dental position anomalies in various degrees of connective tissue dysplasia severity

CTD severity degree due to the hard palate vault height	Tight incisor position	Vestibular position of upper canines	Palatine position	Mesio position	Protrusion	Linguaposition	Tortoanomaly	Multiple	Supraposition	Retroversion pf upper canines	Reverse occlusion	Macrodontis of incisors	Retroversion of low incisors	Disturbance of bigeminy, sequence of eruption	Primary edentulism	Failure of premolar eruption	Lateral position	Retention	Protrusion	Dystopy	Direct occlusion
Mild	17.83±0.41[1,2]	7.48±0.46[2]	12.47±0.44[2]	8.24±0.46[2]	1.39±0.49[2]	2.63±0.49[1]	19.28±0.40[1,2]	0	0	0.47±0.52[2]	8.31±0.46[2]	12.82±0.44[2]	0.71±0.48[2]	0	1.35±0.50[2]	1.94±0.49	2.74±0.49[2]	1.34±0.49[2]	0	0.55±0.53[2]	1.41±0.51[1,2]
Medium	22.03±0.39[1,2]	9.51±0.45[1,2]	5.72±0.47[1,2]	11.57±0.44[1,2]	4.25±0.48[1]	4.51±0.47[1,2]	17.34±0.41[1]	3.01±0.49	0.30±0.50	1.50±0.49[1,2]	2.86±0.49[1,2]	7.59±0.46[1,2]	1.66±0.49[2]	1.79±0.49	2.15±0.49	0.81±0.50[2]	0.77±0.52[1]	0.84±0.50[2]	0.30±0.50	1.20±0.50	3.68±0.48[1,2]
Severe	37.86±0.31[1,2]	14.24±0.43[1,2]	4.59±0.48[1,2]	9.67±0.45[1,2]	6.47±0.47[1,2]	1.52±0.49[1,2]	15.63±0.42[1,2]	0	0	5.03±0.48[1,2]	0.37±0.57[1,2]	7.28±0.46[2]	5.11±0.48[1,2]	0	2.35±0.49	0.72±0.50[2]	0.27±0.25[2]	4.31±0.48[1,2]	0	2.18±0.49[2]	0
Average values	25.75±0.25	10.41±0.05	7.59±0.06	9.79±0.09	4.03±0.04	2.88±0.02	17.41±0.17	3.01±0.49	0.30±0.50	2.33±0.03	3.84±0.02	9.23±0.09	2.49±0.01	1.79±0.49	1.95±0.01	1.57±0.01	1.26±0.01	2.16±0.02	0.30±0.50	1.31±0.01	2.54±0.01

vault height in various degrees of CTD severity (Table 3), the average indicators of close incisors prevailed, where their average value was at the level of 25.75+0.25%, followed by tortoanomalies - 17.41+0.0, 17% and vestibuloposition of upper canines - 10.41+0.0.5%, followed by mesioposition of first molars - 9.79+0.09%, palatine position of upper second incisors - 7.59+0.06% and less frequently other anomalies of tooth position were revealed, where the average values ranged from 0.30+0.50% to 5.58+0.02%.

It should be emphasized that there is a definite pattern of increase depending on the severity degree in some anomalies of tooth position. Thus, an increase of 1.23 times compared to the mild CTD degree and an increase of 1.72 times compared to the moderate degree of severity in the data of close incisor positioning. This trend was found for vestibuloposition of upper canines, protrusion of upper first incisors, retrusion of upper incisors, retrusion of lower incisors, primary adentia of lower jaw incisors and dystopia of upper canines, where the rates were 1.21 and 1.50 times, 3.06 and 1.52 times, 3.19 and 3.35 times, 2.33 and 3.08 times, 1.59 and 1.09 times, 2.18 and 1.82 times respectively.

It should be stressed that a certain group of dental position anomalies revealed a decrease in their indicators depending on the CTD severity. Thus, the index decreases by 2.18 times compared to the mild degree, and by 1.25 times compared to the moderate degree in the severe degree in the palatine positioning data of the upper second incisors. Such a situation was observed in upper and lower jaw incisor tortoanomalies, upper incisor reverse occlusion, incisor macrodentia, premolar eruption timing disorder, and lateroposition of upper second incisors, where the rates were 1.11 and 1.10 times, 2.90 and 7.73 times, 1.69 and 1.04 times, 2.39 and 1.12 times, 3.56 and 2.85 times respectively.

It should be noted that other anomalies of tooth position have been identified that do not tend to increase or decrease in their frequency depending on the severity of CTD. It includes mesioposition of the first molars, lingual position of premolars, supercomplex teeth in the area of the upper incisors, supraposition of the upper first incisors, violation of pairing and sequence of eruption, retention of lower premolars, protrusion of upper first incisors, and direct occlusion of upper first incisors, where their averages were 9.79+0.09%, 2.88+0.02%, 3.01+0.49%, 0.30+0.50%, 1.79+0.49%, 2.16+0.02%, 0.30+0.50%, and 2.54+0.01%.

It is important to emphasize that various aspects of the local and general manifestations of CTD, which are represented by a wide range of symptoms, have been studied. However, studies of dentoalveolar anomalies are conducted without taking into account the CTD severity and hard palate height data.

Pearson correlation analysis revealed the presence of correlation between the gothic palate (r=0.64) and anomalies of teeth position (r=0.73) and narrowing, deformation of dental arches (r=0.85) and anomaly of occlusion (r=0.82), which characterizes the presence of direct correlation between the hard palate vault height and dentoalveolar anomalies at various degrees of CTD severity.

Conclusion. Our research has established the presence of actual pattern of increased prevalence of occlusal anomalies depending on the CTD severity, taking into account the hard palate vault height (distal occlusion, mandibular retroversion and deep traumatic incisal overlap), as well as dental deformities (narrowing of the maxillary and mandibular arches, deep traumatic incisal overlap, underdevelopment of the maxilla and trims) and anomalies of the teeth position (close position of incisors, vestibuloposition of upper canines, protrusion of the upper first incisors, retrusion of upper incisors, retrusion of lower incisors, primary adentia of lower jaw incisors and dystopia of upper canines). In addition, a pattern of decreasing prevalence of occlusal anomalies (reverse incisal occlusion, crossbite, mesial occlusion, retroposition of the lower jaw) as well as dental position anomalies (palatinoposition of the upper second incisors, tortoanomalies of upper and lower incisors, upper incisors backward occlusion, macrodentia of incisors, premolar eruption timing disorders, lateral position of upper second incisors) taking into account the hard palate vault height in different degrees of CTD severity. For the first time, the established regularities of frequency and structure of dentoalveolar anomalies at different degrees of CTD severity due to the hard palate vault height will have a positive effect in the timely development of an individual plan of complex medical and social rehabilitation of school-age children with congenital collagenopathy.

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DOI 10.25789/YMJ.2023.81.06

УДК 57.084.1

STUDY OF THE EFFECTS OF VARIOUS CRYOPRESERVATION METHODS ON VIABILITY OF HUMAN GASTROINTESTINAL TUMOR XENOGRAFTS IN *IN VIVO* MODELS

Background. Modern research requires access to tumor models for various types of studies on a single patient xenograft. Biobanks store tissue fragments and cell cultures for various studies, and it is necessary to form protocols for cryopreservation of various tumor fragments and create collections of biomaterial. **Material and methods.** The study was performed on tumor material from patients with malignant gastrointestinal tumors (esophagus, stomach, and colon). An experiment included 90 Balb/c Nude mice. The third generation of a PDX model was used for cryopreservation. The effectiveness of 3 protocols for cryopreservation of tumor tissue was evaluated using RPMI nutrient medium, fetal bovine serum (FBS), dimethyl sulfoxide (DMSO) and Mr. Frosty containers. 90 days after the freezing of the tumor nodes, the samples were thawed and implanted to animals; engraftment was noted, and the onset of tumor nodes in animals was recorded. At the end of the experiment, the data were analyzed statistically. **Results.** Protocol 1 was the least suitable for preserving fragments of esophageal, colon, and gastric tumors. Protocols 2 and 3 showed higher engraftment rates. The lowest engraftment rate was registered for reanimated gastric cancer PDXs. **Conclusions.** Protocols 2 and 3 with slow freezing of samples should be used for cryopreservation of human esophageal and colon cancer xenografts. Gastric cancer PDXs require other cryopreservation methods due to the low efficiency of the existing ones.

Keywords: cryopreservation, PDX, esophageal cancer, stomach cancer, colon cancer, in vivo.

Introduction. Patient-derived xenograft (PDX) models are increasingly used for various *in vivo* studies. These models reproduce the morphological and biological characteristics of the disease close to human ones [3]. Modern research requires access to biological models designed on tumor material obtained from a single patient. Such studies require a collection of biological material, such as fragments of tumor nodes, certified cell lines, and primary cell cultures [4]. This collection allows using previously generated PDX models in future studies. Its creation is based on the development and testing a tumor fragment freezing procedure and an assessment of its viability after reanimation from cryogenic freezing [10]. With patient-derived xenografts, this procedure allows the formation of a biobank of early-generation PDXs with the possibility of their thawing and re-implantation at any time depending on demand [1, 7].

The purpose of this study was to test three methods of cryopreservation of tumor material obtained from a patient and to assess the engraftment of these samples after rehabilitation.

Material and methods. *Tumor material.* The study was performed on tumor material from patients with malignant gastrointestinal tumors (esophagus, stomach, and colon). All patients gave their written informed consent for the biological material transfer. The study was approved by the ethical committee (protocol №4 from 15.02.2021).

The patients were diagnosed with:

- Esophageal cancer – squamous cell cancer T3N1M0.
- Stomach cancer – adenocarcinoma T3N1M0.
- Colon cancer – adenocarcinoma T3N0M0.

Biological material was collected immediately after excision of the tumor nodes; the tissue was placed in a DMEM nutrient medium with an antibiotic (gentamicin) and delivered to the laboratory. Prior to implantation, tumor fragments were cleaned of necrosis, blood vessels, and connective tissue and divided into fragments 1x1x1 cm.

Animals. The experiment included 90 Balb/c Nude mice (females) aged 4-5 weeks, weighing on average 23 g. The animals were kept in the SPF vivarium at the Experimental Laboratory Center, National Medical Research Centre for Oncology.

The animals were divided into groups and subgroups:

1. Esophageal cancer – 36 mice divided into 3 subgroups with different cryopreservation protocols, 12 animals each;
2. Colorectal cancer – 36 mice divided into 3 subgroups with different cryopreservation protocols, 12 animals each;
3. Stomach cancer – 18 mice divided into 3 subgroups with different cryopreservation protocols, 6 animals each.

The study was approved by the bioethical committee.

Tumor model creation. The third generation of subcutaneous PDX tumors of the human gastrointestinal tract was used for cryopreservation of biological material; the generation was obtained in previous successive inoculations and characterized by 100% transplantability. The recipient animals received anesthesia with 2 stages: introduction of the muscle relaxant xylazine (15 ml/kg of body weight of Balb/c Nude mice); introduction of general anesthesia - zoletil (67.5 ml/kg of body weight of Balb/c Nude mice). An incision was made on the side of the mouse above the rear paw, a pocket of adipose tissue was formed in the cavity of the surgical wound, and a fragment of the donor tumor was placed into it.

Cryopreservation. When the xenografts reached a volume of 400 mm^3, the tumor node was isolated from the animal body, and connective tissue, necrosis,

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and blood vessels were removed; then it was divided into fragments 3x3x3 mm and placed in a sterile cryogenic tube with a mixture for cryopreservation.

The study assessed the effectiveness of 3 protocols for cryopreservation of tumor node fragments.

1. Protocol 1. Medium for cryopreservation: 80% of RPMI 1640 medium; 10% FBS; 10% DMSO.
2. Protocol 2. Medium for cryopreservation: 90% FBS; 10% DMSO. Samples were freezed and stored in Mr.Frosty containers (Thermo Fisher) at -80°C.
3. Protocol 3. Medium for cryopreservation: 80% of RPMI 1640 medium, 10% FBS, 10% DMSO. Samples were freezed and stored in Mr.Frosty containers (Thermo Fisher) at -80°C.

Cryopreservation lasted on average for 90 days for all protocols.

Assessment of tumor engraftment after cryopreservation. 90 days after freezing of the tumor nodes, the samples were thawed in accordance with standard protocols by rapid heating in a 37°C water bath for 90-120 seconds to achieve maximum viability [8]. After reanimation, the samples were washed in a nutrient medium to remove DMSO and implanted into animals. The number of engrafted implants was noted, and the onset of tumor nodes in animals was recorded.

Statistical analysis of the data. Differences between groups were determined using parametric Student's test.

Results and discussion. We analyzed the engraftment rate of xenografts after cryopreservation. The data showed that protocol 1 was the least suitable for preserving fragments of esophageal, colon, and gastric tumors. Protocols 2 and 3 showed higher engraftment rates. The highest engraftment was observed for esophageal cancer PDXs: 41.6%; 83.3%; 83.3% for protocols 1, 2, 3, respectively. The lowest engraftment rate was registered for reanimated gastric cancer xenografts. The data on the engraftment rates are shown in Figure 1.

In addition to engraftment, we evaluated time to the growth of xenograft tumor nodes, since it can suggest the degree of cell viability after cryopreservation. The longest time to tumor growth was registered for protocol 1: the average time was 70±2.9 days for esophageal cancer, 81±1.0 days for gastric cancer, and 74±3.2 days for colon cancer. Protocols 2 and 3 led to similar results. Time to growth of xenografts of esophageal and colon cancer in protocol 2 was on average 41 days ±1.9; ±2.3, respectively, and tumor nodes of gastric cancer formed

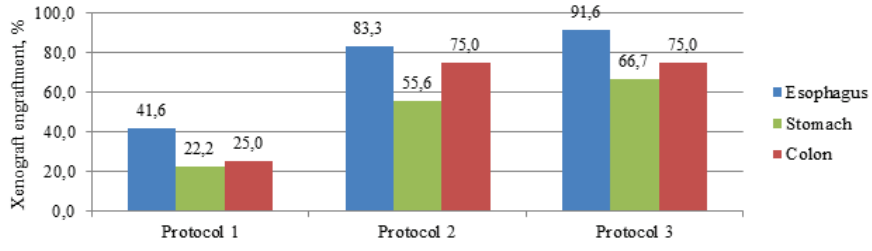

Fig. 1. Engraftment rates for xenografts of esophageal, stomach and colon cancer after cryopreservation

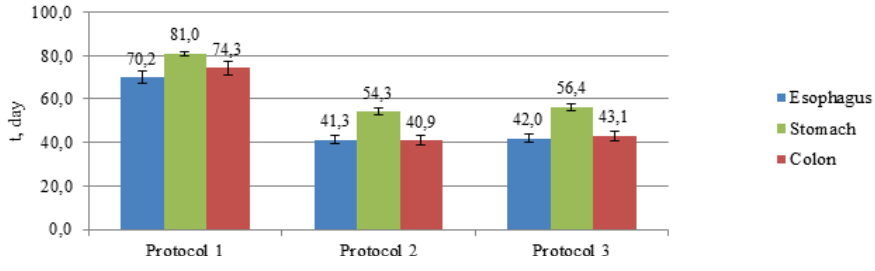

Fig. 2. Time to growth of xenografts of esophageal, stomach and colon cancer after cryopreservation

Comparison of the onset rates of esophageal, stomach and colon cancer after cryopreservation according 3 different protocols

Tumor	Protocol 1/protocol 2 p-value	Protocol 1/ protocol 3 p-value	Protocol 2/ protocol 3 p-value
Esophagus	0.002	0.002	0.84
Stomach	0.1	0.08	0.39
Colon	0.016	0.018	0.63

after 54 days ± 1.7 from the time of implantation. The growth of esophageal tumors in protocol 3 was noted at 42±1.9 days; gastric tumors on average 56±1.6 days; and colon on average 43±2.3 days from the time of implantation. The data is shown in Figure 2.

Statistical analysis of tumor formation rates showed significant differences between protocols 1 and 2, and protocols 1 and 3 in groups with xenografts of esophageal cancer and colon cancer (Table 1). The comparison of protocols 2 and 3 did not demonstrate significant differences in all groups. The results for three cryopreservation protocols did not differ significantly in the group with gastric cancer xenografts.

Discussion. Some studies report the development of various protocols for cryopreservation of tissue samples derived from animals and humans. Munroe et al. determined the advantage of DMSO as a cryoprotectant on the cells of the marine sponge Dysidea etheria in comparison with other tested substances [6]. Faltus et al. studied the thermal properties of various cryoprotectants; as a result, they also recommended DMSO as a cryoprotective agent for freezing cell suspensions [2]. Based on these studies, we chose DMSO as a cryoprotectant for freezing PDX fragments. In mixtures, it ensured the preservation of cell viability after thawing of the samples. However, in protocol 1 (freezing in a mixture of 80% RPMI 1640 culture medium, 10% FBS, and 10% DMSO) showed the lowest engraftment rate and longer time to tumor growth, compared with the other two protocols. Probably, this difference was due to the higher freezing rate in the first case than in the other two. The rate of freezing of samples directly affects their viability after reanimation, since slow freezing is accompanied by the outflow of intracellular fluid preventing the formation of ice in the cell [5]. In our study, the necessary rate was provided by the Mr. Frosty freezing container filled with isopropyl alcohol, it provided a freezing rate close to -1°C/min. However, all protocols demonstrated poor engraftment for human gastric cancer samples. According to Yan et al. creating a comprehensive collection of gastric cancer biological material, tumor fragments were subjected to instant freezing in order to successfully preserve

cell viability, which prevented the formation of ice crystals that injured cell walls [9]. Apparently, slow freezing, suitable for esophageal and colon cancer, was not applicable to the stomach tumor tissue. We will take it into account in the further development of cryopreservation protocols.

Conclusions. Protocols 2 and 3 with slow freezing of samples should be used for cryopreservation of human esophageal and colon cancer xenografts. Gastric cancer samples require other cryopreservation methods due to the low efficiency of the existing ones.

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GENETIC AND IMMUNOLOGICAL MARKERS OF THE FORMATION OF METABOLIC SYNDROME IN SCHOOL CHILDREN (ON THE EXAMPLE OF THE PERM REGION)

DOI 10.25789/YMJ.2023.81.07

УДК 612+613.9

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The problem of the formation of metabolic syndrome in children is becoming more and more urgent every year, which is associated with excess nutrition, physical inactivity, increased psycho-emotional stress, therefore, timely identification of immune and genetic markers of predisposition to the development of this pathology will allow identifying possible health risks at an early stage and preventing their implementation in adulthood. **The aim of the study:** To evaluate the indicators of immune status and genetic polymorphism of candidate genes as markers of the development of metabolic syndrome in school children (on the example of a secondary school in Perm). **Materials and methods.** The study involved 214 school-age children. Three groups were formed, ranked according to the body mass index criterion: observation group1 with metabolic syndrome (BMI SDS >2.0), observation group2 with excess body weight (BMI SDS >1.0 <2), comparison group – absence of excess body weight (BMI SDS <1.0). The evaluation of immune (IL 1B, IL 4, CD19+), neuroregulatory (leptin), metabolic (glucose, HDL, triglycerides), genetic (ADRB rs1042413, PPARA rs4253778) indicators was carried out. **Results and discussion.** It was found that the group of children with metabolic syndrome and excess body weight in relation to the comparison group was characterized by an increase in CD19+ expression by 1.3 times, a decrease in the content of anti-inflammatory cytokine IL4 by 1.5 times, overexpression of pro-inflammatory cytokines (IL1b by 1.9 times), leptin by 2.0 times, an imbalance of lipid-carbohydrate metabolism (reduction of HDL by 7%, against the background of an increase in triglyceride levels by 17% and glucose levels by 8%), significant changes in the frequencies of genotypes associated with metabolic syndrome (increased frequency by 2.7 times of the typical AA genotype of the ADRB2 gene rs1042713, OR=3.79 CI:1.25-11.47; p<0.05, as well as by 4.6 times of the variant CC genotype of the PPARA gene rs4253778 OR=5.00; CI:0.97-25.89; p<0.05). **Conclusion.** Candidate immunological (CD19+, IL 1b, IL4) and genetic (ADRB2 rs1042713, PPARA rs4253778) markers are recommended to be used as indicators for identifying early signs of metabolic syndrome in school-age children living in the Perm region.

Keywords: metabolic syndrome, body mass index, PPARG gene, ADRB2 gene, cytokines, CD, schoolchildren.

Introduction. The peculiarities of the development of the child's body, as well as bad habits, sedentary lifestyle, excess nutrition, genetic predisposition, increased emotional stress lead to the formation of metabolic syndrome in children become risk factors for the development of cardiovascular diseases in adulthood. If earlier the diagnosis of metabolic syndrome was applicable only to the adult

population, today the manifestations of this syndrome are noted in children and adolescents with greater frequency [1].

Metabolic syndrome combines a complex of symptoms including metabolic, hormonal and psychosomatic disorders. For children, the exogenous contributing factors to the development of this syndrome are inactivity, excessive food intake and stress, and the markers accompanying this change are carbohydrate and lipid imbalance, hormonal and immune disorders [2,3].

Russian statistics show that the percentage of children with obesity and malnutrition is growing, and overweight is observed in every fifth child of school age [4].

Physical inactivity of modern children in combination with improper diet and increased psycho-emotional stress during the educational process has a negative impact on the health of children, which is expressed in increased fatigue, irritability, eating disorders, decreased immunity and quality of life of children in general [5].

Excess and unbalanced nutrition contributes to the development of immune disorders. The state of metabolism and the immune system directly depend on the gut microbiome, where an excess of some substances affects the deficiency of others, which disrupts the extraction of energy from consumed foods, contributing additional calories, as well as fueling lipogenesis and gluconeogenesis [6].

The central system regulating the feeling of satiety is the dopaminergic system, where dopamine and leptin act as key neuropeptides in the regulation of metabolism. An increase in leptin in the blood leads to a feeling of satiety and, accordingly, a decrease in the intake of additional energy from food, correlates with the amount of fat mass in the human body, having a long-term effect on brain mechanisms. Thus, with prolonged accumulation of triglycerides, the release of leptin into the blood by adipocytes increases. Pro-inflammatory cytokines and elevated glucose levels also contribute to the release of leptin, while leptin potentiates the pyrogenic effect of IL1. It is known that leptin has a direct relationship with the level of dopamine involved in the regulation of metabolism. Chachhiani I. and co-authors suggest that elevated concentrations of leptin in the first stages of inflammation can stimulate cortisol synthesis, thereby inhibiting the effects of the hypothalamic-pituitary-adrenal axis [7-9].

Genetic predisposition makes a decisive contribution to the development of metabolic syndrome. Thus, the PPARA gene is expressed in tissues with a high level of mitochondrial oxidation (liver, heart, vascular walls), is activated by fatty acids, participates in lipid oxidation and lipoprotein metabolism, thereby counteracting the formation of metabolic syndrome and aging. The PPARG receptor has anti-inflammatory and antiproliferative effects. The expression product of the ADRB2 gene is a lipolytic receptor in fat cells, and is associated with lipid mobilization. The Arg16Gly and Gln27Glu polymorphisms of the ADRB2 gene are associated with the development of metabolic syndrome and pathology of the cardiovascular system in adults [10,11].

The determination of markers associated with the development of metabolic syndrome in children is associated with certain difficulties associated with the peculiarities of the development of the child's body, puberty, mental disorders, and also associated with gender. For example, girls are more susceptible to the development of metabolic syndrome during puberty, which is due to hormonal transformations. The lipid profile may also depend on age, while there is no clearly defined range of insulin norms for children of different ages, especially during puberty.

It is relevant today to identify immunological and genetic markers of the formation of metabolic syndrome in children under conditions of increased psycho-emotional stress associated with the educational process.

The aim of the study: To evaluate the indicators of immune status and genetic polymorphism of candidate genes as markers of the development of metabolic syndrome in school children (on the example of a secondary school in Perm).

Materials and methods. The study involved 214 children (7-17 years old) attending secondary general educational institutions in Perm. The observation and comparison groups were formed based on the assessment of body mass index and divided into groups of children according to the WHO classification: observation group1 – children with metabolic syndrome (BMI SDS >2.0) (12.3±0.7 years), observation group2 - overweight children (BMI SDS >1.0 <2) (11.3±0.5 years), the comparison group – children with no excess body weight (BMI SDS <1.0) (11.6 ± 0.3 years).

The lipid, carbohydrate, and immune profiles were evaluated for the examined children, as well as the polymorphism of candidate genes in the development of metabolic disorders.

The level of triglycerides and HDL was assessed by photometric method, the level of glucose in serum and plasma was determined by glucose oxidase method on the Keylab BPC+Biosed device.

The level of absolute and relative expression of CD19+ B-lymphocytes was assessed by the method of membrane immunofluorescence using FACSCalibur (Becton Dickinson) device.

The level of proinflammatory cytokines TNF and IL4, as well as the level of leptin, were evaluated by the method of enzyme-linked immune blood analysis on the BioTEC Elx808 device.

The polymorphism of candidate genes was evaluated by real-time polymerase chain reaction on a Bio RAD CFX96 device with an assessment of allelic discrimination. The features of polymorphism of candidate genes are investigated: *ADRB2* Arg16Gly rs1042713, *PPARA* G2528C rs4253778.

Statistical analysis was carried out using parametric and nonparametric research models in the Statistica 10.0 program, with an assessment of X-mean, SD-deviation, SE-error, W-normality of distribution, Student's t-test, Mann-Whitney U-test, p- significance level. Statistical analysis of candidate genes was evaluated using multiplicative, general, dominant and recessive inheritance models, with the evaluation of indicators x2- chi-square criterion, OR- odds score, CI – confidence interval, p – significance level. The results were considered significant at $p<0.05$.

Results and discussion. The immune profile of the children of observation group 1 relative to the comparison group was characterized by a significant change in cellular regulation indicators: an increase in the absolute and relative expression levels of CD19+ B-lymphocytes by 1.3 and 1.2 times, respectively.

The lipid profile of the children of the observation group1 relative to the comparison group was characterized by a significantly increased triglyceride level by 1.5 times (1.15±0.20 mmol/dm3 versus 0.78±0.04 mmol/dm3) and a reduced HDL level by 1.2 times, carbohydrate metabolism was characterized by a significant increase in blood glucose by 8% ($p<0.05$) (Tab.1).

The assessment of the immune profile showed that the children of observation group 2, relative to the comparison group, are characterized by an increase in the expression of cytokine IL1b by 1.9 times and a decrease in IL4 by 1.5 times.

In the children of the observation group2, relative to the comparison group, HDL levels were significantly lowered by 8% and triglyceride levels were increased by 18% and leptin by 2.1 times ($p<0.05$).

Thus, it was found that excess body weight in school-age children is accompanied by hormonal dysregulation in the form of hyperproduction of the peptide hormone leptin, which promotes proliferation and activation of monocytes and macrophages, accompanied by increased production of proinflammatory cytokines [12]; features of immune regulation (overexpression of CD19+ B-lymphocytes, IL1beta proinflammatory cytokines, insufficient expression of anti-inflammatory cytokine IL4), however, in the studies of N.Y. Grishkevich and co-authors, analysis of lymphocyte subpopulations in the blood of obese children showed a decrease in absolute and relative expression of B-lymphocytes [13]; an imbalance of markers of carbohydrate and lipid metabolism (high expression of glucose and triglycerides in the blood, a decrease in HDL levels), where the main change in the lipid composition of the blood in metabolic syndrome is precisely an increase in triglycerides and a decrease in HDL [14].

The results of the genetic analysis showed significant changes in the frequencies of alleles and genotypes of candidate genes between the study groups that differ according to the SDS BMI criterion: the PPARA G2528C rs4253778 peroxisome activator gene and the ADRB2 Arg16Gly rs1042713 adrenoreceptor gene (Table 2).

The frequency of homozygous wild genotype AA of the adrenergic receptor gene ADRB2 Arg16Gly rs1042713 was significantly increased in children of the observation group1 relative to the comparison group by 2.7 times, inherited by the dominant type, with allele A (OR=2.31; CI:1.11-4.82; p<0.05) and genotype AA (OR=3.79 CI:1.25-11.47; p<0.05) significantly increase the likelihood of developing metabolic syndrome.

For children of the observation group2, relative to the comparison group, an increase in the frequency of the variant homozygous CC genotype of the PPARA G2528C rs4253778 gene by 4.6 times, inherited by recessive type, was found, and the odds assessment indicates the likely participation of this genotype in metabolic disorders and the formation of excess body weight (OR=5.00; CI:0.97-25.89; p<0.05).

Thus, it was found that the variant allele of the PPARA gene is associated only with excess body weight, when the modified oxidation-peroxidation program can be adjusted by diet, which is sufficient to stop the increase in clinical manifestations of obesity. The transcription factor PPARA regulates the expression of several dozen genes involved in the regulation of cellular differentiation, inflammatory response, glucose and lipid metabolism [15]; whereas the polymorphism of the ADRB2 catecholamine regulation gene under the conditions of the intensity of the educational process triggers a hormonal mechanism associated with leptin overexpression, which leads to more pronounced changes in BMI towards its increase with the development of metabolic syndrome and the need for its drug correction. According to Mitra S. research and the co-authors showed an association of polymorphism of the ADRB2 gene with changes in HDL levels, and the AA genotype of the gene was associated with higher blood glucose levels in children [16].

Conclusion. The present study of the immunological and genetic profile of school-age children (7-17 years old), living in the Perm region, exposed to psycho-emotional stress of the academic load has substantiated the indicator indicators of early diagnosis of the formation of metabolic syndrome and overweight, characterizing violations of immune and metabolomic regulation, including markers of cellular regulation B-lymphocytes

Table 1

The results of a comparative analysis of the immune and metabolomic profile of the studied groups of children according to the SDS BMI criterion.

Indicator	Standard	Observation group1	Observation group2	Comparison group	p1	p2
CD19+ abs	0.09-0.66 (*10^9/л)	0.38±0.04	0.30±0.02	0.29±0.01	0.0320	0.5370
CD19+ rel	6-25 (%)	14.43±0.84	12.97±0.56	11.91±0.27	0.0060	0.1040
ИIL1b	0-6 (пг/мл)	2.97±0.65	4.81±0.97	2.54±0.21	0.5460	0.0320
IL4	0-4 (пг/мл)	1.77±0.36	1.50±0.15	2.28±0.23	0.2560	0.0080
Glucose	3.33-5.55 (ммоль/дм3)	5.018±0.09	4.72±0.08	4.64±0.04	0.0010	0.4180
Triglycerides	0.3-1.7 (ммоль/дм3)	1.15±0.09	0.91±0.07	0.77±0.02	0.0000	0.0590
HDL	0.8-2.2 (ммоль/дм3)	1.46±0.08	1.59±0.04	1.72±0.03	0.0040	0.0290
Leptin	1.1-27.6 (нг/мл)	33.31±9.78*	15.75±1.15	7.49±0.78	0.1320	0.0000

Note: *- significant difference with the norm, abs- absolute, rel- relative, p1- significant differences between groups of observation1/comparison; p2-significant differences between groups of observation2/comparison.

Table 2

The results of a comparative analysis of the frequencies of alleles and genotypes of candidate genes in the studied groups of children differing by the SDS BMI criterion

Groups	Gen	Genotype	X^2(p)	OR(CI)	allele	X^2(p)	OR(CI)
Observation1/ Comparisons	*ADRB2* Arg16Gly rs1042713	AA	6.44 (0.0112)	3.79* (1.25-11.47)	A	5.23 (0.0222)	2.31* (1.11-4.82)
		AG		0.76 (0.26-2.19)			
		GG		0.46 (0.14-1.47)	G		0.43 (0.21-0.90)
Observation2/ Comparisons	*PPARA* G2528C rs4253778	GG	4.73 (0.0296)	1.00 (0.46-2.16)	G	0.42 (0.5164)	0.81 (0.43-1.53)
		GC		0.72 (0.31-1.64)			
		CC		5.00 (0.97-25.89)	C		1.23 (0.65-2.33)

Note: X2 is chi-squared, p - significance level, OR – odds ratio, CI - confidence interval, * -reliability of the results.

CD19+, pro- and anti-inflammatory cytokines IL1b, IL4, an indicator of neuropeptide regulation (leptin) associated with polymorphism of alleles and genotypes of candidate genes of the adrenoreceptor gene ADRB2 rs1042713 (A allele and AA genotype) and the PPARA peroxisome receptor gene rs4253778 (CC genotype).

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THE FREQUENCY OF METABOLIC SYNDROME AND ITS COMPONENTS IN THE NON-INDIGENOUS POPULATION OF SOUTH YAKUTIA

DOI 10.25789/YMJ.2023.81.08

УДК 616-01/09

A one-stage study of the working non-indigenous population of South Yakutia was conducted. A high incidence of abdominal obesity, lipid-metabolic disorders has been shown. Dyslipidemia, arterial hypertension and metabolic syndrome, mainly represented by a three-component, were most often registered in men compared to women. The relationship of blood pressure with triglyceride and glucose levels was obtained.

Keywords: metabolic syndrome, dyslipidemia, arterial hypertension, non-indigenous population, South Yakutia.

Metabolic syndrome (MS) remains a global epidemic in the XI century, increasing its growth rates, causing terrible complications such as type 2 diabetes, stroke, myocardial infarction, etc. Often, the presence of MS exacerbates the course of cardiovascular pathology, increasing the risk factors for its development. Its prevalence in the world ranges from 10 to 30% according to foreign and domestic authors, depending on various criteria for its diagnosis [4;9;10]. According to some estimates, it reaches up to 1/3 of the world's population [7]. The total cost of treatment and economic losses associated with this syndrome are estimated in trillions. The study of MS in the working non-indigenous population of the North is relevant, research in this field is extremely scarce. The spread of MS and its patients becoming younger is the main reason for the relevance of the study. It is also due to the high risk of developing cardiovascular diseases and their mortality in the industrial cities of the Far North.

The aim of the study was to assess the frequency of occurrence of metabolic syndrome and its components in non-indigenous residents of South Yakutia.

Materials and methods of research. A one-time population study of the working population of non-indigenous nationality in the Aldan district of the Republic of Sakha (Yakutia) was conducted. According to the list of employees of industrial and social spheres, every 3rd employee was invited for examination. The response rate was 75%. 174 residents of the Aldan district of working age were analyzed, 66 of them were men, whose

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median age (Me) was 45.0 [34.5-53.0] years, and 108 were women, Me 43.0 [36.0-51.75] years.

All study participants were interviewed, complaints were collected, anamnesis was taken, an anthropometric study with the determination of height, body weight, measurement of waist circumference (WC) and hips, measurement of blood pressure (BP), blood was taken from the ulnar vein in the morning on an empty stomach with an 8-12-hour interval after the last meal, a clinical examination by a cardiologist was also conducted. Respondents signed a voluntary consent to the study according to the Protocol of the Ethics Committee of the YSC CMP.

To detect abdominal obesity, the circumference was measured from a standing position, patients were in underwear. The measurement point is the midpoint of the distance between the top of the iliac crest and the lower lateral edge of the ribs. It does not necessarily have to be at the navel level. When WC > 94 cm in men and > 80 cm in women, it can be assumed that the patient has abdominal type of obesity (criteria of the VNOK 2009).

Blood pressure was measured twice with an automatic tonometer "OMRON M2 Basic" (Japan) in a sitting position with the calculation of average blood pressure with a limit of permissible measurement error of ± 3 mm Hg (ESH/ESC, 2013). Arterial hypertension (AH) was assumed at a blood pressure level≥140/90 mmHg or if the patient was taking antihypertensive drugs during the examination period (ACC/AHA Guideline, 2017).

Laboratory analyses were carried out by the enzymatic method on an automatic biochemical analyzer "Labio" using "Analyticon" reagents (Germany). Laboratory research methods included: determination of the lipid spectrum (total cholesterol (TC), low-density lipoprotein cholesterol (LDL cholesterol), high-density lipoprotein cholesterol (HDL cholesterol), triglycerides (TG)) and glucose.

For diagnosing lipid disorders, the Russian recommendations of the VII revision of the Russian Society of Cardiology 2020, compiled taking into account the European Recommendations of 2019, were applied. Hypercholesterolemia (HCH) was established at the level of TC ≥ 5.0 mmol/l (190 mg/dl), taking into account the cardiovascular risk on the SCORE scale, elevated LDL cholesterol - LDL cholesterol 3.0 mmol/l (115 mg/dl) low, 2.6 mmol/l, moderate, 1.8 mmol/ll, high, 1.4 mmol/l, very high risk on the SCORE scale. Hypo-α-cholesterolemia (Hypo-α-CS) is the level of HDL cholesterol < 1.0 mmol/l (40 mg/dl) in men and 1.2 mmol/l (46 mg/dl) in women. Hypertriglyceridemia (HTG) was attributed to a TG level of 1.7 mmol/l (150 mg/dl). Hyperglycemia (HG) on an empty stomach was established at a glucose level of 6 mmol/l. Also included are patients with these disorders receiving specialized medical treatment for these conditions.

The criteria of metabolic syndrome (MS) were applied according to the recommendations for the management of patients with MS [6]:

The presence of the main component of the disease – abdominal obesity (AO) and 2 additional criteria: an increase in blood pressure > 140/90 mmHg, an increase in TG>1.7 mmol / l, a decrease in HDL cholesterol (in men <1.0, in women < 1.2 mmol / l), an increase in LDL cholesterol>3.0 mmol / l, hyperglycemia (glucose > 6 mmol/ L) or impaired glucose tolerance (glucose 2 hours after the glucose tolerance test >7.8 and < 11.1 mmol/ L).

Statistical analysis was carried out using the SPSS STATISTICS software package (version 26.0). Qualitative variables are described by absolute and relative frequencies (%), quantitative variables are described using the mean and standard error of the mean, median (Me) and interquartile range (Q1—Q3). The share comparison of the groups was carried out using the nonparametric Spearman criterion χ2. The odds ratio (OR) and 95% confidence interval (95% CI) were calculated. The correlation analysis was carried out using the Spearman coefficient. The statistical significance of the differences (p) was assumed to be equal to 5%.

The work was carried out under the research project of the YSC CMP "Regional peculiarities of biochemical, immunological and morphological indicators in the indigenous and non-indigenous population of the Republic of Sakha (Yakutia) in normal conditions and pathology" (FGWU-2022-0014) and the research of the Academy of Sciences of the Republic of Sakha (Yakutia) "Assessment of radiation exposure of the population of the Aldansky district due to natural sources of radiation and recommendations for carrying out protective measures to reduce it."

Results and discussion. A comparative analysis of clinical, laboratory and anthropometric indicators in the non-indigenous population of South Yakutia was carried out.

Comparing the average concentrations of biochemical and immunological parameters depending on gender, we obtained significant differences in the average values of TC, HDL, LDL and uric acid (Table 1). Thus, in men, compared to women, the average concentrations of TC, LDL and uric acid significantly prevailed.

Table 1

Average concentrations of laboratory parameters of residents of the Aldan district (M±m)

Parameter	all	men (66)	women (n=108)	p_{m-w}
TC (mmol/L)	5.40±0.10	5.74±0.23	5.20±0.08	0.012
HDL cholesterol (mmol/L)	1.97±0.04	1.70±0.06	2.14±0.05	0.000
LDL cholesterol (mmol/L)	2.57±0.09	3.12±0.19	2.24±0.09	0.000
TG (mmol/L)	1.88±0.10	2.02±0.20	1.80±0.11	0.324
glucose (mmol/L)	5.43±0.12	5.57±0.26	5.34±0.11	0.350
uric acid (mkmol/L)	324.06±5.97	358.64±9.72	302.94±6.85	0.000

Table 2

Comparative characteristics of lipid and carbohydrate metabolism disorders depending on gender

Parameter	all		men		women		χ^2	p_{m-w}
	n	%	n	%	n	%		
HCH	90	51.7	43	65.2	47	43.5	7.67	0.005
HCL LDL	57	32.8	32	48.5	25	23.1	11.93	0.000
Hypo-α-CS	5	2.9	2	3.0	3	2.8	0.009	>0.05
HTG	73	41.9	31	46.9	42	38.9	1.09	>0.05
GG	25	14.4	8	12.1	17	15.7	0.436	>0.05
Hyperuricemia	43	24.7	16	24.2	27	25.0	0.013	>0.05

A correlation analysis of SBP with biochemical parameters of blood was carried out. The relationship of SBP with TG (r=0.306, p=0.000) and glucose (r=0.192, p=0.011) was obtained. In a gender comparison, a significant correlation of SBP in men was obtained only with the level of TG (r=0.254, p=0.040), in women - with the levels of TG (r=0.336, p=0.000), glucose (r=0.209, p=0.030) and uric acid (r=0.224, p=0.020). For the rest of the parameters, no reliable relationship was obtained.

Lipid spectrum disorders were more often recorded in men (Table 2). HCH was found in more than half of the men examined, its atherogenic fraction - in almost half. These values had statistically significant differences in men compared to women.

Atherogenic HCH and HTG were also common, and were registered in almost half of the men. There was no significant difference in the HTG indicator. There are some concerns about the high incidence of HTG in the examined individuals, especially in men. The data revealed by us significantly exceeds the data of a large-scale epidemiological study of PROMETEUS, where HTG is present in 29.2% of the Russian population, where its level is also 1.25 times higher in men compared to women [8].

Metabolic disorders', such as HG, frequency was 14.4%, and were slightly more common in women. In the study in the open urban population of Western Siberia, men accounted for 7.7% and women - 11.9%.

Thus, in men of non-indigenous nationality of South Yakutia, unlike women, dyslipidemia, represented by HCH, including its atherogenic fraction, and HTG, was most often detected.

When analyzing anthropometric indicators, namely the WC criterion, a high incidence of abdominal obesity (AO) was shown in both men (n=42 or 63.6%) and women (n=75 or 69.4%), there were no significant differences between them (χ2=0.694, p=0.405). The results obtained showed higher AO figures compared to the data of studies by Russian scientists in Tyumen, where, according to the criteria of the 2009 VNOK, the frequency of AO in men was 38.8% and 49.8% in women [1;3]

The mean SBP did not differ, in men it was 129.09±2.40 mmHg and 129.91±2.20 mmHg in women. More than half of the respondents had hypertension (56.3% of the total number of surveyed). In gender comparison, it was most often registered in men (n=41 or 62.1%), compared with women (n=57 or 52.8%), no significant differences were obtained (p=0.228). According to the degrees of AH, the same distribution was obtained: AH of the 1st degree 32 people, or 18.4%, 2nd – 34 or 19.5%, 3rd – 32 or 18.4%, respectively. The gender attribute did not have any differences. In the open urban population of Tyumen, the frequency of hypertension was 61.3% in men and 36.8% in women [1;3]. The data obtained in men of non-indigenous nationality are consistent with the research, in women of our pilot study, the frequency of hypertension exceeds the results almost twice as much. We conducted an analysis of the correlation relationship between AO and SBP, during which a direct correlation was obtained between these parameters (r=0.448, p=0.000), confirming the close relationship between hypertension and obesity, which was confirmed by earlier studies of ESSE-RF [5].

Metabolic syndrome (MS) is a combination of the most important risk factors for the development of cardiovascular diseases. The analysis of the prevalence of MS according to the recommendations for the management of patients with MS [6]. As a result, MS was detected in 51 respondents, which amounted to 29.3% of the total number of study participants. The data obtained by us are coordinated with Russian researchers on the study of MS in the framework of ESSE-RF-2 [4]. The gender analysis did not show significant differences, in men the frequency of MS was 33.3% (n=22), in women 27.8% (n=30) (χ^2=0.603, p=0.437). Our data are consistent with previously conducted studies of the working non-indigenous population in the western territory of Yakutia in the industrial city of Mirny, where it is shown that 30.5% of men and 25.9% of women also had MS according to the 2009 VNOK criteria [2].

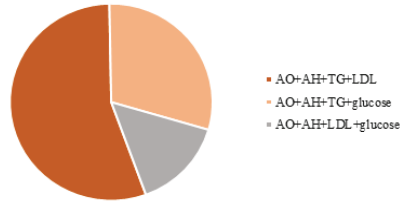
The analysis of the combinations of the main and two additional criteria of MS showed that the most frequent combination were: AO+AH+TG (n=43; 24.7%), AO+AH+LDL (n=25; 14.4%); AO+LDL+TG (n=17; 9.8%). The greatest contribution to the development of MS, in addition to AO, was made by such parameters as hypertension, elevated concentrations of TG and LDL.

Gender comparison shows that men have 4.57 times more chances of developing metabolic syndrome than women with a combination of 3 components - AO+LDL+TG (p<0.003) and 1.97 times more with a combination of AO+AH+LDL (p>0.05), proving atherogenic HCH and HTG, which are significantly often observed among them (Table 3). It should be noted that women most often have elevated glucose levels in MS combinations compared to men, increasing the odds ratio by 2.52 times with a combination of AO + LDL + glucose (p>0.05).

Table 3

Distribution of combinations of metabolic syndrome parameters depending on gender

Combination of components MS	men		women		OR [CI 95%]	p
	n	%	n	%		
AO+AH+TG	16	24.2	27	25.0	0.96 [0.47-1.95]	0.910
AO+AH+LDL	13	19.7	12	11.1	1.97 [0.83-4.60]	0.117
AO+LDL+TG	12	18.2	5	4.6	4.57 [1.53-13.67]	**0.003**
AO+AH+glucose	4	6.1	10	9.3	0.63 [0.19-2.10]	0.451
AO+TG+glucose	3	4.5	7	6.5	0.68 [0.17-2.75]	0.594
AO+ LDL+glucose	3	4.5	2	1.9	2.52 [0.41-15.51]	0.302
AO+TG+HDL	1	1.5	1	0.9	1.64 [0.10-26.77]	0.723
AO+AH+HDL	1	1.5	0	0	-	
AO+HDL+glucose	0	0	1	0.9	-	
AO+HDL+LDL	0	0	0	0	-	



Distribution of 4-component combinations of metabolic syndrome

The analysis of the occurrence of 4-component MS in the general population depending on gender was also carried out. Fig. 1 shows the share distribution of the most common combinations of 4 MS components. The most common variant of MS was a combination of AO+AH+TG+LDL, which accounted for 6.3% of the total number of respondents. It was registered more often in men (10.6%) compared with women (3.7%), no significant differences were obtained (OR 3.08, 95%CI [0.86-10.97], p=0.069). In second place is the combination of AO+AH + TG + glucose (3.4%), in third place – AO +AH +LDL + glucose (1.7%). There were no significant differences by gender. Five-component version of MS (AO +AH+LDL+TG+glucose) was registered only in one man. There were no such variants of 5 combinations of MS in women.

Thus, among the working non-indigenous population of the Aldan district, MS is mainly represented by 3 components, where AH, TG and LDL occupy leading positions among additional criteria. 4-component MS was registered less frequently, it was mainly registered in men with no significant difference.

Conclusion. A comprehensive medical examination of the health status of the working population of South Yakutia of non-indigenous nationality showed a high incidence of abdominal obesity, lipid-metabolic disorders. Dyslipidemia, arterial hypertension and metabolic syndrome were most often registered in men compared to women. The relationship of blood pressure with triglyceride and glucose levels was obtained. Considering that the examined contingent belonged to a non-indigenous or "alien" population, the syndrome of chronic adaptive overstrain can be assumed. Perhaps the reason for this is the low level of medical care, low coverage of medical examinations or the lack of highly qualified specialists. Primary health care, including general practitioners, as well as health schools for patients with hypertension and type 2 diabetes mellitus, play an important role in the early detection of metabolic syndrome and prevention of risk factors for major chronic non-communicable diseases, and timely effective medical care. Among the steps to improve the health of the population are improving urban planning, encouraging an active lifestyle, sports, promoting the principles of healthy eating, subsidizing whole-grain products, limiting the advertising of unhealthy food in the media, etc.

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DIAGNOSTIC AND TREATMENT METHODS

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EFFECTS OF INTRAOPERATIVE USE OF BETA-ADRENOBLOCKERS IN ENDOSCOPIC RHINOSINUS SURGERY UNDER GENERAL ANESTHESIA

DOI 10.25789/YMJ.2023.81.09

УДК 616-089.5-031.81

Functional endoscopic sinus surgery (FESS) provides not only optimal access with a sufficient overview of the surgical field, but also allows to preserve with minimal trauma the functionality of the ostiomeatal complex zone, to provide ventilation and drainage through the natural respiratory tract. To perform FESS procedures it is important to minimize bleeding in the surgical area, since even a small amount of blood can deteriorate the endoscopic view.

The aim of the study: to evaluate the effects of beta-blockers as component of general anesthesia in functional endoscopic sinus surgery.

Materials and methods: 110 patients were included in a single-center prospective cohort study. FESS procedures were performed under general anesthesia. 3 groups of patients were intraoperatively isolated: without administration of beta-blockers (BB) (control group, C) (n=40); with intravenous metoprolol (M) (n=35) 1-2 mg each until a heart rate of 50-60 beats /min, but no more than 15 mg; with intravenous esmolol (E) (n=35) with loading dose of 0.5 mg/kg during 1 min., then 0.05 – 0.15 mg/kg/min. The same type of general anesthesia was performed in all groups The intraoperative intensity of bleeding (IB), heart rate (HR bpm), noninvasive systolic (SBP), diastolic (DBP) and mean blood pressure (MBP) (mm Hg.), perfusion index (PI) were assessed. The study points were the 10th, 30th and the 60th minute of the procedure.

Results and discussion: HR in group C at all points of the study was statistically significantly higher compared to group M and E. MBP significantly differed at the 10th minute of the procedure between the groups, at the 30th minute the levels of MBP were the same in all compared groups, and at the 60th minute in the group M recorded high MBP compared to group E. At the 30th and 60th minutes of the procedure, PI was lower in groups M and E compared to group C. The IB was convincingly lower at all points of the study in groups M and E compared to group C and did not differ between groups M and E. In the prognostic model when assessing factors that may affect the development of intraoperative bleeding the fact of the use of BB in groups M and E leads to a decrease IB at all points of the study. An increase in IB is predicted with an increase in HR and SBP at the 10th and 60th minutes of the procedure.

Conclusion: 1. Intraoperative use of beta-blockers in addition to reduction of the heart rate leads to a moderate decrease in mean blood pressure and a decrease in intraoperative bleeding during functional endoscopic sinus surgical procedures under general anesthesia. 2. Metoprolol and esmolol have the same effectiveness for reducing the intensity of bleeding during FESS procedures. 3. The use of esmolol causes a more expressed decrease in heart rate and mean blood pressure compared to metoprolol.

Short summary. The effects of beta-blockers as component of general combined anesthesia in functional endoscopic sinus surgery were studied. It was found out that intraoperative use of beta-blockers in addition to decreasing heart rate leads to a moderate decrease in mean blood pressure and a decrease in intraoperative bleeding. Metoprolol and esmolol are equally effective, but the use of esmolol causes a more significant decrease in heart rate and mean blood pressure compared to metoprolol.

Keywords: general anesthesia, beta-blockers, metoprolol, esmolol, bleeding control, functional endoscopic sinus surgery, FESS.

Introduction. Functional endoscopic sinus surgery (FESS) is the main method of surgical treatment of chronic rhinosinusitis. FESS provides a sufficient overview of the surgical field, minimal injury, preservation of the functionality of the ostiomeatal complex zone, ventilation and drainage through the natural respiratory tract [7]. The conditions for performing these procedures require minimal bleeding in the area of the operation [4]. Control of intraoperative bleeding provides visibility of the surgical field and reduces the risk of complications [3]. Bleeding in the FESS depends on mean blood pressure (MBP) and heart rate (HR) [9, 10, 12, 14]. However, it is known that a decrease in blood pressure by more than 20% from the baseline increases the risk of myocardial ischemia, acute kidney injury and stroke. MBP less than 60-70 mmHg is accompanied by acute myocardial and kidney damage and an increase in 30-day mortality, systolic blood pressure less than 100 mmHg - by myocardial damage and increased mortality, damage is proportional to the depth and duration of hypotension. [5]. Some researchers believe that at a HR of 60 beats/min there is no need to significantly reduce MBP to improve visibility during FESS procedures [4]. Beta-blockers (BB) can be administered to maintain HR at the level of 60-70 bpm [6]. A controlled decrease in HR ensures hemodynamic stability in FESS [8]. According to the national recommendations of the All-Russian Scientific Society of Cardiology from 2011 it is recommended to continue the therapy of BB in patients taking drugs before hospitalization with dose correction until the target heart rate is reached. It is not indicated the routine use of BB, especially in high doses, on the eve of surgery, since the first administration of metoprolol 2-4 hours before the procedure increased the incidence of strokes and overall mortality [6]. Currently, the use of BB to improve visualization is poorly studied in FESS: there are few publications, but there are no studies of the effectiveness of drugs in this group and recommendations for their choice.

The aim of the study was to evaluate the effects of beta-blockers as component of general combined anesthesia in functional endoscopic sinus surgery.

Materials and methods. 110 patients were included in a single-center prospective cohort study. FESS procedures were performed under general anesthesia in the Otorhinolaryngological clinic of the I.P. Pavlov Institute of Surgery and Emergency Medicine in the period from January 2021 to February 2022. Surgical procedures were performed when conservative treatment of chronic pathology

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of the paranasal sinuses (sinusitis, ethmoiditis, frontitis, sphenoiditis, polyposis of the nose and sinuses) was ineffective. Inclusion criteria: scheduled FESS procedures in patients examined according to the protocol adopted at the clinic. Criteria for non-inclusion: constant usage of BB, grade III obesity (body mass index ≥40), severe lung pathology (severe and uncontrolled bronchial asthma, treatment by stage 4-5; chronic obstructive pulmonary disease of severe degree), ischemic heart disease with signs of angina pectoris, hypertension of stage III on the background of uncontrolled hypertension, signs of decompensation of diseases of the cardiovascular system, pronounced pathology of the kidneys and liver, pathology of the blood coagulation system, taking disaggregants and anticoagulants. Patients were randomized by random numbers into 3 groups: a group without intraoperative administration of BB (control group, C) (n=40); the group with intraoperative intravenous (IV) administration of metoprolol (M) (n=35) and the group with intraoperative IV administration of esmolol (E) (n=35). General anesthesia was performed in all study groups, the same type of premedication was performed on the operating table: IV fentanyl 0.00125–0.004 mg/kg, atropine 0.005 – 0.01 mg/kg, as needed in case of HR <50 bpm. Induction of anesthesia was performed with IV propofol 2.5-3 mg/kg. The classic LMA No. 4-5 was installed after the development of anesthesia, muscle relaxants (rocuronium bromide 0.3-0.6 mg/kg) were injected as needed. Mechanical lung ventilation was carried out by the Dreger Primus (Germany) ventilator in volume control mode with automatic flow control. The leaktightness of the airways was assessed by the indicator of the volume of leakage of the respiratory mixture, the peak inspiratory pressure on and the exhalated respiratory volume. Anesthesia was maintained with desflurane (4-12 vol%) up to a MAC of 0.8-1.4. Additionally, fentanyl was administered at a dose of 50-100 mcg, depending on the stages of the operation. At the beginning of the operation the infiltration anesthesia of the nasal cavity was performed with officinal 3.4 ml solution of articaine hydrochloride with epinephrine hydrochloride 1:100000. Intraoperative monitoring was performed according to the "Harvard Standard". Surgical procedures were performed by one surgeon who assessed at 10, 30 and 60 minutes (study points) of the operation the intensity of intraoperative bleeding (IB) on a 6-point scale (Fromme-Boezaart Score), in which 0 points corresponds to the absence of bleeding in the area of the surgical field and 5 points to severe bleeding with the inability to visualize the surgical field and the continuation of surgical intervention [10]. Simultaneously with the assessment of the visibility of the surgical field the values of HR (bpm), noninvasive systolic (SBP), diastolic (DBP) and mean blood pressure (MBP) (mmHg), perfusion index (PI, %), anesthetic MAC and the concentration of carbon dioxide on exhalation ($PetCO_2$, mmHg). The duration of the operation, anesthesia, time of postoperative recovery (restoration of consciousness), doses of intraoperatively administered drugs were also noted. All patients in the postoperative period were monitored for 2 hours to assess complaints and somatic condition. In C group, BB was not administered intraoperatively, but to reduce the IB the anesthetic MAC was increased, fentanyl was additionally administered, reducing the level of MBP (controlled hypotension). In M group IV metoprolol 1-2 mg was administered to reduce IB until HR of 50-60 bpm was reached. Additional doses were administered up to a total dose of no more than 15 mg with insufficient effect of the initial dose. We tried not to allow a decrease in HR less than 50 bpm. At HR < 50 beats/min metoprolol administration was stopped, and IV atropine 0.005 mg/kg was administered. In E group a loading dose of IV esmolol 0.5 mg/kg was administered in 1 min., then infusion through a syringe pump 0.05 – 0.15 mg/kg/min, with a decrease in HR < 50 bpm, the dose of esmolol was reduced.

Statistical analysis was performed using the StatTech v. 2.8.8 program (developed by Stattech LLC, Russia). Quantitative values are represented using the median (Me) and the lower and upper quartiles (Q1 – Q3). Categorical data were described with absolute values and percentages. Comparison of three or more groups by quantitative indicator was performed using the Kraskel-Wallis criterion, a posteriori comparisons were performed using the Dunn criterion with the Hill correction. Statistical significance

Table 1

Clinical and anthropometric characteristics of patients and intraoperative indicators in groups of comparison

Indicators		Groups of comparison			*p*
		C	M	E	
Gender, n (%)	M	24 (60.0)	20 (57.1)	18 (51.4)	0.752
	F	16 (40.0)	15 (42.9)	17 (48.6)	
Bronchial asthma, n (%)	Not identified	27 (67.5)	30 (85.7)	30 (85.7)	0.078
	Identified	13 (32.5)	5 (14.3)	5 (14.3)	
Allergy, n (%)	Not identified	32 (80.0)	31 (88.6)	30 (85.7)	0.576
		8 (20.0)	4 (11.4)	5 (14.3)	
Age, year		34 (22.0;47.0)	29 (22.5;47.5)	34 (27.0;43.5)	0.697
BMI (kg/m2)		23 (21.0;25.5)	24.4 (21.5;26.7)	23.4 (22.3;25.2)	0.293
Time of procedure (min)		71 (66;74)	70 (64;75)	73 (67;82)	0.158
Time of recovery (min)		14 (12;16)	12 (10; 13)	11 (9;13)	<0.001* PE – M < 0.001 PE – C < 0.001
Fentanyl (mcg)		400 (400.0;550.0)	300 (250.0;400.0)	300 (200.0-400.0)	< 0.001* PC – M < 0.001 PC – E < 0.001

Note. The data is presented in the form of n (%) - the absolute value (the number of percentages of the total) and Me (Q1; Q3) - the median (lower quartile; upper quartile). Comparison groups: C - control, M - metoprolol, E - esmolol. BMI is the body mass index. * - The Kraskel–Wallis criterion.

was determined by the level of p <0.05, confidence intervals – 95%.

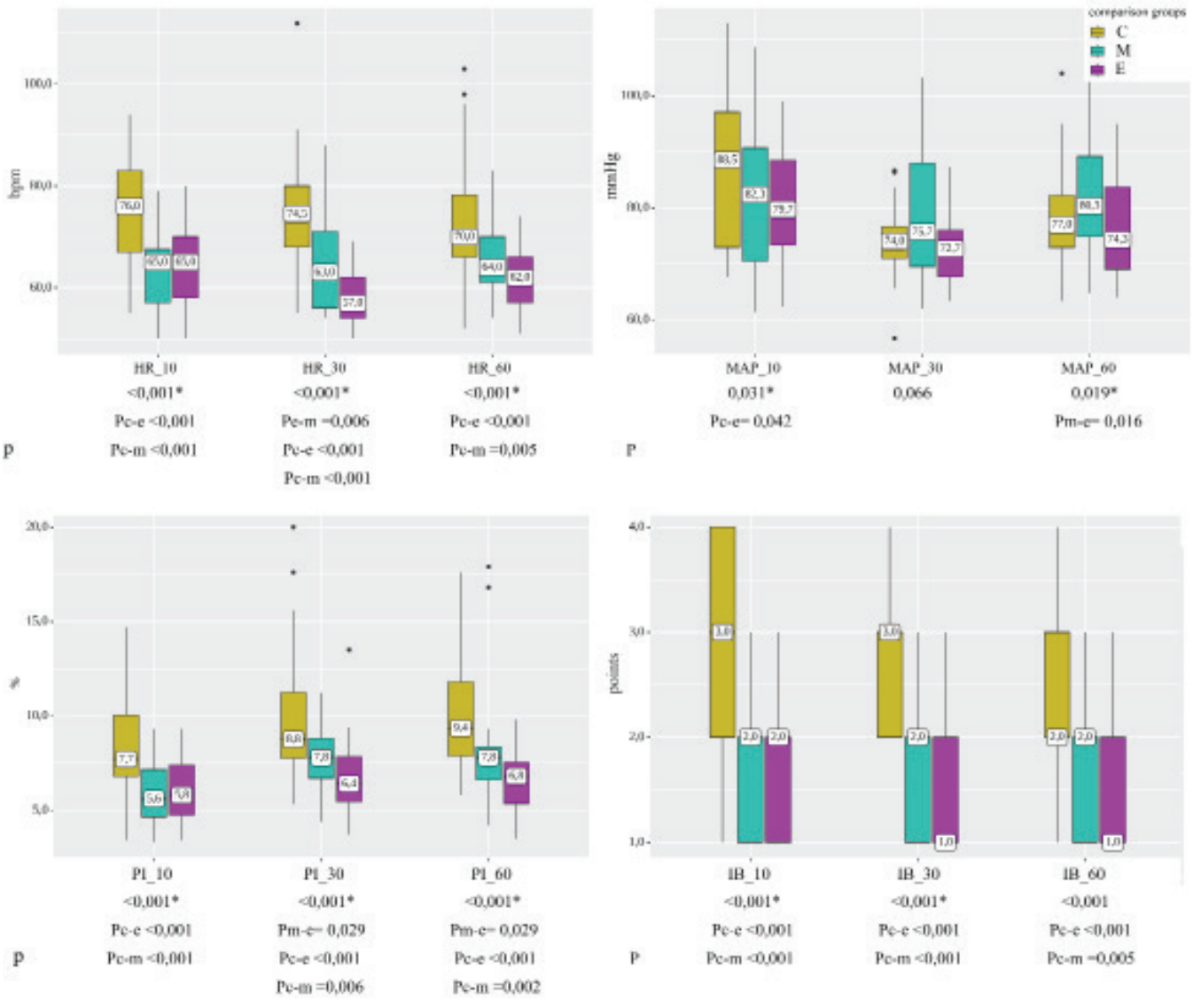
The results of the study. Comparison of the study groups revealed no statistically significant differences in anthropometric indicators, concomitant pathology, the groups were homogeneous (Table 1).

The operation time was the same in all study groups, the wake-up time in C group was significantly greater than in M and E groups. The amount of fentanyl required to provide analgesia was significantly higher in C group. The analysis of hemodynamic parameters and the intensity of bleeding at the study points revealed significant differences (Figure).

The HR in C group at the 10th minute of procedure was statistically significantly higher compared to group M and E, there were no differences between M and E groups. At the 30th minute, the HR in C group was significantly higher than in M and E groups, and the HR in E group was significantly lower compared to M group. At the 60th minute of procedure the HR in M and E groups were significantly lower compared to the C group, and the HR in E group was lower compared to M group as well as at the 30th minute of the operation. MBP values significantly differed at the 10th minute of the procedure between C and E groups with a low level of significance. At the 30th minute of the operation MBP levels were the same in all the compared groups and at the 60th minute, high MBP values were recorded in M group compared to E group. There were no significant differences between C and E groups. PI values at the 10th minute of procedure were significantly lower in groups M and E compared to C group. At the 30th and 60th minutes of surgery PI values were lower in groups M and E compared to C group, as well as at the 10th minute, but still significantly they also differed between M and E groups. The IB was convincingly lower at all points of the study in M and E groups compared to C group and did not differ between M and E groups.

To assess the effect of intraoperative hemodynamic parameters (HR, SBP, MBP, PI) and the use of BB on the intensity of intraoperative bleeding a multivariate analysis by linear regression was performed at all points of the study (Table 2).

As a result of multivariate analysis at the 30th minute of the operation it was shown that if the patient belongs to M group, a decrease in IB by 1.437 points



Comparison of heart rate (bpm), MBP (mmHg), PI (%) and IB (points) in C, M and E groups at the study points. Note. Comparison groups: C - control, M- metoprolol, E- esmolol. Heart rate – HR, MBP – mean blood pressure, PI – perfusion index, IB – intensity of bleeding. * - The Kraskel–Wallis criterion.

Table 2

Results of multivariate analysis of the prognostic model of intraoperative bleeding intensity at the 10th, 30th and 60th minutes of surgery

	B	Std. error	t	p
Characteristics of the predictive model at the 10th minute of the study				
Intercept	-0.452	0.628	-0.719	0.474
Group M	-0.902	0.179	-5.050	< 0.001*
Group E	-0.859	0.173	-4.951	< 0.001*
HR 10	0.021	0.009	2.332	0.022*
SBP 10	0.014	0.006	2.457	0.016*
Characteristics of the predictive model at the 30th minute of the study				
Intercept	0.649	0.690	0.941	0.349
Group M	-1.437	0.163	-8.839	< 0.001*
Group E	-1.445	0.154	-9.358	< 0.001*
HR 30	0.022	0.007	3.376	0.001*
Characteristics of the predictive model at the 60th minute of the study				
Intercept	-1.586	0.711	-2.230	0.028*
Group M	-0.477	0.174	-2.747	0.007*
Group E	-0.424	0.178	-2.386	0.019*
HR 60	0.031	0.008	3.926	< 0.001*
SBP 60	0.014	0.006	2.353	0.020*

Note. Groups of comparison: M- metoprolol, E – esmolol; HR – heart rate, SBP – systolic blood pressure. * - differences in indicators are statistically significant ($p < 0.05$).

should be expected and to E group by 1.445 points. An increase in IB by 0.022 points is predicted with an increase in SBP. At 1 mmHg the obtained regression model is characterized by a correlation coefficient rxy = 0.728, which corresponds to a high closeness of the connection on the Cheddock scale. The model was statistically significant ($p < 0.001$). The resulting model explains 53.0% of the observed IB variance. Similar results were obtained at the 10th and 60th minutes of the operation (see Table 2). In addition, the probability of an IB raising increased by 0.021 and 0.031 points at the 10th and 60th minutes, respectively, with an increase in HR by 1 bpm.

Discussion. The necessary anesthetic care of FESS includes adequate anesthesia, respiratory tract protection, hemostasis control, prevention of postoperative complications, including delayed bleeding [4, 11]. The development of bleeding is determined by several factors: the value of blood pressure, HR and the state of hemostasis [1, 10, 13]. Controlled hypotension is a common method of reducing intraoperative bleeding, however, excessive hypotension can lead to a decrease in blood flow in organs sensitive to fluctuations in perfusion pressure (heart, brain) [13]. It is established that IB depends on MBP and HR [15]. Some researchers believe that with a HR of 60 bpm, there is no need to reduce MBP, since during procedures with controlled hypotension it is not always possible to reduce IB due to peripheral vascular dilation and reflex tachycardia. A decrease in HR reduces the filling of the capillaries of the tissues of the nasal cavity, since venous outflow improves due to an increase in the diastole phase [13, 15]. In our study the use of BB made it possible to reduce IB without a significant decrease in MBP (Fig. 1). The IB in M and E groups was significantly lower at all points of the study, while the MBP values at the 30th and 60th minutes of the operation did not significantly differ from those in C group. The use of BB significantly reduced HR and in E group the effect was more obvious only at the 30th minute of the procedure compared with M group. We believe that this is due to the method of administration of BB with the introduction of a loading dose of esmolol, it is possible to achieve a significant reduction in HR faster without the development of significant bradycardia [2]. The PI values in the control group were significantly higher at all points of the study, since in order to reduce the HR in this group, we increased the concentration of an inhalation anesthetic and additionally administered fentanyl (Table. 1), which led to an increase in peripheral blood flow. We have previously shown that PI >10% is associated with increased tissue bleeding during FESS procedures [1]. Relatively low PI values in E group compared to M group are most likely also associated with a lower need for narcotic analgesics. The decrease in IB in the M and E groups was achieved by a decrease in HR without a significant reduction in MBP. When assessing the factors that may affect the development of bleeding, we found out in the prognostic model that the use of BB in the M and E groups leads to a decrease in IB at all points of the study, but most significantly at the 30th minute of procedure (Table 2). With an increase in HR and SBP an increase in IB is predicted at the 10th and 60th minutes of procedure, which is associated with increased perfusion of peripheral tissues and corresponds to the studies of other authors [8, 13, 14, 15]. In the prognostic model at the 30th minute of surgery the HR losts a significant effect on the IB. Most likely this is due to the stabilization of hemodynamic parameters in all study groups.

Conclusions.

1. Intraoperative use of beta-blockers, in addition to reduction of heart rate, leads to a moderate decrease in mean blood pressure and a decrease in intraoperative bleeding during FESS procedures under general anesthesia.
2. Metoprolol and esmolol have the same effectiveness for reducing the intensity of bleeding during FESS procedures.
3. The use of esmolol causes a more significant decrease in heart rate and mean blood pressure compared to metoprolol.

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CHANGES IN THE PHYSICAL AND CHEMICAL PROPERTIES AND FATTY ACID COMPOSITIONS OF THE BLOOD SERUM IN PATIENTS WITH DIFFERENT COMMON PERITONITIS AS ONE OF THE CRITERIA FOR ASSESSING THE SEVERITY OF THE INFECTIOUS-INFLAMMATORY PROCESS

DOI 10.25789/YMJ.2023.81.10

УДК 616.381-002-089

The **aim** of this study was a clinical assessment of the diagnostic value of determining changes in the fatty acid composition and values of the surface tension of blood serum in patients with peritonitis in the light of assessing the severity of the course of the infectious and inflammatory process. In the course of the study it was found that the most informative indicator of the severity of the course of an infectious-inflammatory process is a sharp and prolonged decrease in the blood serum of patients with CCP in the level of γ-linolenic, dihomo-γ-linolenic fatty acids, as well as a persistent decrease in STC values. The results of the clinical study presented by us allow us to recommend, as a method of choice, to assess the severity of the course of peritonitis with the help of a comprehensive assessment of changes in the fatty acid composition and STC values of blood serum.

Keywords: peritonitis, fatty acids, surface tension coefficient.

Introduction. Despite the long history of studying peritonitis, various issues of its treatment remain one of the most difficult and not completely resolved problems of abdominal surgery. Given the large number of syndrome complexes developing in response to inflammation in the abdominal cavity, the approach to treatment is multidisciplinary in nature and includes a large set of measures aimed at both eliminating the source of peritonitis and correcting homeostasis disorders. The pathogenesis of peritonitis is a complex dynamic process of progression of pathophysiological disorders [8]. The main role in the pathogenesis of this severe complication is assigned to endogenous intoxication, caused by the accumulation in the body of toxins of microbial origin and metabolic products. It is the control of endotoxemia that often determines the outcome the disease.

In recent years, in the world of medical science, there has been an increasing interest in determining the physiological and biochemical status of a person when monitoring pathological processes developing in tissues, organs, and organ systems [1]. The physiological and biochemical status is determined by the presence at a certain stage and a certain situation of the concentration of biologically active compounds - proteins, fats and carbohydrates, as well as their metabolites [4]. In addition, many physiological and biochemical processes occur at the interface. A very important feature in the physical sense of the structural organization of living systems is a wide variety of dynamically stable and unstable interfaces [12]. These primarily include various

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membrane formations (cell membrane, lysosomes, mitochondrial, nuclear, and others), as well as the surfaces of blood vessels, visceral and parietal peritoneum, pleura, alveoli, blood cells [10].

As is known, the basis of endogenous intoxication accompanying peritonitis is the process of appearance in the bloodstream of substances with a pronounced detergent effect [3,9]. These substances primarily include, in particular, fatty acids (FA). It is known that fatty acids, especially polyunsaturated fatty acids (PFA), are involved in lipid peroxidation as substrates [5]. Due to the fact that in critical conditions, which include peritonitis, massive lipolysis occurs with an increase in the content of free fatty acids (FFA) in the blood serum, this leads to destabilization of cell membranes [6]. In turn, surface phenomena at the phase boundary also change, one of the physical indicators of which is the coefficient of surface tension (CST). However, data on the role of detergents and changes in the course of the infectious-inflammatory process of surface phenomena in the blood of patients with peritonitis are contradictory, which currently requires additional research and generalization of the data obtained.

The **aim** of the study. Clinical assessment of the diagnostic value of determining changes in the fatty acid composition and values of the surface tension of blood serum in patients with peritonitis in the light of assessing the severity of the infectious and inflammatory process.

Material and methods. The presented material is based on a clinical analysis of the results of treatment of 50 patients with common purulent peritonitis (CPP) who were treated in surgical hospitals of the Republican Hospital № 2 – Center for Emergency Medical Care (CEMP) of the Republic of Sakha (Yakutia) in the period from 2020 to 2023. The diagnosis of CPP was established on the basis of a standard clinical examination. The mean age of the patients was 35,6±5.1 years; there were 29 (58,0%) men and 21 (42,0%) women. To classify sepsis, in this case abdominal sepsis (AS), the criteria proposed by the conciliation conference of the American College of Pulmonologists and the Society for Critical Medicine Specialists ACCP/SCCM [1] were used. The choice of the spectrum for the determination of fatty acids was based on their prevalence and frequency of occurrence during destabilization of animal cell membranes. Hydrolysis and methylation of the presented fatty acids was carried out by gas-liquid chromatography [7]. Acid hydrolysis by Kenichi Ichihara and Yumeto Fukubyashi was used to obtain FA methyl esters [11]. For this purpose, 100 ml of blood serum were placed in sealed containers, 1 ml of 2,5% methanolic solution of H2SO4 was added and placed in a thermoshaker at 80°C and 1000 rpm for one hour. After cooling to room temperature (20°C), 1 ml of 0,9% NaCl was added to the resulting solution. Next, FA methyl esters were extracted with 0,5 ml of hexane. The resulting mixture was placed in a shaker for 1 min, then centrifuged for 1 min at 6,5 g. Methyl esters of FA were collected by decantation from the supernatant. 200 µl were taken for analysis. The hexane extract of FA esters was placed into the autosampler of a MAESTRO 7820/5975 chromatograph built on the basis of an Agilent 7820 gas chromatograph (USA) and a 5975 mass spectrometric detector from the same manufacturer. An HP-INNOWax capillary column was used for separation. Identification of FA methyl esters was carried out using a set of standards from Sapelco. 37-Component FAME mix (cat. no. 18919-1MP) and using the NIST database. Data collection was carried out using the Agilent ChemStation software. The concentration of methyl esters of fatty acids was determined from the area of chromatographic peaks of the corresponding compounds by the method of internal normalization. Data processing software used: Xcalibur (Thermo); spectral libraries: Mainlib; Microsoft Excel 2010. The static Du-Nouy method (on a Lauda TD1 tensiometer) was used to determine serum CST [2]. The inclusion criteria were: the presence of CCP, the presence of AS, the immediate causes of CCP were inflammatory and destructive diseases of the abdominal organs, the absence of a lethal outcome during the first 72 hours after the primary operation, the initial severity of the condition according to the Mannheim peritoneal index II-III degree. The exclusion criteria were: acute destructive pancreatitis with the development of peritonitis, neoplastic processes in the abdominal cavity, mesenteric thrombosis, initial severity of the condition according to the Mannheim peritoneal index less than grade II, death from peritonitis during the first 72 hours after surgery, the presence of fistulas.

Statistical processing of the material was carried out using the SPSS.Statistica.v22 software package. To determine the hypothesis and determine the type of distribution of the values of the studied features, the Shapiro-Wilkins test was used. In the groups to be compared, the mean values (X), standard deviation (s), confidence intervals and their fluctuations were determined. To study the relationship of quantitative traits, the Spearman correlation analysis method was used, since one of the variables, CST, did not obey the normal distribution law. The critical level of significance (p) when testing statistical hypotheses was taken equal to 0,05.

Results and discussion. Based on the results of the analysis of the FA profile and the assessment of the CST value of blood serum in patients operated on for CCP, it was found that in patients with severe CCP, the development of abdominal sepsis, the content of monounsaturated fatty acids (MFA) and PFA decreased sharply. At the same time, the level of unsaturated fatty acids (UFA) exceeded the control figures and averaged ΣUFA (77,22±1,1%). The high level of UFA was mainly due to the predominance of stearic [C 18:0] (49,19±0,5%), palmitic [C 16:0] (25,10±1,4%), myristic [C 14:0] (2,1±0,1%) and lauric [C 12:0] (0,83±0,2%) fatty acids, respectively. A parallel study of changes in the physicochemical properties of blood serum showed that with an increase in the severity of the condition of patients, there was a decrease in serum CST. A positive correlation was noted between the level of CST values and the severity of the condition (rs = +0,75), respectively.

The assessment of FA levels and CST values in patients with various types of abdominal sepsis showed that the nature of changes in the FA profile and the physicochemical properties of blood serum directly depended on the severity of the disease and developing complications. Thus, in the first 48 hours after surgery in patients with heavy sepsis (HS) and in the first 72 hours in patients with septic shock (SS) and multiple organ failure (MOF), the level of UFA in the blood serum exceeded the control figures by several times (especially significantly in patients with SS and MOF) and was in the blood serum of patients with HS - ΣUFA (78,17±1,4%), in the blood serum of patients with MOF – ΣUFA (81,15±1,6%). The increase in the level of UFA was mainly due to: stearic [C 18:0], margarine [C 17:0], palmitic [C 16:0], myristic [C 14:0] and lauric [C 12:0]. Along with an increase in the level of UFA, one could note consistently low numbers of CST in the blood serum. Thus, in the first 48 hours after surgery in patients with HS and in the first 72 hours in patients with SS and MOF, the level of CST values averaged: in patients with HS 41,2±1,1 mN/m, in patients with SS and MOF – 38,1±0,8 and 35,4±0,7 mN/m, respectively. The

dynamics of the content of fatty acids and the level of CST are presented in table.

When monitoring the level of UFA in the postoperative period, it should be noted that it largely depended on the effectiveness of complex therapeutic measures. So, with a favorable course of the postoperative period (the absence of a sluggish process, complications from the surgical wound or abdominal cavity), a gradual decrease in the level of UFA in the blood serum was observed and, on the contrary, an increase in the level of MFA and PFA. In cases where the level of MFA and PFA remained low for a long time (more than 72 hours), this always indicated an unfavorable course of the infectious-inflammatory process. Often in this case, progression of peritonitis or the development of severe complications with organ decompensation was observed. Similarly, there were changes in the physicochemical properties of the blood serum of patients with CCP. With a favorable course of the disease, the CST values of blood serum gradually approached the control figures. In cases of a complicated course, after some fluctuations in values, there was a progressive trend towards a decrease in serum CST. When considering the concentrations of some PFA, it was found that the level of ω3-PFA, such as cis-5,8,11,14,17-eicosapentaenoic [C 20:5Δ5,8,11,14,17] and cis-11.14,17-eicosatrienoic [C 22:3Δ11,14,17] decreased faster and more significantly than others in the case of an unfavorable course of the disease. Their concentrations were practically "trace" (0,0002±0,1% and 0,007±0,2), respectively. At the same time, it was possible to note an increase in ω6-PFA, mainly due to arachidonic [C 20:4Δ5,8,11,14] in comparison with other ω6-PFA and control values in patients with purulent peritonitis.

Thus, the total level of ω6-PFA was increased in more severe CCP (SS and MOF). A significant decrease in the coefficient ω3-PFA / ω6-PFA, mainly due to cis-5,8,11,14,17-eicosapentaenoic [C 20:5Δ5,8,11,14,17] and cis-11.14,17-eicosatrienoic [C 22:3Δ11,14,17] FA was observed during the entire period when AS events were present. Thus, the ratio of the coefficient ω3-PFA/ω6-PFA significantly decreased in patients with severe AS. More than 3 times in HS ($p<0,05$), more than 7 times in SS and MOF ($p<0,05$). Our data indicate that in patients with a more severe course of the disease, there are more pronounced disorders of the fatty acid composition of blood serum, mainly due to ω3 and ω6. At the same time, there is an increase in the UFA / MFA ratio, which is most pronounced during the first 72 hours after the operation. Such changes seem to be associated with the mobilization of unsaturated fatty acids, which are the first to be oxidized. Summing up the analysis of the FA profile in CCP, I would like to dwell on some features in the behavior of PFA at individual stages of treatment. When analyzing the concentrations of ω6-PFA, we encountered an unusual behavior of some of them. So, at admission and in the first 72 hours after surgery, the level of γ-linolenic [C 18:3Δ6,9,12], dihomo-γ-linolenic [C 20:3Δ8,11,14] FA in patients with HS and SS was extremely low, and in MOF they were present in the form of "trace" concentrations. In the case of a favorable course of the disease on days 7-10 from the moment of surgery, the level of γ-linolenic [C 18:3Δ6,9,12], dihomo-γ-linolenic [C 20:3Δ8,11,14] FA increased and already averaged 0,5 μg/ml and 1,7 μg/ml, respectively. This pattern was not observed in the behavior of other FA, in particular, UFA, MFA, and PFA. In our opinion, these changes are apparently associated with the features of the biosynthesis of unsaturated fatty acids. In addition, from literary sources [6], we know that γ-linolenic [C 18:3Δ6,9,12], dihomo-γ-linolenic [C 20:3Δ8,11,14] fatty acids in the human body are formed from linoleic [C 18:2Δ9,12] acid, which belongs to ω6-PFA. This transformation process requires the enzyme delta-6-desaturase (D-6-D), often the activity of which is suppressed by the excessive content in the blood of a large number of under-oxidized metabolic products, as well as the vital elements of microorganisms and their toxins. In addition, a frequent unfavorable sign of compensatory processes in CCP is a persistent increase in blood glucose levels and a decrease in blood insulin levels. There is evidence [9] that an excess of glucose in the blood blocks the activity of the D-6-D enzyme,

The content of fatty acids and the level of CST in the blood serum of patients common purulent peritonitis (% of the total fatty acids M±s)

Methyl ether FA and physico-chemical index	SIRS-3,4	HS	SS	MOF	Control
Linolenic, [C18:3Δ9,12,15]	0.21±0.03*	0.05±0.1*	0.04±0.1*	0.01±0.01*	0.27±0.02
cis-5,8,11,14,17-eicosapentaenoic, [C20:5Δ5,8,11,14,17]	0.002±0.1*	0.001±0.5*	0.0009±0.5*	0.0004±0.5*	0.032±0.5
cis-11-14-17-Eicosatrienoic, [C22:3Δ11,14,17]	0.01±0.5**	0.001±0.5**	0.009±0.01**	0.008±0.05**	0.03±0.1
γ-Linolenic, [C18:3Δ6,9,12]	0.001±0.07*	0.00096±0.15*	0.00089±0.15*	0.00037±0.07*	0.13±0.01
Linoleic, [C18:2Δ9,12]	10.33±0.01**	9.15±0.01**	7.05±0.01**	5.75±0.04**	16.11±0.05
Arachidon, [C20:4Δ5,8,11,14]	8.22±0.7**	10.33±0.3**	12.44±0.3**	15.66±0.5**	3.82±0.04
cis-8,11,14 -Eicosatrienoic, [C23:3Δ8,11,14] Dihomo-γ-linolenic	1.55±0.1**	2.00±0.1**	2.21±0.1**	3.59±0.6**	0.94±0.1
cis-13-16-Docosadiene, [C22:2Δ13,16]	0.0019±0.05*	0.0016±0.07*	0.0015±0.07*	0.0011±0.01*	0.02±0.03
cis-11-14-Eicosadiene [C20:2Δ11,14]	0.009±0.03*	0.013±0.03*	0.015±0.03*	0.019±0.01*	0.006±0.02
Σω3-PFA	0.22±1.1*	0.04±0.1*	0.03±0.1*	0.01±0.9*	0.62±0.01
Σω6-PFA	21.11±0.02*	22.71±0.05*	24.70±0.05*	25.02±0.01*	21.02±0.02
Σω3-PFA/Σω6-PFA, units	0.01±0.03*	0.003±0.01*	0.001±0.01*	0.0004±0.06*	0.03±0.01
Σ UFA	75.56±1.8*	75.17±1.4*	73.17±1.4*	72.94±1.1*	76.10±1.0
Σ MFA	1.11±0.03*	0.08±0.04*	0.05±0.04**	0.03±0.01*	2.26±0.01
CST (mN/m)	43±1.8*	41.2±1.1*	38.1±0.8*	35.4±0.7*	46.0±0.9

Note. * - the indicator significantly differs from the control ($p<0.05$), ** - the indicator significantly differs from the control ($p<0.01$).

followed by a critical decrease in the level of both γ-linolenic [C 18:3Δ6,9,12] and dihomo-γ-linolenic [C 20:3Δ8 ,11,14] FA. It should also be taken into account that γ-linolenic [C 18:3Δ6,9,12] and dihomo-γ-linolenic [C 20:3Δ8,11,14] fatty acids are involved in the synthesis of eicosanoids (prostaglandins) [10]. Prostaglandins are localized in almost all tissues and organs and are lipid mediators. Prostaglandins are synthesized from UFA and have a diverse effect, often directly opposite. In the course of the conversion of linoleic acid [C 18:2Δ9,12] into arachidonic acid [C 20:4Δ5,8,11,14], there are two steps in the cascade of prostaglandin formation. The first, in this case the key one, is carried out with the help of the enzyme D-6-D. The second, with the help of the enzyme delta-5-desaturase (D-5-D). With an increase in the blood level of underoxidized metabolic products, as well as microbial toxins, the D-6-D enzyme is inhibited, as a result, the synthesis in the cascade of γ-linolenic → dihomo-γ-linolenic UFA → anti-inflammatory prostaglandins (PG1) is disrupted. At the same time, the D-5-D enzyme is activated, which leads to the formation of pro-inflammatory prostaglandins (PG2).

Conclusions. Our observations showed that: 1. The total level of fatty acids in CCP was increased by 5-7 times relative to the control ($p<0,05$). At the same time, the coefficient of the ratio Σω3-PFA/ω6-PFA was significantly reduced in patients with HS by 2 times ($p<0,05$), and in patients with SS and MOF by more than 7 times ($p<0,05$). 2. The results of the study clearly show that in patients with a complicated course of the disease, there are more pronounced changes in the fatty acid composition of the blood serum due to ω3 and ω6 fatty acids, which persist throughout the entire period of existence of signs of abdominal sepsis. At the same time, the ratio of UFA/MFA increases with the severity of the course of the disease. Apparently, this is due to the fact that MFA are the first to be oxidized during lipolysis. 3. The most informative indicator of the severity of the infectious-inflammatory process is a sharp and prolonged decrease in the blood serum of patients with CCP in the level of γ-linolenic [C 18:3Δ6,9,12], dihomo-γ-linolenic [C 20:3Δ8,11,14] FA, as well as a persistent decrease in the values of the surface tension coefficient.

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DOI 10.25789/YMJ.2023.81.11

УДК 616-089.873

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TRANSANAL ENDOSCOPIC RESECTION OF RECTAL NEOPLASMS

Based on a retrospective analysis of the treatment results of 23 patients of the coloproctology department of the Surgut District Clinical Hospital for the period from 2018 to 2021, the effectiveness of transanal endoscopic resection of rectal benign tumors was evaluated. The study found that the technique of transanal resection of large tumors of the rectum has a number of advantages in comparison with traditional approaches: preservation of the function of the rectal closure apparatus, a small number of postoperative complications and preservation of the patient's quality of life at a high level. The results of the study indicate the effectiveness of using the technique of transanal endoscopic surgery in patients with malignant rectal tumors. The feasibility study ensured the radical removal of the formations of the TisN0M0 and T1N0M0 stage with minimal invasiveness of the procedure and the absence of early signs of recurrence of the disease.

Keywords: rectum, benign tumors, transanal endoscopic resection, rectal cancer.

In recent decades, there has been an increase in the number of neoplasms of the digestive tract and, in particular, the colon. The proportion of them during screening studies can reach up to 32%. Especially important is the fact that in Eastern European countries the frequency of colorectal cancers cases in the structure of malignant neoplasms is at least 11.5% and occupies 42.6% among intestinal neoplasms [1-5]. The social significance of the problem of diagnosis and treatment of tumors of the digestive tract related with the fact that every year there is an increase in the number of colonic neoplasms among people younger than 55 years [4]. At the same time, the worst prognosis is associated with rectal neoplasms [5].

Biopsy of villous tumors of rectum makes it possible to detect malignancy focuses in them in 45% of the examined [1-13]. Therefore, the planning of surgical intervention should take into account the possibility of simultaneously obtaining a full-layer fragment of the rectal wall to obtain a reliable morphological analysis or the possibility of performing radical surgical intervention.

Removal of large villous neoplasms using traditional endoscopic techniques is impossible in some cases. For surgical treatment of neoplasms of the rectum, among others, the Mason (excision of the neoplasm transsphincterally) or Kraske (transcoccigeal) techniques were successfully used [11]. This was accompanied by a high level of complications - syndrome of chronic pelvic pain after coccygectomy, the formation of fistulas and anal incontinence [5, 11]. Various resection technologies are traditionally considered to be radical methods of treatment of rectal tumors. These interventions are associated with a high risk of intra- and post-operative complications, damage to the sphincters, and also often require the imposition of a permanent intestinal stoma. This leads to a violation of the patient's labor and social adaptation and sometimes disability [2-5].

The technique of transanal endoscopic microsurgery (transanal endoscopic microsurgery or operation - TEM, TEO) has been introduced into the clinical practice of Russian surgeons in recent decades. It was proposed by the German surgeon G. Buess in 1983 for the removal of epithelial neoplasms of the rectum [6, 7] To date, the TEO technique in large villous polyps and early non-invasive forms of rectal cancer treatment shows good results due to the development and improvement of surgical instruments and the development of a standardized approach to its implementation [1-13]. Nevertheless, the frequency of complications associated with transanal endoscopic intervention, according to large multicenter studies, ranges from 1.7% to 21,9% [1-4, 9, 11, 13].

In this regard, the study of the effectiveness of transanal endoscopic resection of rectal neoplasms is an up-to-date topic of scientific research.

Objective: to evaluate the effectiveness of transanal endoscopic resection of rectal neoplasms

Materials and methods. A retrospective analysis of the treatment results of 23 patients of the coloproctology department of the Surgut District Clinical Hospital for the period from 2018 to 2021 was performed. The inclusion criteria were the presence of a neoplasm in the rectum at a distance of up to 20 cm from the toothed line, with a size of more than 20 mm, a wide base or a prostrate type of neoplasm (Isp, Is, IIa types according to S.Kudo classification). There were 13 (56.5%) males and 10 (43.5%) females in the analyzed group. The average age of the patients in the study group was 58.4 ± 5.6 years.

Before hospitalization, all patients were examined, which included a survey, physical examination, analysis of clinical and biochemical parameters. The list of instrumental studies included colonoscopy with mandatory biopsy of the neoplasm, assessment of its location and size, as well as magnetic resonance imaging of the pelvic organs to exclude invasive growth.

Before surgery, all patients were assessed for their general condition according to the Charlson comorbidity index with the Deyo correction [8]. The average index in the group of surveyed was 4 (3;6).

According to the preoperative pathohistological study, tubulo-villous adenomas with low–grade intraepithelial neoplasia (IEN) were detected in 8 (34.8%) patients, with moderate IEN - in 10 (43.5%) people, with high–grade IEN - in 5 (21.7%) patients (Table 1).

In the study group, neoplasms located at a distance of up to 8 cm from the dentate line were detected in 12 (43.5%) patients, from 9 to 15 cm – in 9 (39.1%) patients. Formations proximal to 15 cm from the dentate line were detected in 2 (8.7%) people.

Localization of the neoplasm on the posterior wall of the rectum was observed in 14 (60.8%) patients, on the anterior –

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in 7 (30.4%) patients, on the lateral – in 2 (8.7%) people.

Antibiotic prophylaxis was carried out in all patients in accordance with the results of monitoring the sensitivity of the nosocomial flora. It was carried out by intravenous infusion of semisynthetic wide-spectrum antibiotics of the inhibitor-protected penicillins group - ampicillin + sulbactam at a dosage of 1.5 grams or amoxicillin + clavulanic acid at a dosage of 1.2 grams. The drug was injected intravenously to all patients once 30 minutes before the beginning of operation.

To date, there is no consensus among specialists performing transanal operations on the preparation of the intestine for surgery [10]. However, according to M Sailer et al., phosphate enemas improve visualization and contribute to a potential reduction in the risk of infection in the area of surgical intervention [12]. In the described group, preparation was carried out on the eve of the operation according to a single-stage scheme using preparations of polyethylene glycol (macrogol). Intraoperatively, such preparation of the intestine for surgical intervention was assessed by us as satisfactory.

The technique of transanal endoscopic surgery was described by Professor G. Buess in 1983 and has been revised over time in order to improve its effectiveness, as well as to modernize the equipment for its implementation.

Before the introduction of the rectoscope, the patient underwent a finger anus divulsion. The next stage was the introduction of an operational rectoscope with an obturator, taking into account the distance to the distal edge of the formation. After visualization, a needle-shaped monopolar electrode was used to mark the postoperative field, while the distance between the formation and the resection cream was at least 10 mm. Full-layer resection of the formation was performed using the Harmonic power system, additional hemostasis was carried out using a monopolar electrode. After the extraction of the drug, the postoperative wound was sutured with a absorbable monofilament material. There are publications in foreign literature about the absence of the need for suturing postoperative wounds that occupy less than 30% of the area of the intestinal lumen. However, this entails an increase in the risk of bleeding in the early postoperative period, and this method was not used by us during the study.

All 23 (100.0%) patients underwent transanal endoscopic removal of the tumor. The patient's position on the operating table was determined by the localization of the disease. With its location on the posterior semicircle, 14 (60.8%) people were operated in the position for stone cutting. When placed on the anterior semicircle in a position on the abdomen - 7 (30.4%) patients. With the lateral location of the formation - on the left side – 2 (8.7%) patients.

As an anesthetic aid, epidural anesthesia was used in 19 (82.6%) patients, endotracheal anesthesia – in 4 (17.4%). The average operation time was 65 (40;100) min. In the postoperative period, patients did not need to stay in the department of anesthesiology and intensive care. Therefore, after the stabilization of vital functions, patients were transferred to the coloproctology department.

The management of patients in the postoperative period was carried out according to the developed protocol for the management of patients after interventions on the colon and rectum. This protocol included, in addition to antibiotic prophylaxis and prevention of thromboembolic complications, refusal of prolonged use of the urinary catheter, early activation of patients and the start of eating per os on the first day after the intervention.

Morphological examination of the surgical material was carried out after cutting on a sledge microtome, sections 4-5 microns thick were prepared for paraffin sections. The staining of the preparations was carried out with hematoxylin – eosin. Microscopy of histological preparations

Table 1

Results of preoperative pathohistological examination (n=23)

Type of morphological structure	Abs, pers.	%
Tubulo-villous adenoma with low grade IEN	8	34.8
Tubulo-villous adenoma with moderate grade IEN	10	43.5
Tubulo-villous adenoma with high–grade IEN	5	21.7
Total	23	100.0

*IEN – intraeptive neoplasia

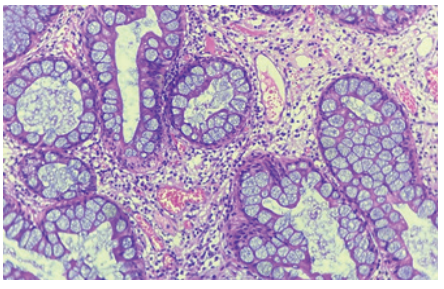

Fig. 1. Patient Sh., 59 years old. Tubulo-villous adenoma of the colon with low-grade intraepithelial neoplasia. Color: hematoxylin-eosin. X40

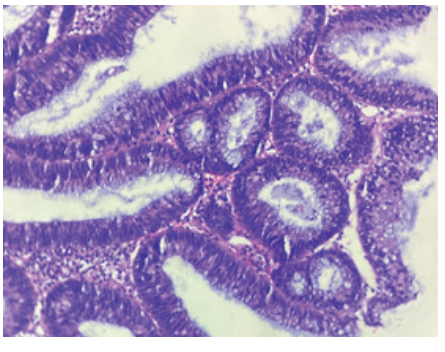

Fig. 2. Patient N., 66 years old. Tubulo-villous adenoma of the colon with moderate intraepithelial neoplasm. Color: hematoxylin-eosin. X40

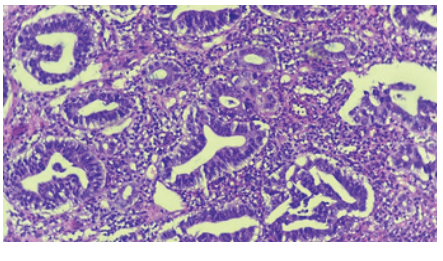

Fig. 3. Patient N., 62 years old. Tubulo-villous adenoma of the colon with intraepithelial neoplasia of high (high-grade)

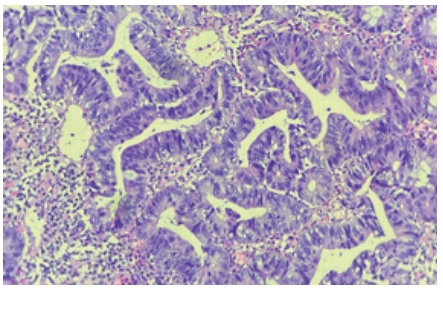

Fig. 4. Patient N., 67 years old. fragments of tuber-villous adenoma of the colon with morphological indicators of widespread glandular intraepithelial neoplasia of high degree (severe dysplasia, Cancer in situ) in several areas small foci of intramucosal adenocarcinoma Color: hematoxylin-eosin. X40.

Table 2

Distribution of TEO complications in the postoperative period (n=23)

Degree of complication according to the Clavien-Dindo	Abs., pers.	%
I degree	1	4.3
II degree	0	0.0
IIIa degree	0	0.0
IIIb degree	2	8.7
IVa degree	0	0.0
IVb degree	0	0.0
V degree	0	0.0
Total	3	13.0

was carried out at 10, 20, 40x magnification using a Zeiss Primo Star light microscope.

To assess the effectiveness of transanal endoscopic surgery, an analysis of the course of the early postoperative period, the degree of radicality of surgical intervention was performed. Descriptive statistical processing of the obtained results was performed by the standard statistical software package SPSS 21.0 for Windows and Microsoft Office Excel 2013.

Results and discussion. The appearance of active peristalsis in patients in the postoperative period was noted on 1 (1;2) day, the discharge of gases – on 1 (1;2) day. The appearance of an independent stool - on the 3rd (2nd; 4th) day after the operation.

In the study group, a complicated course was observed in 3 (13.0%) patients: 1 (4.3%) complication developed in the intraoperative period, 2 (8.7%) cases of postoperative complications. Intraoperative perforation of the rectal wall was noted in 1 (4.3%) patient with a large villous polyp located at a distance of 15 cm from the dentate line. This required emergency surgical intervention in the scope of laparotomy, loop sigmostomy.

In 1 (4.3%) patient in the postoperative period, bleeding developed from the site of removal of the formation of the rectum. Conservative therapy was prescribed to relieve the resulting complication, no surgical treatment was required. In 1 (4.3%) patient, sutures erupted in the area of the postoperative wound with retraction of the proximal edge of the flap of the rectal mucosa and the development of pelvic phlegmon. This complication was the reason for the opening of the phlegmon with perineal access, laparotomy and the imposition of a loop sigmostomy.

Table 3

The results of the final pathohistogical study (n=23)

Type of morphological structure	Abs., pers.	%
Highly differentiated adenocarcinoma in situ	3	13.0
Tubulo-villous adenoma with low grade IEN*	5	21.7
Tubulo-villous adenoma with moderate IEN*	8	34.8
Tubulo-villous adenoma with IEN* of high degree	7	30.4
Total	23	100.0

*IEN – intraeptive neoplasia

There were no fatal outcomes in the group of patients after transanal endoscopic operations. Table 2 shows the distribution of complications by Clavien-Dindo classification.

The duration of hospitalization in this group of patients was 7 (6;13) days, while with an uncomplicated course, the stay of patients was 7 (6;8) days and 11 (9;13) days in the presence of intra- and postoperative complications.

After discharge from the hospital, patients were observed by coloproctologist until the final result of a pathohistological examination of the removed tumor was obtained. When performing a video colonoscopy in the long–term postoperative period - after 6 months of discharge from the hospital - there was no recurrence of the disease at the site of localization of removed neoplasms. 12 months after the surgical intervention, when performing outpatient endoscopic examinations, polypoid formations up to 5 mm in size were detected and removed during biopsy in 2 (8.7%) patients of the observation group. According to the pathohistological examination of the removed neoplasias, signs of hyperplastic colon polyp were found in 1 (4.3%) patient, signs of granular inflammation and fibrosis were found in 1 (4.3%) patient.

In 3 (13.0%) observations in the removed preparation, signs of highly differentiated adenocarcinoma in situ and with the germination of the submucosal layer were revealed, which corresponds to the TisN0M0 and T1N0M0 stages according to the TNM classification. At the same time, there were no signs of malignant growth in the edges of the resection. This group of patients was referred for consultation by an oncologist. After examination, there were no signs of metastasis of malignant formations, and patients are observed by an oncologist in the III clinical group. 3 and 6 months after the surgical intervention, there was no recurrence of the disease.

The distribution of patients according to the histological structure of the removed formations is shown in Table 3, the coincidence of pre- and postoperative diagnoses was 78.3%.

The technique of transanal endoscopic surgery made it possible to successfully perform primary radical intervention in 3 (13.0%) at the initial stages of rectal cancer. At the same time, the complicated course of the intra- and postoperative period was noted in 3 (13.0%) patients.

Conclusion.

Thus, the technique of transanal endoscopic resection has proven itself in clinical practice as an effective way of treating epithelial benign neoplasms of the rectum. The feasibility study has a number of advantages over traditional open surgical interventions and methods of endoscopic removal of rectal formations: preserving the function of the rectal closure apparatus, a small number of postoperative complications and ensuring the patient's quality of life at a high level.

According to modern scientific publications, the five-year survival rate of patients with rectal cancer after transanal endoscopic surgery is 90%. The results of the study indicate the effectiveness of the application of the technique of transanal endoscopic surgery in patients with malignant tumors of the rectum. The feasibility study ensured the radical removal of the formations of the TisN0M0 and T1N0M0 stages with minimal invasiveness of the procedure and the absence of early signs of recurrence of the disease.

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DOI 10.25789/YMJ.2023.81.12

УДК 616.61-006.694

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RENAL CANCER RESECTION WITH TARGETED BALLOON CHEMOEMBOLIZATION

The purpose of the study was to evaluate the content of endothelial vascular growth factor in the tissues of the kidney parenchyma, in the thickness of the tumor and in the blood serum of a patient during partial nephrectomy with intra-arterial administration of an anti-angiogenic drug.

Materials and research methods. The present study was carried out on the basis of an analysis of the results of surgical treatment of patients with kidney cancer. 8 patients with renal cell carcinoma in stage T 1 a N 0 M 0 organ-preserving surgery was performed in the amount of kidney resection with intra-arterial injection of the targeted drug Bevacizumab into the kidney artery. The concentration of vascular growth factor in the tumor, in the renal parenchyma, and in venous blood from the kidney was studied before the renal artery was clamped, during renal ischemia, and after the injection of Bevacizumab into the renal artery.

Results and discussion. With a sudden cessation of blood flow, the tumor releases the amount of vascular growth factor several times higher than the initial values: an increase in the concentration in the thickness of the tumor by 3 times, in the kidney parenchyma - 1.5 times, and in the venous blood - 3.5 times higher than before ischemia. Inactivation of the growth factor by the targeted drug caused a decrease in its level in the tumor tissue by 25%, in the kidney parenchyma by 10% and in the blood serum by 85.35%.

Conclusion. Intraoperative administration of a targeted drug at the time of acute tumor ischemia irreversibly binds the vascular growth factor released during hypoxia, and thus prevents neoangiogenesis in potential metastatic foci.

Keywords: kidney cancer, vascular endothelial growth factor, kidney resection.

Introduction. The discovery of the mechanisms of oncoangiogenesis has led to the creation of new approaches in the treatment of malignant neoplasms. Targeted drugs have firmly entered the routine practice of pharmacotherapy for various types of cancer. Various combinations of targeted and chemotherapy show ambiguous results, which encourages the search for new, non-trivial solutions in the fight against oncology. With regards to renal cell carcinoma, the situation is exacerbated by the peculiarities of the pathogenesis of kidney cancer. There is a lot of evidence in the literature of aggressive metastasis of kidney cancer - up to 30% of all newly diagnosed patients at the time of diagnosis have metastatic lesions of varying severity [2, 4, 10], and, despite the radical nature of the operation performed, these metastases progress to 20-40% of cases of all operations [1, 7]. Such an aggressive course of the disease requires systemic postoperative therapy [3]. In addition, after organ-preserving surgery, a third of patients may experience a relapse [8].

Thus, the treatment of renal cell carcinoma is a complex, completely unresolved problem of both surgical treatment and conservative therapy, since long-term targeted therapy has numerous side effects [9], affecting a wide variety of organs and systems. In some cases, these side effects can lead to death [5].

The threat of activation of kidney cancer metastases or tumor recurrence led to the invention of a new combined method of surgical treatment of malignant tumors of the renal parenchyma [6], which provides additional anti-relapse and antimetastatic protection of the body in organ-preserving surgical treatment of kidney cancer. It is well known that during acute ischemia, a cancerous tumor secretes special biologically active substances that promote the growth of additional vessels to improve the blood supply to the ischemic tumor. The group of these substances includes vascular endothelial growth factor (VEGF), which has the most active effect on neoangiogenesis. In this regard, substances were synthesized that inactivate VEGF by irrevers-

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ible binding to it, and thus prevent further growth of tumor vessels. Local injection of a targeted angiogenesis inhibitor into an artery feeding a segment of the kidney with a tumor at a precisely chosen time of acute ischemia of the tumor during resection of the tumor will lead to the fact that the released growth factor will be immediately inactivated by irreversible binding to the targeted drug, which will minimize the possibility of growth pathological vessels for tumor recurrence or growth of metastatic foci.

Materials and methods. To assess the effectiveness of intra-arterial administration of a targeted drug at the time of resection of a kidney tumor, the content of VEGF was measured in the thickness of the tumor tissue, in the renal parenchyma adjacent to the tumor, and in the venous blood serum taken from the lumen of the renal vein. The studied samples were taken 3 times during lumbotomy resection of a kidney with a malignant tumor: in the native state - before clamping the vascular pedicle of the kidney, a fragment of tumor tissue measuring 5 x 5 x 5 mm, a similar size area of the renal parenchyma, in close proximity to the tumorous tissue and venous blood aspirated with a syringe in a volume of 3 ml from the renal vein. Immediately after taking the first batch of material, a clamp was applied to the vascular pedicle, stopping the blood flow in the kidney. After an exposure time of 5 minutes, the second batch of laboratory material was taken: similar samples were taken from the thickness of the tumor mass, from the kidney tissue and venous blood by puncture of the renal vein distal to the clamp. After the removal of the material was completed, the lumen of the renal artery distal to the clamp was punctured, and the targeted drug Bevacizumab 2.5 mg, dissolved in 10 ml of saline, was injected into the artery. solution. After 5 minutes of exposure with a fringing incision, 5 mm away from the edge of the tumor, the formation was resected within healthy tissue. After suturing the wound of the parenchyma of the kidney with P- and Z - shaped interrupted sutures, before starting the blood flow, the renal vein was repeatedly punctured and 3 ml of venous blood from the kidney was drawn into the syringe.

The obtained material was labeled in test tubes and sent to a specialized laboratory in its native state.

The above study was performed in 8 patients with a diagnosed kidney tumor stage T 1 a N 0 M 0, in whom, according to the results of computed tomography, malignant neoplasms were found, 2.4–4.8 cm in size, with signs of contrast accumulation, subject to organ-preserving surgery. All patients gave their consent to conduct a scientific study and signed the ethical protocol of the North-Eastern Federal University named after M.K. Ammosov.

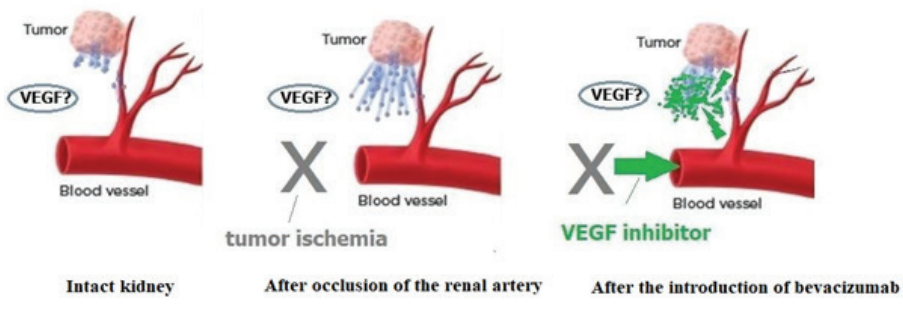

Fig. 1. Scheme for assessing the level of endothelial growth factor in the experiment

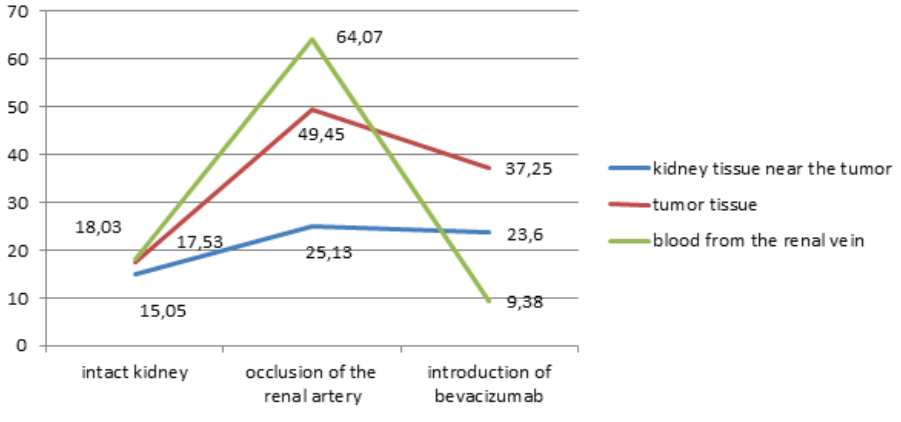

Fig. 2. Dynamics of VEGF content in preparations during the experiment (pg/ml)

Histological examination of the surgical material in all resected formations revealed a clear cell variant of kidney cancer. The early postoperative period was uneventful, and all patients after control studies were discharged for outpatient follow-up.

Quantitative assessment of the VEGF content in the studied samples was performed by enzyme immunoassay using diagnostic reagents HEA 143 Hu (Cloud - Clone Corp., USA). The standard sample preparation consisted in separating the material in a centrifuge with an acceleration of 1000 G for 20 min at a temperature of plus 4°C. The separated serum was analyzed immediately or, if necessary, kept at minus 20°C for a week.

The material obtained before grinding in a homogenizer was washed with chilled phosphate-buffered saline according to the instructions (Invitrogen , USA) and mixed with lysis buffer IS 007 (Cloud - Clone Corp. , USA). The resulting suspension was homogenized on ice using Qsonica ultrasound. Q 125 (Qsonica , USA) with parameters of pulse duration of 5 min, amplitude of 50% and a break of 5 min 2 times. The homogenizate was separated in a centrifuge with an acceleration of 1000 G for 5 min at a temperature not exceeding 4°C. The supernatant after sample preparation was subjected to enzyme immunoassay immediately or delayed, with storage conditions at t -20 °C for no more than 7 days. The results obtained by enzyme immunoassay were

VEGF levels in preparations (pg/ml)

	Intact kidney	Occlusion of the renal artery	Introduction of bevacizumab
Kidney tissue near the tumor	15.04	25.13	23.6
Tumor tissue	17.53	49.45	37.25
Blood from the renal vein	18.03	64.07	9.38

recorded with an SLT plate photometer. Spectra II (Tecan , USA) according to the instructions, with data processing by the MultiCalc software package (Wallac, Finland) .

The analysis of the obtained data was carried out using SPSS statistical packages (Windows version 7.5.2). The significance of differences between quantitative indicators was assessed by Student's t test for normally distributed values. Differences were considered significant at $p < 0.03$.

Results and discussion. The histological conclusion of all operated tumors showed the presence of a clear cell variant of renal cell carcinoma.

The obtained concentration of vascular growth factor in the course of experimental work is presented in Table No. 1 and the dynamics of its changes is shown in Figure No. 2. At the initial point, without any effect on the organ with the tumor, the levels of the growth factor in the tumor, in the peritumorous renal parenchyma and in the venous blood are at the same level (15.4-18.03 pg/ml). As mentioned above, acute ischemia of a cancerous tumor leads to a sharp hyperproduction of VEGF by oncocytes in a state of hypoxia: an increase by 182.08% is noted in the tumor itself, by 66.97% in the parenchyma tissue adjacent to the neoplasm, and an increase by 255.35%. Such an uneven increase in vascular endothelial growth factor is associated with the peculiarity of the redistribution of the produced substance under conditions of cessation of blood flow - the most active deposition occurs in venous blood, its least diffusion occurs in the tissue of the surrounding parenchyma of the kidney.

Based on the mechanism of its interaction, the targeted antiangiogenic drug bevacizumab, injected into the renal artery, irreversibly binds to the released vascular growth factor. It should be noted that the receptors of the monoclonal antibody produced by VEGF were immediately captured by the receptors - already 5 minutes after the administration of the drug, a significant decrease in the level of growth factor was recorded in the studied tissue samples: in the renal vein, a decrease by 85.35%, in the tumor tissue - by 24.67% and in peritumorous in the kidney parenchyma, the concentration of growth factor is reduced by 6.08%.

In the postoperative period, all operated patients did not show any shifts from the usual course of the postoperative period; the performed control studies of general clinical analyzes did not differ from the preoperative ones. All patients were discharged home in a satisfactory condition after removal of postoperative sutures.

The study clearly demonstrates the process of triggering oncological neoangiogenesis - acute hypoxia of cancer cells immediately leads to the production of substances aimed at the growth of new blood vessels to improve tumor nutrition. The obtained evidence of immediate activation of tumor angiogenesis explains the reason for the increased growth of metastases in 30% of cases after organ-preserving surgery: acute ischemia of the tumor during the temporary shutdown of blood flow in the operated organ is accompanied by a sharp release of VEGF , the start of blood flow after the completion of the main surgical procedure spreads the growth factor accumulated in the kidney throughout body and promotes additional vascularization of possible metastatic lesions.

Taking into account the above time factor of the mechanism of activation of possible metastases or relapses of a malignant neoplasm, it is advisable to introduce a commercial antiangiogenic drug into the arterial bed of the operated organ immediately at the time of acute ischemia during surgical treatment.

To achieve this goal, a technique for balloon chemoembolization and resection of malignant tumors of parenchymal organs has been developed and patented [6]. The essence of the technique is as follows: immediately before the operation, by means of selective renal angiography, a segmental branch of the renal artery is selected, feeding the segment with the tumor. Its location and diameter are estimated. Under x-ray control, a coronary balloon catheter with a coaxial channel is inserted into the established segment of the segmental artery (Figure No. 3). A solution of bevacuzimab, a monoclonal antibody that binds to vascular endothelial growth factor, is injected through the canal into the segment of the kidney with the tumor (Figure 4). Immediately after the injection of the drug, the balloon is inflated and thereby occludes the lumen of the artery, stopping the blood flow in the segment. Acute tumor ischemia, as mentioned above, entails a sharp release of VEGF , which irreversibly binds to the injected target substance. After achieving chemoembolization of the kidney parenchyma, the tumor is resected within healthy tissues by laparoscopic access (Figure No. 5). After reaching the final hemostasis, the balloon is deflated and removed from the artery, resuming the blood supply to the kidney (Figure #6).

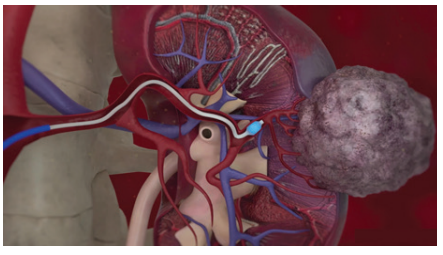

Fig. 3. Superselective balloon embolization of the renal artery

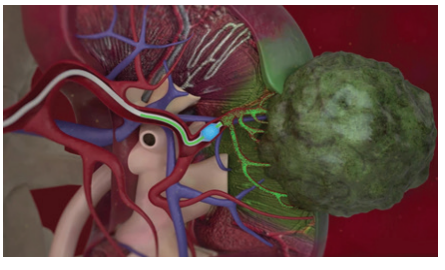

Fig. 4. Introduction of a targeted drug into a segment of the kidney with a tumor

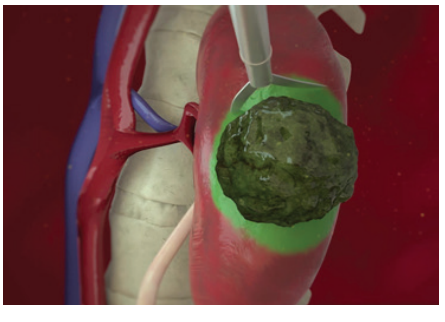

Fig. 5. Resection of the kidney tumor

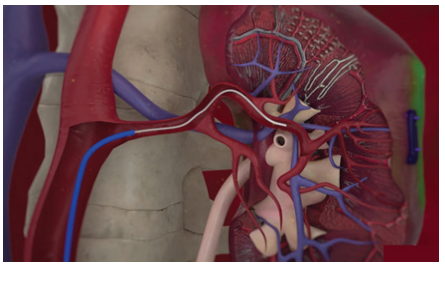

Fig. 6. Removing the balloon and starting blood flow

Thus, the presented method of resection of kidney tumors with preliminary balloon embolization and with the introduction of a targeted drug into the tumor provides an opportunity for minimally invasive, low-traumatic, functionally oriented, radical and carcinoprotective removal of a malignant formation of the renal parenchyma, based on the principles of laparoscopic surgery using transluminal endovascular aids, supplemented targeted, dosed and accurately timed administration of a chemotherapy drug that has an anti-relapse and anti-metastatic effect.

Conclusion. The study showed an immediate massive release of endothelial vascular growth factor by tumor tissue in the event of acute ischemia, and its equally immediate inactivation by a targeted drug, which indicates the need for timely, targeted and dosed administration of a chemotherapy drug. The proposed method of targeted chemoembolization most fully meets the above criteria and, together with superselective balloon embolization, allows the most minimally traumatic, with maximum preservation of organ function, safely, economically, and at the same time, radically and carcinoprotectively, to remove a malignant tumor of the kidney. In the context of the growing incidence of kidney cancer throughout the world, with the expansion of the arsenal of radiation diagnostic methods, the need for organ-preserving operations on the kidney increases every year, and this technique can significantly help in solving this problem.

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ORGANIZATION OF HEALTHCARE, MEDICAL SCIENCE AND EDUCATION

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MEDICAL AND DEMOGRAPHIC SITUATION IN THE REPUBLIC OF SAKHA (YAKUTIA)

DOI 10.25789/YMJ.2023.81.13

УДК 614.2

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In the Republic of Sakha (Yakutia) for the period 2015-2020, an increase in mortality and a decrease in fertility were noted, as well as higher rates of primary morbidity, including in the class of respiratory diseases, 1.4 times higher than in the Russian Federation, while the incidence rate of COVID-19 was higher; also, higher incidence of digestive diseases was noted (1.7 three times higher than in the Russian Federation). Indicators of primary morbidity of children and adolescents are higher than those of the total population in almost all classes of diseases for all 6 years of analysis (2015-2020). Differences in the indicators of primary morbidity in uluses (regions) of the Republic of Sakha (Yakutia) are higher by 3.3 times, which is associated with difference in availability of medical care in different areas of residence. The information obtained is important for development of management decisions at the regional level.

Keywords: medical and demographic situation, mortality, morbidity, age groups, uluses.

Introduction. The key objectives of the national project «Demography» are to improve the medical and demographic situation, reduce the morbidity of the population, increase the birth rate and healthy life expectancy [7, 4, 12, 8].

Geographical, climatic, territorial, national and ethnic features in Russia determine the inequality in the provision of medical care to the population [9]. The Far Eastern Federal District (FEFD) is the largest and most strategically important region in Russia and in the Asia-Pacific area. Since the 1990s, there has been a decline in population at a catastrophic rate in the Far Eastern Federal District, including the Sakha Republic (Yakutia) [11, 3, 10].

The COVID-19 pandemic has exacerbated the challenges of rising noncommunicable diseases (NCDs); scientific studies have shown that NCDs are risks of poor outcome [2, 6]. The way out of the demographic crisis requires an increase in the effectiveness of assistance, taking into account the regional characteristics of the territories [5].

Purpose of the Study. Based on the study of demographic indicators and a comparative analysis of the incidence in the Sakha Republic (Yakutia), in the Far Eastern Federal District and in the Rus-

sian Federation, recommendations are given for making managerial decisions at the federal, regional and municipal levels.

Materials and methods of research: statistical, analytical. The materials of official state statistics of the Ministry of Health of the Russian Federation, Rosstat and Sakha (Yakutia) (statistical bulletin) were used - URL: https://sakha.gks.ru/folder/53475 (date of access 03/23/2022), collection "The incidence of the entire population of Russia", FGBU "Federal Research Institute for Health Organization and Informatics of Ministry of Health of the Russian Federation "(TsNIIOIZ), 2016-2021).

Results and discussion. The Sakha Republic (Yakutia) is the largest region of Russia, the largest administrative-territorial unit in the world, larger than Argentina, the eighth state in the world in terms of area. The territory of Yakutia is characterized by low population density, the average population density is ten times lower than in the European regions of Russia. Representatives of more than 120 nationalities live in the Sakha Republic (Yakutia).

The population of the Sakha Republic (Yakutia) comprised of 992,115 people on January 1, 2022. The percentage of the urban population is 64.1%, rural - 35.9%. Between 2015 to 2020 in the Sakha Republic (Yakutia), the working-age population has decreased, and the number of people older than working age has increased, both in urban and rural settlements. In 2020 the provision with doctors in the Sakha Republic (Yakutia) amounted to 52.1 per 10 thousand population (in the Russian Federation - 38 per 10 thousand population), the provision with paramedical personnel in the Sakha Republic (Yakutia) amounted to 115.8 per 10 thousand population (in the Russian Federation - 85, 3).

In the Sakha Republic (Yakutia), an increase in mortality rates over the period of analysis (2015-2021) was noted from 8.5 to 10.7 per 1000 population, the birth rate decreased from 17.1 to 12.3 per 1000 population, while the natural increase population decreased from 8.8 to 1.6 per 1000 population.

The study showed that the incidence of newly diagnosed morbidity in the Sakha Republic (Yakutia) (2020) was 90425.5 per 100 thousand of the population, in the Far Eastern Federal District the figure is lower - 74596.5‰00, in the Russian Federation - 75840.1 (Table. 1).

In all regions of the Russian Federation in 2020, high rates of primary morbidity in the class Respiratory diseases were noted, which is associated with the COVID-19 pandemic, while in the Sakha Republic (Yakutia) this figure is 1.3-1.4 times higher than in Far Eastern Federal District and in the Russian Federation. The incidence of COVID-19 in the Sakha Republic (Yakutia) is higher and amounted to 4831.4‰00, in the Far Eastern Federal District - 3394.9 and in the Russian Federation - 3384.5, respectively.

In the second place in the frequency of primary morbidity in the Sakha Republic (Yakutia) is the class of injury, poisoning and some other consequences of external causes. In the Sakha Republic (Yakutia), this indicator is higher (9643.2‰00) than in the Far Eastern Federal District and the Russian Federation (8563.9 and 8114.7, respectively).

In the Sakha Republic (Yakutia), for the first time a high incidence of digestive diseases - 4365.3 ‰00 (1.7 times higher than in the Russian Federation), higher rates than in the Russian Federation, in the classes of respiratory diseases - by 36%, injuries, poisoning and some other consequences of external causes - by 18.8%, diseases of the nervous system - by 17.2%.

Table 1

Frequency of newly diagnosed morbidity in the population of the Far Eastern Federal District, the Sakha Republic (Yakutia) and the Russian Federation by disease classes, 2020 (per 100,000 population)

Disease classes (ICD-10)	RF	FEFD	Sakha Republic (Yakutia)
Total (including)	*75840.1*	*74596.5*	*90425.5*
I. Some infectious and parasitic diseases	2043.9	2165.9	1627.4
II. Neoplasms	981.3	807.0	697.3
III. Diseases of the blood, hematopoietic organs and individual disorders involving the immune mechanism	327.2	255.3	270.6
IV. Diseases of the endocrine system, eating disorders and metabolic disorders	1101.9	813.7	638.8
V. Mental and behavioral disorders	346.1	381.1	316.7
VI. Diseases of the nervous system	1251.5	1102.8	1466.4
VII. Diseases of the eye and adnexa	2389.5	2110.9	2304.3
VIII. Diseases of the ear and mastoid process	2049.8	1787.7	1505.3
IX. Diseases of the circulatory system	2931.9	2220.2	1902.0
X. Diseases of the respiratory system	*36983.9*	*37192.7*	*50266.1*
XI. Diseases of the digestive system	*2627.0*	*3496.8*	*4365.3*
XII. Diseases of the skin and subcutaneous tissue	3392.9	3187.7	3363.8
XIII. Diseases of the musculoskeletal system and connective tissue	2495.8	2031.0	2381.1
XIV. Diseases of the genitourinary system	3589.9	3103.9	2796.8
XIX. Injuries, poisoning and some other consequences of external causes	8114.7	8563.9	9643.2
COVID-19	*3384.5*	*3394.9*	*4831.4*

Table 2

Primary morbidity of the population of the Sakha Republic (Yakutia) by age groups in the dynamics of 2015-2020

	2015	2016	2017	2018	2019	2020
0-14 age	221864.6	231081.5	232991.1	233579.1	234784.1	183819.6
15-17 age	155390.6	153268.0	144461.7	144483.7	148806.9	121566.7
Over working age	62939.5	62251.9	57212.5	55261.9	58978.4	59242.4
Adults	61903.4	61008.0	57653.6	56627.0	58908.9	59057.3
Total	102664.3	102664.3	102191.0	101667.3	103558.1	90425.5

The first detected incidence of the entire population in the Sakha Republic (Yakutia) for 6 years of analysis (2015-2020) decreased from 102664.3 to 90425.5‰00. Over the past year, there has been a decrease in indicators in all age groups due to the COVID-19 pandemic, which is associated with a weakening of preventive and dispensary work with the population (Table 2).

It is worth paying attention to the high rates (2020) of the primary incidence of children (0-14 years old) - 183819.6 per 100 thousand of the corresponding population and adolescents (15-17 years old) - 121566.7‰00, respectively. Higher rates of primary morbidity in children and adolescents were noted in almost all classes of diseases over the entire period of analysis, these are, first of all, diseases of the blood and hematopoietic organs, diseases of the endocrine system, eating disorders and metabolic disorders, diseases of the digestive system, nervous system, mental diseases and others.

The study indicates the need for an in-depth analysis of the incidence of adolescents and children and strengthening of preventive work with this age group. It is necessary to develop federal, republican programs of preventive work, introduce new organizational technologies and forms of work with children and adolescents, and it is also necessary to strengthen the Health Modernization Program of the Sakha Republic (Yakutia) with a focus on the health of the future generation.

An analysis of the primary morbidity of the population in the uluses of the Sakha Republic (Yakutia) showed that the highest rates were noted in the Suntarsky ulus - 15384.9‰0. In second place is the Srednekolymsky ulus - 15189.4‰0, in third place - Tomponsky ulus - 13629.1‰0, in fourth place - Amginsky ulus 13141.0‰0). These uluses had higher rates of primary morbidity for all years of analysis. Low rates of primary morbidity were noted in Vilyuisky - 4726.0‰0, in Verkhoyansk - 5832.1‰0, in Verkhnekolymsky uluses - 6237.0‰0 and others.

The existing differences in primary morbidity rates in the uluses of the Sakha Republic (Yakutia) are 3.3 times and associated with different detection of diseases and the availability of medical care. At the same time, the high incidence in uluses indicates the need to develop management decisions at the regional level.

Discussion and conclusion. Over 6 years of analysis (2015-2020) in the Sakha Republic (Yakutia) there was an increase in mortality, a decrease in the birth rate and a decrease in natural population growth. In the Sakha Republic (Yakutia) in 2020, a high primary incidence in the class of respiratory diseases was noted, this figure is 1.3-1.4 times higher than in the Far Eastern Federal District and the Russian Federation. The incidence of COVID-19 in the Sakha Republic (Yakutia) is also higher and amounted to 8431.4‰00, in the Far Eastern Federal District - 3394.9 and in the Russian Federation - 3384.5. In the Sakha Republic (Yakutia), a higher primary incidence was noted than in the Russian Federation in the following classes: diseases of the digestive system (in 1.7), respiratory diseases - by 36%, injuries, poisoning and some other consequences of external causes - by 18.8% and others

The analysis revealed differences in primary morbidity rates in the uluses of the Sakha Republic (Yakutia) by 3.3 times, which indicates the need for an in-depth study of the causes of morbidity, taking into account the medical and demographic characteristics of the territories. Higher rates of primary morbidity in children and adolescents were revealed in almost all classes of diseases over the entire 6-year period of analysis, which determines the need to strengthen preventive work with this age group.

Conclusions. The information obtained is important and should be used to develop management decisions to improve the availability and quality of medical care for the population in different areas of residence in the Sakha Republic (Yakutia).

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HYGIENE, SANITATION, EPIDEMIOLOGY AND MEDICAL ECOLOGY

Sh.G. Gasanova

EPIDEMIOLOGICAL CHARACTERISTICS AND DYNAMICS OF BRUCELLOSIS INCIDENCE AMONG PEOPLE IN AZERBAIJAN (2017-2021)

DOI 10.25789/YMJ.2023.81.14

УДК 619.616

Relevance. The development of preventive measures and the improvement of the system of medical care for the treatment of zoonotic infections depend on the identification of the incidence rate and important risk factors. The present study aims to determine some epidemiological indicators and trends in the incidence of brucellosis among people in Azerbaijan from 2017 to 2021.

Methods. Descriptive cross-sectional retrospective study was carried out on the basis of the Electronic Surveillance System for Infectious Diseases of the Ministry of Health of the Republic of Azerbaijan for 2017-2021. Epidemiological reports on each case of the disease were used to collect data on demographic and background characteristics, risk factors, laboratory test results.

Results. In total, 1,711 newly reported cases of brucellosis have been registered in Azerbaijan from 2017 to 2021. The highest (5.3) and lowest (2.2) incidence rates per 100,000 population were observed in 2019 and 2021, respectively. The highest rates during the observation period were in the cities of Baku and Sumgayit, Shemkir region. The cumulative percentages of the disease were estimated for various variables by the following indicators: by sex: 70.6% for men; by age groups: 10.7% for the age group 26-30; by occupation: unemployed 45.8%; by place of residence: 81.6% for residents of districts and villages.

Conclusions. Despite the general decrease in the number of newly reported cases of brucellosis by years and cumulative characteristics, a detailed descriptive analysis revealed epidemiological features of the spread of cases by years depending on risk groups.

Keywords: brucellosis, incidence, trends, zoonosis, Azerbaijan.

Introduction. An analysis of the incidence of brucellosis among the population of Azerbaijan in the early years showed that over the past 10 years, the number of cases has gradually increased with a peak in 2019 (550 confirmed cases) [3, 5]. In 2020 and 2021, the number of confirmed cases of brucellosis among humans dropped sharply (by 2.8 times). This fact can be explained by quarantine measures during the COVID-19 pandemic in Azerbaijan and, accordingly, by the low rate of people applying to medical institutions. It can also be assumed that the promotion of veterinary vaccination, public education and other measures have been effective in reducing the detection of new cases of brucellosis. The study of the epidemiological characteristics and dynamics of the incidence of brucellosis among people in Azerbaijan at the present stage is of interest. Identification of changes in the prevalence of this zoonotic disease in humans will allow a better assessment of the public health measures and management practices needed to address the current situation. Despite the downward trend observed in recent years, the geographical position of Azerbaijan and its proximity to countries endemic for brucellosis, such as Iran, Georgia, are important risk factors for the reappearance and spread of this infection. [4, 5]. Therefore, it is important to conduct continuous monitoring of this zoonosis both among humans and among animals.

The purpose of the study: To determine some epidemiological indicators and dynamics of the incidence of brucellosis among people in Azerbaijan in 2017-2021.

Material and methods. The epidemiological descriptive assessment of brucellosis rates and dynamics included elements related to demographic and background characteristics such as age, sex, occupation, and area of residence. The data was obtained from the database of the Electronic Surveillance System for Infectious Diseases for 2017-2021. Inclusion criteria were human cases of newly reported brucellosis meeting the country's standard case definition, with a final classification of "Confirmed" by the date of final classification.

The standard definition of a suspected case includes a case with musculoskeletal pain and fever lasting more than 5 days and at least 5 of the following clinical features [2]:

- general signs of an infectious pro-

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cess: fever, sweating, chills, weakness, malaise, headache, lack of appetite, nausea;

- abdominal manifestations: hepatomegaly, splenomegaly, hepatitis;

- lesions of the musculoskeletal system: arthralgia, arthritis, myalgia, back pain, spondylitis, sacroiliitis, tendovaginitis, fibrositis;

- specific organ lesions: orchiepididymitis, miscarriages in women, endocarditis, neurological manifestations (meningoencephalitis, autonomic dysfunction, lesions of the peripheral nervous system).

Probable case: Signs of a suspected case and in addition one of the following: assisting in childbirth with an animal, contact with a sick animal, consumption of unpasteurized milk and dairy products from a sick animal; the use of meat subjected to insufficient heat treatment; epidemiological link to the outbreak; laboratory contact with material suspected of carrying the causative agent of brucellosis.

Confirmed case: Signs of a suspected or probable case and in addition one of the following: a positive result of a blood sample (serologically: Haddelson test, Rose Bengal test, Wright test, bacteriologically).

Study variables of interest to our analysis included age, gender, location, laboratory results (serological tests). Incidence rates per 100,000 population were calculated. Data were analyzed using descriptive statistical methods and presented by frequencies and percentages using Epi Info version 7.0 (Centers for Disease Control and Prevention, Atlanta, USA) and Microsoft Office Excel.

Results and its discussion. Figure 1 shows the absolute figures and the rate per 100 thousand of the population for 5 years (2017-2021). As can be seen from the graph over the past 5 years, there has been a steady downward trend in the incidence of brucellosis among humans. The linear trend line confirms this fact (R^2=0.625). The number of cases of newly reported brucellosis in 2021 is 2.3 times less than in 2017.

An analysis of the incidence distribution among the urban (Baku and Sumgayit) and rural populations showed that the incidence of brucellosis among the rural population is significantly higher than among the urban population (p<0.005). (Fig. 2) The same dependence is noted in the works of other authors [6].

The distribution of cases of brucellosis by district during the study period varied. So, in 2017, not a single case of brucellosis was registered in 8 districts (Astara, Balaken, Gusary, Kelbejar, Lachyn, Shusha, Siyazan, Zardob). Only 2 cases were registered in Balakan in 2019, and no cases have been registered in Gobustan and Shirvan regions since 2019. The largest number of cases over five years was registered in Baku and Nakhichevan Autonomous Republic (an average of 50.2 ± 3.5 cases per year). In the Siyazan region and Gusar, from 0 cases in 2017, up to 4-5 cases per year were recorded in subsequent years of observation. The areas with a high incidence of brucellosis among people include Ab-

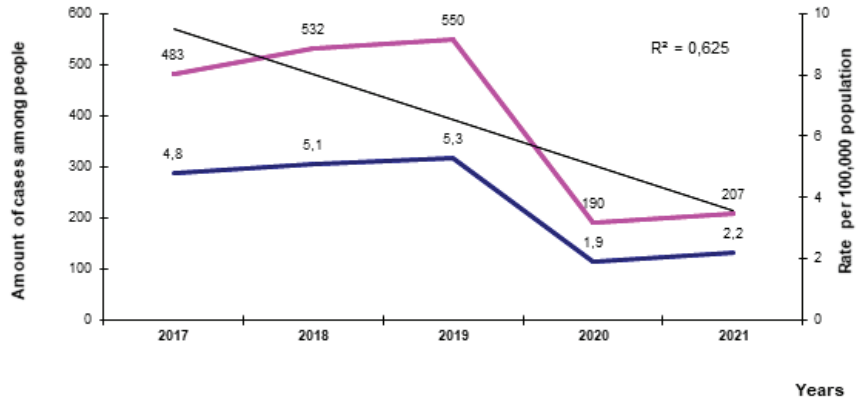

Fig 1. Number of cases of brucellosis among humans and rate per 100,000 population in Azerbaijan, 2012-2021

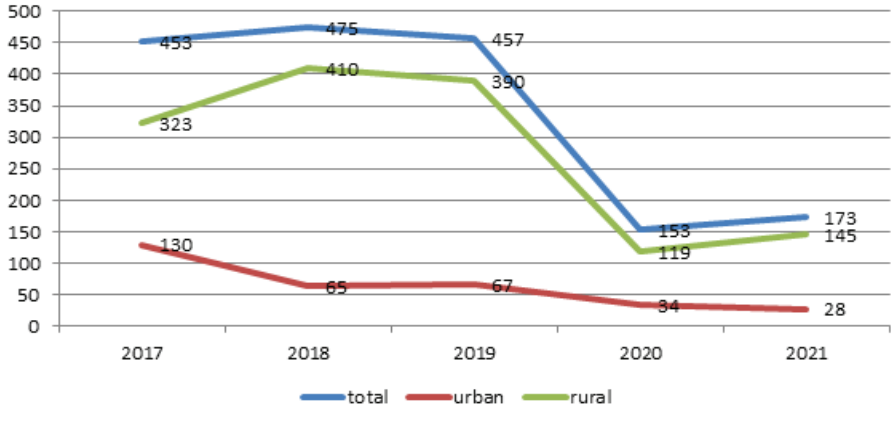

Fig. 2. Distribution of the incidence of brucellosis in large cities (Baku and Sumgayit) and regions of Azerbaijan, 2017-2021

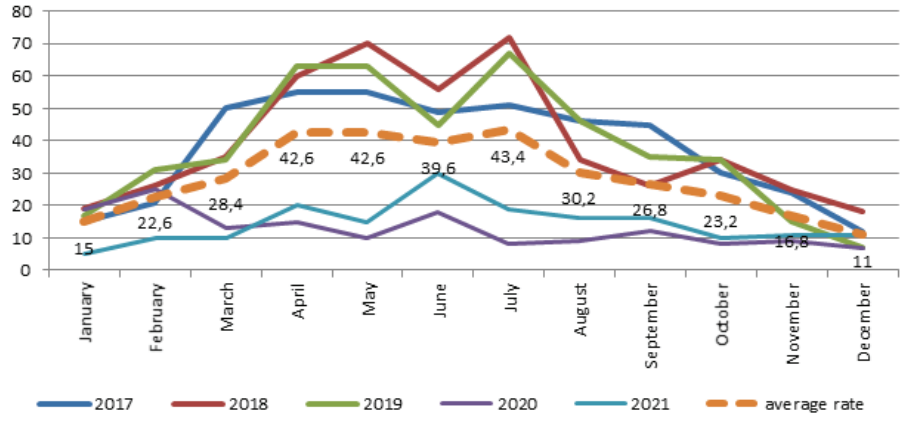

Fig.3. Seasonal distribution of cases of brucellosis in Azerbaijan, 2019-2021

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Analysis of the seasonality of the incidence of brucellosis for 2017-2021 (see Figure 5) showed an increase in the registration of cases in the spring, summer-autumn period. The peak incidence in 2017 falls on April, in 2018-2019. - for April and June, in 2020-2021 - for the month of June. The average annual monthly rate for 5 years also indicates that the peak of the increase in the number of newly reported cases of brucellosis occurs in the spring (April - 42.6 cases) and summer (July - 43.4 cases) months.

The revealed seasonality in the incidence of brucellosis in people can be explained by intensive human economic activity, namely: care for domestic animals, mainly for small cattle. Since brucellosis is mainly an occupational disease, infection occurs through direct contact with animals during childbirth. [1,6] Our results can be used to plan and evaluate interventions based on risk groups.

A study of the distribution of cases of brucellosis by sex showed that, on average, the proportion of newly reported cases of brucellosis among men is 2.37 times higher than among women.

An analysis of the distribution of cases by age group showed differences by year. So, for example, in 2017, the largest proportion of cases occurred in the age group of 6-10 years (16%), the smallest - in people over the age of 70 (0.7%) (Fig. 4). However, among men in the age group of 6-10 years, 15.8% of cases were noted. In 2018, a large proportion of cases of brucellosis incidence occurs in the age group of 26-30 years (12.8%). However, among men in the age group of 16-20 years, the percentage of cases is higher than in the rest - 11.4%. In 2019, the age of patients increases again, showing the maximum value in the age groups of 26-30 and 31-35 years (11.6% each). In 2020, the age groups 11-15 and 41-45 account for 11.1% of all registered. However, among men, a high proportion of cases occurs in the age group of 16-20 years (15.5%), among women - in the age group of 41-45 years (12%). In 2021, the largest number of cases were in the age group of 11-15 years (12.1% of all newly reported cases). By gender, the proportion of people with brucellosis differs - 11.2% of men aged 16-20 years and 12.5% for the age groups of 6-10 and 31-35 years among women.

The average age of people with newly reported brucellosis ranged from 28.4 years in 2017 to 33.9 years in 2019.

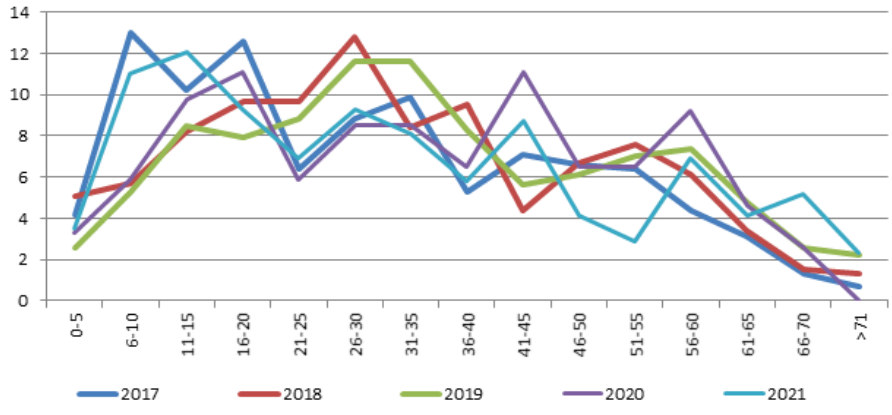

Fig 4. Distribution of cases of brucellosis by age groups in Azerbaijan for 5 years (2017-2021)

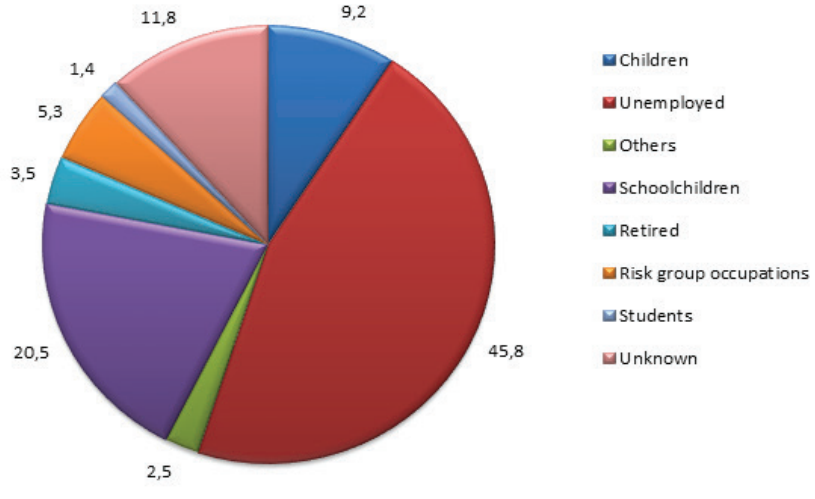

Fig 5. Distribution of cases of initially notified brucellosis by employment (2017-2021)

Young and working age people may have more contact with livestock and animal products, especially in rural areas. [2, 5].

An analysis of the distribution of cases of initially reported brucellosis by employment for the study period (2017-2021) showed that up to 45.8% of cases are the unemployed population (Fig. 5). One fifth of all cases are schoolchildren (20.5%).

Over the years, the distribution of the noted forms of employment differed greatly. Moreover, the share of the "unknown" field gradually increased (from 9.9% in 2017 to 16.8% in 2021). The proportion of preschool children ranged from 16.2% (in 2019) to 25.6% (in 2017). Over the years, there is a noticeable increase among the first reported cases of brucellosis in patients employed in risk group professions. Yes, in 2017. share was 2%, then in 2021. increased by 2 times – 4.1%.

Contact transmission may be related to occupation. It is not clear what duties housewives performed that put them at risk of contracting brucellosis; they may have been directly involved in activities such as cow milking and food preparation, during which they were likely to come into direct contact with brucella-contaminated milk or meat.

An assessment of the percentage distribution of newly reported cases of brucellosis by place of residence and employment showed some regularities. For example, in 2021, the proportion of children with brucellosis in the cities of Baku and Sumgayit increased significantly (17.9%). The same trend was also typical for pensioners in the cities of Baku and Sumgayit (4.5 times in 2021 the proportion of cases is higher than in previous years of observation).

Conclusions and recommendations. For the first reported brucellosis among humans in Azerbaijan in 2017-2021. the following epidemiological characteristics were inherent:

- 70.6% - the proportion of men infected with brucellosis;
- 10.7% - the share attributable to the age group of 26-30 years;

- 45.8% of all reported cases are unemployed and housewives;
- 81.6% - the share of residents of districts and villages.

Given the high incidence of the disease among males, as well as the unemployed, teaching these people how to prevent disease when in contact with animals can help control the disease. Measures such as educating about the importance of proper handwashing with soap and water, using gloves and face masks when dealing with infected livestock and when cleaning pens for affected livestock, and proper air conditioning can prevent infection in these categories of people.

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DOI 10.25789/YMJ.2023.81.15

УДК 574.2; 613.27

FEATURES OF THE ELEMENTAL COMPOSITION OF THE HAIR OF CHILDREN LIVING IN AREAS WITH DIFFERENT ANTHROPOGENIC LOAD

Biological environments of the body serve as a reliable bioindicator reflecting the state of human health and its environment. The aim of the study was to study the accumulation of trace elements in the hair of children from birth to 6 years old, living in areas with different types and degrees of anthropogenic pressure. The content of (Ca, Mg, Fe, Zn, Cu, Cr, Ni, Mn, Pb, Cd) in the hair of newborns was determined by atomic absorption spectrometry; the dynamics of the content of heavy metals in the hair of children from birth to six years of age was assessed; a comparative analysis of the level of trace elements in the hair of preschool children living in different regions of the Republic of Bashkortostan was carried out. By the age of six, the hair of Ufa children revealed a reduced content of essential elements: Fe, Mn and Zn; toxic metals: Pb and Cd; conditionally essential: Ni and Cr compared with the average physiological level. In the hair of children living in a region with a developed mining industry, on the contrary, there is an accumulation of essential elements: Fe and Mn; conditionally essential element - Cu and Ni and toxic - Pb.

Keywords: heavy metals, biological media, children's hair, macroelements, microelements.

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Introduction. One of the key characteristics of society is the state of health of the population, which depends on the adverse effects of the environment [7, 8]. Every year, millions of tons of pollutants enter the atmosphere of settlements from various sources of emissions. The leading place among ecotoxicants belongs to heavy metals: lead, cadmium, chromium. Potential sources of environmental pollution with chemical elements are oil refineries, mining and processing plants, ore deposits, the industrial development of which contributes to environmental pollution and their accumulation in toxic concentrations in biological media [9].

The organism of children is most susceptible to adverse environmental influences, it has an increased sensitivity to insufficient or excessive intake of chemical elements, both toxic and essential, from outside [2, 5]. The routes of entry of chemical elements into the child's body are diverse, mainly metals are supplied with food and water, less with inhaled air and through the skin [14].

To assess the impact of a habitat with a high content of heavy metals on the human body, elemental analysis of hair is used [1, 4, 6, 10]. A characteristic feature of the child population is a more pronounced reaction to harmful environmental factors due to the low threshold of sensitivity to the effects of heavy metals, low mobility and greater attachment to a particular area.

Purpose of the work: to study the accumulation of elements, both essential and toxic, in the hair of children from birth to 6 years old, living in the Republic of Bashkortostan in areas with different ecological and natural geochemical situations.

Materials and methods. 834 samples of children's hair were analyzed by atomic absorption spectrometry, and a

quantitative assessment was made of the accumulation of 10 microelements in the hair of preschool children living in various regions of the republic.

Hair samples of newborn children (n=129) were taken in maternity hospitals, hair samples of children of the first year of life (n=19) - in children's medical institutions, hair of children 3-6 years old (n=686) - in preschool institutions. Informed consent was obtained from the heads of institutions and parents of children to participate in the research. Analysis of hair samples was carried out in an accredited Testing Center of the Institute in accordance with current regulations. The content of chemical elements was determined by atomic absorption spectrometry on devices with flame and electrothermal atomization. The results of the analysis of the hair of newborn children were compared with the control group (Birsk), children aged 3-6 years - with the reference values given in the works of A.V. Skalny [12].

Statistical calculations were performed using the IBM Statistics 21.0 software package (IBM, USA). The distribution was checked for normality using the Kolmogorov-Smirnov test. The data obtained during the analysis were processed using one-way analysis of variance. Differences were considered statistically significant at $p<0.05$.

Results and discussion. The paper studied the hair of newborn children living in a large industrialized city - Ufa, a mining geochemical province - the city of Sibay and a small city with a favorable environmental situation - Birsk (control group). The results of the determination are presented in table 1.

The ecological situation in Birsk is much better than in Ufa, but drinking water in the city is highly hard. This is confirmed by the peculiarities of accumulation of calcium and magnesium in the hair of children, revealed in our studies, depending on the place of residence. Due to the increased level of calcium in the drinking water of Birsk, this element accumulates in the hair of newborns - 1.8 times higher than the content of this element in similar samples from the cities of Ufa and Sibay. In addition to calcium, the hair of newborn children from the city of Birsk and Ufa accumulates magnesium, the content of which is 2.2 times higher than in the hair of children living in the city of Sibay.

Zinc is the most important element, its biological role was established more than 100 years ago, it is part of the composition of enzymes involved in all types of metabolism: skin regeneration processes, hair and nail growth, secretion of sebaceous glands, maintaining the body's immune defense [1, 13]. With a lack of zinc in children, spatial thinking is disturbed, memory and learning ability deteriorate, as protein and nucleic acid synthesis slows down. Excess intake of zinc is accompanied by a decrease in the level of calcium not only in the blood, but also in the bones, while the absorption of phosphorus is disrupted; which leads to osteoporosis. In the studied hair samples, there is a difference in the content of zinc. There is 3.7 times more zinc in the hair of newborns from the city of Ufa than in the hair of children from the city of Birsk. The maximum content of zinc in the hair of newborns in Sibay is two times lower than in Birsk, eight times lower than in Ufa, but the minimum concentration is three times higher than in Birsk, and Ufa - 12 times.

Iron is a critical element that is involved in a number of biological reactions: energy release processes, enzymatic reactions, cholesterol metabolism [1]. Both deficiency and excess of iron adversely affect human health. Iron deficiency in children leads to iron deficiency anemia, in which there is developmental delay and behavioral abnormalities, an increased risk of atherosclerosis, liver and heart disease, arthritis, and diabetes [1, 3]. Excess iron can be caused by a genetic defect and also occur in some types of anemia or porphyria. An imbalance of copper and nickel can lead to low iron levels. The average content of iron in the hair of newborn children in Sibay is 2.5 times lower than in Ufa and almost 5 times lower than in Birsk. Iron deficiency states in newborns can be associated with the corresponding state of the mother, or due to living in a geochemical province.

Chromium plays an important biological role in the body: biomolecules containing chromium are involved in the regulation of fat synthesis and carbohydrate metabolism, interact with insulin in carbo-

Table 1

The content of chemical elements in the hair of newborns. mcg/g

Element	Statistical parameters				
	Locality	M	±m	Min	Max
Ca	Sibay	717.0	60.0	217.10	1239.9
	Birsk	1339.0	258.6	351.4	3366.7
	Ufa	729.8	18.9	19.8	2483.8
Mg	Sibay	700.9	54.9	163.8	1052.7
	Birsk	1554.5	6.8	327.0	3983.3
	Ufa	1547.0	4.5	93.11	5205.5
Fe	Sibay	28.9	5.8	10.5	122.6
	Birsk	132.6	2.6	18.5	716.7
	Ufa	71.9	2.6	14.3	229.3
Zn	Sibay	184.3	10.2	98.3	268.1
	Birsk	148.3	12.6	29.9	558.3
	Ufa	550.5	11.4	8.1	2202.7
Cu	Sibay	7.10	0.36	3.94	9.69
	Birsk	8.06	1.20	1.07	30.60
	Ufa	5.76	1.32	0.19	21.20
Ni	Sibay	3.00	0.41	0.86	6.68
	Birsk	4.23	0.90	0.38	20.60
	Ufa	5.88	0.46	0.38	23.80
Cr	Sibay	61.00	8.03	11.74	140.36
	Birsk	47.49	0.90	6.66	200.00
	Ufa	54.73	0.09	0.59	179.50
Mn	Sibay	1.21	0.21	0.18	3.11
	Birsk	2.92	0.10	0.31	16.60
	Ufa	2.64	0.87	0.22	75.90
Pb	Sibay	2.30	0.37	0.83	7.29
	Birsk	1.30	0.09	0.37	5.90
	Ufa	12.08	0.11	0.00	40.50
Cd	Sibay	0.090	0.010	0.020	0.240
	Birsk	0.210	0.001	0.010	1.540
	Ufa	0.080	0.040	0.000	0.730

hydrate metabolism, and regulate blood sugar levels [1]. Chromium deficiency can provoke the development of diabetes mellitus, lead to the development of atherosclerosis, coronary heart disease, anxiety, insomnia, and headaches. The toxic effect of chromium depends on its valency: hexavalent chromium is more dangerous for the body, it has a general toxic, nephrotoxic and hepatotoxic effect. An excess of chromium in the body leads to asthmatic bronchitis, bronchial asthma and cancer [3]. The increased concentration of chromium in the biological environment of newborns usually decreases rapidly during the first months of life. In the hair of children from Sibay, the concentration of chromium is 1.3 times higher than in the control group. The minimum concentration of chromium in the hair of newborns in Sibay is almost twice as high as in Birsk, and 20 times in Ufa.

Copper - plays an important role in maintaining a healthy immune system, is part of vitamins, hormones, enzymes, and is involved in processes that strengthen bone tissue. It increases the body's resistance to infections, binds microbial toxins and enhances the action of antibiotics, promotes the absorption of iron [1]. Copper deficiency develops against the background of prematurity, malnutrition, treatment with iron and zinc preparations and can cause iron deficiency anemia, osteoporosis, arterial aneurysms. A comparative assessment of the copper content revealed that the average content of copper in the hair of newborns from Ufa is 1.4 times less than in similar samples of the control group. The maximum copper content in the hair of newborns in Sibay is 2-3 times lower than in Birsk and Ufa, the minimum concentration is three times higher than in Birsk, and in Ufa - 20.7 times.

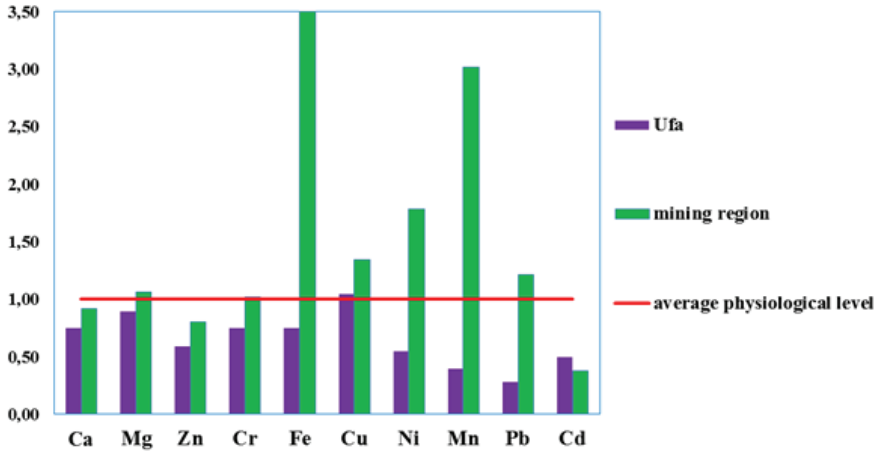
Lead is one of the most toxic trace elements with the ability to accumulate in the human body. Elevated lead levels negatively affect the nervous and cardiovascular systems, kidneys. Excess lead leads to a decrease in calcium, iron, zinc, selenium in human organs and tissues. Since zinc and calcium are lead antagonists, its elevated concentrations displace zinc, calcium, iron, disrupting their physiological role in the body. With the simultaneous intake of zinc and lead, the accumulation of lead in the body decreases [11].

Lead was found at a high level (12.08 μg/g) in hair samples from children from the city of Ufa, which is 9.3 times higher than the content in biological samples of children's hair in the control group (1.30 μg/g). Since lead is a calcium antagonist,

Table 2

The content of metals in the hair of newborns and preschool children living in Sibai, mcg/g

Element	Statistical parameters				
	Age	M	±m	Min	Max
Ca	At birth	717.0	60.0	217.1	1239.9
	A year later	1315.8	258.6	138.1	6434.6
	At the age of 3-6 years	522.1	18.9	492.8	567.5
Mg	At birth	700.9	54.9	163.8	1052.7
	A year later	143.8	6.8	30.3	299.2
	At the age of 3-6 years	43.3	4.5	33.4	51.2
Fe	At birth	28.91	5.8	10.5	122.7
	A year later	10.31	2.6	2.0	34.9
	At the age of 3-6 years	46.55	2.6	42.6	52.1
Zn	At birth	184.3	10.2	98.3	268.1
	A year later	135.0	12.6	58.3	259.7
	At the age of 3-6 years	106.10	11.4	82.5	128.1
Cu	At birth	7.1	0.4	3.9	9.7
	A year later	8.5	1.2	3.5	14.7
	At the age of 3-6 years	10.2	1.3	7.8	13.3
Ni	At birth	3.0	0.4	0.9	6.7
	A year later	2.2	0.9	0.3	6.1
	At the age of 3-6 years	1.5	0.5	0.6	2.3
Cr	At birth	61.0	8.0	11.7	140.4
	A year later	5.5	0.9	2.1	10.5
	At the age of 3-6 years	1.1	0.1	1.0	1.3
Mn	At birth	1.2	0.2	0.2	3.1
	A year later	0.9	0.1	0.3	2.2
	At the age of 3-6 years	3.5	0.9	2.4	5.7
Pb	At birth	2.3	0.4	0.8	7.3
	A year later	4.9	0.1	0.2	16.0
	At the age of 3-6 years	2.7	0.1	2.6	3.0
Cd	At birth	0.10	0.01	0.02	0.24
	A year later	0.400	0.001	0.030	1.690
	At the age of 3-6 years	0.10	0.04	0.07	0.23



The level of trace elements in the hair of preschool children, in shares of the average physiological level

Table 3

The content of chemical elements in the hair of children 3-6 years old living in a large industrial city and in a region with a developed mining industry, mcg/g

Element	Hair of children 3-6 years old		
	Average physiological level (by A.V. Skalny, 2003)	Large industrial Ufa	A region with a developed mining industry, Sibai
Ca	498.2	372.0	457.9
Mg	47.0	42.1	49.9
Fe	26.0	19.4	91.1
Zn	138.7	81.9	111.2
Cu	9.5	9.9	12.8
Cr	0.99	0.74	1.01
Ni	0.55	0.30	0.98
Mn	1.01	0.40	3.05
Pb	2.66	0.74	3.23
Cd	0.24	0.12	0.09

with an increased lead content in the hair of Ufa newborns, there is a reduced accumulation of calcium. It is important to note that at a low concentration of iron in the body (in the studied hair samples of newborns from Ufa and Sibay, it was contained less than in the control group), the risk of toxic effects of lead increases (lead content in bioassays of children's hair in the city of Ufa is increased).

Manganese protects the body from the harmful effects of peroxide radicals, is responsible for the stability of the structure of cell membranes, muscle and connective tissue. The role of manganese in cell metabolism and enzymatic reactions is known. Manganese deficiency causes anemia, growth retardation, and weight loss. Hypomanganosis in children leads to a violation of carbohydrate metabolism, which is manifested by allergies, dermatitis, impaired muscle tone, lethargy, fatigue, stunted hair and nail growth. [11]. In the hair of newborn children of Sibay, the concentration of manganese is 1.7 times lower than in the control group.

Nickel belongs to conditionally essential elements, but at the same time is the most dangerous environmental pollutant. The concentration of nickel in the biological media of children from the city of Ufa is 1.7 times higher than in the control group, and in the hair of children from the city of Sibay it is 1.4 times lower. Petrochemical enterprises are a source of nickel entering the environment. This may explain the high concentrations of this metal in the hair of newborns in Ufa compared with the control group.

If an excess or deficiency of macroelements and essential microelements can still be considered physiological or associated with the activation of redox processes in the body of a newborn during growth and development, then the accumulation of toxic metals (cadmium, lead) is rather pathological. In samples of children's hair in Sibay and Ufa, the content of cadmium was 2.3-2.6 times ($p<0.05$) less than in similar samples of the control group. Hair is an additional excretion pathway for cadmium. The high concentrations of cadmium in the hair of children gradually decrease over the course of life.

For further in-depth study of macro- and microelements in the body of children, we conducted a study of their content a year after the initial study and at the age of 3-6 years, analyzed the dynamics of their changes. The main statistical parameters of the distribution of metal content in the hair of a child were studied using the example of children living in the city of Sibay. Data on the content of metals in the hair of children from birth to preschool age (3-6 years) are presented in Table 2.

Judging by the average concentrations, by the end of the first year of life, the content of ecotoxicants and metals in the hair of children increased due to living in a geochemical province, i.e. lead, cadmium and copper. But by preschool age, the content of lead and cadmium decreases by 1.8 and 3.1 times, respectively. Perhaps there is an adaptation of the body to the environment.

In children, by the age of 3-6 years, there is a decrease in the content of chromium, nickel, magnesium and zinc. The average level of chromium decreased by almost 11 times by the first year of life and 55 times by the age of 3-6 years; magnesium is almost five times a year and 16 times by the age of 3-6 years; nickel - about 2 times; zinc - 1.7 times. The average copper content increased 1.4 times. The average level of lead, cadmium and calcium increases by the first year of life, but gradually decreases by 3-6 years. The level of iron and manganese in the child's hair decreases by the end of the first year of life, but increases again by preschool age, which may be due not only to an increased need for iron in the body, but also to the formation of other "tissue" iron depots.

Table 3 presents the results of studying the elemental composition of the hair of preschool children and the average physiological level according to the literature data.

A comparative characteristic of the level of trace elements in the hair of preschool children living in a large industrial city (Ufa), in a region with a developed mining industry, was carried out in comparison with the reference values obtained by A.V. Skalny [12]. The results obtained are shown in Figure 1.

In the hair of Ufa children, a reduced content of iron (by 6.6 times), lead (by 3.6 times), manganese (by 2.5 times), cadmium (by 2.0 times), nickel (by 1.8 times) was revealed, zinc (1.7 times), calcium (1.3 times) and chromium (1.3 times) compared with the average physiological level.

In the hair of children living in a region with a developed mining industry, an increased content of iron (3.5 times), manganese (3.0 times), copper (1.3 times), lead (1.2 times) was found and somewhat reduced - zinc. The revealed high level of iron in hair samples may be associated with a low content in the blood due to malnutrition. The elevated level of copper is probably associated with the geochemical features of the region under study.

The present study did not analyze the morbidity of the child population associated with elemental homeostasis and the sex of the child. These issues may be the subject of further study.

Conclusion. The conducted studies revealed the features of the elemental homeostasis of the child population living in regions of the republic with different ecological conditions. The elemental composition of children's hair depends on the region of residence, the degree and type of environmental pollution, the quality of drinking water, the synergy and antagonism of elements in the body, and changes with the age of the child.

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TOPICAL ISSUE

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SARS-CoV-2 VIRAL LOAD IN NEWBORNS WITH COVID-19

DOI 10.25789/YMJ.2023.81.16

УДК 616.2 (616.9)

The aim. Investigation of nasopharyngeal SARS-CoV-2 viral load in newborns with COVID-19 of different severity.

Materials and methods. The main group was composed of 44 newborns with RT-PCR confirmed COVID-19. 168 children aged from 1 month to 17 years old with RT-PCR confirmed COVID-19 were included in the group of comparison. SARS-CoV-2 viral load was measured as amount of viral RNA copies in 1 ml of nasopharyngeal mucosa using the regression model and presented as lg of the amount. The results presented as Me[Q1;Q3].

Results. SARS-CoV-2 viral load in newborns was detected significantly higher as compared to children aged 1-17 years: 3,2×106[5,7×104; 7,8×107] and 1,3×105[2,6×104; 1,2×107] respectively and no association has been revealed between nasopharyngeal SARS-CoV-2 viral load and disease severity, lung injury and the type of feeding.

Discussion. Nasopharyngeal SARS-CoV-2 viral load presumably reflects epidemiological circumstances and tends to decrease as the disease develops. It can be due to virus dissemination to lung tissue, vessel walls and other organs that followed by fall of number of viral particles in upper respiratory tract. While elimination of virus from mucosa on the early stages after infection probably depends on efficiency of innate immunity (which mechanisms can kill and/or impede virus invasion before immune response develops), viral load in blood and internal organs tissues, as well as favorable course of the disease, mainly depends on 'proportionate' immune response. So that, assessing the viral load and its significance for disease development should be performed considering the day after infection.

Conclusions. High level of nasopharyngeal SARS-CoV-2 viral load in newborns along with mostly mild COVID-19 course can be based on age-correlated features such as: immature immunity mechanisms, low expression of ACE2 receptors, the absence of comorbidity and intake of innate immunity factors while breastfeeding.

Keywords: newborns, COVID-19, viral load, SARS-CoV-2, children, new coronavirus infection.

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Introduction. COVID-19 pandemic challenged worldwide healthcare system. Since understanding of the patterns of infection spread and mechanisms of its interaction with the human body are incomplete, it is of particular importance to search for reliable and accessible clinical and/or laboratory criteria, allowing to predict the features of the course and outcomes of the disease in a particular person.

The children's population is also susceptible to COVID-19 infection, but, unlike adults, it is more heterogeneous in the context of immune response characteristics. In general, the clinical experience accumulated during the fight against the new coronavirus infection suggests a milder course of COVID-19 in children: as acute respiratory viral infection and nasopharyngitis. The fact does not reduce the importance of identifying predictors of severe forms, such as pneumonia, acute respiratory distress syndrome, as well as multisystem inflammatory syndrome [3-5, 18, 27], which could contribute to the early diagnosis of the adverse development of the disease. As polymerase chain reaction (PCR) is generally accepted method for COVID-19 confirming, data on the amount of viral genetic material on the mucosa of the upper respiratory tract – nasopharyngial viral load (VL) can be used as an available prognostic criterion. In addition, this indicator, unlike the clinical picture, has not been studied in newborns, which makes the presented materials unique. The work continues a series of clinical and epidemiological studies of COVID-19 infection in the pediatric population [1, 2, 7-9, 15].

The aim. To study the level of SARS-CoV2 viral load in newborns with COVID-19 infection.

Material and methods. Two groups of children with a positive SARS-CoV-2 PCR test of a nasopharyngeal smear were examined. The main group included 44 newborns. The comparison group included 168 children aged from 1 month to 17 years, of which: 5 – children from 1 month to 1 year, 12 – children 1-3 years, 28 – children 3-6 years, 55 – children 7-11 years and 70 – children from 12 to 17 years. The nasopharyngeal VL of SARS-CoV-2 was determined in both groups. In children of the main group, the severity of the disease and objective signs of lung damage were additionally established, in accordance with the data of computed tomography (CT) of the chest or radiog-

raphy (Rg) of the lungs. The newborns were selected from children admitted to the “City Ivano-Matreninskaya Children's Clinical Hospital” Irkutsk, Russia in 2020-2021yy. with a diagnosis of a New Coronavirus Infection (COVID-19).

Baseline characteristics of the main group: boys predominate in the sexual structure – 65.9% (29/44); mean *(M)* age at admission is 4±3.1 days; weight and body length Me[Q1;Q3] at birth was 3210[2720;3600] grams and 51[49.5;54] cm, respectively, body weight at admission – 3300 [2780;3660] grams. The majority of newborns in the group were born full-term 88.6% (n=39), with a gestational age of 39[38.2;40] weeks, were immediately breastfed and 65.9% were breastfed during the treatment (n=29).

During inpatient treatment, the condition of 43.2% (n=19) newborns were assessed as mild, 38.6% (n=17) were of moderate, and 18% (n=8) were severe. CT- or Rg-signs of lung tissue injury ("ground glass", "cobblestone pavement", "reverse halo" symptoms) were identified in 54.5% (n=24). The most frequent clinical symptoms were fever 34±13.9% (n=15) and a runny nose of 29.5±13.4% (n=13). There were no cases of respiratory distress syndrome, multisystem inflammatory syndrome and deaths among the examined newborns.

The isolation of the genetic material of nucleic acids from the samples was performed with a set of reagents "RealBest extraction 100". RT-PCR studies were carried out using "Real-Best RNA SARS-CoV-2" reagents and with the Real-time CFX96 Touch (BioRad) amplifier according to the manufacturer protocol. The viral load (concentration of genome-equivalents of the SARS-CoV-2 virus in 1 ml of nasopharyngeal fluid) was determined according to the method described earlier [1].

Comparison between the groups was performed using Statistica 6.0 software (StatSoft, USA) using a nonparametric Mann-Whitney U-test, the differences were considered significant (*p) at $p<0.05$. Tabular data are presented in absolute numbers as median and interquartile interval – Me[Q1;Q3]. The data in the figures are presented as a decimal logarithm (lg) of the normalized number of copies of viral RNA in 1 ml of nasopharyngial mucus [1].

The study was approved by the Ethics Committee of the "Scientific Center for Family Health and Human Reproduction" (Protocol No. 6.1 of 06.19.2020). All participants or their legal representatives have signed an informed consent.

Results and discussion. The viral load of SARS-CoV-2 for all examined children was $1,3\times10^5$[$2,6\times10^4$; $1,2\times10^7$]. In the group of newborns – 3.2×10^6/ml (Table 1). that was significantly higher than in the pediatric population as a whole (according to Orlova E.A. et al.) (Figure 1) [1].

There were no differences of VL in groups of newborns with varying degrees of severity of the new coronavirus infection (Table 2).

Among children of all age groups, SARS-CoV-2 VL was maximum in the group of newborns; this indicator was significantly lower in children 3-11 years old (Figure 2).

The level of SARS-CoV-2 VL in breastfed newborns (n=23; $1,6\times10^6$[$5,8\times10^5$; $7,9\times10^7$]) did not differ (p=0.7) from VL in formula fed children (n=9; 1.8×10^7[7.7×10^5; $6,6\times10^8$]) (Table 4).

Additionally, a comparison of the LV level in newborns with CT or Rg signs of lung tissue injury and without them was performed (Table 3). There was no connection between the level of VL and the presence of lung injury (p=0.3) (Figure 3).

The accumulated clinical experience indicates a relatively low incidence of severe and complicated variants of COVID-19 in newborns. An asymptomatic or mild course does not exclude the fact of infection of children with the SARS-CoV-2 virus, which is confirmed by measuring the nasopharyngeal viral load, which is significantly higher than in the pediatric population as a whole [1, 6].

Today in the scientific literature, controversial data can be found concerning the relationship of the VL level with the severity of the inflammatory process. A number of studies reveal a positive correlation, however, most of the them indicate the absence of a clear linkage between VL and the severity of the disease,

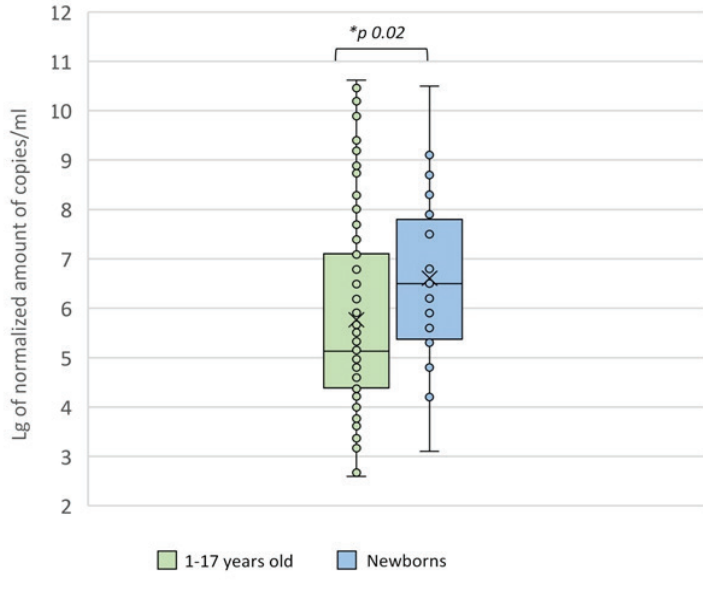

Fig. 1. SARS-CoV-2 viral load in 1-7 years old children and in newborns

Table 1

SARS-CoV-2 viral load in newborns

Me [Q1;Q3] copies/ml	Min-Max
3.2×10^6 [5.7×10^4; 7.8×10^7]	1.2×10^3 – 3.3×10^{10}

Table 2

SARS-CoV-2 viral load in newborns with COVID-19 of different severity

Disease severity	Viral load Me[Q1;Q3], copies/ml	Significance
Mild, n=19	2.4×10^6 [4.8×10^5; 2.5×10^8]	
Moderate, n=17	3.4×10^7 [1.5×10^7; 7.9×10^7]	$p^1=0.9$
Severe, n=8	2.2×10^5 [1.8×10^4; 3.2×10^6]	$p^1=0.2$; $p^2=0.2$

p^1 – as compared to Mild group (Mann-Whitney U-test), p^2 – comparison between Moderate and Severe group (Mann-Whitney U-test); the differences were considered significant at $p<0,05$.

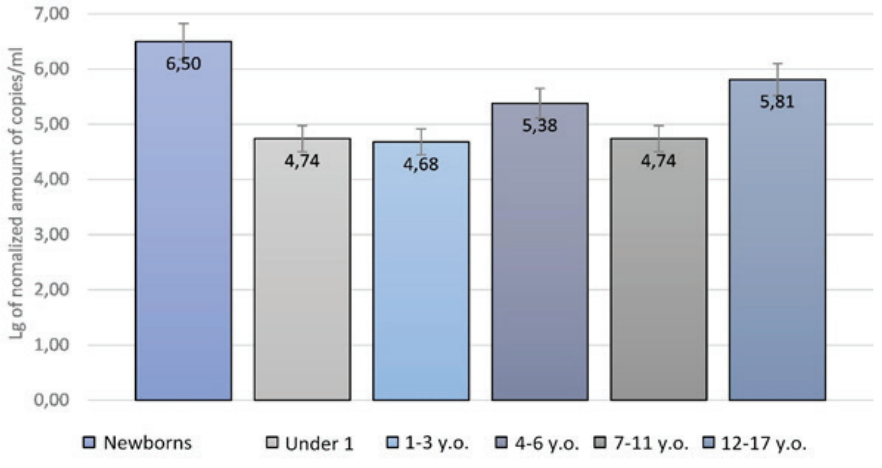

Fig. 2. SARS-CoV-2 viral load in children of different age

Table 3

SARS-CoV-2 viral load in newborns with and without lung injury

Group of newborns	Viral load Me [Q1;Q3] copies/ml	Min-Max
Lung injury, n=19	3.2×10^6 [7.6×10^5; 4.9×10^8]	3.2×10^5 – 1.3×10^9
No lung injury, n=9	1.5×10^6 [1.8×10^4; 3.4×10^7]	1.2×10^3 – 3.3×10^{10}

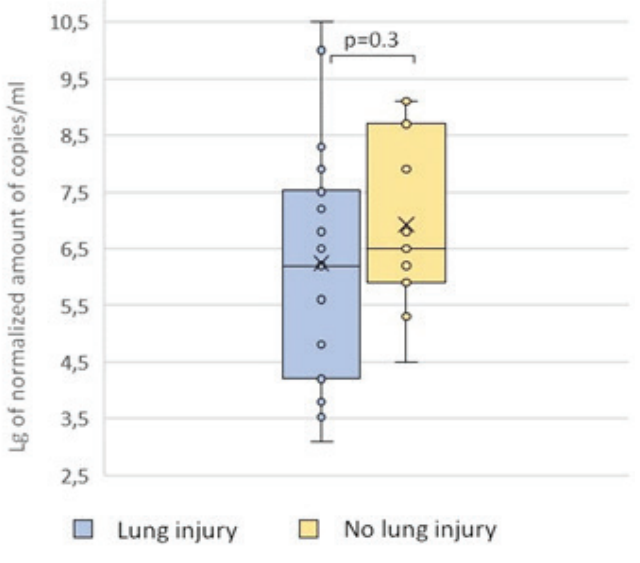

Fig. 3. SARS-CoV-2 viral load in newborns with lung injury and without lung injury

Table 4

SARS-CoV-2 viral load according to newborns`s type of feeding

Type of feeding	Viral load Me[Q1;Q3], copies/ml	Significance
Breast feeding, n=23	1.6×10^6[5.8×10^5; 7.9×10^7]	
Formula feeding, n=9	1.8×10^7[7.7×10^5; 6.6×10^8]	p=0.7

Сравнение выполнено с использованием U-теста Манна-Уитни, отличия значимы при p<0,05.

which is consistent with the data obtained in this work [19-24].

The results obtained suggest the following: VL level tends to decrease with the development of the disease, both in the case of positive dynamics and recovery, and in the case of an unfavorable course. After infection, viral particles accumulate in large quantities on the mucous membrane of the nasopharynx – the entrance gate of infection, therefore, asymptomatic and patients in the early stages of the disease are the most dangerous epidemiologically. Over time, the virus invades the cells of the body, replicate and disseminate into various tissues, which leads to a decrease in the number of viral particles on the mucous membrane of the upper respiratory tract, but an increase in VL in target organs (for example, in lung tissue or in the vessel wall), thus, inducing damage of specific systems [11, 12].

At the same time, the severity and outcome of the disease are determined not by the VL, but by the characteristics of individual reactivity, immune, in particular (considering the influence of comorbidity) [16, 17]. It follows that the level of nasopharyngial VL is, predominantly, a reflection of the epidemiological situation and effectiveness of nonspecific resistance mechanisms of the mucosa, while the number of viral particles in the internal environment depends mainly on the effectiveness of the immune response. Also it can indicate insufficiently studied, genetically determined features of somatic and/or immune cells, for example, the receptor apparatus, which makes some individuals prone to severe course [2, 12, 23-26].

This assumption is confirmed by the results of a number of studies aimed at studying the immune response in COVID-19: in asymptomatic patients and in patients with mild infection, specific antibodies to SARS-CoV-2 antigens are not detected or are present in low levels, unlike patients with a pronounced clinical manifestation [12, 13, 15, 16]. The fact allows hypothesize about the elimination of the virus from the mucous membrane before the development of a complete immune response. The period of time – from infection to the collection of biomaterial, also matters, as significantly affects the interpretation of the results.

Conclusions. The SARS-CoV-2 VL in newborns is significantly higher than in the pediatric population as a whole. At the same time, there is no evidence of the correlation between clinical severity and nasopharyngeal VL. The predominantly mild course of COVID-19 in newborns

may be due to age-related features: immaturity of their own immune mechanisms, low expression and functional activity of angiotensin-converting enzyme 2 receptors, which is necessary for invasion into the target cell, the absence of comorbidity and the consumption of a number of resistance factors with breast milk, including secretory antibodies [5, 10, 14, 18, 28].

Conflict of interests. The authors declare no conflict of interests.

Acknowledgment. The authors express their gratitude to the staff of the Department of Newborns Pathology of the "City Ivano-Matreninsky Children's Clinical Hospital", and also to the deputy chief physician for medical work Belkova Tatiana Yurievna.

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DISTRIBUTION OF CLINICAL FORMS OF NEWLY DETECTED PULMONARY TUBERCULOSIS AMONG ADULT POPULATION OF THE SAKHA REPUBLIC (YAKUTIA) DURING THE PANDEMIC OF NOVEL CORONAVIRUS INFECTION

DOI 10.25789/YMJ.2023.81.17

УДК 616.24-002.5-053:616.98:578.834.1 (571.56)

With an aim to study the impact of restrictive measures against COVID-19 pandemic on epidemiologic situation with tuberculosis (TB), we analyzed key epidemiological data and distribution of clinical forms of newly detected pulmonary TB (PTB) among adult population of the Sakha Republic (Yakutia), with a separate analysis for the Arctic zone, as an example of a territory with low population density and limited transport accessibility.

For the study purpose, we used data from federal statistical recording and reporting forms, and data from TB medical patient database of the Sakha Republic (Yakutia). Rates for adult newly diagnosed PTB cases were analyzed for two pandemic years (2020-2021) against two pre-pandemic years (2018-2019). Statistical methods of choice were Pearson’s correlation coefficient and data weighing by chest x-ray coverage trend.

Study findings showed that the period of strengthened anti-epidemic and sanitation measures against novel coronavirus infection (COVID-19) was followed by substantial decrease in epidemiologic rates for TB, but, at the same time, profoundly deteriorated situation with delays in TB detection.

In the Arctic zone of Yakutia, epidemiologic rates for TB were showing decrease during restrictive measures, but generally remain higher than mean republic rates.

Increase in proportion of new adult patients with advanced disease forms was observed, together with the fact the TB detection rates in the Arctic zone were lower compared to mean republic detection rates.

The scope and quality of preventive checkups for TB correlated in a statistically significant way with the distribution of clinical forms of TB among newly diagnosed patients.

In conclusion, to reduce the rate of detections of MTB-positive destructive TB forms, prevention and detection measures will need to be strengthened, and should include universal annual chest x-ray screening of the population aged 15 and above.

Keywords: pulmonary tuberculosis, detection, novel coronavirus infection, COVID-19, Yakutia, Arctic.

Introduction. Steadily for the last years, the Sakha Republic (Yakutia) has been implementing advancements in public TB services aimed to meet local needs caused by difficult transport accessibility. These efforts, which included the organisation of centralized treatment for patients with multidrug- and extensively drug-resistant (MDR, XDR) *M.tuberculosis* (MTB), high-tech surgical treatment, and early population screening for tuberculosis (TB), have led to appreciable improvement and stabilization of the epidemiologic situation with TB.

During the pandemic of novel coronavirus infection (COVID-19), global wide anti-epidemic restrictive measures caused numerous readjustments in TB case detection, diagnosis and treatment routines (2,3,5,10). Several authors have commented on lower rates of coverage with preventive checkups for TB in 2020-2021 as a cause of decrease in TB incidence rates, although, at the same time, decrease in coverage with unscheduled exams for TB among TB case contacts was noticed likewise (1,7-9).

Novel coronavirus infection pandemic that emerged in 2019 demanded complete reset of healthcare systems across the world, and emphasized the importance of mass anti-epidemic measures aimed at reducing the threat of infectious disease spread. TB services during the pandemic faced the need to adjust to restrictive measures, which were developed and implemented primarily with the purpose of deescalating the threat of infection spread, but ended up largely neglecting the algorithms of proper dealing with socially significant diseases (1,4,10). These circumstances have urged the leading federal and regional TB centers in Russian Federation to estimate risks and develop their own interventions and workflow schemes adapted to meet local needs, but they proved insufficient to maintain the stable decreasing trend in TB incidence and mortality achieved during previous years (3,8). Restrictive measures against the spread of coronavirus infection resulted in major reduction of population coverage with preventive checkups for TB, and, eventually, steep decrease in TB incidence (8,10-13,15).

A number of authors predicted the risks associated with the upsurge in numbers of advanced and sputum-positive TB cases, the groups presenting the highest epidemiologic hazard, and, as it turned

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out, these concerns were confirmed by our study findings (9,11,14).

Epidemiologic situation with TB in Sakha Republic (Yakutia) showed a gradual improvement trend until the onset of COVID-19 pandemic. During 10 pre-pandemic years (2009 to 2019) TB incidence had decreased by 30.3%, mortality from TB had decreased by 58.5%, presence of necrotic lesions in newly identified cases had decreased by 28.5%, while coverage with preventive chest x-rays for TB had increased by 23.2%.

By the Decree of the Head of the Sakha Republic issued on March 17, 2020, a heightened preparedness regime was announced in the republic, mandating measures to be taken to counteract the spread of novel coronavirus infection (COVID-19). Anti-epidemic measures included discontinuation of national health checkup program (dispensarization) and mass preventive checkups (chest x-rays), which substantially diminished the threat of infection spread (coronavirus, and other communicable infections, TB as well), but caused a new threat – late detection of active TB cases. Two years before the pandemic, mean total incidence of TB over Yakutia was 47.9/100 000. During pandemic, the rate had decreased by 39.5%, but the proportion of destructive TB forms had escalated by 15.9%. Total population coverage with preventive checkups for TB had reduced by 18.1%, which caused changes in the distribution of clinical forms of newly diagnosed TB.

The present study was focused on the changing incidence of adult TB, while the consequences of work incapacity and mortality among adult population are directly associated with economic losses of a country, and were demonstrated in our previous study (2).

Material and methods. For this study, we used rates from federal statistical recording and reporting forms ('Form 33', 'Form 30', 'TB-03'), patient lists from 'Contingent' and 'Treatment control' databases maintained within 'Barclay' HDBMS (health database management system), and data from Federal TB Register of the Sakha Republic (Yakutia). Rates for adult patients (aged 18 or above) newly diagnosed with pulmonary TB (PTB) were analyzed for two pandemic years (2020-2021) against two pre-pandemic years (2018-2019). Data for Arctic zone of Yakutia were analyzed separately, to focus specifically on territories with low population density (0.01-0.08/km2) and limited transport accessibility. Relationships between changes in variables over time were assessed using Pearson's correlation coefficient and Kendall's Tau rank correlation coefficient. To assess associations between trends, variables were weighed by values of chest x-ray coverage. New TB case was considered an 'advanced case' if the patient was both MTB-positive and had necrotic lesions in the lungs. Calculations were performed using Microsoft Office Excel 2019, and IBM SPSS Statistics software.

Results. During COVID-19 pandemic, restrictive measures were brought to effect in Sakha Republic (Yakutia), which negatively impacted population coverage with mass chest x-rays for TB, further leading to changes in the rates reflecting effectiveness of TB detection by primary care health facilities.

Pearson's correlation analysis was used to assess the significance of changes in variables over time, during pre-pandemic (2018-2019) and pandemic (2020-2021) periods (see Table 1).

Adult population coverage with chest x-rays decreased by 18.6% (in the Arctic zone by 14.9%) (Fig. 1). Alongside with that, incidence of PTB among adult population decreased sharply, both over Yakutia and in Arctic zone: by 41.3% and 32.7%, respectively. Fig. 1 shows upsurge in mean proportions of newly diagnosed adult PTB cases with necrotic lesions, both over the entire republic (by 19.1%) and in the Arctic zone (by 5.3%). Also, rates in the Arctic zone exceeded all-republic rates by 16.6% before pandemic, and by 4.3% during pandemic.

Strong and meaningful linear cor-

Table 1

Correlation analysis of variables for adult population; Sakha Republic (Yakutia); Periods 2018-2019 and 2020-2021

Variable	Decline/growth rate (%)	Pearson's r-coefficient	Two-tailed significance level
Sakha Republic (Yakutia)			
Incidence	-41.3	0.530558	0.001043
Chest x-ray coverage	-18.6	0.776775	0.000000
Proportion of patients avoiding chest x-ray for ≥2 years	11.1	0.767547	0.000000
Proportion of advanced PTB cases	69.9	0.491074	0.002556
Arctic zone			
Incidence	-32.7	0.534530	0.059842
Chest x-ray coverage	-14.9	0.808062	0.000831
Proportion of patients avoiding chest x-ray for ≥2 years	160.0	0.839557	0.000331
Proportion of advanced PTB cases	48.6	0.264876	0.381808*

* Pearson's correlation did not show meaningful correlation (due to presence of outliers). Whenever Kendall's τ coefficient was employed, coefficient value was -0.536925, significance level was -0.013316, which were considered as the presence of association between attributes

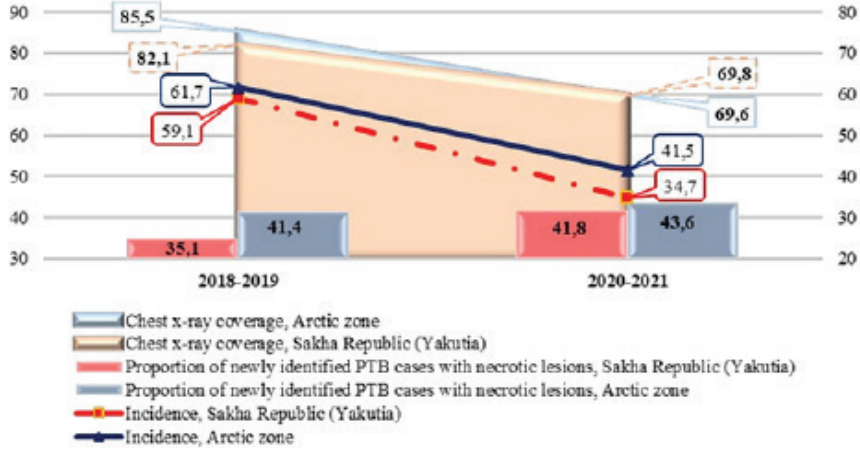

Fig. 1. Incidence of pulmonary TB (PTB), coverage with chest x-rays, and proportions of new adult PTB cases with necrotic lesions; Sakha Republic (Yakutia) and Arctic zone; Years 2018-2021

relation was observed between PTB incidence decline rates and chest x-ray coverage decline rates, among the adult population, analyzed in breakdown by municipal entities (Table 2). There was a linear correlation between chest x-ray coverage decline rates and the proportions of new PTB cases with necrotic lesions, visible in breakdown by municipal entities, but the correlation was inverse and negligible. At the same time, a strong correlation was observed between chest x-ray coverage rates and the proportions of advanced PTB cases among newly diagnosed patients (Table 2).

It is calling for attention, that during pandemic years, proportions of population avoiding chest x-rays for 2 or more years had increased by 11.1% in entire republic, and by a factor of 2.6 in the Arctic zone (Table 2). Proportion of new TB cases detected by visit to primary care facility had increased by 46.8% in the republic overall, and by a factor of 2.6 in the Arctic zone (Fig. 2).

Over time, mean proportions of advanced cases among new patients increased by 69.9% in the republic, and by 48.6% in the Arctic zone. Importantly, the proportion of patients with caseous pneumonia among newly diagnosed advanced cases had risen by 35.4% in the republic, and by 98.4% in the Arctic zone (Fig. 3).

Rates describing the proportions of MTB-positive (MTB+) patients and proportions of patients with MDR/XDR TB were controversial, and require further evaluation. In the Arctic zone, these rates showed negative trend (decrease by 24.0 and 29.6%, respectively), compared to mean all-republic rates (decrease in proportion of MTB+ cases by 9.1%, and increase in proportion of MDR/XDR cases by 29.6%). After subjecting the data to correlation analysis, we found no significant differences in variable changes over time periods (Table 2).

Presented in Table 2 is paired correlation analysis of variables with their significance levels, for chest x-ray coverage, incidence of TB, and delays in TB detection.

Reduced population coverage with preventive chest x-ray was a statistically significant cause of reduced TB incidence, and was related to the rise in proportion of advanced cases among new PTB cases, both over the entire republic, and over Arctic districts. Increase

Table 2

Paired correlation analysis of decline/growth rates for new adult cases of pulmonary TB (PTB); Sakha Republic (Yakutia) and Arctic zone; Periods 2018-2019 and 2020-2021*

Paired variables (decline/growth rates)	M±m	Pearson's r-coefficient	Two-tailed significance level
Sakha Republic (Yakutia)			
Chest x-ray coverage/Incidence	19.3±0.29 83.4±4.51	0.122412***	0.004817
Chest x-ray coverage/ Proportion of advanced PTB cases	19.3±0.29 91.7±5.17	0.289103***	0.000000
Proportion of population avoiding chest x-rays for 2 or more years/ Proportion of new cases with necrotic lesions	109.7±10.72 35.7±5.17	-0.124006***	0.004290
Proportion of population avoiding chest x-rays for 2 or more years/ Proportion of cases discovered by visit to health facility	109.7±10.72 51.5±4.39	0.143629***	0.000925
Incidence/ Proportion of advanced PTB cases	83.4±4.51 91.7±5.17	-0.091671**	0.035070
Incidence/ Proportion of MDR/XDR cases	83.4±4.51 5.5±3.38	-0.120161***	0.005661
Proportion of cases discovered by visit to health facility/ Proportion of advanced PTB cases	51.5±4.39 91.7±5.17	0.520253***	0.000000
Arctic zone			
Chest x-ray coverage/Incidence	20.5±0.50 60.8±6.37	0.355492***	0.000000
Chest x-ray coverage/ Proportion of advanced PTB cases	20.5±0.50 133.3±10.01	0.760004***	0.000000
Proportion of population avoiding chest x-rays for 2 or more years/ Proportion of new cases with necrotic lesions	220.5±24.23 -5.93±4.51	0.407058***	0.000000
Proportion of population avoiding chest x-rays for 2 or more years/ Proportion of cases discovered by visit to health facility	220.5±24.23 71.7±6.45	-0.073506 (нет корреляции)	0.302107
Incidence/ Proportion of advanced PTB cases	60.8±6.37 133.3±10.01	0.169259**	0.016838
Incidence/ Proportion of MDR/XDR cases	60.8±6.37 -5.4±4.73	-0.423332***	0.000000
Proportion of cases discovered by visit to health facility/ Proportion of advanced PTB cases	71.7±6.45 133.3±10.01	0.386985***	0.000000

* Data sets were subjected to weighing. ** Correlation was significant at 0.05 (two-tailed significance). *** Correlation was significant at 0.01 (two-tailed significance).

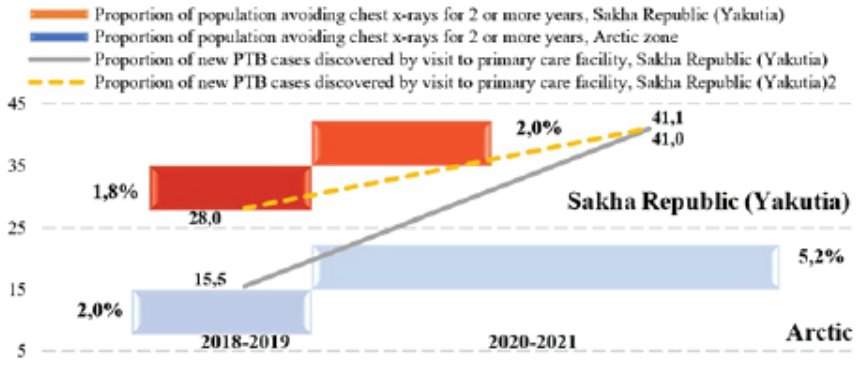

Fig. 2. Proportions of population avoiding chest x-rays for ≥2 years compared to proportions of new PTB cases discovered by vizit to primary care facility; Sakha Republic (Yakutia) and Artic zone; Periods 2018-2019 and 2020-2021

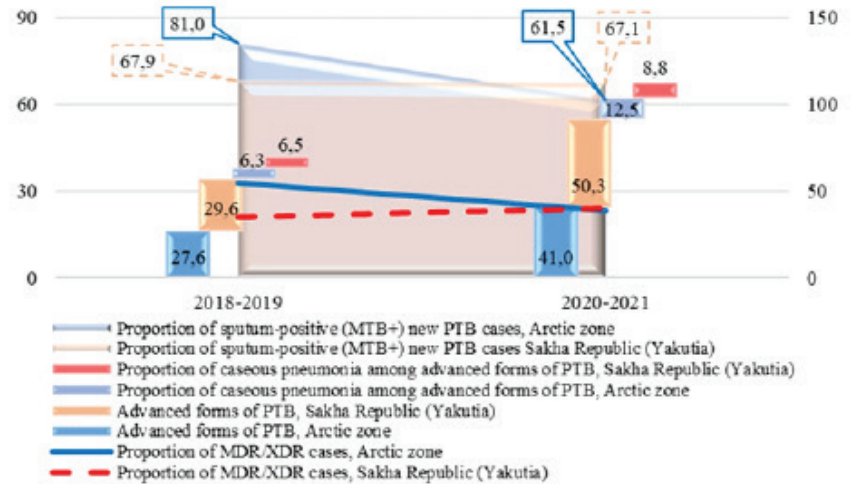

Fig. 3. Changes in clinical forms of new adult (aged 18 and above) pulmonary TB (PTB) cases over time; Periods 2018-2019 and 2020-2021 (%)

in proportion of population avoiding chest x-rays for 2 or more years was significantly associated both with the proportion of new cases with necrotic lesions, and with the proportion of cases discovered by visit to health facility. In the Arctic zone, the latter two rates showed no correlation.

Correlations presented above point at statistically significant association existing between reduced population coverage with chest x-ray screening and lower quality of TB detection. Furthermore, a marked aggravation among new cases of PTB was observed in our study, which was reflected by meaningful correlation between TB incidence rates and both the proportions of advanced cases, and proportions of patients with MDR/XDR TB.

Conclusion. Presented study results together with summarized experience reported by other authors showed that restrictive measures caused by COVID-19 pandemic had, without doubt, prevented the spread of this highly dangerous infection and the spread of other communicable infections as well, including TB. But the aspect largely missed out was that discontinuation of mass preventive checkups for TB fueled the threat associated with late TB detection, emergence of hidden (undetected) infection sources, including infection with MDR/XDR MTB as causative agent, all of which can adversely impact the incidence of TB and the prevalence of advanced clinical forms of the disease.

COVID-19 pandemic in the Sakha Republic (Yakutia) was associated with noticeable reduction in epidemiologic rates for TB incidence, mortality, and prevalence. But simultaneously, we observed escalation in proportions of patients with advanced forms of TB (rise in detections of necrotic lesions and MDR/XDR MTB), and in proportions of patients with TB discovered by visit to primary care facility.

In our view, to reduce the rate of detections of PTB forms presenting high epidemiological danger, and to reduce mortality from TB, we will need to strengthen efforts for early detection of TB by universal chest x-ray screening of the population aged 15 and above.

Further analysis of trends in epidemiologic rates and distribution of clinical forms of PTB is needed to assess the performance of primary care services in terms of TB prevention and early TB detection, and next, to adopt necessary managerial decisions at the level of regional healthcare authorities.

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ASSOCIATION OF DELETION POLYMORPHISMS OF THE *GSTM1* AND *GSTT1* GENES WITH THE DEGREE OF LUNG DAMAGE IN ELDERLY PEOPLE AFTER COVID-19

DOI 10.25789/YMJ.2023.81.18

УДК 159.963.2; 591.481.3

A feature of the SARS-CoV-2 virus, unlike other respiratory infections affecting the human body, is a rather high virulence and mortality. It has been established that severe forms of the disease are more common in elderly people with concomitant diseases. It has been established that free radical lipid oxidation plays an essential role in the pathogenesis of COVID-19. The body's antioxidant defense system prevents damage to cells and tissues from initiating free radical reactions. The family of enzymes glutathione-S-transferase (GST; EC 2.5.1.18) is interesting for research. The aim of this work was to analyze the association of polymorphisms of the *GSTM1* and *GSTT1* genes with the degree of lung damage in elderly people who had COVID-19. A survey of 51 elderly volunteers who had coronavirus infection aged 60 to 75 years (average age: 64.470± 0.602 years) was conducted. Informed consent to the study was obtained from all participants of the study (according to the protocol of the Ethics Committee of the YSC CMP No. 52 dated March 24, 2021, decision 1). Identification of samples by the GSTT1 and GSTM1 genes was carried out using polymerase chain reaction (PCR) according to the method described in the work of Zehra et al. (2018). According to the data obtained by us, 74.50% of all surveyed elderly people suffered a coronavirus infection with a mild degree of lung damage, and 25.49% with a severe degree. The results of our study show that the combination of zero deletion genotypes GSTM1 and GSTT1 are a risk factor for the development of severe lung lesions in elderly people in Yakutia.

Keywords: glutathione-S-transferase, *GSTM1* and *GSTT1* genes, deletion polymorphisms, COVID-19, SARS-CoV-2.

Introduction. The SARS-CoV-2 virus differs from other respiratory infections by its rather high virulence and mortality. Many researchers have found that severe forms of the disease are more common in elderly people with concomitant diseases: diabetes mellitus, cardiovascular, etc. [4].

It has been established that free radical lipid oxidation plays an essential role in the pathogenesis of COVID-19 [1]. The SARS-CoV-2 virus initiates the production of free radicals and inhibits antioxidant protection by suppressing the expression of the transcription factor Nrf2 (nuclear factor E2-related factor 2) [17]. Toxic products of lipid peroxidation are involved in damage to cells and tissues. Neutralization of toxic products of lipid peroxidation is carried out by the enzyme glutathione S-transferase, reducing the intensification of free radical oxidation of lipids [6,15].

Glutathione-S-transferases (GST; EC 2.5.1.18) are a large and widespread family of enzymes that are divided into three main groups: cytosolic; mitochondrial; microsomal. In humans, GST enzymes are mainly represented by the cytosolic family. There are 7 classes of cytosolic GST enzymes (α, μ, π, θ, σ, ω, ζ), which include 17 isoforms of the enzyme, each encoded by a separate gene or a group of genes located on different chromosomes [2]. The enzymes encoded by the GSTM1 and GSTT1 genes are the most studied, well expressed in human lung tissues, their genes are located on chromosomes 1p13.3 and 22q11.23, respectively [14]. A feature of these *GSTM1*

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and *GSTT1* genes is the presence of extended deletions in them, characterized by the absence of expression of the corresponding enzymes. Deletion polymorphisms of the *GSTM1* and *GSTT1* genes occur with high frequency in many human populations. Carriers of homozygous null deletion polymorphisms of the *GSTM1* and *GSTT1* genes have an increased risk of developing multifactorial diseases associated with oxidative stress, including respiratory, cardiovascular, oncological and other diseases [7,18,20].

The aim of this work was to analyze the association of polymorphisms of the *GSTM1* and *GSTT1* genes with the degree of lung damage in elderly people who have undergone COVID-19.

Material and methods of research. A survey of 51 elderly volunteers aged 60 to 75 years (average age: 64.470±0.602 years) was conducted. All the examined patients had a coronavirus infection, were discharged from the hospital in the period from August to September 2020, the examination and collection of the material was carried out in March 2021. Informed consent to the study was obtained from all participants of the study (according to the protocol of the Ethics Committee of the YSC CMP No. 52 dated March 24, 2021, decision 1).

Since coronavirus infection is associated with the development of pneumonia in patients, the diagnosis of lung damage (inflammation) was assessed by the percentage of destruction of lung tissue based on computed tomography. In our study, patients were divided into two groups: group 1 – patients with mild lung damage (CT1-2), group 2 – patients with severe lung damage (CT 3-4). Clinical indicators during the disease, such as the degree of damage assessed on CT, were taken from a medical record statement. All participants were personally interviewed, filled out a series of questionnaires. The general characteristics of the examined patients are presented in Table 1.

For genotyping, DNA was isolated from whole blood by the standard two-stage method of phenol-chloroform extraction. DNA samples were sampled by deletion polymorphisms of the biotransformation genes: *GSTT1* and *GSTM1*, which encode the glutathione S-transferase enzymes θ1 and μ1, respectively. Identification of samples by *GSTT1* and *GSTM1* genes was carried out using polymerase chain reaction (PCR) according to the method described in the work of Zehra et al. (2018).

The results were visualized electrophoretically in 3% agarose gel, with the addition of ethidium bromide. The PCR results were viewed in transmitted UV light on a transilluminator. The presence of deletion polymorphisms of the *GSTM1* and *GSTT1* genes was determined by the absence of the corresponding fragments: 219 bp – for *GSTM1* and 459 bp – for *GSTT1*. The presence of these fragments indicates the presence of at least one normal (without deletion) copy of the genes. β-globulin with a fragment of 268 bp was used as an internal control. Evidence of successful PCR analysis was the presence of an amplification of 268 bp, the β-globulin gene.

Statistical processing was carried out using the software package SPSS 11.5 for Windows and Microsoft Excel. The relationship between the degree of lung damage and genotypes in COVID-19 survivors was assessed by odds ratio (*OR*) with a 95% confidence interval (*95% CI*). Comparison of genotype frequencies in groups of sick and healthy individuals was carried out using the Fisher criterion. The differences were considered statistically significant at $p < 0.05$.

Results. According to the data obtained by us, 74.50% of all surveyed elderly people suffered a coronavirus infection with a mild degree of lung damage, and 25.49% with a severe degree (Table 1).

The distribution of deletion genotypes *GSTM1* and *GSTT1* in patients with mild and severe lung lesions is shown in Table 2. The frequency of $GSTM1^{-/-}$ and $GSTT1^{-/-}$ genotypes (null genotypes) was higher among volunteers with severe lung damage, compared with volunteers with a milder degree (76.92% vs. 55.26%; 69.23% vs. 44.73%, respectively), but the differences did not reach statistical significance.

Individuals who had COVID -19 with a combination of two null genotypes ($GSTM1^{-/-}$ / $GSTT1^{-/-}$) showed a sufficiently high risk of developing severe

Table 1

General characteristics of the examined patients who have had a coronavirus infection

Indicator:	Values
Number of examined	51
Men / Women	59/102
Disease severity status (CT stage):	
1-2	38 (74.50%)
Age, years:	63.947±0.673
3-4	13 (25.49%)
Age, years:	66.000±1.260

Table 2

The frequencies of *GSTM1* and *GSTT1* genotypes and their relationship with the degree of lung damage in elderly people who have had COVID-19

Genotypes		Group	n (%)	p	*OR (95% CI)*
GSTM1	$M^{+/+}$	1	17 (44.74)	0.20	2.69(0.63-11.38)
	$M^{+/+}$	2	3 (23.08)		
	$M^{-/-}$	1	21(55.26)		0.37(0.08-1.56)
	$M^{-/-}$	2	10(76.92)		
GSTT1	$T^{+/+}$	1	21 (55.26)	0.20	2.77(0.72-10.61)
	$T^{+/+}$	2	4 (30.77)		
	$T^{-/-}$	1	17 (44.73)		0.35(0.09-1.37)
	$T^{-/-}$	2	9 (69.23)		
GSTM1/GSTT1	$M^{+/+}/T^{+/+}$	1	9 (23.68)	0.70	1.70(0.31-9.17)
	$M^{+/+}/T^{+/+}$	2	2 (15.38)		0.58(0.10-3.15)
GSTM1/GSTT1	$M^{-/-}/T^{-/-}$	1	9 (23.68)	**0.02**	0.19(0.05-0.74)
	$M^{-/-}/T^{-/-}$	2	8 (61.54)		5.15(1.34-19.77)
GSTM1/GSTT1	$M^{-/+}/T^{-/+}$	1	12 (31.58)	0.47	2.53(0.48-13.27)
	$M^{-/+}/T^{-/+}$	2	2 (15.38)		0.39(0.07-2.06)
GSTM1/GSTT1	$M^{+/-}/T^{+/-}$	1	8 (21.05)	0.41	3.20(0.36-28.42)
	$M^{+/-}/T^{+/-}$	2	1 (7.69)		0.31(0.03-2.77)

lung damage by 5.15 times (61.54% vs. 23.68%), which is evidence of a reliable association of a combination of null genotypes with the development of more severe forms of lung damage in elderly people who had COVID-19 ($p< 0.05$).

Discussion. The pathogenesis of SARS-CoV-2 in COVID-19 disease is associated with the way the virus enters the human body. The SARS-CoV-2 virus enters the cell by interacting with the protein receptor - ACE2 (angiotensin converting enzyme 2). SARS-CoV-2 blocks the work of the ACE2 protein, stimulates its internalization. The loss of ACE2 receptor activity leads to a rapid drop in the production of angiotensin-1–7 (Ang 1-7), and consequently the accumulation of angiotensin II (Ang II). Imbalance between angiotensin II (hyperactivity) and angiotensin 1-7 (deficiency) may play a role in the occurrence of an acute increase in blood pressure [12].

In addition, the accumulation of Ang II octapeptide leads to an increase in the expression of transcription nuclear factor-kB (NF-kB). The results obtained in the work of a group of researchers Blanco-MeloD, Nilsson-PayantBE, LiuWC, (2020) in vitro on a model of human bronchial epithelial cells are an indirect confirmation of the expression of NF-kB. The results showed that when cells are infected with the SARS-CoV-2 virus, overexpression of proteins is observed: CCL20, CXCL1, IL-1B, IL-6, CXCL3, CXCL5, CXCL6, CXCL2, CXCL16 and TNF. These proteins can lead to chemotaxis of neutrophils into virus-affected tissues (lung tissues) and a strong inflammatory reaction. Neutrophils, in virus-infected tissues, intensively generate ROS, thereby shifting the prooxidant-actioxidant equilibrium towards the intensification of free radical processes [9]. The shift of the pro-oxidant-antioxidant equilibrium towards lipid peroxidation is evidenced by studies by other authors [10,13].

Authors Khomich O A, et.al . (2018) showed that a high level of ROS and a violation of the redox balance of the host is of great importance for the replication of viruses and the occurrence of the disease. Fuentes E, et.al. (2018) showed that the SARS-CoV-2 virus activates platelets [5]. Platelets, in turn, induce neutrophils to produce neutrophil extracellular traps, which play a key role in the development of thrombotic complications leading to acute respiratory failure in lung tissues, lead to the development of hypoxia, which further initiates free radical oxidative processes [8].

Glutation-S transferase enzymes neutralize the products of free radical oxidation, thereby they are inhibitors of the development of oxidative stress. The presence of deletion polymorphisms in the *GSTM1* and *GSTT1* genes leads to a higher risk of initiation of free radical reactions and the development of oxidative stress. In turn, oxidative stress plays an important role in susceptibility to SARS-CoV-2 infection and increases the risk of developing a large number of complications in COVID-19 [16].

Our study showed that patients carrying a combination of zero genotypes $GSTM1^{-/-}$ and $GSTT1^{-/-}$ who have had COVID-19 have a higher risk of developing severe lung lesions. In a study by Saadat (2020), it was shown that people with a zero genotype of the *GSTT1* gene had a higher risk of COVID-19 infection compared to people without deletion polymorphism.

The results of our study show that the combination of zero deletion genotypes *GSTM1* and *GSTT1* are a risk factor for the development of severe lung lesions in elderly people in Yakutia.

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FUNCTIONAL FEATURES OF THE CARDIOVASCULAR SYSTEM IN COVID-2019 CHILDREN

DOI 10.25789/YMJ.2023.81.19

УДК 159.963.2; 591.481.3

Relevance. The consequences of COVID-19 are a serious problem and a cause of health problems for children. Identifying post-COVID-19 health problems to develop rehabilitation and treatment options is an important public health challenge. One of the urgent problems is the violation of the functional parameters of the cardiovascular system in children.

Material and methods. The functional state of the cardiovascular system of children who had COVID-19 in school conditions at the age of 8-12 years was studied. A total of 64 children (32 girls and 32 boys) were studied, 3-6 months after suffering COVID-19. The children were examined by copy-pairs. The study included children in the age range (8-12 years), in which functional indicators have the same reference values according to the methods used. The functional indicators of the cardiovascular system were studied according to the electrocardiogram data using the analysis of heart rate variability according to R.M. Baevsky, as well as with the help of the Martinet-Kushelevsky functional test. The following were studied: adaptive potential, response quality index, Kerdo autonomic index, coefficient of variation, systolic and diastolic blood pressure, pulse pressure, heart pumping function by assessing the stroke and minute volume of the heart, the index of functional changes, the stress index, the total power of the spectrum.

Results. It was shown that one of the mechanisms of functional disorders of the cardiovascular system in the period from 3 to 6 months after COVID-19 is a syndrome of autonomic dysfunction. The manifestations of autonomic dysfunction in children are hypertensive changes in the cardiovascular system in the absence of pronounced clinical manifestations, the presence of functional tension of regulatory systems, unsatisfactory functional status, decreased rates of increase in heart rate power in response to load, low values of sympathetic regulation mechanisms and centralization of the cardiovascular system regulation circuit, a large proportion of the influence of the peripheral regulation circuit.

Conclusion. One of the pathogenetic mechanisms for reducing the functional parameters of the cardiovascular system in children who have had a coronavirus infection is endothelial dysfunction syndrome.

Keywords: children, COVID-19, cardiovascular system, functional parameters, endothelial dysfunction syndrome.

The SARS-COV-2 virus (COVID-19), which has caused the pandemic coronavirus infection, has several features that lead to the development of a multisystemic inflammatory syndrome [1]. According to various authors, the condition does not exclude the paediatric population [6]. According to foreign studies, autonomic dysfunction syndrome should be excluded in children with abdominal pain, signs of gastrointestinal disturbances, respiratory or neurological symptoms of unclear etiology [19].

According to some authors [18, 19, 20, 21], the over-infected patients (COVID-19) have multiple organ involvement, myocardial dysfunction, coagulopathies, and increased inflammatory markers [4]. Often, serious cardiovascular abnormalities in children are asymptomatic and are not recognised in time (6).

Therefore, the study of the effect of coronavirus infection on the health and functional outcomes of children is highly relevant.

Purpose of the study: to identify functional changes in the cardiovascular system in children with coronavirus infection.

Material and methods of investigation. Children aged 8-12 years who had had coronavirus infection during the last 3 months and a control group were investigated. The children in the study group had a mild to moderate coronavirus infection. The disease was diagnosed by an outpatient clinic doctor according to the diagnostic criteria of COVID-19 (main clinical manifestations: nasal congestion, sneezing, headache, weakness, fever) and was confirmed by laboratory PCR test of the mouth and nasopharynx. Children with severe forms of the disease, with clinical manifestations of multinflammatory syndrome, with myocardial infections, and carriers of infection were not included in the study group. Children in the control group were matched by the copy-pair method and had no documented contacts with COVID-19 patients during the last 3 months before the examination. Carriage in children in the control group was excluded by the results of rapid tests administered at school to all students in the follow-up group (8-12 years old) during the period from the beginning of the pandemic to the end of the study. No blood was drawn for antibodies in the observation group. Copies were matched for age and sex. The age group 8-12 years old was investigated, taking into account the grouping of the functional indicators of the techniques that were used, i.e. the age norms of these indicators in children 8-12 years old fell within one age range of the age norm. All children attending the Irkutsk Education Center No. 47. All children of the control and studied groups had no somatic (and other) pathology, including vegetative dysfunction and were in the 1st health group. A total of 64 children were investigated, 32 children who had had a coronavirus infection within the next 3-6 months, and 32 children in the control group (who had not been ill themselves and had no family history of coronavirus infection in their relatives since the beginning of the pandemic). Children who became ill with COVID-19 were not allowed to attend classes im-

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mediately after the first signs of illness. Each group consisted of 16 girls and 16 boys, distributed in copy-pairs.

Electrocardiography (ECG) [11] and the Martinet-Kushelevsky functional test [9] were used to study the functional parameters of the cardiovascular system.

Electrocardiogram was recorded using 12-channel electrocardiograph POLI-SEKTR-8/EX (Neurosoft Ltd.) equipped with modules for heart rate variability (HRV), stress-ECG, pulse wave velocity (PWV), Q-T dispersion, detection of late ventricular potential (LEP) [5, 6]. ECG was assessed using heart rate variability (HRV) analysis method according to R.M. Baevsky [11], using software included into the module. The system is included in the standard of equipment of functional diagnostics departments according to the orders of the Ministry of Health of the Russian Federation No. 92n dated March 7, 2018 "On approval of the Regulations on organization of primary health care for children" and No. 997n dated December 26, 2016 "On approval of the Rules for functional studies". Six standard leads (according to W. Einthoven and E. Goldberger) [5, 7, 12] were recorded first at rest and then in orthopposition.

The Martinet-Kushelevsky test was performed in a standardised manner with a dosed load of 20 squats for 30 seconds, followed by measurements during recovery.

Functional indices were studied according to age groups [5, 7, 12]. The following parameters were studied: systolic and diastolic blood pressure (SBP and BP), pulse pressure (PPP) were studied by Korotkoff method; ECG analysis by pulsometry according to R.M. Baevsky assessed the heart pump function (by estimating the stroke volume (SBV) and minute blood volume (MOB)), functional change index (FDI), tension index (TI), total power spectrum (TP), adaptive potential (AP), response quality index (RQI), Kerdo vegetative index (CI), coefficient of variation (CV) [5, 12]. All studied indices were validated in Russia [5,7,12].

Shock volume was calculated:

YO=80+0.5×PD - 0.6×(DAD - C),

where AP is pulse blood pressure (mmHg), DAP - diastolic blood pressure (mmHg), B - age (years).

Minute blood volume according to the formula:

MOC = YO × HSF [5, 6],

Index of functional changes by the formula:

IFI = 0.011HSS + 0.014SAD + +0.008DAD + 0.014V + 0.009MT - -0.009R - 0.27 [7, 12],

where HR - heart rate, bpm, SAD - systolic blood pressure, mmHg, DAP - diastolic blood pressure, mm Hg, B - age, years, MT - body weight, kg, P - body length, cm, 0.27- independent coefficient.

The regulatory tension index, which reflects the degree of centralisation of heart rhythm control, was determined according to the formula:

IN=AMo/(2BP×Mo)) [7,12],

Adaptation potential was determined according to the formula:

AP = 0.011HP + 0.014SAD + +0.008DAD + 0.014V + 0.009MT - -(0.009P + 0.27) [7,12],

where B - age, years, MT - body weight, kg, R - height, cm, SBP - systolic blood pressure, mmHg, DAP - diastolic blood pressure, mm Hg, HR - pulse rate per 1 min.

The response quality index in the Martin-Kushelewski test:

PCR = (RD2 - RD1)/(P2 - P1) [7,9,12],

where P1 is resting pulse, PD1 - pulse pressure at rest, P2 - heart rate after exercise, PD2 - pulse pressure after exercise).

The good functional state of the cardiovascular system was taken at PKR = 0.5 to 1.0.

The Kerdo index is calculated according to the formula:

IC = (1-DAD / HSF)×100[7,12],

where DAP is diastolic pressure, HR is heart rate [5].

The basic orientation of the autonomic nervous system (ANS) tone was classified into 5 types according to IR [5, 11]:

1. IR > -31: predominance of parasympathetic tone - marked parasympathicotonia.

2. IR between -16 and -30: intermediate state between normal and parasympathic tone - parasympathicotonia.

3. IR between -15 and +15: sympathetic and parasympathetic balance - normotonia.

4. IR between +16 and +30: intermediate state between normal and sympathetic tone - sympathicotonia.

5. AC > +31: predominance of sympathetic tone - marked sympathicotonia.

In addition, we used clinical pediatric examination to define cardiac boundaries and auscultation.

Statistical processing was performed using Statistica Base 10 for Windows. Statistical processing included arithmetic mean (M), standard deviation (s), and error in arithmetic mean (m). Prior to statistical analysis we assessed the distribution of the signs for normality using the Harker-Bera test. Statistical significance of differences in quantitative characteristics having normal distribution was analysed by Student's t-test in the confidence interval > 95%. In case of non-normal distribution of the variation series, the statistical significance of the differences was analysed using Mann-Whitney test. The statistical significance of differences in qualitative variables was analysed using the χ2 test. Dependence between two variables was assessed using Spearman's correlation coefficient. The critical level of significance for statistical hypothesis testing was 0.05.

Results of the study. It was found that the main blood pressure parameters such as systolic and diastolic pressure, pulse pressure and heart rate tended to increase in the COVID-19-treated children, but no statistically significant differences could be found (Figure 1 and Figure 2). This was evident when the values were measured both at rest and after exercise.

There was a trend towards increased stroke and minute blood volume in children with COVID-19 (Table).

Studying the degree of adaptability, functional reserves of organism and predicting negative changes of health by studying the index of functional changes (IFI) in the investigated group was higher than 2,1 conventional units, which shows the presence of functional tension of regulatory systems ($p < 0,05$). Such indexes of RSI require elimination of risk factors and rehabilitation of children. In the control group, the index was 1.9±0.3 standard units, which corresponds to the norm ($p > 0.05$) [5].

The study of cardiac rhythm spectrum power index in children, which characterizes the total absolute level of regulatory systems, showed that the index (TP) at rest in the control group was 1.5 lower than in the study group ($p < 0.05$).

Indicators of shock and minute blood volume in children

Indicator	Study group n=32	Control group n=32	Value of p
Shock volume (SV). beats per minute	65.4±8.4	64.8±6.4	$p > 0.05$
Minute blood volume (MBV). millilitres per minute	5967.5±1020.8*	5495.3±883.9	$p < 0.05$

*($p < 0.05$).

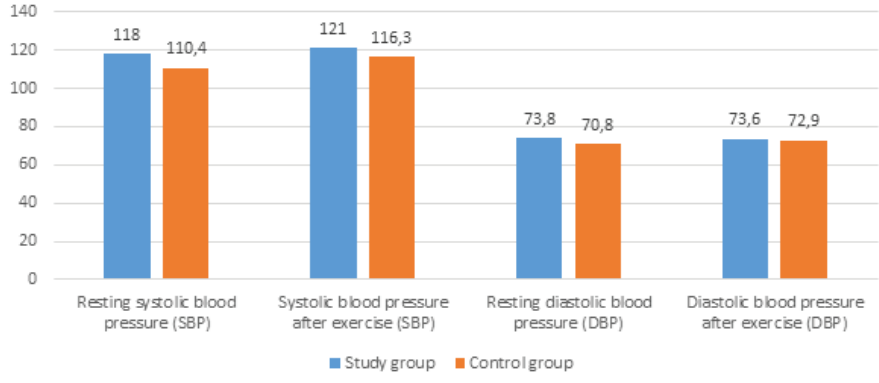

Fig. 1. Indicators of systolic and diastolic pressure in children

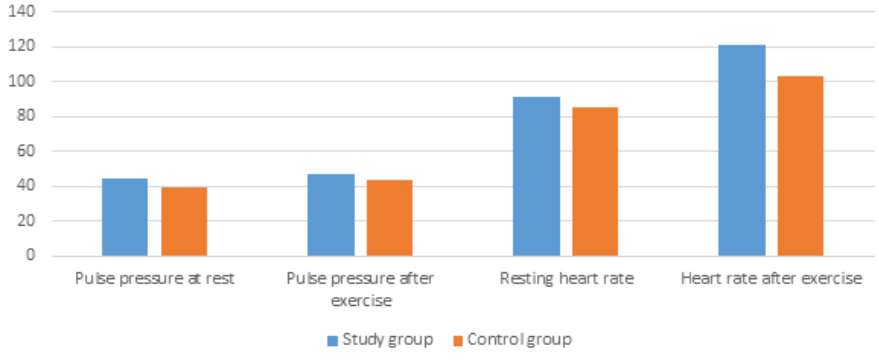

Fig. 2. Indicators of pulse pressure and pulse in children

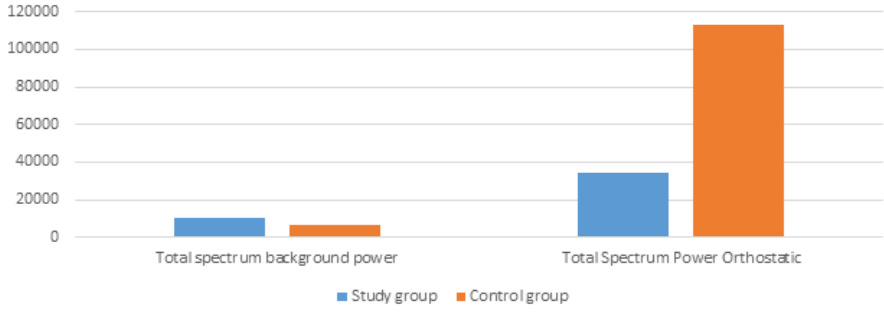

Fig. 3. Spectrum power indicators in children (p < 0,05)

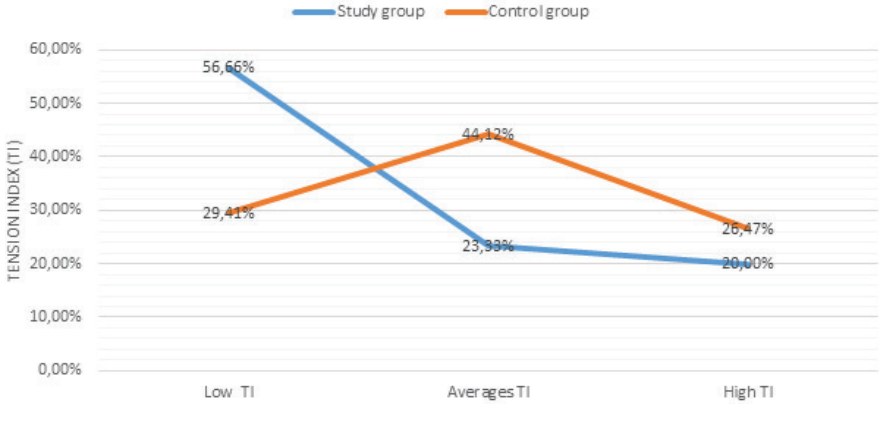

Fig. 4. Tension Index Levels in Children

This indicated the absence of tension of regulatory systems in children without COVID-2019 and insignificant tension at rest in the studied group. During orthostatic test the power of the spectrum increased significantly in the control group (16.5 times) ($p < 0.05$), which indicated effective mobilization of regulatory systems in response to the load. While in the studied TP increased only 3.3 times ($p < 0.05$), i.e. the mobilization of regulatory systems was 5 times lower compared to the control group[5].

The stress index of regulatory systems, which characterizes the activity of sympathetic regulation mechanisms, the state of the central circuit and is calculated on the basis of analysis of the diagram of cardiointervals distribution demonstrated that in the studied group the low activity of the central circuit of sympathetic regulation was registered in most children. The AN level increases with increasing endurance of the organism, the indices of AN level (see Fig. 4) characterized the decrease of endurance and the decreased role of the central circuit regulation in the group of children who underwent COVID-19. In our study, IN values were divided in the groups into low, medium and high. The high IN values were similar in both groups, but the low IN values predominated in the study group, whereas the medium IN values predominated in the study group ($p < 0.05$) [5].

The index of reaction quality in the Martine-Kushelevsky test revealed an unsatisfactory functional state in children in the studied group and was 0.4±1.4 conventional units, while in the control group this index corresponded to a good functional state (0.5±1.4 conventional units) [11].

Adaptation potential is an index of vital functions, the formation of which level depends on a complex of changes in physiological systems of a human organism (state of nervous, pituitary and adrenal hormones, cardiovascular, respiratory and other systems) as well as under the influence of stress factors (physical and mental load, atmospheric pressure, temperature changes, etc.) [11].

In our study, the level of adaptive potential was 3.6±0.8 in the COVID-19 group and 3.2±0.7 in the control group, which corresponds to a more pronounced stress of adaptation mechanisms ($p<0.05$).

After Kerdo index calculation, the autonomic nervous system (ANS) state types were determined, which were classified into 5 tone types

1. IR > -31: predominance of para-

sympathetic tone - pronounced parasympathicotonia.

2. IR between -16 and -30: intermediate state between normal and parasympathic tone - parasympathicotonia.

3. IR between -15 and +15: sympathetic and parasympathetic balance - normotonia.

4. IR between +16 and +30: intermediate state between normal and sympathetic tone - sympathicotonia.

5. IR > +31: predominance of sympathetic tone - expressed sympathicotonia.

In this case the IR values were distributed as follows (see Fig. 5) [5].

Fig. 5 shows that sympathicotonic responses were less pronounced in children in the study group than in the control group ($p < 0.05$).

Coefficient of variation (CV) in its physiological sense is an index normalised to heart rate and reflects a less artifact-dependent and ectopic heart rate variability (Figure 6) [5].

Figure 6. Coefficient of variation of heart rate (CV) ($p < 0.05$).

It was shown that there were practically no changes of heart rate variability at rest and during exercise in children in the studied group. In the control group, rhythm variation was well expressed in relation to the load received ($p < 0.05$) [5].

Clinical paediatric examination, auscultation and measurement of the boundaries of absolute and relative cardiac dullness showed no abnormalities.

Discussion of results. The study showed that in children who had received COVID-19 there was an increase in the main haemodynamic indices (BP, BP, BP, HR, OI, IOC), indicating hypertensive changes in the cardiovascular system. However, this increase has no statistically significant differences between the study and control groups. A tendency towards hypertension has also been shown by many authors in COVID-19 sufferers [15,17].

The absence of clinical manifestations, changes of cardiac boundaries and auscultatory abnormalities of the cardiovascular system showed that the changes were of a functional nature. This was evidenced by an increase in the index of functional changes (IFI), which determined the presence of functional stress of the regulatory systems in children who had had COVID-19, which is consistent with the findings of other authors [14].

Reaction quality index in the Martine-Kushelevsky test revealed a poor functional state in children in the study group compared to the control group ($p < 0.05$).

Changes in the level of adaptive potential in the COVID-19 group of children also indicated a marked strain on adaptation mechanisms.

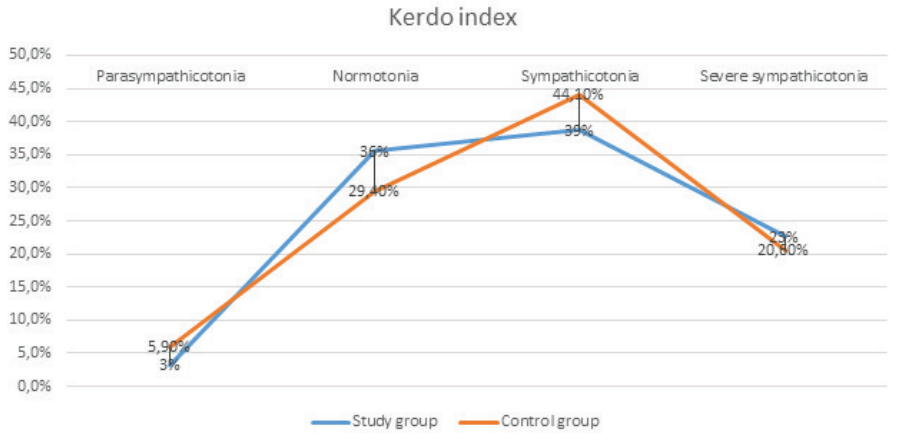

Fig. 5. Vegetative Kerdo index

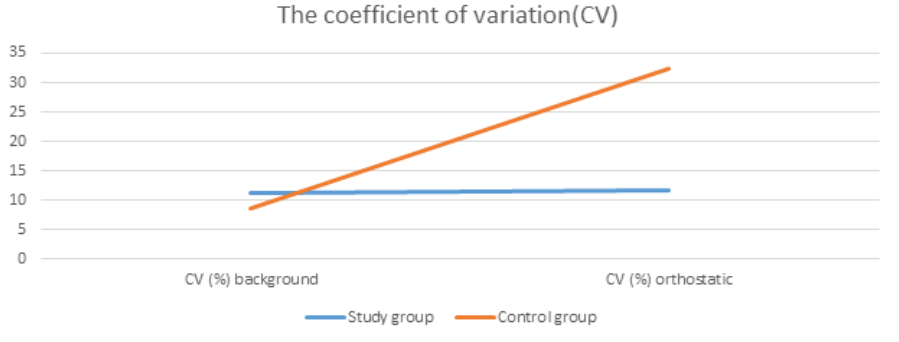

Fig. 6. Coefficient of heart rate variation (CV)

Functional changes in the study group were associated with an initial increase in total spectrum power, which increased 5-fold less in response to exercise than in the control group ($p < 0.05$). Such values of total spectrum power may be associated with depletion of mechanisms (or structures) responsible for an adequate response to load.

At the same time, the stress index of regulatory systems related to the activity of sympathetic regulation mechanisms and the state of central regulation circuit in the studied group showed lower values in comparison with the control group, which can be connected with the contribution of a greater part of regulatory mechanisms by the peripheral regulation circuit, and thus related to the state of the vascular system [14].

This phenomenon was also indicated by less pronounced sympathicotonic responses in the children in the study group, where the Kerdo index showed a flatter curve when allocating the children to groups according to the type of autonomic tone (see Fig. 5). The absence of variation in heart rate variability at rest and during exercise in children in the study group indirectly confirmed the depletion of central regulatory mechanisms and the predominance of adverse peripheral influences [3].

In this regard, it can be assumed that the main impairments of functional parameters on the part of the cardiovascular system in children who have undergone COVID-19 are associated with changes in the nervous system (general toxic lesion, cerebrovascular disorders, hypoxia) [16], the heart muscle (adenosine-converting enzyme-2 mediated cardiac lesion, hypoxia, cardiovascular disorders, systemic inflammatory response syndrome) [14] and the vascular bed (endothelial dysfunction syndrome, increased blood coagulation) [2, 3, 20, 21].

If we assume that the main mechanism of functional disorders of the cardiovascular system in our study is toxic action or hypoxia, then hypotensive reactions would be observed. If systemic inflammatory response syndrome was the main mechanism in this process, then indirect signs of inflammation of the heart muscle (changes in heart boundaries, cardiac murmurs) could have been observed. However, this was not observed in our study, due to the fact that it was conducted between 3 and 6 months after the disease.

Our study has shown the importance of investigating the functional state, physical and neuro-psychological development of children who have had coronavirus infection. This problem is of particular importance in the context of school intensification [8,16].

Conclusion. Our study revealed functional dysfunction in children with COVID-19. This is indicated by the pattern of changes in the functional parameters of the cardiovascular system in children between 3 and 6 months after COVID-19. The findings will help to suggest effective treatments for functional impairment in children.

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FREQUENCY AND OUTCOMES OF EXTREMELY EARLY PRETERM BIRTH IN PREGNANT WOMEN WITH NEW CORONAVIRUS INFECTION COVID-19

DOI 10.25789/YMJ.2023.81.20

УДК 618.39-036-06:616.98:578.834.1

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The frequency of extremely early preterm birth (ERPR), their obstetric and perinatal outcomes, morphological features of the placenta in pregnant women in the city of Chelyabinsk and the Chelyabinsk region (CO) with a new coronavirus infection (NCI) in periods 1-2 and 3-4 waves of the COVID pandemic were studied. -19. The results obtained indicate that there is no increase in the frequency of ERPR in pregnant women with NCI. Delivery by caesarean section is associated mainly with the increase in acute respiratory distress syndrome (ARDS) of the mother. In the period of waves 3-4 of the COVID-19 pandemic, an increase in the perinatal mortality rate was noted compared to the period of waves 1-2 without statistical significance of these indicators. At the same time, antenatal fetal death during the 3rd-4th wave of the NCI pandemic was observed statistically significantly more often (p=0.033). The features of placental damage to the placentas in patients with NCI with ERPR and antenatal fetal death during the 3rd-4th wave of the pandemic are severe maternal and fetal vascular malperfusion and severe acute inflammatory lesions of the placenta (2,3 stages and 2,3 degrees).

Keywords: new coronavirus infection, extremely early preterm birth, obstetric and perinatal outcomes, placental damage.

Introduction. Presently specialists continue to actively study the effect of NCI COVID-19 on the course, outcomes of the pregnancy and fetal development. It is known that NCI of various degree of severity could be associated with the adverse obstetric and perinatal outcomes. In particular, it increases the risk of preterm birth (PB) [1, 2, 3, 8]. Extremely early PB (EEPB), that make up 5% in the structure of the general population, but are accompanied by higher rates of perinatal losses and disablement of the surviving babies, are especially unfavorable [5, 10, 12]. Data have been published on the decrease in the frequency of EEPB against the background of preventive and protective measures taken in different communities in the period of lockdown to decrease the transmission of the SARS-COV-2 virus. For the first time this was outspoken by Danish scientists based on the results of the national study (from 2.19 to 0.09 per 1,000 births, p<0.001) [11]. Similar data have been presented by specialists from the Netherlands, Japan, Italy, USA. In a number of other papers (California, Philadelphia, Israel, Spain and Great Britain (London), Sweden (national study) no data were obtained on the decrease in the EEPB frequency in the period of NCI COVID-19 pandemic [14]. There are more and more studies devoted to the analysis of the specifics of morphological changes in placenta against the background of NCI, their role in adverse pregnancy outcomes [1, 7, 9, 13, 16, 17]. It should be noted that EEPB frequency in pregnant women with NCI COVID-19, obstetric and perinatal outcomes in such cases have been studied insufficiently, including the studies performed in the territory of the Chelyabinsk region (ChR).

Objective of the study: to determine the frequency of EEPB, their obstetric and perinatal outcomes in pregnant women with NCI COVID-19 in Chelyabinsk and Chelyabinsk region.

Materials and Methods. A retrospective comparative analysis of the data from the medical records (labor and delivery records, individual health records of pregnant and puerperant women, neonatal records, reports of antemortem pathology study of the biopsy (surgical) material) has been performed for 237 patients with NCI and PB, hospitalized to the maternity hospital of the Chelyabinsk Reginal Clinical Hospital №2. This hospital was repurposed to a hospital providing medical assistance to pregnant, parturient, and puerperant women with NCI COVID-19, as well as to their newborn children in the territory of the Chelyabinsk city and the Chelyabinsk region. During the 1st-2nd wave of NCI pandemic (April 2020 – May 2021) 98 patients with NCI and PB underwent treatment. Within the period of the 3rd -4th wave (June 2021 – December

2021) – 139 women with NCI and PB. Frequency and outcomes of EEPB (22 weeks 0 days – 27 weeks 6 days) in the period of the 1st – 2nd and 3rd – 4th waves of COVID-19 pandemic were analyzed. The 1st study group consisted of 10 patients with NCI and EEPB in the period of the 1st – 2nd waves of pandemic. The 2nd group comprised 14 women with NCI and EEPB in the period of the 3rd – 4th waves of COVID-19 pandemic.

The inclusion criteria were: confirmed NCI COVID-19 (U07.1), gestational age – 22 weeks 0 days (22/0 weeks) – 27 weeks 6 days (27/6 weeks), check up in the maternity welfare clinic, available medical records to collect necessary information on the pregnancy course and outcomes. Exclusion criteria: possible/suspected NCI COVID-19 (U07.2/Z03.8) case. To compare the obtained results the authors used the data on the EEPB frequency and outcomes in pregnant women without NCI over the studied period from April 2020 through December 2021 inclusive, and over the 2019 year, that were provided in the annual reports of the main external obstetrician-gynecologist of the Ministry of Health of the Chelyabinsk city and ChR.

The classification proposed in 2014 by Amsterdam Placenta Workshop Group was used in the description of the placental lesions. It includes the vascular (maternal and fetal), inflammatory (inflammatory infectious and immune) and other (placental abnormalities in location, shape, umbilical cord insertion) placental lesions [15].

Statistical processing of data was performed using the statistical software package IBM SPSS Statistics-19. Standard methods of non-parametric statistics were used in the analysis of data depending on their type (Mann–Whitney U test, Fisher's exact test and Pearson chi-squared test). In checking the statistic hypotheses, the critical level of significance was assumed to be 0.05.

Results and Discussion. In general, the EEPB frequency in pregnant women with NCI (10.1% - 24/237) over the period from April 2020 through December 2021 in Chelyabinsk and ChR did not differ statistically significantly either from EEPB frequency in pregnant women without NCI over the same period of time (9.6% - 577/6,002) (p=0.793), or from EEPB frequency in the previous 2019 (8.2% - 280/3,433) (p=0.287). The median of the NCI manifestation time in group 1 and 2 was 26/5 (25/8; 27/3) weeks and 26/2 (23/9; 27/2) weeks, respectively (p=0.278). EEPB in pregnant women with NCI in the period of the 1st – 2nd waves occurred in 10 cases (10.2%), during the 3rd – 4th waves – in 14 cases (10.1%) with no statistically significant differences (p=0.974). Information on the frequency of EEPB against the background of the manifestation of NCI COVID-19 in various regions of the Russian Federation is limited in the available literature, and their comparative analysis is difficult. In the study by A. V. Everstova et al. (2021) EEPB in pregnancy outcomes was not registered according to the results of a retrospective analysis of 82 individual records of pregnant women and puerperas with confirmed NCI (Republic of Sakha (Yakutia)) [4]. According to A. E. Esedova et al. (2022), in the republics of the North Caucasus Federal District, among pregnant women with mild and moderate NCI, EEPB was observed with a frequency of 0–1.4%. Pregnant women with severe NCI were not included in this analysis [6].

In our study, groups were comparable in medical and social characteristics. For example, median age of the pregnant with EEPB in the 1st and 2nd groups was 34.0 (33.0; 38.0) and 32.5 (27.5; 36.3), respectively (p=0.378). In terms of ethnicity the patients were mainly Slavs – 6 (60.0%) and 12 (85.7%) cases in the 1st and 2nd group, respectively (p=0.056). As for the place of residence, 6 (60.0%) and 7 (50.0%) lived in Chelyabinsk, 2 (20.0%) and 0 (0.0%) - in Magnitogorsk, 0 (0.0%) and 2(14.3%) - in other big towns of the ChR, in small towns and settlements of the ChR - 2 (20.0%) and 5 (35.7%) of the pregnant women from the 1st and 2nd group, respectively with no statistically significant differences (p=0.462). The majority of them had higher (5 (50.0%) and 8 (57.1%) of women) or secondary professional (4 (40.0%) and 3 (21.4%) women) education (p=0.661). As for marital status, 9 (90.0%) and 13 (92.9%) (p=0.999) women were married in the 1st and 2nd group, respectively. As for the employment pattern, 6 (60.0%) and 8 (57.1%) women were people in work, 4 (40.0%) and 6 (42.9%) were homemakers in the 1st and 2nd group, respectively (p=0.999). Patients from both groups were predominately multigravida (10 (100.0%) and 13 (92.9%), p=0.999), multipara (8 (80.0%) and 11 (78.6%), p=0.999) in the 1st and 2nd group, respectively.

Preterm birth in the past medical history was observed in 1 (10.0%) and 1 (7.1%) (p=0.999), artificial abortions – in 4 (40.0%) and 5 (35.7%) (p=0.999), spontaneous abortion – in 3 (30.0%) and 2 (14.3%) (p=0.615), extrauterine pregnancy – in 0 (0.0%) and 1 (7.1%) cases (p=0.999) in the 1st and 2nd group, respectively. Uterine scar was registered in 4 (40.0%) and 4 (28.6%) patients in the 1st and 2nd group, respectively (p=0.673). In four (28.6%) cases in the 2nd group current pregnancy occurred as a result of the assisted reproductive treatment. There were no such patients in the 1st group (p=0.114).

There were no statistically significant differences in terms of frequency and nature of the somatic pathology: 7 (70.0%) and 11 (78.6%) cases in the 1st and 2nd group (p=0.665), and specifically: chronic tonsillitis was registered in 2 (20.0%) and 1 (7.1%) cases (p=0.550), disease of cardio-vascular system (chronic arterial hypertension) – in 3 (30.0%) and 2 (14.3%) (p=0.615), syndrome of the connective tissue dysplasia (mitral valve prolapse) - 1 (10.0%) and 0 (00.0%) (p=0.417), lower extremity varicose veins – in 1 (10.0%) and 1 (7.1%) (p=0.999), myopia – in 1 (10.0%) and 0 (00.0%) (p=0.417)), thyroid disorders (asymptomatic hypothyroidism) – in 0 (0.0%) and 2 (14.3%) (p=0.493), urinary system pathology (non-acute chronic pyelonephritis) – in 0 (0.0%) and 2 (14.3%) (p=0.493), chronic iron-deficiency anaemia – in 1 (10.0%) and 2 (14.3%) (p=0.999), diabetes mellitus – in 0 (0.0%) and 1 (7.1%) (p=0.999) cases in the 1st and 2nd group, respectively.

Statistically significant differences in the body weight were revealed between the groups. Normal weight was observed in 5 (50.0%) and 2 (14.3%), excess weight – in 0 (0.0%) and 7 (50.0%), obesity – in 5 (50.0%) and 5 (35.7%) women in the 1st and 2nd group, respectively (p=0.017). There were no statistically significant differences in terms of Body Mass Index (BMI). Yet, there were more patients with BMI≥25 (excess weight and obesity) during the 3rd and 4th waves of pandemic - 5 (50.0%) and 12 (85.0%) women in group 1 and 2, respectively (p=0.085).

Threatened miscarriage (TM) was observed in 1 (10.0%) and 2 (14.3%) (p=0.999), placenta praevia – in 3 (30.0%) and 0 (00.0%) (p=0.059), chronic placental deficiency (ChPD) – in (10.0%) and 2 (14.3%) (p=0.999), fetal growth restriction – in 1 (10.0%) and 0 (00.0%) (p=0.417), amniotic fluid pathology (oligoamnios) – in 2 (20.0%) and 0 (00.0%) (p=0.163), cervicovaginal infections – in 4 (40.0%) and 2 (14.3%) (p=0.192) cases in studied group 1 and 2, respectively.

No statistically significant differences in NCI degree of severity were observed among the patients with EEPB in group 1 and 2 (p=0.942). Mild NCI course was registered in 2 (20.0%) and 2 (14.3%), moderate severity course – in 3 (30.0%)

and 3 (21.4%), severe course – in 3 (30.0%) and 6 (42.9%), extremely severe – in 2 (20.0%) and 2 (14.3%) cases in in the 1st and 2nd group, respectively. Groups were similar in terms of pneumonia development (1st group – 8 (80.0%), 2nd group – 12 (85.7%) cases, p=0.999). Groups did not differ in the percentage of lung damage based on the findings of the computer tomography in dynamics either (1st group – 4 (40.0%), 2nd group – 7 (50.0%) cases, p=0.697).

EEPB at the 22-24/6 weeks occurred in 1 (10.0%) and 4 (28.6%), at 25-27/6 weeks – in 9 (90.0%) and 9 (71.4%) cases in the 1st and 2nd group, respectively (p=0.358). EEPB were spontaneous in 3 (30.0%) and 2 (14.3%) cases in the 1st and 2nd group, respectively (p=0.615). In terms of clinical course there were no differences between the pandemic periods. They started with premature rupture of membranes in 1 (33.3%) and 1 (50.0%), with spontaneous labor – in 2 (66.7%) and 1 (50.0%) cases in the 1st and 2nd group, respectively (p=0.999).

All the spontaneous EEPB ended as vaginal delivery. Medically induced vaginal EEPB occurred only in the 2nd group in 2 cases (14.3%). The induction indication was intrauterine fetal death. Medically induced EEPB via caesarean section were performed in 7 (70.0%) and in 10 (71.4%) cases in the 1st and 2nd group, respectively (p=0.999). Main indication for caesarean section was isolated acute respiratory distress syndrome (ARDS) of the mother: 5 (71.4%) and 9 (90.0%) cases (p=0.360).

The weight of the newborns in the 1st and 2nd group, respectively was 917.5 g (712.5; 990.0) and 775.0 g (611.3; 1007.5), (p=0.229), length – 34.0 cm (32.8; 37.0) and 33.0 cm (29.5; 35.3), (p=0.157), i.e. no statistically significant differences were observed.

Important parameters of the work of obstetric-gynecologic service are stillbirths and intrauterine deaths (IUD). Frequency of stillbirths was 3 (30%) and 7 (50%) cases in the 1st and 2nd group, respectively, without statistically significant differences (p=0.421). In all the cases (3 (100%)) in group 1 intranatal fetal death occurred; in the 2nd group - 1 (14.3%) case of intranatal fetal death and 6 (85.7%) - intrauterine fetal death (p=0.033). This testifies to a statistically significant predominance of intrauterine fetal death in the period of the 3rd – 4th wave of NCI pandemic.

A wide range of placental lesions was identified in all the patients in both groups in case of EEPB and NCI. They were different in the degree of manifestation and prevalence, variety of combinations involving maternal and fetal malperfusion, maternal and fetal inflammatory response. Moderate or massive intervillous thrombosis with massive fibrin accumulation, fibrin agglutination and villous infarction, and thrombosis of the vessels of chorionic plate stand out particularly.

If there was an intrauterine fetal death, in all the cases a combination of marked maternal and fetal vascular malperfusion and severe acute inflammatory damage of the placenta (2nd, 3rd stage and 2nd, 3rd degree) occurred both for maternal and fetal inflammatory response. It resulted in decompensated placental deficiency. At the same time when there were no intrauterine deaths, the signs of the maternal and fetal stromal – vascular lesions of the placenta were manifested to a lesser degree. In half of the cases they were combined with chronic forms of placental inflammation (basal deciduitis, intervillusitis).

Conclusion. Thus, no statistically significant increase in the EEPB frequency is observed in pregnant women with NCI COVID-19 of various degree of severity in the Chelyabinsk city and the Chelyabinsk region in the period of the 1st – 2nd and 3rd – 4th waves of the NCI pandemic. The level of EEPB in patients with NCI throughout the whole period under study remains stable. It does not statistically significantly exceed the EEPB level either among the population of the pregnant women without COVID-19 who were under observation from April 2020 through December 2021 (p=0.793), or the EEPB level registered in 2019 before the onset of the pandemic (p=0.287). Frequency of spontaneous EEPB in the pregnant women with NCI in the 1st and 2nd group does not differ statistically significantly and makes up 30% and 14.3% cases (p=0.615).

Medically induced vaginal EEPB in patients with NCI were observed only in the 2nd group in case of intrauterine fetal death. Surgery EEPB in the pregnant women with NCI were associated mainly with the increase in maternal ARDS. An insignificant increase in the intrauterine mortality rate is observed in the period of the 3rd – 4th wave of pandemic in case of EEPB relative to the period of the 1st – 2nd wave. Moreover, in the period of the 3rd – 4th wave of pandemic in case of EEPB, intrauterine fetal death occurs statistically significantly more often (p=0.033) in patients with NCI.

Morphological specific features of the placenta in case of the intrauterine fetal death in the period of the 3rd – 4th wave of pandemic in patients with NCI of various degree of severity are characterized by a marked maternal and fetal vascular malperfusion and severe acute inflammatory damage to placenta (2nd, 3rd stage and 2nd, 3rd degree) in comparison to the morphological characteristics of the placenta without intrauterine losses, when in half of the cases a chronic inflammation in placenta is registered, whereas signs of the maternal and fetal stromal-vascular lesion of the placenta are manifested to a lesser degree.

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ARCTIC MEDICINE

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PROTEIN MARKERS OF NEGATIVE EFFECTS IN CHILDREN UNDER COLD EXPOSURES

DOI 10.25789/YMJ.2023.81.21

УДК 613.1: 616.1

Currently, a promising study is the identification of changes in the level of expressed proteins (omic markers) in the body under the influence of adverse factors, including climatic ones, reflecting the destabilization of homeostasis. The purpose of the study was to identify protein markers of negative effects in children living under the influence of adverse factors of the subarctic climate.

Materials and methods. A study of the proteomic profile of the blood plasma of children was carried out; statistical evaluation of the values of the relative volume of identified protein spots; establishing and evaluating a probable relationship between the change in the relative volume of identified protein spots and the impact of adverse factors of the subarctic climate.

Results and discussion. Under the influence of adverse factors of the subarctic climate in children of the observation group, relative to the indicators in children of the control group, there was a significant change in the volume of proteins (prothrombin, vitronectin, hemoglobin beta subunit, apolipoproteins A1, C-II and C-III, amyloid proteins A-1 and A- 2, P2Y purinoreceptor 12, transthyretin), the expression or decrease in production of which can cause a violation of the cascade of reactions of the blood coagulation system, a change in the development of mature forms of erythrocytes, a violation of the regulation of reverse cholesterol transport, and damage to endothelial cells.

Conclusion. The study made it possible to establish a relationship between the impact of adverse factors of the subarctic climate and the expression of proteins (apolipoprotein C-III, transthyretin, prothrombin, vitronectin, and hemoglobin β-subunit) identified in the blood plasma of children exposed to this effect. The established omic markers make it possible to predict the development of negative effects in the form of impaired hemostasis mechanisms, intracellular cholesterol esterification, insufficient oxygen supply to tissues, and endothelial dysfunction. The obtained results should be used for predicting, early detection and prevention of the development of possible diseases of the cardiovascular system, blood and hematopoietic organs associated with prolonged exposure to natural cold.

Keywords: harmful factors of subarctic climate, omic markers, predicted negative effects, children.

Introduction. In the Russian Federation approximately 40% of all regions are located in the Arctic Zone beyond the Polar circle or are considered to have similarly harsh natural and climatic conditions. These conditions are rather severe since there are considerable temperature fluctuations, long winter, short summer, and high wind speed [6, 13]. Cold is a predominant non-specific factor which is typical for the climate on these territories. Several research works have established that exposure to cold produces certain effects on peripheral skin receptors and epithelium in the upper airways and induces specific thermoregulation reactions of the sympathetic nervous system which prevent overcooling [1, 7, 24]. When a person is exposed to natural cold, his or her body reacts to it, and this reaction involves depletion of some sections in the endogenous antioxidant protection system and excessive lipid peroxidation; also, cold receptors produce their impulses in a different way. All this leads to systemic vascular resistance, impairs vascular permeability and regulation of vascular tone [14]. It should be noted that there are also considerable changes in hemodynamics as a component of thermal homeostasis [12]. Homeostatic systems in the body undergo complicated restructuring, and functional disorders occur in barrier organs (liver, kidneys, spleen, lungs and the immune system) [8]. As a result, there may be a growth in chronic population morbidity and cold is among basic factors causing it.

At present, there is a promising trend in tackling problems related to early detection of health disorders. This trend is identification of changes in levels of expressed proteins (omic markers) in the body under exposure to harmful factors [18, 19, 21], climatic ones included,

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which indicate that homeostasis is destabilized. Results produced by a proteomic examination of protein structure of human blood plasma make it possible to assess and predict changes in homeostasis mechanisms at the molecular-cellular level and risks of developing negative effects associated with exposure to extremely cold climate in the Arctic regions.

This research work is a part of the whole cycle of studies conducted by the Federal Scientific Center for Medical and Preventive Health Risk Management Technologies with the focus on identifying omic markers in protein profiles of children under exposure to various environmental factors [4, 5].

The goal of the present work was to identify protein markers of negative effects in children living under exposure to harmful factors of subarctic climate.

Materials and methods. This study was conducted as part of the Industry Research Program of Rospotrebnadzor for 2021-2025. «Scientific substantiation of the national system for ensuring sanitary and epidemiological well-being, managing health risks and improving the quality of life of the population of Russia», paragraph 5.5.3 "Molecular profiling, including based on proteomic and metabolomic analysis, and the study of molecular and cellular mechanisms of involvement of the transformed profile in the pathogenesis of priority non-communicable diseases associated with exposure to environmental factors" (Reg, №. NIOKTR 121032300225-5). We examined 35 blood plasma samples of children living under exposure to natural cold on territories with subarctic climate (the test group) and children who lived on a territory with milder continental climate (the reference group). Both groups were comparable as per age (3-5 years), social and living conditions, absence of any burdened hereditary case history, and minimal or practically absent ambient air pollution with chemicals on the territories where they lived.

The sampling of whole blood from the cubital vein in the morning (on an empty stomach) into a vacutainer for subsequent obtaining of blood plasma from children included in the sample was performed by a procedural nurse on the basis of preschool educational institutions selected for the study. The study was conducted in accordance with the ethical principles of the Declaration of Helsinki (64th WMA General Assembly, 2013) and approved in the prescribed manner by the Ethics Committee of the Federal Scientific Center for Medical and Preventive Technologies for Public Health Risk Management (Minutes of the meeting № 1 from 06.02.2021). The legal representatives of the children participating in the survey signed a voluntary informed consent to the use of biological material for scientific purposes. The studies conducted did not infringe on the rights, did not jeopardize the well-being of the subjects of the study, and did not harm their health.

Basic data on climatic factors were taken from open databases on climate. We modeled exposure to climatic factors as their complex influence on a person that lasted 11 months each year during 70 years (an average lifetime), excluding 1 month of a vacation which is spent every year beyond a territory where a person lives permanently. The climate was subarctic on the test territory with the average air temperature being -9.4 °C (the cold peak is −50 °C or lower). Temperatures are well below zero for approximately 280 days a year. The climate was continental on the reference territory was continental with the average air temperature being +1.6 °C.

The study was conducted in full conformity with the ethical principles of scientific medical research on human subjects and was approved as per the established procedure by the Committee on biomedical ethics of the Federal Scientific Center for Medical and Preventive Health Risk Management Technologies. Legal representatives of all children participating in the research gave their written informed voluntary consent to use of biological materials for scientific purposes.

We identified proteins in blood plasma using analytical technologies for examining proteome with chromate-mass-spectrometry. The procedure involved determining amino acid sequences of fragments of individual proteins as per UniProt freely accessible database with a sampling made as per Homo Sapience taxon. A gene which determined expression of a specific protein was identified using HGNC database of human gene name (https://www.genenames.org/).

We comparatively assessed relative

Table 1

Results produced by identifying protein fractions in blood plasma of the examined children

Protein	Protein stain intensity, int				U-test	Z-test	Validity of differences as per mean values, p≤0.05
	Test group		Reference group				
	$\bar{X}$(SEM)	SD	$\bar{X}$(SEM)	SD			
Hemoglobin subunit β	223.50 (149.69)	473.37	1447.90 (111.83)	353.64	4.000	-3.439	0.001
Apolipoprotein A-I	0.00 (0.00)	0.00	66.50 (27.17)	88.91	30.000	-2.110	0.035
Apolipoprotein C-III	3189.90 (165.72)	524.07	1663.41 (72.10)	228.00	3.000	3.515	0.0004
Apolipoprotein C-II	1504.50 (228.02)	721.07	2326.70 (130.46)	412.54	17.000	-2.457	0.014
Prothrombin	387.30 (151.36)	478.64	1724.60 (20.93)	66.20	6.500	-3.250	0.001
Vitronectin	340.4 (167.93)	531.04	1844.10 (63.03)	199.33	2.000	-3.591	0.0003
Transthyretin	2687.40 (329.60)	1042.30	1541.52 (87.26)	275.95	21.000	2.154	0.031
Serum amyloid protein A-1	711.60 (278.68)	881.26	61.80 (26.38)	196.00	21.000	2.154	0.031

volumes of identified protein stains in children from the test and the reference group as per conventional statistical procedures (simple mean ($\overline{X}$), standard error of mean (SEM) and standard deviation (SD)) using Statistica 10 software package. We applied non-parametric Mann-Whitney U-test (U ≤ Ucr) to determine significance of differences between two independent samplings. The significance level was taken at p ≤ 0.05 when statistical hypotheses were tested.

We identified and assessed a probable correlation between a change in relative volums of identified protein stains and exposure to harmful factors of subarctic climate by calculating odds ratio (OR) and its confidence interval (CI). OR ≥ 1 and the bottom limit of CI≥1 indicated there was an authentic correlation [15].

Results and discussion. Having examined proteomic blood plasma profiles of the examined children from the test group, we identified approximately 30 protein fractions. 8 out of them were authentically different from those identified in blood plasma of children from the reference group as per the intensity of a protein stain (Table 1).

Children from the test group who lived under exposure to harmful factors of subarctic climate had authentically lower volumes of prothrombin (F2 gene), vitronectin (VTN gene) and hemoglobin subunit beta (HBB gene) than children from the reference group, by 4.5 times, 5.4 times and 6.5 times accordingly (p=0.0003-0.001). Reduced production of the identified proteins indicates that there might be certain disorders of the cascade of enzyme responses by the plasma blood clotting system [16, 23] and vascular-thrombocyte homeostasis [25, 26]; there may also be some reduction in development of mature erythrocytes which results in oxygen deficiency in tissues [2, 10].

We detected multi-directional changes in levels of apolipoproteins in blood of children from the test group. Thus, there was a decrease in apolipoprotein A1 (APOA1 gene) and apolipoprotein C-II (APOC2 gene) levels, by 1.5 times, and an increase in apolipoprotein C-III (APOC3 gene) level, by 2.0 times (p=0.0004-0.035) against the reference group. Given the exposure to cold climate, these changes indicate that there is probable deregulation of reverse cholesterol transport from peripheral tissues into blood flow and, subsequently, an increase in some lipid spectrum indicators (total cholesterol, triglycerides) [6, 20]. Besides we established an elevated amyloid protein A-1 level (SAA1 gene) which was by 11.5 times higher in children from the test group against the reference one (p=0.031). We identified amyloid protein A-2 (SAA2 gene) and P2Y purinoceptor 12 (P2RY12 gene) which were not detected in proteome blood plasma profiles of children from the reference group. Amyloid proteins A-1 and A-2 are prone to deposit in interstitial tissues of organized insoluble amyloid fibrils [17]; P2Y purinoceptor 12 is able to activate vasoconstriction of vessels [11]. Progressing accumulation of lipoproteins in blood, expression of amyloid proteins and P2Y purinoceptor 12 can induce damage to endothelial cells resulting in adhesion molecules occurring on their surface. They also stimulate further penetration of monocytes and thrombocytes into sub-endothelial space which is accompanied with endothelial dysfunction [9].

Body overcooling is known to excite and activate the neuroendocrine system; given that, attention should be paid to the transthyretin (TTR gene) concentration which was by 1.7 times higher in blood plasma of children from the test group against the reference one (p=0.031). There are ambiguous opinions about a role transthyretin plays in the body. On one hand, expression of this protein re-

Table 2

Assessment of the correlations between changes in the identified proteins in blood plasma and exposure to harmful factors of subarctic climate

Omic markers of a predicted negative effect	Group	Response to exposure		Odds ratio (OR)	95 % confidence interval (CI)
		yes	no		
Impaired regulation of cholesterol transport					
Decrease in apolipoprotein A-I	test group	9	1	1.00	0.25-6.75
	reference group	1	9		
Increase in apolipoprotein C-III	test group	6	4	13.50	1.20-152.22
	reference group	1	9		
Decrease in apolipoprotein C-II	test group	3	7	3.86	0.33-45.57
	reference group	1	9		
Disorders in blood clotting					
Decrease in prothrombin	test group	7	3	21.0	1.78-248.11
	reference group	1	9		
Decrease in vitronectin	test group	6	4	13.50	1.20-152.22
	reference group	1	9		
Reduced development of mature erythrocytes					
Decrease in hemoglobin subunit beta	test group	8	2	36.00	2.72-476.30
	reference group	1	9		
Deposition of organized insoluble amyloid fibrils in interstitial tissues					
Increase in transthyretin	test group	6	4	13.50	1.20-152.22
	reference group	1	9		
Increase in serum amyloid protein A-1	test group	2	8	2.25	0.17-29.77
	reference group	1	9		

sults in insoluble fibril glycoprotein depositing in intercellular space [11, 22]; on the other hand, some experts consider a possible role transthyretin plays in the peptide neuroprotection by activating the retinol-tiroxin medicated system which implements synaptic plasticity and neurogenesis [3].

We assessed correlations (by calculating odds ratio) between detected changes in the levels of the identified proteins in blood plasma profile under exposure to harmful factors of subarctic climate. The assessment revealed that a possibility of apolipoprotein C-III and transthyretin expression was by 13.5 times higher for children from the test group against the reference one; a possibility of reduced prothroimbin production, by 21.0 times; possibility of reduced vitronectin production, by 13.5 items; and possibility of reduced hemoglobin subunit beta production, by 36.0 times (Table 2).

Predicted negative effects in the examined children are well in line with results produced by scientific research which indicate that there are changes in mechanisms of adaptation restructure developing under exposure to severe climatic conditions in the Arctic regions [2, 6, 10, 12, 13, 14].

Conclusion. Our research established the correlation between exposure to harmful factors of subarctic climate and expression of proteins (apolipoprotein C-III, transthyretin, prothrombin, vitronectin and hemoglobin subunit β) identified in blood plasma of exposed children. These established omic markers give an opportunity to predict developing negative effects including impaired homeostasis and intracellular cholesterol etherification, insufficient oxygen supply to tissues, and endothelial dysfunction. It is advisable to use these research results to predict, detect in due time and prevent various cardiovascular diseases and diseases of blood and blood-making organs associated with long-term exposure to natural cold.

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INTERCELLULAR INTERACTIONS AND THE LEVEL OF AGGREGATION OF BLOOD CELLS IN THE AREA OF INFLAMMATION IN PEOPLE LIVING IN THE EUROPEAN NORTH

DOI 10.25789/YMJ.2023.81.22
УДК 612.112:616-002

The immunological results of 242 people with bronchitis and 198 people with colitis, as well as 47 practically healthy people aged 21 to 55 years at the time of examination were analyzed in order to study intercellular interactions and the relative proportion of neutrophil aggregation in the area of inflammation in people living in the North. Against the background of neutropenia in the area of inflammation, the level of migration of monocytes and lymphocytes is less pronounced. In conditions close to physiological, in the exudate of the "skin window", no significant differences in the level of leukocyte migration were revealed. With neutropenia in peripheral venous blood, the content of neutrophil aggregates in the area of inflammation is higher. Aggregation of neutrophils leads to significantly more active exosecretion, degranulation and lysis of aggregate cells, which may be one of the mechanisms for the formation of neutropenia in people living in the North.

Keywords: aggregation, neutrophils, monocytes, lymphocytes, neutropenia, bronchitis, colitis.

Introduction. In response to any change in the intercellular environment that carries a potential threat to the cell or blood loss, a reaction of changes in the microcirculatory unit develops with a change in blood filling, blood flow velocity. In this case, the cells release a complex complex of biologically active substances that cause reactions from a number of humoral effector systems. The set of lysosomal enzymes is highest in polymorphonuclear neutrophil granulocytes. Neutrophil granules of neutrophil include lysozyme, lactoferrin and alkaline phosphatase, active only in a neutral medium. Reducing the pH value to 4 is optimal for the activity of enzymes of azurophilic granules – myeloperoxidase and acid hydrolases. Azurophilic granules contain a large amount of elastase, which can be a factor of destruction; two metalloproteinases (collagenase and gelatinase) can cause degradation of the extracellular matrix [22, 28].

An increase in the content of proteolytic enzymes of active substances in the intercellular medium and blood in the area of trouble causes a reaction from the plasma protease system with the activation of Hageman factor, the conversion of precallikrein into kallikrein and the subsequent formation of plasma bradykinin and tissue kinin (lysyl-bradykinin or kallidin). An increase in the content of kinin causes the expansion of the vessel lumen in the microcirculation area with an increase in permeability, by contracting smooth muscles and endothelial cells.

Serotonin, histamine, kinins and prostaglandins are involved in the formation of edema during inflammation. The further continuation of the reaction involves the complement system; vasodilation and elevation of the vascular wall reproduces the product of activation of the C1, C4, C2 complement system (C-kinin). C-kinin differs from kinins in that it is inactivated rather than initiated by trypsin. The complement system also supplies the main chemotaxis factor C5, which is formed by neutrophil enzymes and enhanced by platelet reaction.

Tissue hypoxia with a decrease in O2 partial pressure, changes in microcirculation and vascular permeability cause an increased level of neutrophil adhesion in venules [24-26]. The adhesion of leukocyte blood cells is enhanced by a whole series of biologically active substances produced by activated neutrophils themselves [19].

In the study of intercellular interactions in peripheral venous blood in practically healthy residents of high latitudes, it was previously found that the activity of aggregation of peripheral venous blood cells in Arctic residents is 1.5-1.7 times higher than that of people living in more favorable climatic conditions. Erythrocytes and platelets aggregate most often (20.92 and 18.95%, respectively), peripheral blood leukocytes form aggregates actually 2 times less often (neutrophil granulocytes in 10.45%, lymphocytes in 7.19%) [21]. The level of neutrophil aggregation activity is associated with a decrease in the content of these cells in the circulating blood and an increase in the concentration of sCD56 adhesion molecules. The facts of the release of specific leukocyte granules into the pseudopod region associated with increased secretion of adhesion molecules, selectins, their ligands and chemotactic receptors in the adhesion region are known [7, 20, 29, 31, 34]. In residents of high latitudes, the content of the extracellular pool of signaling molecules, receptors and their ligands is significantly higher than in people living in more favorable climatic conditions. The dependence of this phenomenon on northern climatic conditions is demonstrated by the highest levels of extracellular pool content in residents of Arctic regions [2, 13]. Even in practically healthy people living in the North, the concentrations of free adhesion molecules and the L-selectin ligand (respectively sCD54, sCD62 and sCD62L) are significantly higher.

A higher level of adhesion activity and subsequent migration of activated neutrophil granulocytes in the inhabitants of the North is necessary due to the low level of vascular permeability and clearance of metabolic products [5, 8]. Neutrophils are involved in the clearance of cell waste products and their apoptosis. Phagocytosis of apoptotic bodies by granulocytes occurs very quickly and does not cause inflammatory reactions [4]. A high level of chemoattractants contributes to a constant migration flow of granulocytes, which is often manifested by a decrease in the content of circulating and actively

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phagocytic neutrophils in the blood [18]. If there is a sufficient concentration gradient of the chemoattractant, the location of the receptors on the surface of the cell membrane becomes asymmetric, concentrates on one of the poles in the form of a cap (capping) and determines the direction of its movement [23].

Leukocytes normally have a fairly significant viscosity, compared, for example, with the level of this property in erythrocytes and platelets. Studies have shown that the mouth of the capillaries is not so rare, even normally, leukocytes clog, but after a short time the blood flow is restored. With inflammation, such effects are recorded much more often [16]. During inflammation, accumulations of leukocytes are recorded near the walls of blood vessels (venules), which is associated with an unfavorable course of the inflammatory process. The phenomenon of granulocytes sticking to the vascular endothelium in the area of inflammation with the formation of cell aggregates is the earliest manifestation of the neutrophil reaction to changes in homeostasis or tissue damage. The phenomenon of clumping (aggregation) of granulocytes has been proven in vivo in pulmonal vascular leukostasis, interstitial edema and pulmonary insufficiency, as well as in Arthus phenomenon and complications of hemodialysis [24-26]. Irreversible aggregation of neutrophils is involved in the formation of leukostasis and leukopenia due to a decrease in the content of cells in the circulating pool and their transition to marginal. A high level of aggregation (clumping) involving C5a is already associated with neutrophil lysis and can lead to granulocytopenia [16].

In connection with the above, the aim of the study was to study intercellular interactions and the relative proportion of neutrophil aggregation in the area of inflammation in people living in the North.

Materials and methods. The immunological results of the preanalytical and analytical stages of the examination of 242 people with bronchitis and 198 people with colitis living in Arkhangelsk who applied to the Biolam center for professional diagnostics with a previously established diagnosis, as well as 47 practically healthy people aged 21 to 55 years at the time of the examination were analyzed. The survey was carried out with the written consent of the respondents in compliance with the basic norms of biomedical ethics in accordance with the document "Ethical principles for medical research involving human subjects" (WMA Declaration of Helsinki 1964, amended in 2013), and also approved and approved by the Commission on Biomedical Ethics at the Institute of Physiology of Natural Adaptations of FECIAR UrB RAS (Protocol No. 5 of 11.02.2022).

Peripheral venous blood, sputum, and feces were examined. A hemogram was determined in peripheral venous blood using the XS-1000i hematological analyzer (Sysmex, Japan). The deficiency of neutrophil granulocytes (neutropenia) was established at a content of <2.0×109 cells/l in peripheral venous blood. Preparations for microscopy of feces were prepared after preliminary preparation of an emulsion with a saline solution (1/1), both the sediment and the supravaginal fluid were examined [10]. Cytological examination of sputum was studied after centrifugation of 1 ml of sputum and application of 0.5 ml of sediment to a slide [12]. Cytogram and phagocytosis were studied in smears stained according to Romanovsky-Giemse and Gram; counting was performed at the rate of 100 cells. Leukocyte aggregation was studied by light

Table 1

Comparative analysis of the content of cells and their phagocytic activity in peripheral venous blood and biomaterial from the area of inflammation, depending on the presence or absence of neutropenia in the examined (M±m)

Parameters studied	Bronchitis		Colitis	
	Neutropenia, n=124	Normal neutrophil content, n=118	Neutropenia, n=135	Normal neutrophil content, n=63
1	2	3	4	5
	Venous blood			
Neutrophils, %	45.93±0.38	56.18±0.54	46.29±0.43	59.25±0.37
% of active phagocytes	44.32±0.57**2-3	55.37±0.52	42.55±0.68**4-5	59.31±0.56
Phagocytic number	8.61±0.27*2-3	6.23±0.32	12.23±0.31**4-5	7.46±0.44
Monocytes, %	10.29±0.21	6.87±0.25	12.69±0.45	8.34±0.46
% of active phagocytes	19.29±0.13	18.87±0.25	23.23±0.34	21.32±0.46
Phagocytic number	8.52±0.22*2-3	5.34±0.33	9.85±0.31*4-5	6.71±0.41
Lymphocytes, %	29.42±0.58	27.51±0.53	37.39±0.48	29.22±0.53
	Sputum		Feces	
Neutrophils, %	31.36±1.22**2-3	36.24±0.78	32.42±1.23**4-5	39.23±1.35
% of active phagocytes	51.24±1.53**2-3	58.36±1.24	49.83±1.12**4-5	56.45±1.32
Phagocytic number	9.53±0.56*2-3	7.32±0.43	11.64±0.62**4-5	8.32±0.79
Monocytes, %	9.52±0.47***2-3	19.36±0.52	15.23±0.51**4-5	21.43±0.65
% of active phagocytes	22.24±1.05**2-3	29.53±0.73	25.37±0.66*4-5	28.42±0.69
Phagocytic number	6.23±0.26*2-3	5.08±0.32	7.93±0.34*4-5	6.32±0.42
Lymphocytes, %	29.45±0.61	25.31±0.67	34.42±1.21*4-5	38.23±1.65
Aggregation of neutrophils, %	25.86±0.92***2-3	13.64±0.63	31.34±1.53**4-5	23.48±1.45

* p<0.05, ** p<0.01, *** p<0.001.

microscopy [3, 9]. The study of neutrophil migration in physiological conditions was carried out by the "skin window" method [33].

Statistical processing of the obtained data was carried out using the application software package "Statistica 10.0" (StatSoft, USA). The level of statistical significance of the differences (p) in the work was assumed to be 0.05.

Results and discussion. At the initial stages of the development of inflammation, neutrophils and mononuclears are mobilized, which can be manifested by a decrease in the total content of leukocytes in the blood due mainly to neutrophils and monocytes.

It was of interest to study in comparative terms the content of leukocytes and their phagocytic activity in the area of inflammation (table 1).

The inflammatory exudate clearly shows the ability of cells to stick together with the formation of aggregates of 3-5 cells. When calculating the number of neutrophil aggregates per 200 neutrophils, it was found that with neutropenia, the ability of granulocytes to stick together is noticeably higher. In sputum smears of patients with bronchitis, the differences in the activity of the formation of aggregates from neutrophils are on average almost 2 times greater (fig. 1, 2). Against the background of neutropenia in the area of distress, the level of migration of monocytes and lymphocytes is less pronounced. It is known that the rate of chemotaxis of polymorphonuclear granulocytes into the area of trouble is at first much higher, and then it drops rapidly; the process of penetration of monocytes into the lesion is slow, but longer. The migration of lymphocytes cannot actually be differentiated by morphological methods of investigation from the recirculation that these cells are capable of [35].

Under conditions close to physiological, in the exudate of the "skin window", no significant differences in the level of leukocyte migration were revealed (table 2). Consequently, the significant decrease in the migration activity of neutrophils and monocytes to the focus of inflammation in neutropenia is associated with a decrease in the content of their circulating pool. An increase in the content of aggregated neutrophils and monocytes in neutropenia indicates an increase in the level of irreversible aggregation or inability to dissociate aggregates under these conditions. Since the dissociation of the aggregate occurs before the secretion of acid hydrolases and cathepsin [30, 32], it can be assumed that the process of reversibility of aggregation is disrupted in neutropenia. Glucocorticoids are involved in this process, which prevent the degranulation of enzymes working in an acidic environment and the release of cathepsin and elastase.

Table 2

Comparative composition of blood cells in the exudate of the "skin window" of practically healthy people 12 hours after scarification, depending on the level of neutrophils in the blood (M±m)

Indicator	Neutropenia, n=22	Normal neutrophil content, n=25
Neutrophils, %	67.34±2.18	62.77±2.38
Monocytes, %	14.52±0.38*	17.43±0.33
Lymphocytes, %	16.23±0.64*	18.65±0.52
Eosinophils, %	1.41±0.28	1.15±0.22
Aggregation of neutrophils, %	22.13±0.27***	14.53±0.19
Aggregation of monocytes, %	19.53±0.31***	11.23±0.26

* p<0.05, ** p<0.01, *** p<0.001.

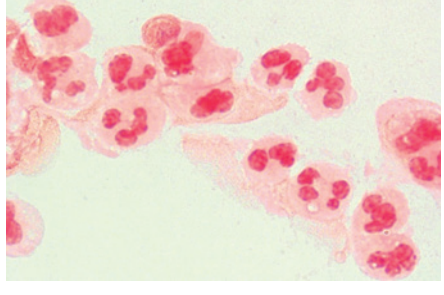

Fig. 1. A smear of sputum at bronchitis with neutropenia. Gram staining. × 1000. Neutrophil aggregation

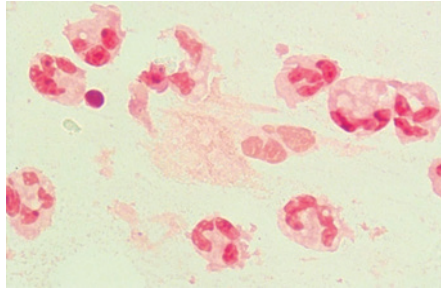

Fig. 2. A smear of sputum at bronchitis with normal neutrophil content. Gram staining. × 1000. Neutrophils

Cell adhesion on the surface of the endothelium or other surface of the extracellular matrix and their aggregation may be related processes. The adhesion of leukocytes to the endothelium, which regulates the size of the marginal pool, on the one hand, is initiated by the aggregation of cells on the surface of the endothelium, on the other hand, is determined by the production of various adhesion molecules, selectins and their ligands by endotheliocytes [7, 29, 34]. Adhesion ensures the attachment of neutrophils to the endothelium and other surface of the extracellular matrix, then aggregation is assimilated with the release of biologically active substances by neutrophils with subsequent activation of enzymatic systems, including the complement system.

The formation of sockets is based on the interaction of cells under the influence of C3 activation products, which initiate not only adhesion, conglutination, but also the formation of sockets. Activating factors can be trypsin-like enzymes, lipopolysaccharides, polysaccharides, C-reactive protein, Fc-aggregated Ig. Cluster-forming cells can be neutrophils, monocytes and lymphocytes [1, 11]. Upon contact of cells with cluster-forming cells, proteolytic and hydrolytic enzymes and reactive oxygen species are released [6, 15, 36]. Enzymes and biologically active substances in cases of rosette lysis are also isolated by platelets (hydrolases, cathepsins, collagenases) [14]. Intercellular interactions of neutrophils, monocytes, erythrocytes and platelets are one of the forms of participation of these cells in preventive reactions and inflammation. Cytolysis and phagocytosis, which follow the formation of rosettes, are a mechanism for protecting organs and tissues from the damaging influence of factors and actions that activate the complement system [17, 27].

Conclusion. So, with neutropenia in peripheral venous blood, the content of neutrophil aggregates in the area of inflammation is higher. Aggregation of neutrophils leads to significantly more active exosecretion, degranulation and lysis of aggregate cells, which may be one of the mechanisms for the formation of neutropenia in people living in the North.

The study was performed under the program of fundamental scientific research, № 122011800217-9.

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THE CONTENT OF THE MAIN CHEMICAL ELEMENTS IN BLOOD SERUM OF PRESENT-DAY EVENKS, THE INDIGENOUS ETHNIC GROUP OF THE RUSSIAN ARCTIC

DOI 10.25789/YMJ.2023.81.23

УДК 616.517:577.12(048)

The Evenks are an indigenous people of the North of Russia. The study included 103 Evenks living in the rural area of Jilinda in the Oleneksky district of Yakutia. The concentration of 4 major elements was determined in the blood serum by mass-spectrometry: sodium (Na), magnesium (Mg), phosphorus (P), calcium (Ca). The study revealed a reduced level of calcium (72.8 mg/L) and an increased concentration of phosphorus (130.3 mg/L) in the serum of Evenks compared to the literature data, which may affect the development of diseases in this ethnic group.

Keywords: major elements, serum, indigenous peoples of the North, Evenks, Arctic, sodium, magnesium, phosphorus, calcium.

Introduction. In the conditions of the fragile nature of the Russian Arctic, 45 indigenous peoples of the North live. In Russia, a comparative analysis of the 2002 and 2010 censuses showed that 15 ethnic groups showed a slight increase in numbers, all other ethnic groups showed a sharp decrease: "Kereks" - 4, "Enets" - 227, "Uilta (Oroks)" - 295, "Chulms" - 365, "Tofalars" - 762, and "complete assimilation" of the Alyutors and Izhors was also recorded [2, 3, 8]. According to the All-Russian census of 2010, 958.5 thousand people live in Yakutia, of which the indigenous population is: Yakuts - 466.5 (48.7%), Evenks - 21.0 (2.2%), Evens - 15 ,1 (1.6%), Dolgan - 1.9 thousand people. (0.2%) [7].

Indigenous peoples of the North of Russia do not adhere to the traditional way of life in the conditions of modern socio-economic development, which entails a deterioration in health. The morbidity and mortality rates of the population of the northern regions have increased [5]. Currently, according to the Federal State Statistics Service of the Russian Federation, there is no data on the health status of the indigenous population of the North, since this information is recorded within the framework of an administrative entity.

According to the results of the All-Russian census of 2010, the number of Evenks was 37,800 people, of which 21,000 people live in Yakutia [7]. Currently, the Evenks lead a sedentary lifestyle, living in isolation in the tundra in national villages. A small number of men are engaged in reindeer herding, hunting and fishing. There is a sharp decrease in the number of domestic deer. A quarter of the population does not every day consume venison and fish products, which traditionally represented the basic part of the diet of the indigenous peoples of the North. The Evenk diet consisted of bakery, pasta and sugar [10].

The reference values of the main elements that make up human biological fluids and tissues are determined: calcium, magnesium, sodium, potassium, phosphorus, sulfur, fluorine and chlorine. Their content at different ages of a person, their role in metabolism and pathogenesis of diseases have been studied [6]. Methods of biochemical analysis have been introduced into Russian healthcare practice to determine the content of macroelements in the blood serum of patients. Unfortunately, these routine tests are carried out in the laboratories in large cities for medical reasons. Indigenous peoples of the North, living in remote villages, do not have access to such types of medical research.

In this regard, the purpose of this study was to determine the regional basic level of macroelements in the blood serum of the Evenks and to search for the dependence of the content of elements with age in the adult population.

Materials and research methods. The study involved 103 Evenks of the village Jilinda. The village Jilinda in the Oleneksky district of Yakutia is located beyond the Arctic Circle in the forest-tundra on the banks of the Malaya Kuonapka River, a tributary of the river Anabar. The road to the nearest village Olenek is only 300 km by winter road, there are no regular helicopter flights. The population is made up of indigenous peoples of the North - Evenks, 400 adults, 200 children. They are engaged in reindeer breeding and commercial hunting. The inhabitants of the village have lost the Evenk language, communication is conducted in the Yakut language. Written informed consent was obtained from residents prior to inclusion in the study. The survey was conducted in accordance with the principles and ethical standards established by the Declaration of Helsinki (Local Committee on Biomedical Ethics of the Yakut Science Center of Complex

Table 1

Age and gender distribution of Evenk people who participated in this study

Age, years	Male	Female	Total
25-29	1	2	3
30-39	7	10	17
40-49	8	12	20
50-59	15	19	34
60-69	8	18	26
70-79	-	3	3
Total	39	64	103

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Medical Problems (Yakutsk, Russia, Protocol No. 46, 2017).

Serum was separated and stored in aliquots frozen at -40°C. The content of major elements in serum was quantified by inductively coupled plasma mass spectrometry (ICP MS) using an Elan 9000 instrument (Perkin Elmer, USA) at the Institute of Tectonics and Geophysics (Khabarovsk). The content of the following 4 elements was studied: sodium (Na), magnesium (Mg), phosphorus (P), calcium (Ca). Samples were decomposed in glassy carbon crucibles by an open method. An aliquot of 0.5 ml was taken to determine the concentration. To decompose the sample, 1 ml of conc. HNO_3 and H_2O_2, after evaporation, 10 ml of 10% HNO_3 was added to the dry residue and heated until the precipitate was completely dissolved. After that, the solution was cooled to room temperature, transferred to a measuring tube, and adjusted with 2% HNO_3 to a volume of 50 ml. Calibration straight lines were built using three points: 0; 20 and 40 μg/dm^3, for which multielement standard solutions from Perkin Elmer were used. To reduce the influence of the matrix effect on the determination of element concentrations, the internal standard method was used, which was the indium isotope ^{115}In, which was additionally added to all samples at a concentration of 40 μg/dm^3. Whenever possible, the most common isotopes with minimal isobaric and polyatomic interferences were selected to determine the concentration of elements. The content of the studied chemical elements in the blood serum was expressed in milligrams per liter (mg/L).

Statistical processing of the obtained results was carried out using the SPSS 19.0 application package. Checking the normality of the distribution of quantitative traits was carried out using the Shapiro-Wilk test. A descriptive analysis of the numerical characteristics of the traits was carried out (Me (Q25-Q75) - median (interquartile range 25 and 75). When comparing differences in groups, non-parametric assessment criteria were used (U-test according to the Mann-Whitney method). To analyze the relationship between quantitative traits, we used correlation analysis with calculation of the Spearman correlation coefficient (rs) The critical value of the significance level (p) was taken equal to 0.05.

Results and discussion. 103 Evenks took part in the study of the macroelement composition of the blood serum, of which 39 (37.9%) were men and 64 (62.1%) were women (Table 1). The age of the subjects was from 25 to 79 years. The mean age with standard deviations for men was 54 (41-59) years, for women - 55 (44-61) years, without statistically significant differences.

The content of the main "structural" human elements, sodium (Na), magnesium (Mg), phosphorus (P), calcium (Ca) in the blood serum of the Evenks is presented in Table 2 and Table 3. The serum content of macroelements in Evenks had a normal distribution in the sample, except for calcium.

Table 3 reflects the median values in the serum content of major elements in Evenks, the values of the literature data studied by the ICP-MS method are given. Serum sodium (Na) and magnesium (Mg) in Evenks were within the limits of literature data. The content of serum phosphorus (P) was at the level of the upper limit of literary values, and content of calcium (Ca) was low.

The content of elements was analyzed separately for male and female (Table 4). When comparing of median macroelements values by gender, no significant differences were found.

Correlation analysis the serum calcium (Ca) from age in both gender was carried out. At the same time, Evenks women showed a tendency to decrease in calcium levels with age (rs=-0.2; p=0.15) (Figure). In Evenk men, no dependence of calcium on age was found (rs=0.0003; p=0.998). The dependence of serum phosphorus (P), sodium (Na), magnesium (Mg) on age in Evenks of both genders did not differ significantly.

Sodium (Na) contains the most electrolytes in the human body. Sodium is present in all body fluids and tissues, but the highest concentration is in the blood and extracellular fluid. It plays a major role in the distribution of fluid between the extracellular and intracellular spaces. Differences in electrolyte concentrations in the cell and extracellular fluid are maintained by the mechanism of active ion transport, which is carried out with the participation of the sodium-potassium pump. Sodium is necessary for the formation of bone tissue, for the transmission of impulses in the nervous system, and muscle contractions. Removal of sodium from the body is carried out mainly with urine. In the kidneys, the ion is reabsorbed in the tubules after glomerular filtration. The activity of reabsorption of Na ions is significantly affected by the concentration of aldosterone in the body, the secretion of which by the adrenal cortex is under the control of the renin-angiotensin system [6]. For humans, the source of sodium is salt. Most get their daily intake of this element from salt. In Russian medical laboratories, the determination of sodium concentration in blood serum is carried out by the ion-selective method; a reference interval of 136-145 mmol/L (3126.6-3333.6 mg/L) is taken as

Table 2

The serum content of major elements in Evenks, n=103, mg/L

Major element	Median	1st Qu. 25%	3rd Qu. 75%	Mean	SD[1]	Min	Max	Sh-W test[2]
Sodium	3002.3	2801.5	3113.7	2972.0	201.3	2540	3450	0.220
Magnesium	17.8	12.5	22.1	17.7	5.6	7.5	33.7	0.182
Phosphorus	130.3	105.1	159.1	131.3	33.1	58.0	200	0.077
Calcium	72.8	51.0	138.6	111.4	93.7	14.6	414.7	**0.000**

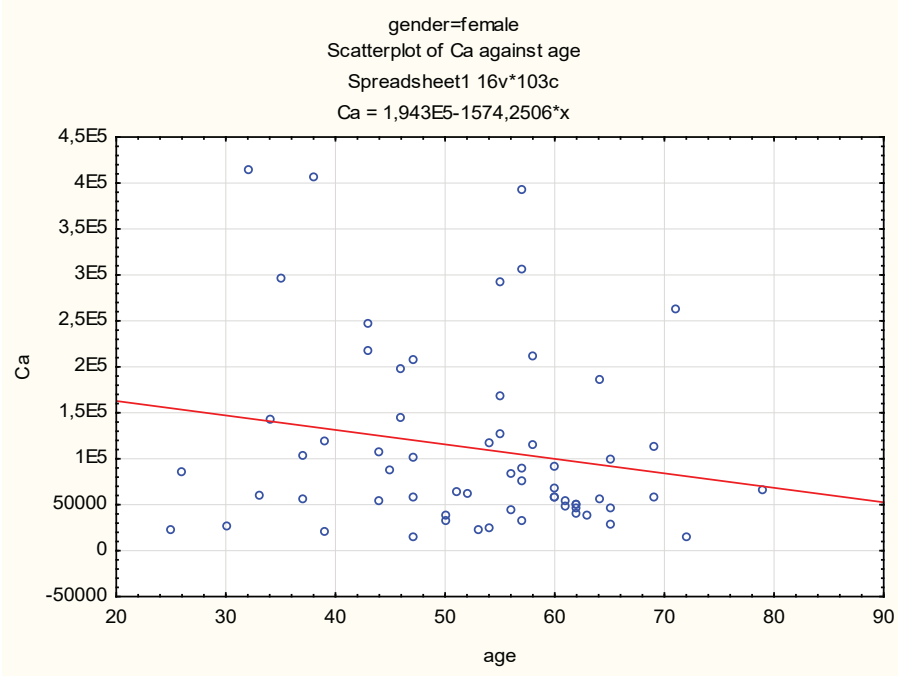
[1]SD – standard deviation.
[2]Sh-W test – Shapiro-Wilk test.

Table 3

The serum content of major elements in Evenks and Literature data, mg/L

Major element	Evenks, n=103	Literature data
	Me (Q25-Q75)	
Sodium	3002.3 (2801.5-3113.7)	2277-4320
Magnesium	17.8 (12.5-22.1)	14.8-34.0
Phosphorus	130.3 (105.1-159.1)	77-133
Calcium	**72.8** (51.0-138.6)	77-125

Me (Q25-Q75) – median (interquartile range 25 and 75)



Dependence of serum calcium in female on age

the norm [16]. In our study, the serum sodium (Na) in Evenks (3002.3 mg/L) was found to be lower when compared with the results obtained by ICP-MS in residents of Novosibirsk, Siberia - 4321 mg/L [11], in healthy adults in Germany - 4190 mg/L [27]. In Spain, in the elderly with age-related cataract, serum Na ranged from 2277-3666 mg/L [12]. The serum Na in Evenks did not differ by gender (Table 4) and did not depend on age.

Magnesium (Mg) is a mineral involved in energy production, muscle contraction, nerve impulse conduction, and bone skeleton construction. It enters the body from food, being absorbed in the small and large intestines. Magnesium is mainly concentrated in bones, cells, and tissues [6]. Its largest part (60%) is contained in the bones, forming their structure in collaboration with calcium (Ca). At the same time, Mg is a natural physiological antagonist of calcium ions. The blood contains about 1% of the total amount of magnesium [4, 6]. In Russian healthcare, the determination of magnesium in blood serum is carried out by the ion-selective method, the reference interval in adults is 0.66–1.07 mmol/L (16.05–26.01 mg/L) [16]. According to the literature data, serum magnesium by ICP-MS was found in residents of Germany - 14.75 mg/L [27], in residents of Shanghai in China - 17.9 mg/L [28], in female students in the city of Yaroslavl, Russia - 19.2 mg/L [9], residents of St. Petersburg, Russia - 19.4 mg/L [1], elderly people with eye diseases in Spain - 23 mg/L [12], population of Novosibirsk, Russia - 28.0 μg/L [11], among the elderly in Switzerland - 34.0 [17]. In residents of Jilinda, serum Mg (17.8 μg/L) is within the literature values, without differences by gender (Table 4) and age.

Phosphorus (P) plays a fundamental role in basic cellular processes, such as bioenergetics, intracellular signaling and mineralization of bones and teeth, and is part of nucleic acids and cell membranes. About 70-80% of phosphorus in the body is associated with calcium, forming the framework of bones and teeth, 10% is in the muscles and about 1% in the nervous tissue. The rest is contained in all cells of the body as an energy reserve. Normally, about 1% of all phosphorus is in the blood [6, 22]. Phosphorus, being a part of many food products, is quickly absorbed in the small intestine. In the laboratories of Russian medical institutions, inorganic phosphorus in serum is determined by colorimetry with ammonium molybdate, while the reference values are 0.74–1.45 mmol/L (22.92–44.91 mg/L) [16]. A comparison was made with the data of the Dolgans, indigenous peoples of the North, living in the village of Yuryung-Khaya. Dolgan survey was conducted by us in April 2017 according to a similar protocol. In our studies using mass spectrometry, the content of the serum macronutrient phosphorus (P) in Evenks (130.3 mg/L) (Table 3) and Dolgans (148 mg/L) [24] turned out to be higher than in mid- latitudes population (86 мг/л [1]; 115 мг/л [27], 116 мг/л [17], 111-133 мг/л [11]). The level of phosphates depends on the amount of parathyroid hormone, calcium and vitamin D. An excess of phosphorus in the blood (hyperphosphatemia) can be caused by excessive intake of the mineral from food and hypocalcemia [21, 22]. According to the literature, high levels of inorganic phosphorus in serum increase vascular calcification [15, 18, 25]. In Dolgans in Yakutia, the serum phosphorus (P) is statistically significantly higher, due to significantly higher levels in Dolgan women (151 mg/L) compared to Evenk women (123 mg/L), while no significant differences were found in men (135 mg/L in Evenks and 143 mg/L in Dolgans) (Table 4 and [24]). When studying in two age groups, in the group of elderly Dolgan women, when compared with young ones, a statistically significant high con-

Table 4

Comparison of median element values in serum Evenks by gender, mg/L

Major element	Male, n=39			Female, n=64			p
	Me (Q25-Q75)	min	max	Me (Q25-Q75)	min	max	
Sodium	3025.4 (2892.5-3128.7)	2540	3330	2966.1 (2767.8-3100.4)	2580	3450	0.133
Magnesium	17.3 (12.0-22.7)	8.3	29.5	18.0 (13.0-22.0)	7.5	33.7	0.624
Phosphorus	135.3 (112.5-164.2)	78.0	198	123.3 (101.3-158.9)	58.0	200	0.143
Calcium	74.7 (54.0-137.2)	19.6	382.5	69.1 (47.6-134.9)	14.6	414.7	0.589

Me (Q25-Q75) – median (interquartile range 25 and 75);
p - statistical significance of differences by the Mann-Whitney U-test.

tent of phosphorus (P) (154.60 mg/L versus 133.91 mg/L, p=0.037) was revealed, while the correlation not identified [24]. In our studies the content of phosphorus in the serum did not have significant correlations with age in both genders of the Evenks.

Calcium (Ca) is one of the most important minerals for humans. It is necessary for the contraction of skeletal muscles and the heart, for the transmission of a nerve impulse, as well as for normal blood clotting (promotes the transition of prothrombin to thrombin), to build the framework of bones and teeth. About 99% of this mineral is concentrated in the bones and only less than 1% circulates in the blood. Total calcium in the blood is the concentration of free (ionized) and its bound forms. Part of calcium leaves the body every day, being filtered from the blood by the kidneys and excreted in the urine. To maintain equality between the release and use of this mineral, about 1 g per day should be supplied [6, 21]. In the practical healthcare of Russia, the level of calcium is determined in the blood serum by the method of colorimetric photometry, the limits of the norm are 2.25–2.75 mmol/L (90.18–110.22 mg/L) [16]. According to the literature data, serum calcium (Ca) by ICP-MS was found to be 77 mg/L in German residents [27], 97.4 mg/L in female students from Yaroslavl [9], in population of the North- Western region of Russia - 83.5 mg/L [1], in the population of Novosibirsk - 122.0 μg/L [11], in the elderly in Switzerland - 125 [17], in population of Shanghai in China - 80.8 mg/L [28]. In the Evenks of Jilinda, serum calcium (Ca) (72.8 mg/L) (Table 2, 4) was found to be lower than the available literature data. This nutritional fact can be associated with a decrease in fish consumption over the past decades, with the lack of dairy products in the diet of the population of Jilinda [10]. Dairy and fish diets have been shown to reduce the risk of fractures [19, 20]. Insufficient calcium intake can be a factor that seriously increases the risk of osteoporosis [14, 23, 26]. The combined effects of inadequate daily calcium intake and vitamin D deficiency have caused low bone mineral density and increased prevalence of osteopenia and osteoporosis in postmenopausal Korean women aged 45 to 70 years [13]. Evenki women show a decrease in serum calcium with age (rs=-0.2; p=0.15) without statistical significance, which is probably related to the onset of menopause (Figure).

Conclusions. The study was conducted to assess the content of the main elements (Na, Mg, P, Ca) in blood serum of the Evenks, an indigenous people of the North of the Russian Federation. Major element concentrations were measured using the ICP-MS method, which allows the study of many elements simultaneously and with high sensitivity. The content of the serum major element calcium (Ca) in the Evenks (72.8 mg/L) is lower than the literature data. At the same time, in Evenk women, a tendency to a decrease in the level of calcium with age was revealed. The content of the serum major element phosphorus (P) in Evenks (130.3 mg/L), as in Dolgans living nearby, turned out to be higher than in residents of temperate latitudes, which may be due to hypocalcemia. Significant dependence of the content of serum phosphorus (P) on age in both genders of the Evenks was not revealed.

Our study revealed a reduced serum level of the element calcium (Ca) and an increase in phosphorus (P), which may affect the development of diseases in aboriginal inhabitants of the Arctic in conditions of reduced insolation and malnutrition.

This work was supported by Russian Science Foundation No 22-25-20095, https://rscf.ru/en/project/22-25-20095/.

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THE RELATIONSHIP OF LIPID PROFILE AND BLOOD PRESSURE IN MEN IN THE EUROPEAN NORTH OF RUSSIA

DOI 10.25789/YMJ.2023.81.24

УДК: 612.12-008.331/153.915-055.1(1-922)

The paper analyzes the relationship of traditional lipid indicators and lipid indices with the level of blood pressure in men living in the European North of Russia. In apparently healthy middle-aged men, normal high blood pressure, abdominal pre-obesity, and compliance of traditional lipid indicators with reference values were revealed. At the same time, a number of lipid indices (atherogenicity coefficient (AC), the ratio of total cholesterol (TC) to high-density lipoprotein cholesterol (HDL-C), atherogenic plasma index (AIP), triglyceride/HDL-C ratio and lipid accumulation index (LAP)) appeared to be elevated.

Correlation analysis revealed the linear relationship of systolic pressure in the brachial artery with the content of Apo A-1, Apo B, TC, low-density lipoprotein cholesterol (LDL-C) and cardiovascular risk (CVR); diastolic pressure with TC, CVR and the waist-hip circumference ratio; heart rate index with waist and hip circumferences and body mass index. More number and greater strength of significant correlations were found between indicators of central hemodynamics and lipid profile. The relationships between age and length of living in the North with peripheral and central hemodynamic parameters turned out to be obvius.

Thus, male northern residents with normal high blood pressure have abdominal pre-obesity, normolipidemia but elevated lipid indices. Apparently, lipid indices are of greater prognostic significance and may be more sensitive predictors of the risk of arterial hypertension at the normal level of traditional lipid profile indicators.

Keywords: blood lipids, lipid indices, peripheral and central pressure, men, Arctic.

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More number and greater strength of significant correlations were found between indicators of central hemodynamics and lipid profile.

The interrelations between lipid parameters and pressure level were revealed: a) positive correlation of AC with peripheral systolic and diastolic pressure, systolic pressure in the aorta, diastolic and pulse pressure, b) TC-non-HDL with systolic and diastolic pressure in the aorta, c) negative correlation of LAP with peripheral pulse pressure.

These correlations indicate that in Arctic conditions, the presence of normolipidemia and pre-obesity may be a prerequisite for the formation of normal high pressure.

Keywords: blood lipids, lipid indices, peripheral and central pressure, men, Arctic.

Introduction. In the pathogenesis of cardiovascular diseases (CVD), an important role is assigned to lipid metabolism disorders, which are determined by the content of traditional lipid indicators in the blood, such as total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C). At the same time, LDL-C is considered as the main predictor of an unfavorable prognosis of atherosclerosis and pathogenetically related CVD [14, 17], while an increased content of HDL-C in the blood is associated with a low degree of cardiovascular risk (CVR) [20]. However, it turned out that a significant increase in HDL-C is not always a protective factor [7]. It is suggested that the functional activity of HDL-C is more determined by the concentration of HC in individual HDL-C subfractions than the level of HC contained in HDL-C.

To more accurately clarify the role of lipid metabolism in the development of atherosclerosis and related diseases, their derivatives, the so-called lipid indices or combined lipid parameters (in the English literature), have increasingly been used along with traditional indicators of lipid metabolism. Most of the known indices reflect the ratio of the main classes of lipids in blood plasma, the relationship within lipid metabolism, the ratio of pro- and anti-atherogenic potential of blood [4]. It is believed that lipid indices represent the greatest strength in predicting CVD, while LDL-C may show a weak connection with the pulse wave velocity or not at all [10, 13, 18, 22].

In atherosclerosis, lipid accumulation in the intima of the arteries modulates vascular stiffness. There are isolated data on the relationship of lipids with vascular stiffness in residents of the North. In particular, it was shown that in young men, residents of Finland, the elasticity of the aorta was not associated with stan-

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dard lipid parameters [16]. At the same time, the ratio of HDL-C/TC did not reveal an association with aortic elasticity, but showed a decrease in the extensibility of the carotid artery in individuals with a low ratio of HDL-C/TC. In another study in patients with arterial hypertension (AH), residents of Nadym, risk factors such as hypercholesterolemia, body mass index (BMI) and smoking did not affect the viscoelastic properties of the common carotid artery wall [3].

Despite the fact that arterial stiffness is a common indicator of AH [19, 21], there is no convincing data on the relationship of lipid parameters and indices with AH.

The results of studies of the lipid profile in residents of the Arctic regions and the facts of early and frequent development of CVD [1, 2, 8], dictate the need to study lipid indices and assess their relationship with the level of blood pressure in Northerners.

Objective: to study the relationship of traditional lipid indicators and lipid indices with arterial hypertension in men in the European North of Russia.

Materials and methods. The study was conducted in the Murmansk region (67´ N) in the autumn-winter period. Men working at a mining and processing plant were examined (n=31). The average calendar age was 42.68±2.44 years, the northern experience was 19.69±2.40 years, the total work experience was 22.38±2.63 years.

Inclusion criteria: no complaints at the time of the study, average age, written consent to participate in the examination and blood donation for biochemical tests.

Exclusion criteria: exacerbation of chronic diseases, presence of familial hypercholesterolemia, oncological, infectious diseases, diabetes mellitus, chronic heart failure, aortic aneurysm, heart and aortic valve diseases, myocardial infarction and stroke in the anamnesis.

Blood sampling was carried out in the BD Vacutainer from the ulnar vein in the morning hours after a night of fasting. After blood centrifugation (3000 rpm, 10 min), plasma was transferred to Eppendorf tubes, frozen and stored at a temperature of –70°C.

Biochemical research. After a single plasma defrosting, the content of TC and TG was determined using Thermo Fisher Scientific kits (USA). To determine LDL-C, HDL-C, apolipoprotein A1 (Apo A1) and B (Apo B) kits from DiaSys (Germany) were used. The lipid content was measured using an AU 480 Beckman Coulter automatic biochemical analyzer (USA).

The atherogenicity coefficient (AC) = (TC – HDL-C)/HDL-C and other lipid indices were calculated using the formulas: Apo B/Apo A-1 ratio; atherogenic plasma index (AIP) = (logTG/HDL-C); atherogenic index (ATH) = ((TC – HDL-C) x Apo B)/(HDL-C x Apo A1); TC-non-HDL (non–HDL) = TC – HDL-C; Castelli index 1 = TC / HDL-C; Castelli index 2 = LDL-C/HDL-C [4]; TG/HDL-C; accumulation index lipids (LAP) = (waist circumference – 65) x TG [5].

The assessment of the total risk of fatal events from CVD, cardiovascular risk (CVR) over the next 10 years was carried out on the SCORE scale (ARSSC, 2011).

Physiological studies. After resting for 15 min in a sitting position, systolic, diastolic and pulse pressure on the brachial artery (bSBP, bDBP, bPP) were measured twice with an interval of 2 min using an automatic cuff oscilloscope device (MT-4, MediTech, USA), heart rate (HR). The parameters of central (aortic) hemodynamics (aSBP, aDBP, aPP) were determined by applanation tonometry using the SphygmoCor apparatus (Atcor Medical, Australia) according to the description [11].

Anthropometric studies. Height (m) and body weight (kg), waist circumference (WC, cm) and hips (HC, cm) were measured by well-known methods, body mass index (BMI) was calculated.

This study approved by the Bioethical committee of FRC FTM was performed in accordance with the requirements of "Ethical principles for medical research involving human subjects" (the Helsinki Declaration of the World Medical Association 1964, as amended in 2013) and "Rules for clinical practice in the Russian Federation" (Order of the Ministry of Health of the Russian Federation № 266 of 19.06.2003).

Statistical processing was carried out using the Statistica v. 10 application software package (Stat Soft Inc., USA). Depending on the normality of the distribution of features in the sample, calculated by the Kolmogorov-Smirnov method, nonparametric analysis methods were used to determine the median (Me), lower, upper quartile (Q25; Q75) and arithmetic mean (M), mean error (m). Correlation analysis was performed by Spearman (*r, p*).

Results and discussion. Men had an excess BMI (27.47 (24.45; 29.94) kg/m^2), which indicates the presence of excess body weight. The measurement results WC (95.00 (89.00; 102.00) cm), HC (103.00 (99.00; 108.00) cm) and their ratio (0.91 (0.87; 0.97)) indicate an android type of adipose tissue distribution, which suggests the presence of an abdominal form of pre-obesity. At the same time, the content of individual lipids (TG – 1.14 (0.82; 2.23) mmol/L; HC – 4.96 (3.98; 5.44) mmol/L; HDL – 1.04 (0.75; 1.35) mmol/L; LDL – 2.63 (2.10; 2.98) mmol/L) and apolipoproteins (Apo A1 – 140.08 (124.52; 160.36) mg/dl; Apo B – 45.16 (36.04; 52.06) mg/dl) corresponded to reference values, indicating normolipidemia. Nevertheless, the results of anthropometry suggest an increased risk of

Relationship of peripheral and central hemodynamic parameters with lipid profile, anthropometric indicators and cardiovascular risk of men, residents of the European North of Russia (r; p)

Parameter	TC	HDL-C	LDL-C	Apo A-1	Apo B	WC	HC	WC/HC	BMI	CVR
bSBP	-	0.43; 0.030	0.40; 0.043	0.43; 0.030	0.42; 0.031	-	-	-	-	0.57; 0.003
bDBP	0.46; 0.019	-	-	-	-	-	-	0.45; 0.017	-	0.52; 0.008
HR	-	-	-	-	-	0.48; 0.007	0.48; 0.007	-	0.43; 0.015	-
aSBP	0.67; 0.0001	-	0.53; 0.003	0.53; 0.003	0.58; 0.001	-	-	-	-	0.74; 0.0001
aDBP	0.59; 0.001	-	0.51; 0.006	0.41; 0.031	0.51; 0.006	0.46; 0.010	-	0.60; 0.001	-	0.65; 0.0003
aPP	0.44; 0.016	0.45; 0.015	0.37; 0.050	0.44; 0.018	0.39; 0.039	-	-	-	-	0.58; 0.001

Note: bSBP, bDBP – systolic, diastolic blood pressure on the brachial artery; HR – heart rate; aSBP, aDBP, aPP – systolic, diastolic, pulse aortic pressure; TC – total cholesterol; HDL-C – high-density lipoprotein cholesterol; LDL-C – low-density lipoprotein cholesterol; WC – waist circumference; HC – hip circumference; BMI – body mass index; CVR – cardiovascular risk

CVD, and the CVR on the SCORE scale (3.36±0.84) predicts a moderate risk. According to the Recommendations of the ARSSC (2008), the indicators of bSBP (134.5 (122.25; 140.00) and bDBP (88.00 (81.25; 90.00) mmHg. art. in the Northerners correspond to high normal pressure. The bPP value was 47.50 (40.00; 50.00) mmHg, HR – 77.00 (69.00; 83.00) beats/min.

9 lipid indices were evaluated, derivatives of traditional lipid indicators reflecting the ratio of atherogenic and anti-atherogenic lipid profile in Northerners. 5 of them were elevated: AC (3.70 (2.45; 5.25)) above 3.0, Castelli index 1 (4.68 (3.43; 6.26)) above 4.0; AIP (0.17 (-0.19; 0.36)) above 0.1; TG/HDL-C ratio (1.49 (0.66; 2.29)) above 0.9 and LAP (36.34 (24.59; 61.02)) is greater than 21.2 relative to the data of other authors [4, 5, 12] obtained in healthy individuals. Other indexes: Apo B/Apo A-1 ratio (0,32 (0,26; 0,40)), ATH (1.16 (0.59; 2.10)), non-HDL (3.77 (3.03; 4.44)) and the Castelli index 2 (2.57 (1.78; 3.29)) did not differ from the data of other authors [4, 9, 15].

There is evidence that in healthy men, residents of Arkhangelsk aged 20 to 59 years, the LAP was 17.8 (9.1; 30.5), in the age group of 40–49 years, the indicator increased to 21.2 (12.3; 33.2) [5], nevertheless, this is significantly lower than in the group of Northerners surveyed by us. The lower values of the LAP index in Archangelgorodians can be explained by lower anthropometric indicators: WC 81.0 (75.0; 87.0) cm, BMI 24.4 (22.1; 26.3) kg/m^2. In Japanese men (n=1720, ages 25 and 55, WC 81.8 cm, BMI 23 kg/m2), LAP did not exceed 21.2, the TG/HDL-C index – 0.9 [12].

Thus, in Northerners with normolipidemia, the revealed high lipid indices may be associated with an increased risk of atherosclerosis and CVD. Since AC reflects the ratio of atherogenic lipoproteins to anti-atherogenic cholesterol, whereas the TC/HDL-C index is the ratio of TC to anti-atherogenic lipoproteins, AC can more accurately predict the risk of developing CVD. Moreover, it has already been shown that the progression of AH and changes in the geometric parameters of the left ventricle were more closely associated with AC than with individual lipids [6].

Indicators of peripheral hemodynamics correlated with indicators of lipid profile and anthropometry. A positive correlation of average strength was noted: bSBP with Apo A-1, Apo B, TC, LDL-C and CVR; bDBP – with TC, CVR and the ratio of WC/HC; HR – with OT, OB and BMI, which reflects the relationship of the pressure level with lipid metabolism, while HR – with anthropometry data (table). These connections are aimed at the possibility of regulating hemodynamics in Northerners by controlling lipid metabolism and abdominal pre-obesity.

A significantly greater number of correlations and greater strength were found between the indicators of central hemodynamics (aSBP 118.00 (106.00; 129.00), aDBP (88.00 (81.00; 92.00), aPP (30.00 (24.00; 34.00) mmHg) and lipid profile. Positive associations were noted: aSBP with Apo A-1, Apo B, TC, LDL-C, CVR; aDBP – with Apo A-1, Apo B, TC, LDL-C, CVR, WC; WC/HC; aPP – with Apo A-1, Apo B, TC, HDL-C, LDL-C, CVR.

Significant positive associations of the age of Northerners with bSBP (r=0.54; p=0.003), bDBP (r=0.48; p=0.010), aSBP (r=0.71; p=0.0001), aDBP (r=0.57; p=0.001), aPP (r=0.60; p=0.0003) and polar experience with bSBP (r=0.51; p=0.006), bDBP (r=0.45; p=0.017), aSBP (r=0.69; p=0.0002), aDBP (r=0.56; p=0.001), aPP (r=0.59; p=0.001).

Correlations between lipid indices and the level of pressure in men deserve attention. In particular, a) positive correlation of AC with indicators of bSBP (0.44; 0.025), bDBP (0.44; 0.023), aSBP (0.62; 0.0003), aDBP (0.56; 0.002), aPP (0.42; 0.022); b) non-HDL with aSBP (r=0.49; p=0.007), aDBP (r=0.48; p=0.010) and c) negative correlation of LAP with aPP (r=-0.51; p=0.015). These correlations indicate that in Arctic conditions, the presence of normolipidemia and pre-obesity may be a prerequisite for the formation of normal high pressure, which, in turn, is realized by the interaction of lipid indices with peripheral and central hemodynamics.

Conclusion. The results of this study showed that in men, residents of the European North of Russia, who have normal high blood pressure, abdominal pre-obesity with normolipidemia, moderate CVR on the SCORE scale, elevated lipid indices – AC, Castelli index 1, AIP, TG/HDL-C ratio and, especially, LAP were found relative to similar indicators of other researchers. Traditional lipid parameters are more closely related to the parameters of central hemodynamics than peripheral, which should be taken into account when developing diagnostic and preventive measures. The determination of lipid indices can increase the detection of latent lipid metabolism disorders even at the normal level of traditional lipid profile indicators in the blood, i.e., lipid indices may be more sensitive predictors of AH risk. The results obtained by us dictate the need for additional studies involving a larger number of subjects among residents of the Arctic region to determine the reference values of lipid indices.

The study was carried out with the partial support of the Presidium of the Russian Academy of Sciences under the basic research Program "Exploratory fundamental scientific research in the interests of the development of the Arctic zone of the Russian Federation".

We used the equipment of Core Facility "Modern Optical Systems" FRC FTM.

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SCIENTIFIC REVIEWS AND LECTURES

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THE ROLE OF COVID-19 IN MODIFIED COGNITIVE FUNCTIONING

DOI 10.25789/YMJ.2023.81.25

УДК 616.517:577.12(048)

SARS-CoV-2 virus impairs cognitive functions during illness and in long-term periods: from 3 months (in 44% of patients) to one year (in 16.2% - 63% of patients) after recovery. Cognitive deficits are more common in patients with severe COVID-19, especially those treated in the intensive care unit, and with infection duration of more than 28 days. Such consequences are associated with direct impact of SARS-CoV-2 on the functioning of brain neurons and changes mediated by endothelial dysfunction due to impaired blood supply to the cerebral cortex. The long-term results of the viral effect on brain neurons are due to immune responses to the virus multiplying in cells and to changes in the epigenetic regulation of gene expression. The immune response leads to inflammation, which is expressed in the form of encephalitis, encephalopathy, anosmia, hypogeusia and is reflected in the development of cognitive deficit. Epigenetic changes are mediated by virus-induced activation of retroelements that have cis- and trans-effects on genes involved in neurogenesis. SARS-CoV-2 promotes the expression of miRNAs that silence the expression of many genes, thus impairing cognitive functioning. The mechanism of these changes is associated with the effect of the virus on retroelements, which are the sources of miRNAs. Reverse transcriptase and endonuclease of retroelements may be involved in the integration of SARS-CoV-2 into the human genome, which may also affect the change in the expression of genes necessary for cognitive development.

Keywords: cognitive functions; microRNA; retroelements; COVID-19.

Introduction. The COVID-19 pandemic remains an urgent problem for all mankind both due to a high mortality rate and due to the consequences, which are developed after recovery. Since individual cognitive functions (CF) inherently determine the ability of complete functioning, professional skills and self-care, the question on the impact of COVID-19 on cognitive functioning remains relevant. Indeed, it is impossible to imagine individual's life without CF [24, 35], that can be adversely affected by the virus. Back in 2020, the analysis of 214 hospitalized patients with COVID-19 in Wuhan, China, showed the development of neurological impairments in 41.1% of patients [34]. Modified cognitive functioning in patients after recovery remains highly relevant [47]. COVID-19 also accelerates neurodegenerative processes in the elderly [2].

The most pronounced cognitive impairments are detected in severe patients who require intensive care (IT) during hospitalization [21]. Thus, the study of 92 COVID-19 patients, who required IT therapy, were characterized by pathological cognitive changes in 44% of cases 3 months after recovery [47]. The duration of infection plays an important role. In the study of 3,762 patients from 56 countries with a confirmed diagnosis of COVID-19, which duration was more than 28 days, cognitive dysfunction and memory problems were identified in all age groups in 88% of patients [11]. Nevertheless, another study of 81,337 patients after COVID-19 recovery demonstrated that cognitive deficit developed even in individuals with asymptomatic course of the disease compared with the control group [19].

Indicators of the frequency of cognitive impairments observed after COVID-19 recovery differ in the studies from various countries. However, significantly en-

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hanced frequency of cognitive changes in infected patients compared to control groups was characteristic for all of them. Longitudinal studies of cognitive functions in 452 patients in the Netherlands indicate the development of psychiatric symptoms in 26.2% and cognitive decline in 16.2% of individuals one year after IT for COVID-19 treatment [21]. In Spain, the assessment of cognitive functions carried out one year after COVID-19 hospitalization revealed the presence of neurocognitive dysfunction in 46.8% and mental illness in 45% of cases [38]. In Italy, cognitive deficits were detected in 13.5% of patients 4 months after IT for COVID-19 treatment and only in 1.2% of patients with mild to moderate illness without hospitalization [36]. The study of 92 COVID-19 patients in Mexico reported the presence of cognitive impairments in 54.4% of patients after 6 months [16]. In the USA, the analysis of cognitive changes conducted in 156 patients revealed at least mild cognitive impairment according to Neuro-Qol in 63% of patients 351 days after COVID-19 recovery [52]. These long-term effects are due to the direct and indirect effects of the virus on brain neurons.

Direct effect of SARS-CoV-2 on cognitive functioning. A direct effect of SARS-CoV-2 on the central nervous system was evidenced in empirical research, which demonstarted that neuronal stem cells (NSCs) were sensitive to the penetration of the virus. Extensive expression of infectious SARS-CoV-2 particles and their proteins was detected in the neurospheres and brain organoids including the cerebral cortex and NSC [56]. It was proved that the virus penetrated into the central nervous system through the olfactory mucosa, and subsequently via thin olfactory sensitive nerve fibers into the brain. The SARS-CoV-2 virus has a tropism to neurons and is distributed to certain neuroanatomic regions, including the respiratory and cardiovascular centers in the medulla oblongata, where it actively multiplies and indirectly causes vascular damage. Morphological post-mortem examination of COVID-19 individuals showed the presence of foci of acute ischemic strokes due to thromboembolism, which regions were characterize by increased levels of immune reactivity to S protein of the SARS-Cov-2 [37]. SARS-CoV-2 induces inflammatory processes in CNS regions responsible for memory, learning and emotional responses due to damage to neurotransmission and neurogenesis [27].

The immune response to the multiplying virus in neurons causes inflammatory reactions in the brain, which is reflected by the development of specific clinical manifestations. The retrospective multicenter analysis of 232 COVID-19 patients in Spain reported that the onset of neurological symptoms, on average, was determined on the 8th day from the infection onset. The development of encephalopathy or encephalitis was observed in 21.9% of the examined patients, while 7.8% had brain lesions diagnosed with MRI and 61.9% - with EEG [1]. Meta-analysis conducted on 3,868 patients confirmed the development of delirium in 27% of COVID-19 patients [45]. A possible consequence of a direct effect of SARS-CoV-2 on cognitive functioning is anosmia detected in 44% of infected patients and hypogeusia (decreased taste sense) - in 43% [9]. The study of 514,459 patients with a positive test for SARS-CoV-2 examined using 6 National digital surveillance platforms revealed the presence of anosmia/ageusia in 43% of COVID-19 patients in the USA, 29% - in the UK, and 14% - in Israel, which was significantly higher compared to individuals with negative PCR tests [51]. A direct effect of SARS-CoV-2 on CNS neurons with a violation of the blood-brain barrier can be confirmed by the detected viral antigens in the cerebrospinal fluid (CSF) of COVID-19 patients [3]. In experiments with mice infected with SARS-CoV-2, a selective microglial reactivation of the white matter of the brain was revealed. Similar changes have been identified in the post-mortem brain tissues of COVID-19 patients. Within 7 weeks after murine infection, an enhanced level of proinflammatory cytokines/chemokines. It was accompanied by suppression of neurogenesis in the hippocampus, a decreased number of oligodendrocytes and loss of myelin in the subcortical white matter [14].

Indirect effect of COVID-19 on cognitive impairment. Impaired cognitive functioning during infection and in the long-term periods after recovery may be due to hypoxia caused by damage of lung tissue. This is evidenced by data on more frequent occurrence of cognitive deficits in severe COVID-19 patients [21, 47], and in those with prolonged course of the disease [11], since they are accompanied by intense oxygen starvation of the brain [16]. The situation is aggravated by concomitant damage of the CNS due to inflammation and endothelial dysfunction. The study based on 749 COVID-19 patients demonstrated an impaired signal intensity in MRI of the cerebral cortex in 37% of patients with neurological symptoms [23]. Long-term impairments (after 6 months) of cognitive functions have been directly related to the level of hypoxemia during COVID-19 [16].

A cause of neurological diseases may be atherosclerosis and endothelial cells (EC) dysfunction. A flow–mediated dilation (FMD) represents one of the indicators of a dysfunction. Impaired FMD is observed with worsened cognitive functioning, especially with respect to attention, executive functions, and memory. Recovering COVID-19 patients are characterized by endothelial dysfunction with a direct correlation between the severity of lung and vascular lesions, which may play an important role in modified cognitive functioning in patient [39]. A retrospective analysis of global data on the COVID-19 consequences demonstrated the development of ischemic stroke in 1.3% of 8,163 infected individuals compared with 1% among 19,513 patients without COVID-19. This indicates a slight but significant increase in the risk of stroke caused by SARS-CoV-2 [46]. The study of 21,483 COVID-19 adult patients, who took a therapy in 107 hospitals in the USA, 0.2% of them reported spontaneous intracranial hemorrhages [30].

Endothelial dysfunction is caused by a direct effect of SARS-CoV-2 on EC, which abundantly express angiotensin-converting enzyme 2 (ACE2). The ACE2 protein is a receptor for the virus, facilitating its penetration into the cells and resulting in the activation of immune response that causes a cascade of coagulation and subsequent vasculopathy [8]. Impaired EC and coagulopathy are related to the inflammatory processes in the brain, as evidenced by the detection of antibodies against SARS-CoV-2 in the CSF of COVID-19 patients [3] in 77% of the studied cases [15]. Activated macrophages, which initiate inflammation through the TLR4-MyD88 signaling pathways, are detected in the brain of patients. As a result, high levels of IL-6, IL-18, CC-chemokine ligand 2 (CCL2), and a soluble cell adhesion molecule (sICAM-1) are detected in CSF of patients [37]. The most expressed increase in proinflammatory cytokines IL-6, IL-10, ferritin and D-dimer in CSF was determined in COVID-19 patients with strokes, which is comparable to similar indicators in post-stroke patients without COVID-19 [15].

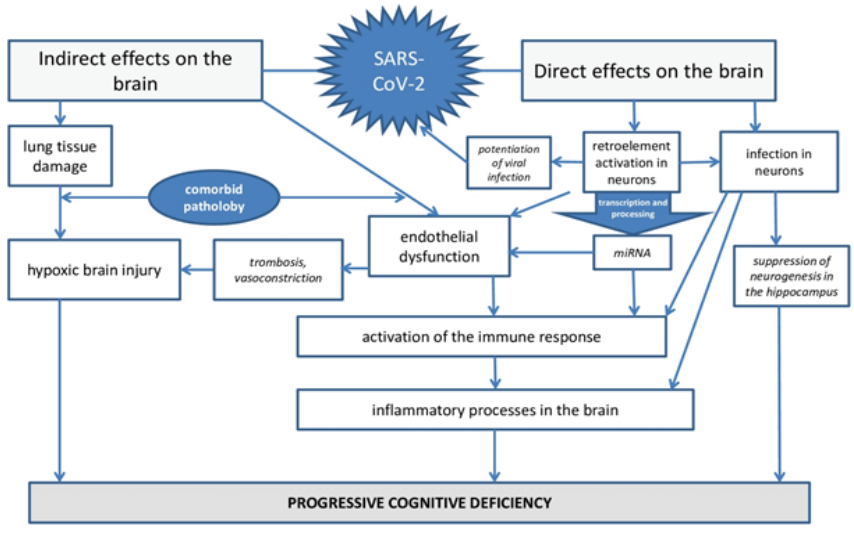
The impact of COVID-19 on CF related to EC dysfunction with long-term consequences can be compare with the progressive dementia in aging population, an important role in it is played by microRNAs such as miR-128, miR-132, miR-134, miR-222, miR-323-3p, miR-382, miR-409-3p, miR-451a, miR-486-5p,

miR-502-3p, and miR-874 [57]. Dynamic changes in microRNA levels regulate the expression of genes involved in CF such as learning and memory [41, 55]. The microRNAs affecting the genes responsible for brain functioning are involved in the pathogenesis of vascular dementia. Thus, the target for miR-124 (inhibits the formation of Aß) is the *BACE1* gene; miR-126 (improves vascular function) – the *MMP-9* gene; miR-132 (protects against chronic cerebral hypoperfusion) – the *Nav1.1,* and *Nav1.2*genes; miR-134-5p (promotes damage to cortical neurons) – the *Snap25* gene; miR-195 – the *APP, BACE1* genes; miR-153 (contributes to the abnormal synaptic plasticity) – the *Snap25, Vamp2, Stx1a,* and *Syt1* genes; miR-181c (enhances cellular adaptation during prolonged ischemia), miR-210-5p (reduces the number of synapses) – the *Snap25* gene; miR-26b (suppresses the inflammatory reaction of microglia) – the *IL6* gene; miR-501-3p (aggravates damage of the blood-brain barrier) – the *ZO-1* gene; miR-9 (induces impaired cognitive functioning) – the *Nav1.1, Nav1.2, BACE1* genes; miR-93 (enhances inflammatory reactions) – the *TLR* gene; miR-96 (inhibits autophagy) – the *mTOR* gene [57]. The most studied microRNAs associated with cognitive functions is the cluster of miR-132/212, which are actively expressed in neurons and localized in synaptodendritic fractions. Their hippocampal levels are significantly increased as a result of cognitive training. The miR-134 suppresses the formation of dendrite spikes due to *Limk1, Creb and Bdnf* silencing. In addition, miR-34a negatively affects dendrites growth and branching, weakening a synaptic plasticity of neurons. The levels of miR-34a and miR-128b in the basolateral amygdala are increased with fear, while miR-34a suppression by microRNA sponges reduces fear memory [55]. The microRNAs miR-140-5p, miR-197-3p and miR-501-3p can be used as biomarkers of cognitive aging [18]. It can be assumed that miRNAs play a role in the development of cognitive disorders in patients after COVID-19 due to the activation of retroelements (REs) caused by SARS-CoV-2, since REs are the most important sources of human microRNAs [31, 54].

The role of retroelements in developing COVID-19 cognitive consequences. A direct effect of SARS-CoV-2 on impaired cognitive functioning is caused by both a direct infectious process in neurons and immune-inflammatory reactions, and the impact on the expression of specific genes involved in CF. It is assumed that this observation is attributed to the activation of mobile genetic elements (TE – transposable elements), which are divided into DNA transposons and REs according to the mechanism of their translocation in the genome. More than 40% of the human genome consists of REs, including 8% are endogenous retroviruses (ERV), which contain LTR (long terminal repeats) [42]. About 33% of human genome consists of non-LTR-containing REs: autonomous LINE1 (L1) and non-autonomous SINE [29]. In the evolution of primates, several LINE retrotranspositions and the formation of novel REs related to SINE and SVA (SINE/VNTR/Alu) occurred, which significantly affected brain development [32]. This process can explain a pronounced activity of L1 in the regions of neurogenesis in the human hippocampus. Somatic transpositions of L1 have a programmed effect on the expression of specific neuronal genes, thus forming unique transcriptomes of individual neurons for the development of cognitive abilities [40]. Therefore, somatic retrotranspositions of L1 are the sources of genetic mosaicism and potential phenotypic diversity of neurons in brain development. The expression of L1 in adults may be due to various environmental influences, affecting neuronal stem cells differentiation. Murine experiments demonstrated the role of L1 expression in the formation of long-term memory, which indicates the importance of REs in cognitive functioning [5]. COVID-19 causes a significant impairment in neurogenesis in the hippocampus [27], which may be attributed to a pathological activation of RE due to the viral exposure [5].

The most common SINE elements in the human genome are Alu (comprise about 11% of all DNA), which require L1 enzymes for their own transpositions. It is assumed that Alu contributed to the formation of cognitive functions in humans, since they play a crucial role in the development of connections between the neurons and in epigenetic regulation of biochemical processes in the brain. However, the involvement of REs in the management of gene expression in the CNS is a subtle and evolutionarily programmed species-specific process. In this regard, specific deviations from it due to pathological activation of REs and nonspecific transposition can cause serious consequences. Thus, non-programmed Alu translocations have been described as the causes of a large number of neurodegenerative diseases [29]. In murine experiments, ERV activation in the CNS resulted in hippocampal-related memory impairment and cognitive deficits [48].

Within the evolution, REs were the sources of various protein-encoding genes (molecular domestication). A comparative genomic and functional analysis has shown the origin of many human genes from ERVs. These genes are involved in development of placenta and immune responses, and in the regulation of cognitive functioning. They include the *Zcchc16, Arc, Mart4,* and *Sirh11* [42]. REs affect cognitive development in several ways. Firstly, the programmed somatic activation of REs in neuronal stem cells determines the specificity of differentiation and subsequent functioning of mature cells. Secondly, genes, which originated from Res in the evolution, are involved in regulatory pathways controlled by various REs. In this regard, pathological activation of REs due to exposure to SARS-CoV-2, can result in



The scheme of mechanisms of COVID-19 effect on modified cognitive functioning

cognitive changes. In particular, in mice, the *Sirh11/Zcchc16* (11/Zinc finger CCHC domain-containing 16) gene, which is a homologueof Sushi-ichi-related RE, is responsible for regulation of cognitive functions including attention, impulsivity and working memory [22].

The Arc protein (encoded by the *IEG* gene – immediate-early gene), which mRNA is specifically located in the synaptic region, thus regulating synaptic plasticity and memory formation, also occurred in evolution through the "domestication" of RE genes [10]. Enhanced Arc expression in hippocampal neurons *in vitro* was shown to increase the number of dendrite spikes, while suppression of Arc synthesis *in vivo* reduced the density of hippocampal neuronal spikes in mice [43]. This protein is required for spatial learning, object recognition, contextual tasks for inhibited avoidance, taste aversion, fear formation, memory reconsolidation, reactions to visual experience and deprivation, network excitability, Alzheimer's and Angelman's diseases, fragile X chromosome syndrome [28]. In addition, a reduced Arc transcription was observed in the neurogenesis region in the hippocampus during aging (in rat models) [44]. The role of mutations in the Arc-encoding gene in the development of autism and schizophrenia has been described. At the same time, an inducing influence of exogenous viral infections could not be excluded, since Arc preserves the properties of exogenous viruses, which are used for information transfer between the neurons and innervated organs. In particular, experiments with drosophila revealed that Arc forms structures similar to the viral capsid, which pack mRNA in the neurons of the brain. Formed structures are loaded into extracellular vesicles, which are transmitted from the motor neurons to the muscles. 3'-untranslated region of the gene contains RE-like sequences required for the loading of capsid structures into vesicles [4]. The mammalian *PEG10* gene, homologous to ERV *gag*, has similar properties. Its encoded protein binds to its mRNA for its secretion into vesicles [49].

Viruses modulate the activity of REs, which affects the expression of the downstream genes of the hosts. The analysis of ChiP-Seq data revealed a differential expression of REs located in the transcription factors binding sites, which regulate the expression of genes involved in the immune response in COVID-19. Enhanced levels of 52 HERV and 40 LINE1 was detected on cell lines infected with SARS-CoV-2 [35]. Since HERVs can be activated in response to infectious agents, causing the development of various immune pathological effects, the analysis of HERV changes in 17 COVID-19 patients was performed. HERV-W was highly expressed in patients infected with SARS-CoV-2 compared to healthy controls. HERV-W levels correlated with the markers of T-lymphocyte differentiation and cytokine levels in the blood (IL-6, IL-17, TNF-α, CCL-2, and CXCL6). The percentage of HERV-W ENV-positive lymphocytes correlated with inflammatory markers and severity of pneumonia in COVID-19 patients, as well as with poor outcomes of hospitalized patients [6]. *In vitro* study demonstrated an increased expression of the HERV-W envelope protein as a result of the introduction of the SARS-CoV-2 spike protein into the leukocyte culture [17]. Comparative analysis of the transcriptome of bronchoalveolar lavage and peripheral blood monocytes of COVID-19 patients and healthy subjects indicated a significant increase in the levels of HERV transcripts in bronchoalveolar fluid in SARS-CoV-2 infected individuals, to a greater extent in elderly [26]. The study of children with COVID-19 revealed a positive correlation between the expression of HERV and *IFN-I, IFN-II, TRIM28, SETDB1* genes, which products are involved in immune responses to the virus [53].

Together with the effect of SARS-CoV-2 on the changes in the expression of REs, it is assumed that the TEs themselves play an important role in modulating COVID-19 infection, since HERVs are involved in the regulation of the immune system and can participate in the mechanisms of infection and viral penetration into the cells. In addition, HERV synthesizes proteins that complement the viral set of ORFs during penetration, infection, replication, packaging and integration of SARS-CoV-2 into the human genome. The products of HERV expression can also modulate the initiation of translation on the ribosome by changing ORFs pattern of SARS-CoV-2 in different cells, which affects the severity of infection [12]. REs can mediate EC dysfunction in COVID-19, since translocation of activated L1 into the novel genomic loci results in reduced proliferation and migration of EC via selective influence on such angiogenic factors as Tie-2 (protein kinase receptor) and VEGF [7].

The phenomenon of long-term detection of SARS-CoV-2 RNA in patients after COVID-19 recovery, made it possible to suggest the integration of viral cDNA into the host genome, which was confirmed on human cell cultures. Duplications of the target site flanking the viral sequence were found together with consensus sequences of recognition by endonuclease L1 in integration sites. The data obtained indicate the mechanism of reverse transcription and retroposition of SARS-CoV-2 via L1. An additional confirmation was the data on the detection of viral sequences in the tissues of COVID-19 patients, which transcribed from integrated DNA copies of SARS-CoV-2, creating chimeric transcripts of the virus and the host [58]. Thus, the role of REs in COVID-19-caused cognitive decline is related to the possible activation of Res by SARS-CoV-2, which aggravate the clinical course of the disease by changing the expression of specific genes responsible for cognitive functioning. Moreover, individual specificty of HERV affect the COVID-19 course by modulating the immune response and viral transcription, while RE-encoded proteins can participate in the integration of SARS-CoV-2 into the human genome, which affects the long-term consequences of infection including the deterioration of brain functioning.

Conclusion. SARS-CoV-2 causes cognitive impairment both by direct impact of the virus on brain neurons and by endothelial dysfunction (Fig. 1). The study of the mechanisms of COVID-19 influence on modified cognitive functioning can become the basis for effective therapy of impaired functioning. Interactive cognitive-motor training can be one of the ways [2]. It is also proposed to use REs and evolutionary derived genes, such as *PEG10*, capable to form virus-like particles exported to extracellular vesicles, for targeted therapy of cognitive impairment caused by exposure to SARS-CoV-2 [49]. This approach is especially relevant due to the role of REs as effectors of changed viral-related brain functioning, and the important role of REs as microRNAs sources [31, 54]. The latter, like the products of *PEG10* [49] and *IEG* [4] genes expression, are exported into extracellular vesicles from neurons. The results of clinical studies of elderly individuals over 65 years indicated that reduced stress could improve cognitive functioning by increasing miR-29 expression (with its immersion in vesicles) and suppressing the production of DNA methyltransferases DNMT3A/3B in neurons [20]. The analysis of the role of specific microRNAs in restoration of cognitive functions can become the basis for both targeted therapy and predicting the significance of certain approaches in patients' therapy. In particular, a favorable role of physical exercises in improved cognitive functioning was reported via

modulating the expression of miR-146a, miR-21, miR-223 in humans [13]. Restoration of CF is proposed to be performed with miR-218, which regulates contextual and spatial memory due to the activating effect on the complement component C3 gene necessary for presynaptic cognitive functioning in the hippocampus [33]. REs and microRNAs can be used to develop novel approaches for COVID-19 treatment. Information analysis revealed 21 human microRNAs homologous to the SARS-CoV-2 genome and capable of inhibition of transmission and replication of viral RNAs. Of these, the most effective were miR-1296, miR-3202, miR-4476, miR-548-1d, miR-651 [50].

The study was supported by the State Contract of the Ministry of Science and Education of RF (№AAAA-A16-116020350032-1) with partial support of the Ministry of Science and Higher Education of the Republic of Bashkortostan and the Russian Science Foundation (project no. 17-78-30028).

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THE ROLE OF *FADS* GENE POLYMORPHIC VARIANTS IN ADAPTATION TO THE NORTHERN CLIMATE AND METABOLIC DISORDERS

DOI 10.25789/YMJ.2023.81.26

УДК 616-01/09; 575.875

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The review summarizes the studies of the role of the *FADS* gene in the metabolism of polyunsaturated fatty acids, as one of the mechanisms of human adaptation to the environmental conditions, in particular, a cold climate. A comparative analysis of the distribution of the most significant for circumpolar ethnic groups polymorphic variants rs7115739, rs174570 of the *FADS* 2-3 genes in various ethnic groups, including the Inuits and Yakuts, was carried out. The results of studies of the *FADS* polymorphic markers effect on lipid metabolism, the risk of cardiovascular diseases and type 2 diabetes mellitus in different world populations are systematized.

Keywords: *FADS* genes, desaturases, polyunsaturated fatty acids, rs7115739, rs174570, metabolism, adaptation, cold, North, Yakuts.

Introduction. Currently, the contribution of *FADS* cluster genes associated with the synthesis of long-chain polyunsaturated fatty acids (LC-PUFAs) in the development of metabolic disorders, increased risk of cardiovascular disease (CVD) and type 2 diabetes mellitus (DM2) is being actively studied. LC-PUFAs are involved in many physiological processes: they are part of cell membranes, serve as a substrate for the synthesis of inflammatory eicosanoids (leukotrienes and prostaglandins), act as signaling molecules, and regulate gene expression [42]. One of the main LC-PUFAs are eicosapentaenoic (EPA), docosahexaenoic (DHA) and arachidonic (AA) acids, the last two are necessary for the full functioning of the central nervous system [43]. These fatty acids are not synthesized in the body *de novo*, therefore, they must be supplied with food, or in the form of their 18-carbon substrates for endogenous biosynthesis (omega-6 linoleic (LA) and omega-3 alpha-linolenic acids (ALA)) [42]. The content of LC-PUFAs and their precursors in the traditional diet of various world populations varies greatly depending on the geography and type of economic activity. EPA and DHA,

found in seafood and fish, are an important component of the diet of coastal populations, especially those in the circumpolar regions.

A key role in the metabolism of LC-PUFAs is played by desaturase enzymes, which catalyze the conversion of a single bond between carbon atoms (C-C) of a fatty acid substrate into double bonds (C=C), and are encoded by genes of the *FADS* family [38]. *FADS* genes regulate numerous physiological processes associated with metabolism: they enrich membrane phospholipids with PUFAs, influence to lipoprotein metabolism and lipogenesis, inflammation, levels of circulating monocytes and T cells, and regulate the functions of macrophages, fatty acids, and cholesterol [13, 38, 42, 50]. It has been shown that the *FADS* genes belong to the genes involved in adaptation to a cold climate, which is associated with energy metabolism and the content of LC-PUFAs in food [18, 29, 30]. Thus, the selection of some polymorphic variants in the *FADS* gene cluster in the Greenlandic Inuit has been shown; the strongest signal is associated with rs7115739 T > G in the *FADS3* gene and rs174570 C > T in the *FADS2* gene [30].

One example of populations adapted to harsh climatic conditions are the peoples of Yakutia, whose traditional diet is dominated by foods characterized by a high content of proteins and fats. The population of the Arctic zone has the features of the so-called northern adaptive type, which is characterized by: dense body type, high basal metabolic rate, high content of high-density lipoprotein cholesterol (HDL cholesterol), low content of triglycerides (TG) and atherogenic index [2, 4, 44]. Currently, the diet of the indigenous population of the North, as well as throughout the world, is undergoing global westernization with an increase in the proportion of carbohydrates in the diet and a change in the ratio of fatty acids, which suggests an imbalance of LC-PUFAs. In this regard, the possible influence of desaturase gene activity on the metabolic health of circumpolar peoples, including Yakutia, is of interest.

The purpose of the study is to evaluate the possible relationship of polymorphic variants of the *FADS 1-2-3* genes with adaptation to the extreme conditions of the North and the development of metabolic disorders in modern conditions based on a review of the literature data.

Methods. The structure of the systematic review and the algorithm for information searching and selection are according to the PRISMA standard. The review includes the main sections: introduction, methods, results, discussion, conclusion, funding. The results of the review include the following sections: the geography of distribution of polymorphic markers of *FADS* genes in world populations and the association of *FADS* genes with the development of metabolic disorders in different ethnic populations.

Studies search and selection strategy. The studies were searched using keywords in the following databases: Russian scientific electronic library: elibrary.ru (https://www.elibrary.ru), Pubmed (https://pubmed.ncbi.nlm.nih.gov/), Google scholar (https://scholar.google.com/). Key words for sources in Russian were: gene* FADS*; in English for the Pubmed database: (FADS) AND (gene) with filters: species - human, language - English; for Google scholar search: human FADS gene, metabolism. The systematic review included studies of the association of polymorphic variants of FADS genes with adaptation and the development of metabolic disorders. The authors independently assessed the titles and abstracts of publications for compliance with the inclusion criteria, all disagreements were resolved through negotiations. A manual search was also carried out in the reference lists of the found articles to identify additional sources on the topic. The last search was carried out on August 15, 2022.

Inclusion Criteria. The studies selection criteria for the systematic review were: 1. language: Russian, English; 2. Type of study: cross-sectional and case-control; 3. Age of the subjects over 18 years old; 4. In case-control studies, individuals with type 2 diabetes mellitus (DM2), metabolic syndrome (MS) and cardiovascular diseases (coronary heart disease (CHD), ischemic stroke (IS)) were taken as cases.

Extraction of research data. In the initial screening, using the search queries described above, 241 publications were selected from the PubMed database and 24,100 results from the Google Scholar database. After assessing titles and abstracts, duplicate publications and publications that did not correspond to the search topic were excluded. After filtering, 160 studies remained that corresponded to the purpose of the review. After detailed examination of the full texts, 11 cross-sectional studies and 12 case-control studies fulfilling the inclusion criteria and theme of review were selected.

Search in Russian-language databases for the keywords: "gen* FADS*" found 8 articles, of which 3 publications were selected on the topic of the study.

Results of a systematic review Geography of Distribution of Polymorphic Markers of *FADS* Genes in Various World Populations. Studies of genetic variants of the long-chain unsaturated fatty acid desaturase gene region have revealed the evolutionary history of the *FADS* gene region. In different human populations, two common and very different haplotypes (A and D) were identified, covering the *FADS* gene region and closely related to the level of LC-PUFA synthesis. Haplotype A is considered to be the ancestral haplotype, while haplotype D is specific only for humans and appeared after the separation of the common ancestor of humans and Neanderthals [22]. Currently, haplotype D is most common in Africa and is closely associated with low levels of linoleic and alpha-linolenic acids, which act as substrates for fatty acid synthesis, and higher levels of production of AA, EPA, DHA, and gamma-linolenic acids [7]. Individuals homozygous for the D haplotype had 24% higher levels of DHA and 43% higher levels of AA in the blood than those homozygous for the ancient A haplotype [22]. It is assumed that the modern D haplotype was formed approximately 85,000 years ago, during the development of the continental part of the African continent, as one of the means of survival, during the period of unavailability of a diet rich in arachidonic and docosahexagenic acids, necessary for the development of the brain [7, 22].

The ancestral haplotype A is now generally less common and has been preserved with the highest frequency in populations whose traditional diet is marine mammals and fish with a high content of LC-PUFAs, while it is associated with lower desaturase activity. The natives of America have the lowest frequency of haplotype D, up to 0.01%. The low frequency of haplotype D in this population indicates that this haplotype may have been lost due to the bottleneck effect during the colonization of the American continent [22], and haplotype A was retained under conditions of a diet rich in essential LC-PUFAs. East Asian populations, in whose diet seafood plays a significant role, also have increased frequencies of polymorphisms, which determine lower desaturase activity, compared with European and African populations. A striking example of the influence of a diet high in LC-PUFAs on the maintenance of haplotype A is the Greenlandic Inuit population, in which its frequency reaches 99.9% [30]. Climatic conditions could also play a role in the preservation of haplotype A, requiring an effective ener-

gy balance in the conditions of available food resources. In 2018, studies were conducted on the distribution of variants of two polymorphisms (rs174546 *FADS1* and rs174568 *FADS2*) in Siberian populations. A wider distribution of variants related to haplotype A was shown with a frequency of 57% in South Siberian populations and 97% in West Siberian and northeastern populations [3].

Matteo Fumagalli et al. showed that 6 SNPs in the Greenlandic Inuit in the *FADS* gene cluster underwent the greatest selection, of which the polymorphic markers rs7115739 (G/T) of the *FADS3* gene, rs174570 (C/T) of the *FADS2* gene [30]. As a result of a meta-analysis involving 10 largest studies among two ethnic groups (Greenland Inuit n= 4584 and Europeans n=207300), an association of these polymorphic variants with body mass index, with insulin, with LDL was revealed. It is also interesting that Caucasoids carrying T alleles of rs7115739 and rs174570 polymorphisms (n = 263451) showed a statistically significant association with short stature, and, conversely, no association with body weight was found. The possible association of these polymorphic variants with metabolic disorders and adaptation to cold climates determined our interest in them. Table 1 shows the frequency of minor alleles of these polymorphic variants in world populations, as well as in representatives of the Yakut ethnic group, which we identified in genome-wide studies.

Analysis of the distribution of rs7115739 of the *FADS3* gene shows a frequency from 0.03 in Europeans, including Russians, to 0.33 in the northern peoples of China, and 0.98 in the Greenlandic Inuit. In Yakuts, the frequency of the minor allele, which is considered to be associated with adaptation to cold, contrary to expectations, is 0.14, which is comparable with South Asian populations [28]. A more logical distribution of the frequency of the rs174570 polymorphism of the *FADS2* gene looks from 0.03 in Africans to 0.38 in East Asians, such as Han Chinese, and 0.99 in Greenlandic Inuit. In some Chinese populations, the frequency of the minor allele reaches 0.44 (Daurs), 0.49 (Evenki), and 0.77 (Dai of South China) [47]. In Yakuts, the minor allele occurs with a frequency of 0.43 [28]. It is suggested that the haplotype associated with low desaturase activity in combination with a traditional diet rich in LC-PUFA plays a protective role in reducing the risk of metabolic syndrome and CVD. This issue is relevant in modern conditions of changing nutrition and lifestyle, and is of great interest to researchers.

The Role of Polymorphic Markers of *FADS* Cluster Genes in the Development of Metabolic Disorders in Different Ethnic Populations. A systematic review of the results of cross-sectional and case-control studies on the analysis of the influence of the *FADS* genotype and desaturase activity on lipid metabolism and associated metabolic disorders revealed several publications with ambiguous results. Genetic polymorphisms in the *FADS 1-2-3* gene cluster are located mainly in intron regions and are linked by linkage disequilibrium. The main alleles conditionally belong to the D haplotype, and the minor alleles to the A haplotype. Table 2 shows the results of cross-sectional studies aimed at studying the relationship between *FADS* genotypes and the level of fatty acids (FA) in blood plasma, erythrocyte membranes, total cholesterol (TC), low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglycerides (TG) and other indicators of metabolic health (body mass index (BMI), waist circumference, carbohydrate metabolism and others). The most studied are polymorphic variants of the *FADS1* gene [5, 6, 12, 15, 16, 19, 20, 25, 27, 32, 33, 45]. Most authors indicate that minor alleles of the *FADS* genes are associated with low desaturase activity, which is determined by the ratio of enzyme products to substrates. The only study we found in which minor alleles of the *FADS* genes showed an association with increased activity of desaturase enzymes was a study of the Eskimos of Alaska [48]. With regard to indicators of lipid metabolism, there is no such clear uniformity in the results of studies. However, most researchers have found lower levels of TC, LDL, and slightly less HDL in carriers of minor alleles [16, 19, 25, 27, 30, 33, 45]. The most conflicting data were obtained regarding the level of triglycerides; in the owners of minor alleles, it can be either increased or decreased [5, 6, 32, 34, 45].

Attempts were made to assess the risk of developing CVD, coronary heart disease, ischemic stroke and T2DM depending on the *FADS* genotype in case-control studies (Table 3). Such work was carried out in Chinese, Indian, Iranian and some European populations. In a number of publications, the association of the *FADS* genotype with CVD and DM2 is not detected or is not detected with all the studied *FADS* polymorphic variants [1, 26, 10, 17, 24, 37, 40, 41]. Some Chinese researchers have found a decrease in the risk of coronary artery disease and DM2 in carriers of minor alleles, accompanied by a reduced level of TC [10, 40]. Other data, also obtained in China, show an increased risk of coronary artery disease and IS in carriers of the minor T allele rs174546 of the *FADS1* gene and rs174601 of the *FADS2* gene [11]. At the same time, the frequency of the minor allele T of these two SNPs was higher than the frequency of the C allele both in the control group and in patients. The T allele in this study was associated with a decrease in serum HDL and ApoAI levels in groups of patients with coronary artery disease and IS [11]. Also, in the Chinese population, an increased risk of CHD was found in carriers of minor alleles of rs174547 in *FADS1* when adopting a recessive model [21]. In another study, on the contrary, carriers of the main allele rs174537 - patients with DM2 had an increased risk of developing coronary artery disease (odds ratio (OR) 1.763; 95% CI 1.143-2.718; p = 0.010) [41]. At the same time, the genotype with an increased risk was associated with an increased level of plasma LDL cholesterol.

The association of the *FADS* genotype with DM2 is much less common. Thus, other Chinese authors in studies of several SNPs showed an association of the minor allele only rs174616 of the *FADS2* gene with a reduced risk of DM2 in both codominant and dominant models after adjusting for age, sex, and BMI [40]. Detailed studies of the rs174575 *FADS2* gene have been carried out in the Indian population. In these studies, the minor allele rs174575 was associated with higher fasting blood glucose and HOMA-IR, while HOMA-β was lower [9]. In the recessive model, carriers of the minor allele rs174575 also had statistically significant elevated levels of total cholesterol, TG, LDL, VLDL [46]. Multivariate models of the rs174575 genotype (carriers of the minor allele) with insulin and the rs174575 genotype with insulin and triglyceride showed an association between the genotype and the risk of type 2 diabetes [46]. In other studies, the association of *FADS* polymorphisms was not revealed [24, 37, 41]. In studies performed by O. V. Kochetova et al. in Bashkir and Tatar women, no association of the polymorphic variant rs174550 (T/C) of the *FADS1* gene with metabolic syndrome was found, but a statistically significantly reduced level of triglycerides was shown in carriers of the minor allele in the recessive model (p =0.02) only in the group of women of the Bashkir ethnicity [1].

Analysis of data from cross-sectional studies of *FADS1-2* genotypes in different populations shows that minor alleles of disequilibrium linked SNPs of this clus-

Ассоциация полиморфизмов генов кластера FADS с риском развития ССЗ и СД2 по результатам исследований случай-контроль в различных мировых популяциях

Популяция (общее кол-во исследованных n)	Случай (кол-во n, возраст, лет); Контроль (кол-во n, возраст, лет)	Исследованные гены *FADS* и их SNP (основная /минорная аллель)	Ассоциация генотипа *FADS* с риском случая	Ассоциация генотипа *FADS* с метаболическими нарушениями	Ссылка
Швеция, городское население (n=24032)	ССЗ (n=2648, 44-74) Контроль (n=21384, 44-74)	*FADS1* rs174546 (C/T)	нет		[26]
Китай (n=992)	ИБС (n=497, 67.0 (18.0)); Контроль (n=495, 58.5(22.0))	вблизи *FADS1* rs174537 (G/T), *FADS1* rs174547 (T/C), *FADS2* rs1535 (A/G), rs174575 (C/G), rs174602 (T/C), *FADS3* rs174450 (A/C), rs7115739, (G/T), rs1000778 (A/G)	↓ риска ИБС у носителей минорного аллеля rs1000778 *FADS3*	↓ ОХ у носителей аллелей rs174575-G *FADS2* и rs174450-C и rs7115739-T *FADS3* в контроле	[10]
Китай, южная популяция (n= 1 669)	ИБС (n = 534, 61.93 ± 10.69); ИИ (n = 553, 62.54 ± 12.11); Контроль (n=582, 61.40 ± 10.54)	*FADS1* rs174546 (C/T), *FADS2* rs174601 (C/T)	↑ риск ИБС и ИИ у носителей аллелей Т двух SNP	↓ ЛПВП и АроАI в сыворотке крови в группах пациентов ИБС и ИИ у носителей аллеля Т двух SNP и генотипа rs174601 ТТ.	[11]
Китай (n=1660)	ИБС (n=515, 66.7±10.0); Контроль с высоким риском (n=524, 50.2±12.5); Контроль с низким риском (n=621, 49.8±12.4)	*FADS1* rs174547 (T/C)	↑ риск ИБС у носителей генотипа rs174547 CC в рецессивной генетической модели	↓ ЛПВП и ↑ ТГ у носителей минорного аллеля С	[21]
Китай (n=872)	ИБС (n=200, 59.47 ± 10.53); СД2 (n=234, 57.74 ± 12.76); СД2 и ИБС (n=185, 60.30 ± 9.73); Контроль (n=253, 59.73 ± 10.06)	вблизи *FADS1* rs174537 (G/T), *FADS2* rs174616 (C/T), rs174460 (T/C), rs174450 (A/C)	↑ риск ИБС у пациентов СД2 с генотипом rs174537 GG (основной аллель)	↑ ЛПНП, АК и дельта-6 десатуразы у носителей генотипа rs174537 GG	[41]
Китай (n=772)	СД2 (n=441, 58 (11.0)); Контроль (n=331, 56 (12))	*FADS1* rs174545 (C/G), *FADS2* rs2072114 (A/G), rs174602 (A/G), rs174616 (C/T)	↓ риск СД2 у носителей минорного аллеля Т rs174616 как в кодоминантных, так и в доминантных моделях после корректировки на возраст, пол и ИМТ	↓ ОХ у носителей минорных аллелей. ↓ ЛПВП у гомозигот по минорному аллелю rs174546 и rs3834458.	[40]
Китай (n=1158)	СД2 (n=758, 58.25±12.31); Контроль (n=400, 51.99 ± 8.41)	вблизи *FADS1* rs174537 (G/C), *FADS2* rs174575 (C/G), *FADS3* rs174455 (G/A)	нет		[24]
Индия (n=429)	СД2 (n=213, 51.03 ± 8.25); Контроль (n=216, 47.44±10.16)	*FADS2* rs174575 (C/G)	Не показано.	↑ глюкоза натощак и HOMA-IR, ↓ HOMA-β у носителей минорной аллели rs174575.	[9]
Индия (n=429)	СД2 (n=213, 51.03 ± 8.25); Контроль (n=216, 47.44± 10.16)	*FADS2* rs174575 (C/G)	↑ риск СД2 в многомерных моделях генотипа rs174575 (носители минорного аллеля) с инсулином и генотипа rs174575 с инсулином и ТГ.	↑ ОХ, ТГ, ЛПНП, ЛПОНП у носителей минорного аллеля rs174575 в рецессивной модели.	[46]
Иран (n=190)	СД2 (n=95, 53.23±10.87); Контроль (n=95, 53.67±8.47)	*FADS2* rs174583 (C/T)	нет		[37]
Германия (n = 2653)	СД2 (n=673, 55.1 ± 7.4); Контроль (n=1980, 50.0 ± 8.9)	*FADS1* rs174546 (C/T)	Нет. Но более низкая активность фермента D6D предсказывала более низкий риск СД2 у носителей минорного аллеля.		[17]
Татары (женщины), n=375; башкиры (женщины), n=166	МС (n=243, 52.92±7.22); Контроль (n=298, 54.14±6.91)	*FADS1* rs174550 (T/C)	нет	↓ ТГ у носителей минорного аллеля в рецессивной модели (p=0,02) только в группе женщин башкирской этнической принадлежности	[1]

ter are associated with low desaturase activity and, probably, a more favorable lipid profile. This conclusion is supported by a Mendelian randomization study to investigate the associations between plasma FA levels of phospholipids and 15 cardiovascular diseases, which showed that carriers of the *FADS1* minor allele rs174547 (T/C) have a reduced risk of ischemic stroke, large artery stroke, and venous thromboembolism, and evidence feedback on ischemic heart disease, abdominal aortic aneurysm, and aortic valve stenosis [39].

In some cases, the *FADS* genotype is not associated with the disease, but the activity of desaturases has a statistically significant association with T2DM. Thus, the work of J. Kroger et al showed a positive relationship between D6D activity, encoded by *FADS2*, and a clear inverse relationship between D5D (*FADS1*) activity and the risk of diabetes, while the relationship with the genotype was not proven [17]. A recent Mendeleev randomized study revealed the overall effects of increased activity of both D6D and D5D on the risk of T2DM and the effect of D6D activity on the risk of CHD [14]. According to the authors, the influence of desaturase activity on the risk of diabetes is probably mediated by changes in the composition of FAs in cell membrane phospholipids, which affect cellular function, including insulin signaling and receptor binding affinity. [14, 17]. In addition, long chain PUFAs can act as biological ligands for PPAR-γ, which is associated with adipogenesis and lipogenesis. PUFAs also block NF-kappa B, reducing inflammation. Together, all factors can increase insulin sensitivity, and when the activity of desaturases changes, lead to metabolic disorders. Another Mendeleev randomized study showed that a decrease in the synthesis of omega-6 LC-PUFAs was not significantly associated with the risk of developing DM2, however, the authors concluded that in a predominantly white European population, the synthesis of omega-6 LC-PUFAs is not a major risk factor for developing DM2 [49].

Discussion. An analysis of the prevalence in world populations of haplotypes covering the region of *FADS* genes and closely related to the level of LC-PUFA synthesis reflects both the historical processes of migration of modern humans and the influence of available food sources and processes of adaptation to climatic conditions on the selection of the most energetically advantageous polymorphisms. The spread of a derivative of the D haplotype with high desaturase activity is associated with the development of agriculture and farming and an increase in the share of plant foods in the diet, which contributed to the development of new continental territories.

The fixation of the ancestral haplotype A in modern Native Americans and circumpolar peoples has been interpreted as evidence of selection from their Siberian or Beringian ancestors [23, 31]. However, it is not excluded that the ancestral haplotype was preserved from the Paleolithic Eurasians, in whom the derived alleles probably did not yet become common until the time of separation of the ancestors of the indigenous inhabitants of America and the Eurasians [35]. In northern populations, this haplotype, which is responsible for a lower level of LC-PUFA biosynthesis, is adapted to the availability of LC-PUFA in the diet and, accordingly, to higher levels of accumulation in the body of 18-carbon precursors and other fats capable of being actively deposited in adipose tissues. of the body as an energy source that provides thermogenesis of the body in cold conditions and energy consumption during traditionally active physical activity.

It should be noted that there is an insufficient number of studies of *FADS* genes in Russian populations, while the diversity of climatic conditions and ethnic groups in Russia could make a significant contribution to the coverage of this topic. As part of a project to study the entire genome of Russian populations, an analysis was made of the frequency of the two most significant polymorphic variants rs7115739 (G/T) of the *FADS3* gene and rs174570 (C/T) of the *FADS2* gene involved in adaptation to cold climates in Yakuts and Russians. It was shown that the frequency of minor alleles rs7115739 and rs174570 in Yakuts is higher (14% and 43%, respectively) than in Russians (3-4% and 7-23%), and is close to that in East Asian peoples [28]. It can be assumed that this is influenced by a common origin with the East Asian peoples, but the frequencies obtained cast doubt on the selective selection for these variants in the Yakut population. However, about half of the Yakut population have a genetically predetermined low desaturase activity in the *FADS2* gene.

A systematic review of the results of studies of the association of polymorphic variants of the *FADS* genes with metabolic disorders in different world populations did not give an unambiguous answer to what extent the effect of the ancestral haplotype A is favorable on lipid metabolism and the risk of developing DM2 and CVD. This haplotype with low desaturase activity can be associated with both a favorable lipid profile and an unfavorable one. It is likely that the relationship between the *FADS* genotype and the risk of metabolic disorders is complex and is affected by diet, lifestyle, and the state of the human immune system. In general, it seems that the carriers of minor alleles, i.e. ancestral haplotype A are more sensitive to lipid metabolism disorders and the associated risk of CHD and IS. The haplotype that causes low desaturase activity, which is compensated by the intake of LC-PUFAs with traditional foods, has a protective effect on CVD, but under conditions of westernization of the diet and low physical activity, it has a negative effect on metabolic health.

Conclusion. In circumpolar ethnic groups, the distribution of an ancestral haplotype with low desaturase activity was shown. Although this haplotype is generally considered favorable for lipid metabolism, a review of studies suggests that its holders may be more vulnerable to CVD. This is probably due to the fact that in modern conditions, with a global shift in the diet towards the Western diet and a decrease in the level of consumed LC-PUFAs, low deaturase activity, combined with a decrease in physical activity, also leads to lipid metabolism disorders. When developing preventive measures and technologies for personalized medicine to reduce the prevalence of risk factors for cardiovascular diseases and alimentary-dependent pathology, genetically determined desaturase activity should be taken into account. As the example of the Yakut population shows, the prevalence of one or another haplotype is determined not only by geographical factors and environmental conditions, therefore, the development and implementation of personalized approaches to each population is more justified.

The study was carried out within the framework of the basic part of the state task of the Ministry of Education and Science of the Russian Federation on the topic "Genetic characteristics of the population of the north-east of Russia: reconstruction of genetic history, mechanisms of adaptation and aging, age-dependent and hereditary diseases." (FSRG-2023-0003).

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POINT OF VIEW

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DOI 10.25789/YMJ.2023.81.27

УДК 616-053.2(571.56)

THE ROLE OF SOCIAL FACTORS IN THE FORMATION OF CONGENITAL HEART DISEASE IN THE REPUBLIC OF SAKHA (YAKUTIA)

The article presents the results of the study of the association of some socio-demographic factors with risk of congenital heart disease in children in the Republic of Sakha (Yakutia). The analysis was carried out on the basis of the Perinatal Center of the Republican Hospital No.1-NCM). The study includes all cases of congenital defects among newborns born alive in two time periods – from 2001-2003 and 2011-2013. In the first period, 697 cases were registered, in the second period there were 1127 cases of congenital heart disease.

The first group included newborns with persistent fetal communications without signs of heart failure, without expansion of the heart cavities and without hemodynamic disorders. The second group was represented by newborns with congenital heart disease with signs of heart failure and functional class of various degrees. This group was divided by severity of heart failure and functional class stages into two subgroups which were 2A and 2B respectively.

The factors such as the education of parents, the number of births in the history, the presence of a full and incomplete family, were analyzed.

The compared groups were not statistically significantly different by age of the parents. The median values of the mothers' age at the time of birth of a child with congenital heart disease were 27 years in the first group, 26 years in the 2A group, and 28 years in the 2B group. The median age of the father in all groups was 29 years.

The structure of categories of social status is represented mainly by employees, non-workers, workers and students. An analysis of the parents of the education factor as a possible predictor of the birth of a child with congenital heart disease was also conducted.

According to the results of the study, social factors affecting the risk of birth of children with congenital heart defects were an incomplete family without a sign of marriage (registered and unregistered marriage), the level of education of the mother in the case of simple heart defects, the number of births and the social status of the mother in complex diseases.

Keywords: congenital heart disease, social factors, parents, statistical analysis.

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Introduction. Children's health is formed under the influence of the interaction of endogenous and external factors. The leading place among the endogenous are genetic factors, parents' health, the course of antenatal and perinatal periods. The external environmental factors also can increase the likelihood of disease [1,9,14].

Congenital heart disease is one of the global problems of modern neonatology and pediatrics. Most of the risk factors influencing the formation of CHD in the fetus can be managed, as evidenced by numerous multicenter studies, which reinforces the importance of preventive measures aimed at preventing further growth of CHD prevalence [2, 7].

The etiology of congenital heart disease is still unclear. In addition to genetic or chromosomal abnormalities, maternal factors such as drug intake during pregnancy, viral infections in the first trimester of pregnancy, smoking, alcohol abuse, and others can contribute to CHD development [4, 5, 6, 8, 13]. There is an evidence of an increased risk of heart defects in children of mothers with diabetes

mellitus or obesity [10, 11]. Some studies have shown that family socio-economic status, age of parents are risk factors for the development of congenital heart disease [4, 8, 12].

The purpose of the research is to assess the association of some socio-demographic factors with the risk of congenital heart disease development in children in the Republic of Sakha (Yakutia).

Material and methods of the study. The retrospective study was conducted at the Perinatal Center of the Republican Hospital No.1 National Center of Medicine (PC RH No.1-NCM). All cases of CHD among newborns born alive in the periods 2001-2003 (period A) and 2011-2013 were included in the analysis. (period B). In period A there were 697 cases, in period B there were 1127 cases of CHD.

The first (1) group (n=1008) involved newborns with persistent fetal communications without signs of heart failure (HF), without dilated heart cavities and without hemodynamic abnormalities (small atrial septal discharge (ASD) without a functioning open arterial duct (OAP) or in combination with OAP with small blood discharge). The second (2) group was represented by newborns with CHD with signs of CHD and functional class (FC) of different degrees. This group was divided by severity of CH and FC stages into two subgroups: 2A and 2B. The subgroup 2A (n=183) consisted of newborn infants with CHD, with no or minimal manifestations of CH, i.e., with signs of CH 1, FC 1. This group included the patients with an average TMB discharge, without or in combination with a functional AVP less than 0.2 cm in diameter. Subgroup 2B (n=625) consisted of newborn infants with CHD and features of CH 1-3, class 2 or more. This group included: 1) moderate discharge on the IAP in combination with an OAP with a diameter of 0.2 cm or more, large discharge on the IAP (0.56 cm -1.0 cm) without or in combination with a functioning OAP of any size, atrial septal defect (ASD) of any size without or in combination with an OAP of any size; 2) complex CHD, without or in combination with a functioning OAP. In all patients of Group 2B the diagnosis was verified by X-ray contrast methods.

To assess the medical and social characteristics of the patients' families, a retrospective analysis of the primary documentation- statistical cards of an inpatient (form №066/-02) and inpatient registers (form №010u) was performed.

Statistical calculations were performed using IBM SPSS Statistics 22 software. Pearson and Kruskal-Wallis criteria were used to compare groups. Logistic regression method was used to assess the contribution of risk factors. The critical value of the significance level for testing statistical hypotheses was assumed to be 5%.

Results and discussion. The compared groups did not differ statistically significantly by parental age. The median maternal age at the time of birth of a child with CHD was 27 years in Group 1 (n=1008), 26 years in Group 2A (n=183), and 28 years in Group 2B (n=625) (p=0.252). The median values of the father's age in all groups (n=862, n=154, n=561) were - 29 years (p=0.915).

The structure of parents' social status categories among the entire sample population and Group 2B was analyzed further (Table 1). The structure of categories of social status of parents was represented mainly by employees, unemployed, workers, students of universities and colleges. In dynamics, over the 10 years of observation, the structure of social categories among mothers remains the same: in the first place - "employees", in the second place - "unemployed", in the third place - "workers". Analysis of the dynamics of the structure of social categories among mothers in periods A (n=147) and B (n=479) among Group 2B CHDs showed an increase in the proportion of unemployed from 19.7% to 27%. In period B, there was a decrease in the proportion of employees from 54.2% to 48.5% and of college students from 6.3% to 3.5%.

The social categories of fathers were slightly different from the structure presented above. Among the fathers in the total sample, A (n=697) and B (n=1127), the proportion of employees was approximately equal to that of workers, at 35%. Furthermore, among fathers, in the total sample population, A (n=697) and B (n=1127), the nonworking category was 11.2% and 13.2%. Students of higher education accounted for 3.0% and 4.2% and students of secondary education accounted for 3.6% and 1.4%.

The structure of social categories among fathers has remained the same over the 10 years of observation: the first place is held by employees and workers, the second one is occupied by unemployed men, and the third place is held by students of universities and colleges. There is no clear difference between the indicators of the general sample and Group 2B, as well as between periods A and B (Table 1).

The analysis of the parental education factor as a possible predictor of birth of a child with CHD was performed (Table 2). The education of both fathers and mothers in the general population was evenly distributed among the three CHD groups, with the exception of incomplete higher education. The second period had a higher proportion of persons with a college degree, but the differences between the total sample and the 2B group of CHDs did not exceed 10% of the total population for each category.

According to the data presented in Table 2, the structure of the educational categories of parents is represented by secondary school, secondary specialized, higher, and incomplete higher education. In period A, among the mothers and fathers in the total sample (n=697), secondary school, secondary specialized, and higher education were relatively evenly distributed, accounting for about 30% (29.5% to 32%.), and incomplete higher education accounted for 8.2% to 9.5%. In period B, there was a decrease in the proportion of mothers and fathers in the total sample (n=1127) with specialized secondary education (23.1% for mothers, 27.7% for fathers). The proportion of mothers with higher education increased (37.7%). Among the sample of mothers with newborns in group 2B, when comparing the two periods, (period A (n=147), period B (n=479)), there was a persistence of a higher proportion of persons with higher education in relation to the proportion of persons with secondary school and secondary special education. There was no such a tendency among fathers.

During the studied periods, there was a decrease in the proportion of individuals with specialized secondary education (23.1% - mothers, 27.7% - fathers) and an increase in the proportion of mothers with higher education of 37.7%. In the sample of CHD group 2B, mothers have a higher share of persons with higher education in relation to the share of individuals with secondary school and secondary vocational education. Among fathers, the share of individuals with higher education decreased.

The social categories of father and mother proved to be statistically significant predictors of CHD development (Table 3). In the first period of the study, the logistic regression data (comparison category - employees) revealed that the risk of having children with simple CHD was higher in mothers, categorized as "college students" (OR = 5.94, p=0.03) and was lower for fathers categorized as "civil servants" (OR = 0.07, p=0.027), "unemployed" (OR = 0.16, p=0.018) and "college students" (OR = 0.0374, p=0.037). Analysis by group showed that the low-

Table 1

Social categories of father and mother in periods A and B. total and among Group 2B CHD cases. n (%)

Category	Mother				Father			
	Period A		Period B		Period A		Period B	
	Total (n=697)	CHD in the 2B group (n=147)	Total (n=1127)	CHD in the 2B group (n=479)	Total (n=697)	CHD in the 2B group (n=147)	Total (n=1127)	CHD in the 2B group (n=479)
Employees	48.0	54.2	49.9	48.5	35.2	40.0	30.5	31.1
Civil servants	2.2	0	0.9	1.1	5.9	0.8	1.6	2.0
Workers	13.8	11.3	11.2	11.7	32.7	33.3	32.5	29.1
Peasants	1.6	2.8	0.2	0.2	0.5	0	1.2	1.5
Unemployed	22.7	19.7	25.9	27.0	11.2	12.5	13.2	11.5
College students	4.1	6.3	3.5	3.2	3.6	2.5	1.4	1.2
Entrepreneurs	0.3	0	1.1	0.9	3.7	1.7	9.3	11.2
Soldiers	0	0	0	0	0.7	0.8	0.9	1.2
Employees of the Ministry of Internal Affairs	0.7	0	1.1	0.6	3.4	2.5	4.8	5.1
School students	0.6	0.7	0.7	0.6	0.2	0.8	0.1	0.2
Church workers	0	0	0	0	0	0	0.1	0.2
People with disabilities	0.1	0	0.4	0.6	0	0	0.2	0.5
University students	5.7	4.9	5.2	5.5	3.0	5.0	4.2	5.1

Table 2

The level of education of fathers and mothers in periods A and B in the total sample and among Group 2B CHD cases, n (%)

The level of education	Mother				Father			
	Period A		Period B		Period A		Period B	
	Total (n=697)	CHD in the 2B group (n=147)	Total (n=1127)	CHD in the 2B group (n=479)	Total (n=697)	CHD in the 2B group (n=147)	Total (n=1127)	CHD in the 2B group (n=479)
secondary school	29.5	21.3	31.1	32.7	30.7	29.1	34.0	35.5
secondary specialized	31.0	28.3	23.1	19.8	31.8	28.2	27.7	28.1
Incomplete higher	9.5	9.4	8.2	9.4	5.3	6.4	6.1	6.1
Higher	30.0	40.9	37.7	38.1	32.2	36.4	32.1	30.3

er risk of having group 2A children was characterized by fathers categorized as "employees" (OR = 0.03, p=0.043) and "non-workers" (OR = 0.01, p=0.017). The lowest risk of having group 2B children was found in fathers classified as "civil servants" (OR = 0.03, p=0.037).

In the second period of the study, a lower risk of having children with simple CHD was typical for fathers from the "worker" category (OR = 0.48, p=0.006), compared to the "employee" category. The lower risk of having children with complex CHD was statistically significantly higher for mothers from the "workers" category (OR = 3.71, p=0.025) and the " entrepreneurs" category (OR = 10.65, p=0.01).

Statistical analysis of the effect of parental education as a predictor of the risk of having a child with CHD was performed. The results are presented in Table 4. In period A, the risk factor for the birth of children with simple CHD (comparison with secondary education) was the mother's higher education (OR = 3.47, p=0.031); in group 2B, the risk of having a child with CHD was lower for mothers with a specialized secondary education (OR = 0.437, p=0.024). In period B, the risk of having children with simple CHD was lower in fathers with incomplete higher education (OR = 0.32, p=0.026) and higher education (OR = 0.486, p=0.006); the risk of having children with complex CHD was higher in mothers with incomplete higher education (OR = 7.06, p=0.013). The risk of birth of CHD in group 2A was lower in fathers with secondary education (OR = 0.41, p=0.03).

Next, the analysis of other factors with possible risk of having a child with CHD, such as the number of repeated births in the anamnesis, the presence of full and single-parent families, and marital status (registered and unregistered marriages) was performed. These factors were statistically significant (Table 5).

According to the data presented in Table 5, in period A, incomplete family (OR = 4.84, p=0.049) was a statistically significant risk factor for having children with simple CHD (compared with the presence of registered marriage). In Group 2B, the risk of CHD was lower in families with registered marriage (OR = 0.187, p=0.046). There was also evidence of an increased risk of complex CHD birth in period B in women with a history of repeated births (OR = 1.51, p=0.014).

Thus, according to the results of the study, in both periods, the highest risk of CHD birth was detected in the categories "single-parent family without paternal residence" and "number of births".

Table 3

Social category of parents and risk of having a child with CHD

Factor	Risk	Period	Odds ratio (OR)	p
Mother is a college student	Simple CHD	A	5.94	0.031
Father is a civil servant	Simple CHD	A	0.07	0.027
Father is unemployed	Simple CHD	A	0.16	0.018
Father is a college student	Simple CHD	A	0.038	0.037
Father is an employee	2A group	A	0.03	0.043
Father is unemployed	2A group	A	0.01	0.017
Father is a civil servant	2B group	A	0.03	0.037
Father is unemployed	2B group	A	0.06	0.044
Father is a college student	2B group	A	0.052	0.025
Father is a worker	Simple CHD	B	0.48	0.006
Mother is a worker	Complex CHD	B	3.71	0.025
Mother is an entrepreneur	Complex CHD	B	10.65	0.011

Table 4

Education of parents and the risk of having a child with CHD

Factor	Risk in groups	Period	Odds ratio (OR)	p
HE of mother	Simple CHD	A	3.47	0.031
SSE of mother	2B group	A	0.437	0.024
IHE of father	Simple CHD	B	0.32	0.026
HE of father	Simple CHD	B	0.486	0.006
IHE of mother	Complex CHD	B	7.06	0.013
SE of father	2A group	B	0.41	0.030
STE of father	2B group	B	1.61	0.053

Notes: HE - higher education; IHE - incomplete higher education; SE - secondary education; SSE - secondary specialized education; STE - secondary technical education; period A is 2001-2003; period B is 2011-2013.

Table 5

Other social factors and the risk of having a child with CHD

Factor	Risk of CHD	Period	OR	p
2)	Simple CHD	A	4.84	0.049
3)	2A group	A	0.026	0.039
4)	2A group	A	0.028	0.019
3)	2B group	A	0.187	0.046
2)	Simple CHD	B	3.09	0.020
1)	Complex CHD	B	1.51	0.014
3)	2B group	B	0.116	0.004
4)	2B group	B	0.108	0.004

Notes: period A is 2001-2003; period B is 2011-2013; 1) - number of births, 2) - single-parent family: father does not live in the family, 3) - families with registered marriage, 4) - families with unregistered marriage.

In both periods, one of the most important risk factors for the birth of children with CHD was the birth of a child in a single-parent family. A single-parent family was a factor with a low risk of having a child with CHD, and the absence of formal marriage had no significant effect. In Group 2A and 2B CHD samples, registered marriage and unregistered marriage were the factors with low risk of having a child with CHD. These data are consistent with those of other researchers [3].

The factors listed appeared to be multidirectional and do not fit into a single obvious concept. In general, the evidence on socioeconomic inequality and the risk of CHD is somewhat contradictory [8]. Education level, employment, socioeconomic status, behavior, and environmental factors are related. In this case, we can also assume that the social category indicated in the medical record is not directly related to the real socioeconomic status of the family.

Summary. All studied groups did not differ statistically significantly by the age of parents at the time of birth of a child with CHD (p = 0.252). Social factors associated with the risk of birth of children with CHD were:

single-parent family, with no significant differences in marital status (registered and unregistered marriages);

in the sample of simple CHD - higher and incomplete higher education of the mother, study of the mother in a specialized secondary educational institution. In both studied periods, in the sample of simple CHD, the factors of higher education, incomplete higher education, and studies at a college were associated with a possible risk of CHD development; the same factors in fathers were associated with a low risk;

in the complex CHD sample, factors in the number of repeated births and the mother's social category of "worker" and "entrepreneur".

Conclusion. According to the research data, it can be assumed that the social category indicated in the medical records may not have been related to the real socioeconomic situation of the family. Among the social factors, in both time periods, the single-parent family category was the most high-risk.

The work was performed within the research theme "Physical development and health status of the child population in the Far North (by the example of Yakutia)" (state registration number: 1021062411641-9-3.2.3), within the state assignment of the Ministry of Science and Education of the Russian Federation (FSRG-2023-0003).

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ASSESSMENT OF THE IMMUNE STATUS IN MEN OF THE SUBRACTIC AND SEMI-ARID REGIONS USING FACTOR ANALYSIS

DOI 10.25789/YMJ.2023.81.28

УДК 612.019

Functional systems, including the immune system in humans, adapt depending on the influencing environmental factors. Factor analysis is considered an important method for identifying latent operating parameters and their contribution to the overall process. The aim of this work is to assess the immune status of men aged 20-60 years old living in the subarctic and semi-arid regions using factor analysis. After determining the concentration of leukocytes by standard methods, and the concentration of lymphoid subpopulations by the method of indirect immunoperoxidase reaction using monoclonal antibodies, factor analysis was carried out by the method of the main component with the determination of coefficient scores of indicators to calculate the contribution of different stages of the immune reaction in the formation of the immune response. The processes of lymphoproliferation and apoptosis play a controlling role over other processes, regardless of the place of residence. The activity of the phagocytosis process increases under semi-arid conditions. The activities of the processes of differentiation and the acquired cellular response are intensified in the subarctic region. At the same time, the balance between the processes of proliferation and apoptosis is disturbed to a greater extent in the subarctic region. Thus, the formation of an adaptive immune response in men of the subarctic region is accompanied by excessive use of the reserve capabilities of immune homeostasis. In men of the semi-arid region, the adaptive immune response is formed more fluently, which contributes to the preservation of reserve capabilities of immune homeostasis and is the most optimal (beneficial) for the body.

Keywords: immune system, factor analysis, phagocytosis, apoptosis, lymphoproliferation, subarctic region, semi-arid region.

Introduction: The impacts of living in different climatic, environmental and technogenic conditions can lead to adaptive functional and systematic changes, including the immune system, The body's reserve capabilities may subsequently be exhausted as a result, which may lead to the development of chronic pathology of a regional nature [1,6].

The air temperature, daylight and solar irradiation, UV index, and air quality index are all different in the subarctic and semi-arid regions. The average temperature in the subarctic region is 16°C lower than in the semi-arid region, and the semi-arid region has 3 hours more sunshine than the subarctic region. The UV index is 2.5 times higher on average in the semi-arid region. The air quality index in the subarctic region (AQI=23) is higher than in the semi-arid region (AQI=41), because the concentration of pollutants, particularly particulate matter (2.5 and 10 microns), is 25 times higher in the semi-arid region than in the subarctic region [9,12].

The evaluation of the functions of the human immune system is based on the development new methods and is important for determining the internal relationship of immunological parameters and the mechanisms of their functioning [11]. At present, the quantitative determination of immunocompetent cells, including cytotoxic, T-helper, B-lymphocytes and natural killers, by microscopic or flow cytometry method gives a good idea of the state of the body's immune homeostasis, normal ranges of cell content, and is also considered an important indicator in the norm. and in pathology [2,3].

Since these parameters are frequently involved in complex variable immu-

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nological mechanisms, the processes of substantiating the results obtained, the mechanisms of action in normal and pathological conditions, and predicting the possibility of developing environmentally dependent diseases are becoming more difficult [5,13]. The factor loadings for each variable within the components can be interpreted as measures of correlation between the observed variable and the underlying unobserved component, which is a feature of factor analysis. Factor analysis can be used in immunological studies to extract information not only about the role of each parameter, but also about the role of the underlying mechanism [5]. Therefore, a comprehensive study of these parameters is necessary in order to build an overall picture that reflects the specificity of the immune status.

The aim of work is to assess the immune status of men aged 20-60 living in the subarctic and semi-arid regions using factor analysis.

Materials and methods: We analyzed the examination results of 63 men aged 20-60 years, 33 people living in the subarctic region (Arkhangelsk, Arkhangelsk region of the Russian Federation) and 30 people living in the semi-arid region (Aleppo, Syria). Participation in the examination was on a voluntary basis; at the time of venous blood sampling, the voluntaries had neither acute nor chronic diseases according to the conclusion of a local clinic doctor. The primary analysis of peripheral venous blood for immune status of Aleppo, Syria residents was conducted in the University of Aleppo's biochemistry laboratory. In the peripheral blood, the number of leukocytes in the Goryaev's chamber, the leukocyte formula in the stained blood smear according to Romanovsky-Giemsa were determined. The lymphocytic subpopulation ($CD3^+$, $CD4^+$, $CD5^+$, $CD8^+$, $CD10^+$, $CD16^+$, $CD20^+$, $CD71^+$, $CD95^+$, $HLA\text{-}DR^+$) were determined by the method of indirect immunoperoxidase reaction using monoclonal antibodies on preparations of lymphocytes of the "dried drop" type using a peroxidase conjugate and staining with a chromogen solution for analysis in immersion microscopy.

Determined parameters are conditionally divided into different stages of the immune response: 1- Phagocytosis (neutrophils, monocytes and eosinophils), 2- Congenital cellular response (natural killers CD16), 3- lymphoproliferation (CD10 and CD71), 4- Differentiation (CD5 and CD3), 5- Adaptive cellular response (CD4, CD8 and HLA-DR), 6- Humoral response (CD20 and HLA-DR), 7- Apoptosis (CD95).

The work was carried out in the Laboratory of Physiology of Immunocompetent Cells of the Institute of Physiology of Natural Adaptations of N. Laverov Federal Center for Integrated Arctic Research of the Ural Branch of the Russian Academy of Sciences, Arkhangelsk, Russia within the State Assignment № 122011700267-5 "Physiological significance of the features of immune homeostasis, functional and receptor activity of immunocompetent cells in people in extremely changing environmental conditions, considering professional status and socially significant diseases among residents of the Arctic region"

The results were statistically processed using Microsoft Excel 2010 and SPSS 20.0 for Windows. Kaiser's statistical test was used to determine the number of significant factor sets for factor analysis. The Bartlett test was used to determine the acceptability of factor analysis. The principal component method was used to select the factors. The factor loadings were rotated using the Varimax method to maximize the correlation coefficients in the factor sets. To identify the contribution of each stage of the immune reaction in the formation of the immune response, the weight value of the immunological parameters of the stages was calculated using the coefficient score of the parameter (K_n), the percentage of intrinsic variance (σ_n) and the total percentage of variance (σ) obtained from the results of factor analysis using the following formula [7,4]:

$$\omega = \frac{X \sum_{n=1}^{u} \sigma_n K_n}{\sigma},$$

where ω is the weight value of the parameter , X is the concentration of the parameter.

The percentage of the contribution of the stage is equal to the total weighted value of all parameters of the stage multiplied by one hundred and divided by the total weighted value of all stages.

The assessment of the significance of differences for paired independent samples between groups was carried out using the Mann-Whitney test, the threshold level of significance was taken as $p<0.05$.

Results and discussion: The Kaiser-Meyer-Olkin (KMO) criterion shows acceptable and satisfactory adequacy in the subarctic and semi-arid regions, respectively, and the Bartlett sphericity criterion confirms that the data are acceptable for factor analysis (Table 1).

Using the principal component method, 3 factors in the subarctic region and 4 factors in the semi-arid region were identified (Table 2). These factors explain 75.32% and 78.11% of the variance in immunological status parameters in men living in the subarctic and semi-arid regions, respectively.

By determining the parameters that represent each factor (Table 3), it was found that in men in the subarctic climatic

Table 1

Acceptability and adequacy of data for factor analysis in men aged 20-60 living in the subarctic and semi-arid regions

		Subarctic	Semi-arid
Kaiser-Meyer-Olkin Measure of Sampling Adequacy		**0.744**	**0.659**
Bartlett's Test of sphericity	χ^2	340.522	256.782
	df	78	78
	P	**< 0.001**	**< 0.001**

Table 2

Explained cumulative variance of the immunological status parameters of men aged 20-60 living in the semi-arid and subarctic regions

Living region	Factor	Eigenvalue	% variance	Total %
Subarctic	1	5.58	**42.93**	42.93
	2	2.96	22.74	65.67
	3	1.26	9.66	**75.32**
Semi-arid	1	5.11	**39.32**	39.32
	2	1.94	14.93	54.25
	3	1.62	12.48	66.73
	4	1.48	11.38	**78.11**

region, the highest loads in the first factor correspond to parameter that reflect humoral immune response activation (activated lymphocytes (HLA-DR$^+$) and B-lymphocytes (CD20$^+$)), lymphoproliferation and mitosis of lymphocytes due to increased expression of transferrin receptors (CD71$^+$), apoptosis of lymphocytes due to marker expression (CD95$^+$), and innate immune response (natural killers (CD16$^+$)). The composition of the 2nd factor includes parameters that reflect the activity of the cellular immune response (cytotoxic T-lymphocytes (CD8$^+$), T-helpers (CD4$^+$)), lymphocyte proliferation due to the expression of the marker of lymphocyte precursors (CD10$^+$), and differentiation of common T- and B-1 lymphocytes (CD5$^+$). The composition of the 3rd factor includes cells that reflect the level of phagocytosis (eosinophils and, to a lesser extent, neutrophils, monocytes) and the maturation of lymphocytes (CD3$^+$).

The analysis showed that in men in the semi-arid climatic region, the greatest loads as part of the first factor correspond to the parameters of the humoral immune response, mainly B-lymphocytes (CD20$^+$), the process of lymphoproliferation due to the transferrin receptor (CD71$^+$) and the process of apoptosis (CD95$^+$). The 2nd factor includes markers that reflect the level of differentiation and maturation mainly (CD3$^+$), cellular immune response (CD8$^+$, CD4$^+$) and, to a lesser extent, innate cellular immune response (CD16$^+$). The third factor includes markers that reflect the level of lymphoproliferation mainly (CD10$^+$), differentiation and maturation due to CD5$^+$, and the level of activation of the immune response (HLA-DR$^+$). And the composition of the 4th factor includes parameters of phagocytosis, mainly eosinophils, monocytes and, to a lesser extent, neutrophils.

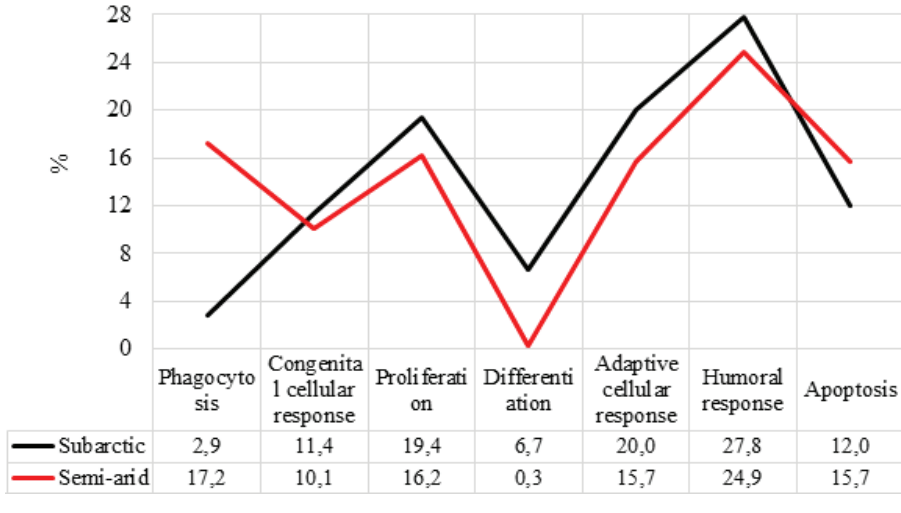
Determining the percentage contribution of different stages of the immune response using the coefficients scores for assessing the variables, a significant difference was revealed (Figure 1) at the stages of phagocytosis, differentiation, cellular reaction and apoptosis ($p<0.01$). In subarctic conditions, the contribution of phagocytosis, as well as apoptosis, to the formation the immune response is almost 6.0 times and 1.3 times lower than in men of the semi-arid region, respectively, which may be explained by a decrease in air temperature and UV index [8,10]. The contribution of the processes of differentiation and cellular acquired reaction is 22.0 and 1.3 times higher than their contribution in men of the semi-arid region, respectively. Thus, it can be assumed that cold weather, photoperiods, and UV radiation deficiency contribute to an increase in the activity of T-lymphocyte differentiation, which in turn enhances the cellular acquired response. The contribution of the process of lymphoproliferation in men of the subarctic region is 1.6 times higher than the contribution of the process of apoptosis, in contrast to the men of the semi-arid region, in which both contributions are practically equal, which reflects the distinctive features of the functioning of the immune system depending on the place of residence of a person and can contribute to the development of secondary ecologically dependent immune imbalances, including autoimmune diseases, oncopathology, etc.

Moreover, determining the percentage contribution of different stages of the im-

Table 3

Structure of factor variables and coefficient scores of immunological status parameters in men aged 20-60 living in semiarid (1) and subarctic (2) regions

Parameter	Region	Rotated Component Matrix				Coefficient scores			
		Factor				Factor			
		1	2	3	4	1	2	3	4
CD20$^+$	1	**0.94**	0.18	-0.03	0.02	0.367	-0.035	-0.085	-0.09
	2	**0.93**	0.09	0.14		0.233	-0.004	-0.092	
CD71$^+$	1	**0.91**	0.12	0.26	0.01	0.347	-0.090	0.071	-0.106
	2	**0.93**	0.04	0.19		0.221	-0.026	-0.046	
CD95$^+$	1	**0.86**	0.11	0.08	0.24	0.319	-0.097	-0.039	0.051
	2	**0.91**	0.10	0.22		0.212	-0.01	-0.033	
CD3$^+$	1	-0.01	**0.90**	0.08	-0.02	-0.123	0.425	-0.054	-0.122
	2	0.26	0.33	0.54		-0.037	0.041	0.289	
CD8$^+$	1	0.16	**0.87**	0.21	0.20	-0.072	0.349	-0.011	-0.017
	2	-0.04	**0.92**	0.21		-0.069	0.286	0.044	
CD4$^+$	1	0.32	**0.85**	0.22	0.22	-0.006	0.315	-0.015	-0.014
	2	0.05	**0.90**	0.02		-0.006	0.298	-0.104	
CD16$^+$	1	0.53	0.58	0.03	0.28	0.120	0.173	-0.099	0.052
	2	**0.88**	0.06	0.29		0.189	-0.031	0.031	
CD10$^+$	1	0.05	0.07	**0.89**	0.19	-0.055	-0.090	0.427	0.027
	2	0.20	**0.90**	0.05		0.028	0.290	-0.106	
CD5$^+$	1	-0.03	0.16	**0.89**	0.01	-0.081	-0.016	0.433	-0.083
	2	0.07	**0.82**	0.16		-0.026	0.255	-0.001	
HLA-DR$^+$	1	0.28	0.18	**0.69**	0.04	0.054	-0.032	0.312	-0.078
	2	**0.94**	0.08	0.08		0.246	0.000	-0.134	
Eosinophils	1	0.02	0.21	0.03	**0.89**	-0.101	-0.009	-0.083	0.506
	2	0.03	0.02	**0.86**		-0.156	-0.094	0.593	
Monocytes	1	0.26	0.05	0.29	**0.79**	0.018	-0.133	0.062	0.421
	2	0.44	0.12	0.55		0.014	-0.034	0.291	
Neutrophils	1	0.04	0.07	0.00	0.58	-0.042	-0.042	-0.058	0.336
	2	0.19	0.08	0.57		-0.058	-0.044	0.354	



Percentage contribution of immune response stages in men aged 20-60 living in the subarctic and semi-arid regions

mune response will help in assessing the immune status of the population, and will also be useful for predicting possible deviations, manifested by the development of possible pathologies that may appear in the future. In addition to the above, we suggest that the line presented in Figure 1 should have a reference form that should be determined for optimal assessment and interpretation of the immune status of the population.

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BIOCHEMICAL PARAMETERS OF BLOOD OF MAS WRESTLER STUDENTS DURING THE TRAINING PERIOD

DOI 10.25789/YMJ.2023.81.29

УДК 616-092.11; 796.071

The purpose of this work was to evaluate the biochemical parameters of blood in the mass-wrestler students during the training period. 28 students of the NEFU named after M.K. Ammosov, indigenous nationality of the Republic of Sakha (Yakutia), including 17 athletes - wrestlers, took part in the survey on the basis of informed voluntary consent. The biochemical parameters of blood aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), creatine kinase, alkaline phosphatase, gamma-glutamyltransferase (GGT), glucose, total cholesterol, HDL cholesterol, LDL cholesterol, VLDL cholesterol, triglycerides, uric acid, urea, creatinine, total protein, albumin by enzymatic method. Calculated indicators were determined: the de Ritis coefficient (AST/ALT), the index of muscle tissue damage (CPK/AST) and the atherogenicity coefficient. According to the results of the study, the excess of normal indicators of CPK, SCHF and the muscle damage index (CPK/AST) of more than 10 units was revealed.

Keywords: mass wrestlers, biochemical parameters, CPK, AST, ALT.

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Introduction. The ability to accurately quantify the physiological effects of exercise on the human body is crucial to understanding recovery needs and to ensure adequate rest before re-training. The use of biomarkers can improve the ability of trainers to assess the recovery period after training and to establish the intensity of subsequent training in the most effective way [6]. The study of the effect of physical exertion on the activity of intracellular enzymatic profiles specific to certain tissues and organs provides additional information not only about the condition of muscles, but also about its biochemical adaptation to the training process of athletes [5]. Analyzing the dynamics of enzymes under the influence of physical exertion, it is possible to vary exercises of different nature and intensity in such a way as not to cause destructive changes in the body systems [5]. In mas-wrestling, athletes, as a rule, perform exercises for a large number of repetitions to develop strength and muscular endurance of the arms, while often using the "to failure" method. However, the inept use of this method leads to excessive local acidification of the muscles of the hands, which ultimately negatively affects the development of strength and muscular endurance of the hands [2].

The aim of the study was to evaluate the biochemical parameters of the blood of the mass–wrestlers during the training period.

Materials and methods. The survey was conducted on the basis of in-

formed voluntary consent by 28 young students of the M.K. Ammosov NEFU of indigenous nationality of the Republic of Sakha (Yakutia), average age 21.06 ± 1.63 years, of which 17 athletes (sport "Mas-wrestling"), 11 students who are not athletes attending physical education classes twice per week (control group). The study was conducted in full compliance with the ethical recommendations of the Helsinki Declaration of the World Medical Association. Studies of blood biochemical parameters were carried out in the laboratory of the Federal State Medical University "YANC KMP" in conditions of constant quality control. Blood sampling for the study was carried out in the morning on an empty stomach from the ulnar vein. Activity of aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), creatine phosphokinase (CK), alkaline phosphatase (ALP), gamma-glutamyltransferase (GGT), glucose levels, total cholesterol, HDL cholesterol, LDL cholesterol, VLDL cholesterol, triglycerides (TG), uric acid The analysis of urea, creatinine, total protein, albumin was carried out by the enzymatic method on an automatic biochemical analyzer "Labio200" of the company "Shenzhen Mindray Bio-Medical Electronics" (China) using reagents "Analyticon" (Germany). Calculated indicators were determined: the de Ritis coefficient (AST/ALT), the index of muscle tissue damage (CPK/AST) and the atherogenicity coefficient. Statistical analysis of the data obtained was carried out using the IBM SPSS Statistics 23.0 software package. The descriptive analysis data are presented in the table in the form of Me (median), Q1 and Q3 (quartiles 25 and 75%). When comparing quantitative indicators of groups, the significance of differences in abnormal distribution was assessed using the Mann-Whitney U criterion. Correlation analysis of the data was carried out using the Spearman method. The results were considered statistically significant at the values of the achieved significance level $p < 0.05$.

Results and discussion. The biochemical parameters of the blood of the examined students are presented in Table.

A comparative analysis of the levels of biochemical parameters of students - mas-wrestlers with the control group revealed significant differences in the levels of AST ($p<0.01$), LDH, CPK, GGT, HDL-C and de Ritis coefficient ($p<0.05$). The biochemical parameters of the blood of students - mas-wrestlers are in the range of normal values, except for the levels of CPK and alkaline phosphatase. The average values of CPK exceed the physiological norms in both groups of the examined, but in the group of students - mas-wrestlers, an excess of more than 2.5 times is noted. The ALP level was high in half of the students-maswrestlers, which was reflected in the average value. VLDL-C is below the normal range in both groups, HDL-C is below normal in the control group and at the lower limit of normal in student mas-wrestlers. The coefficient of atherogenicity was increased in the control group due to a decrease in the level of HDL-C. AST is a characteristic of thermogenesis, ALT-gluconeogenesis, de Ritis coefficient (AST / ALT) - the ratio of cata- and anabolic metabolic fluxes, GGT-indicator of tissue feeding, ALP-regulator of membrane flows and phosphate potential level (macroerg reserve, power of bioenergetic processes), LDH-regulator of blood pH and redox processes, CK-enzyme "stress" [4].

CPK activity is a biochemical marker regularly analyzed by coaches and sports researchers. It should be emphasized that the activity of this enzyme in the blood of athletes does not always increase immediately after exercise. An increase in CPK activity is often observed during recovery [8]. The peak of CPK is reached 24 hours after the end of training, and CPK activity may remain elevated for 48-72 hours [7]. ALP regulates the content of phosphates in the blood. The higher the level of alkaline phosphatase in the blood, the greater the power of bioenergetic processes and the rate of transmembrane flows [4]. Blood ALP activity is a well-known diagnostic marker of bone mineralization and pathological disorders. Changes in ALP activity after exercise may be useful for assessing early symptoms of some vitamin deficiency in the diet of athletes [8]. Diaz et al. [9] described a correlation between ALP activity and daily intake of vitamin B6 and niacin.

With different intensity of metabolic processes, the predominance of cata- and anabolic metabolic pathways can be judged by the de Ritis coefficient (AST / ALT), the adaptive range of which ranges from 1.2 to 1.6, its reference value is 1.5. In our study, the de Ritis coefficient was below the normal range in the control group of students due to the high value of ALT compared to AST, which indicates the predominance of anabolic processes. For students - mas-wrestlers, this indicator is in the normal range.

During life, ALT and AST are in reciprocal ratios. In the process of skeletal

Biochemical parameters of blood, Me (Q25; Q75)

Indicator, reference values	Students-maswrestlers (n=17)	Control group (n=11)	p
LDH, (225-450 U/l)	428.0 (372.504; 478.50)	364.00 (306.04; 417.0)	0.02
CPK, (< 190 U/l)	285.0 (173.50; 700.50)	168.00 (93.0; 224.0)	0.02
ALP, (< 258 U/l)	257.0 (201.5; 343.0)	225.0 (185.0; 268.0)	0.13
TG, (0.5-1.7 mmol/l)	0.65 (0.55; 1.04)	0.93 (0.51; 0.93)	0.40
GGT, (11 – 50 U/l)	23.0 (18.50; 24.50)	26.0 (23.0; 33.0)	0.02
ALT, (< 30 U/l)	24.0 (19.0; 26.0)	20.0 (14.0; 28.0)	0.43
AST, (< 40 U/l)	31.0 (23.5; 40.0)	21.18±5.87	0.01
de Ritis coefficient, AST / ALT (norm 1.3 - 1.5)	1.29 (1.02; 1.82)	1.0 (0.69; 1.06)	0.02
Index, CPK/AST (c.u.)	14.28 (7.18; 18.52)	6.06 (4.89; 11.79)	0.07
Uric acid,	292.0 (254.50; 329.0)	258.0 (226.0; 319.0)	0.37
(men 268-488 μmol/l)	5.67 (5.07;6.78)	5.14 (4.59; 6.19)	0.37
Urea, (5 - 12.1 mmol / l)	105.0 (95.5; 108.5)	98.0 (92.0; 103.0)	0.20
Creatinine, (50 - 120 μmol/l)	4.90 (4.80; 5.40)	5.0 (5.20; 5.50)	0.24
Glucose, (3.3 - 5.5 mmol/l)	75.40 (72.30; 77.65)	76.20 (72.30; 77.70)	0.78
Total protein, (75 - 85 g/l)	43.40 (42.85; 44.75)	44.70 (43.10; 46.30)	0.17
Albumin, (38 - 42 g/l)	3.97 (3.62; 4.32)	4.10 (3.66; 4.92)	0.48
Cholesterol, (3.6-6.5 mmol/l)	0.89 (0.74; 1.12)	0.63 (0.53; 0.84)	0.01
HDL cholesterol, (0.78-2.2 mmol/l)	2.59 (2.24; 3.12)	2.99 (2.53; 3.66)	0.28
LDL-C, (1.68-4.53 mmol/l)	0.34 (0.25; 0.47)	0.42 (0.23; 0.73)	0.57
Ka, (< 3)	3.0 (2.6; 3.35)	3.10 (2.70; 3.90)	0.458

muscle hypertrophy, in obesity or during pregnancy, ALT activity predominates in this pair. And, vice versa, during intense muscle loads, fasting, fever, during aging or against the background of cachexia, the activity of another transaminase, AST, dominates [4]. With chronic physical activity of moderate and submaximal power, a gradual increase in the activity of enzymes in the blood is observed: CC, LDH, AST, ALT, lactic acid content. Correlation analysis showed that the CPK/AST index has a strong positive relationship with the level of LDH (0.657; p=0.000), and a weak one with ALT (0.432; p=0.022). The de Ritis coefficient had a strong direct correlation with LDH (0.585; p=0.001) and CPK (0.502; p=0.006). Hyperenzymemia can be considered as a "functionally optimal" (adaptive) reaction in response to changes in the living conditions of the organism [1]. Depending on the direction of training loads, the release of the enzyme into the blood from the cell can be due to various reasons, the main of which are mechanical damage to the muscles induced by physical activity and metabolic stress caused by the formation of free radicals during training. A significant increase in enzyme activity against the background of rest after exercise acts as a marker of overtraining [3].

Conclusion. The results of the study indicate that students - mas-wrestlers are characterized by high values of CPK and ALP. An increase in CPK and muscle damage index (CPK / AST) more than 10 c.u. e. in student mas-wrestlers, it can be explained by mechanical damage to muscle fibers when exposed to large volumes of training load. High levels of alkaline phosphatase may be associated with an increase in the power of metabolic processes or a deficiency of certain vitamins in the diet of athletes. Control of the biochemical parameters of the blood of athletes is an important marker for identifying the current functional state of the body.

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CLINICAL CASE

DOI 10.25789/YMJ.2023.81.30

УДК 616.314.21

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CLINICAL CASES OF UPPER JAW CONSTRICTION IN CHILDREN AND ADOLESCENTS DUE TO SEVERITY OF CONNECTIVE TISSUE DYSPLASIA

The problems of improving complex medical and social rehabilitation of children and adolescents with connective tissue dysplasia due to its degree of severity (DCT) have not been completely solved up to the present time. At the same time, insufficient information on the diagnosis of dentition anatomical changes depending on DCT severity has been identified in the research. Thus, we present clinical cases of upper dentition cconstriction in children and adolescents with connective tissue dysplasia at various degrees of severity, taking into account the arch height of the hard palate. The **purpose of the research** is to present clinical cases with pronounced upper dentition constriction in children and adolescents with different severity of connective tissue dysplasia based on the clinical and biometric studies. **Discussion**. We've obtained high values of the sum of the four upper incisors width, characterized as macrodentia in the examined children and adolescents with DCT, which has a direct impact on the deformation of the maxillary dentition. Thus, constriction of maxillary dental arches in mild DCT is 19,32+1,47%, moderate - 22,39+0,72 and severe - 28,52+1,70%, which have significant differences (p<0,05), and the average is at the level of 23,41+0,54%. A certain pattern of increased frequency of upper dentition constriction depending on DCT severity has been established. **Conclusion**. The research clinical results characterize local DCT manifestations of the maxillary dental row in the form of incisor macrodentia as well as its constrictions where the tendency of increasing the incidence

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rate depending on its severity degree has been established in the examined age groups of schoolchildren of the North. The established data of the anomalies increase of the frontal teeth group shape and upper jaw narrowings depending on DCT severity in schoolchildren may become the basis for the improvement of treatment, prophylactic and rehabilitative measures.

Keywords: connective tissue dysplasia, phenotypic features, upper dentition, dental anomalies, diagnosis.

Introduction. Connective tissue dysplasia (DCT) refers to congenital pathologies that are associated with changes in the synthesis and assembly of collagen, elastin, leading to their insufficient crosslinking [4, 22]. At the same time, DCT manifests itself in the form of general and local phenotypic signs, where dental anomalies, Gothic palate, TMJ dysfunctions, periodontal diseases, multiple caries, etc. are most often detected in the oral cavity. [1, 2, 10, 11, 15, 16, 17]. Meanwhile, a certain part of syndromic forms of DCT can lead to a persistent deterioration of health in childhood, which is of medical and social importance [7, 9, 14,18]. Today, the issues of diagnosis, treatment, prevention and rehabilitation of patients with DCT, which remain unresolved, are widely studied [3, 6, 13,21].

It should be noted that most often in the structure of local manifestations, changes in the dentition of the maxilla 6 are detected [5, 8, 12]. In this case, these manifestations are often accompanied by the change in the function of speech formation, the respiratory system, the development of the child, the jugular system. In this regard, various studies aim at improving the quality and availability of medical and preventive measures in patients with DCT [19, 20].

Objective of the research is to present clinical cases with pronounced upper dentition constriction in children and adolescents with different severity of connective tissue dysplasia based on the clinical and biometric studies.

Clinical Case of the Treatment of the Patient with Mild DCT # 1. Patient A., 17 years old, consulted a pediatrician at «Yakutsk Specialized Dental Center". He complaints of dental anomalies, posture disorders. The main diagnosis: osteochondrosis of the thoracic department, scoliosis, platypodia.

During the clinical examination, a pediatrician diagnosed a mild connective tissue dysplasia. During the dental examination, the patient was diagnosed with gothic palate (Fig. 1), narrowing of the upper dental arch, close position of the incisors of the upper and lower jaws, tortoanomaly 11 and multiple dental caries.

The pronounced constriction of the upper and lower jaws proved a mild DCT, taking into account the index of the vault height of the hard palate (1.7 cm), the result of measuring the first premolar of the

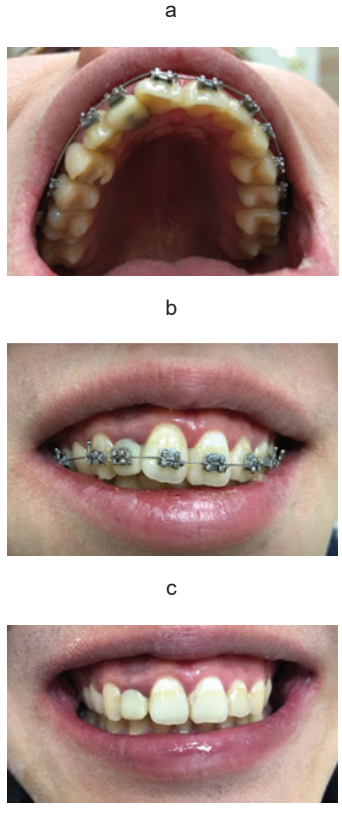

Fig. 1. A patient with narrowing of the upper dentition with a mild degree of severity of DST: a - revealed gothic palate, b - at the stage of corrective therapy with a bracket system on the upper jaw, c - after orthodontic treatment

upper jaw (-2.53 mm), by the first molar (-2,15 mm), the result of measuring the lower jaw between the premolars (-2.22) mm, by the first molar (-3.51 mm).

The braces system was installed with monthly correction during treatment, there was a positive dynamics (Fig. 2). At the end of treatment, the normalization of the occlusion, the expansion of the upper dental arch and the position of the 11 tooth were determined (Fig. 3).

A clinical case of the patient with moderate DCT # 2. Patient B., 15 years old, consulted a pediatrician at "Yakutsk Specialized Dental Center". He complaints of dental abnormalities, postural disorders, hyperextensibility of the skin, epicanthus, chest deformities, adherent earlobes. The main diagnosis: osteochondrosis of the cervical department, vegetative vascular dystonia, epicanthus, scoliosis, platypodia.

During the clinical examination, a pediatrician diagnosed a moderate connec-

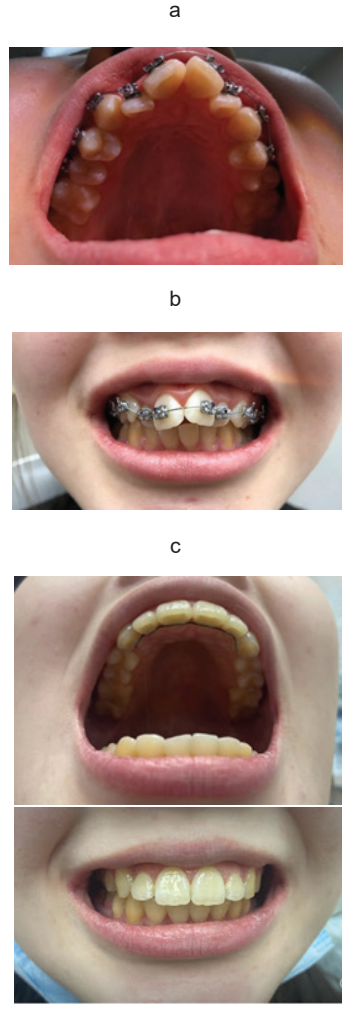

Fig. 2. A patient with narrowing of the upper dentition with moderate severity of DST: a - revealed gothic palate, b - at the stage of corrective therapy with a bracket system on the upper jaw, c - after orthodontic treatment

tive tissue dysplasia. During the dental examination, the patient was diagnosed with gothic palate, distal occlusion, sagittal incisional dysocclusion, narrowing and shortening of the anterior dental arches, close position of the incisors of the upper and lower jaws, macrodentia, multiple dental caries, chronic catarrhal gingivitis and dysfunction of the temporomandibular joint.

The pronounced constriction of the upper and lower jaws was interpreted as the average degree of DCT due to the index of the vault height of the hard palate - 2.1 cm, the results of measurements of the first premolar of the upper jaw - 2.74 mm, according to the first molar -

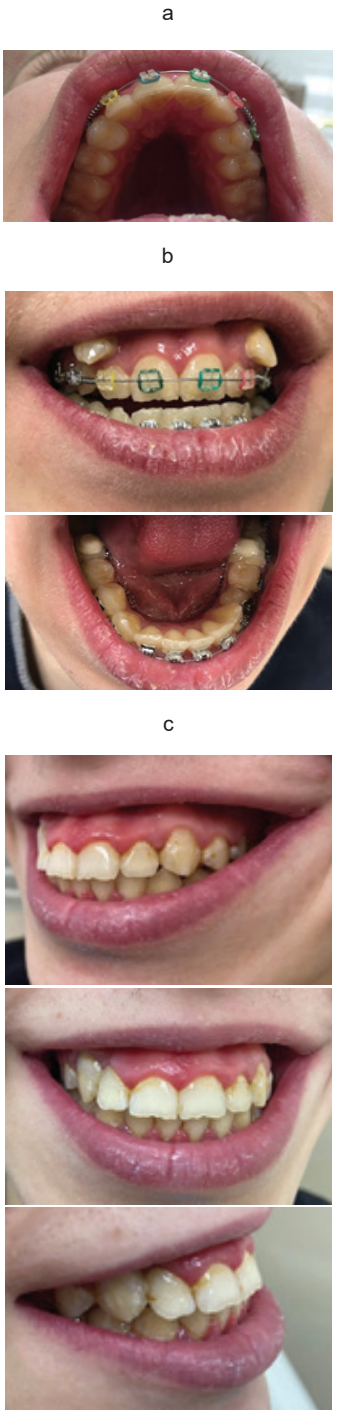

Fig. 3. A patient with narrowing of the upper dentition with severe severity of DST: a - revealed gothic palate, b - at the stage of corrective therapy with a bracket system on the upper jaw, c - after orthodontic treatment

(-2.55) mm, and on the lower jaw the result of measurements between the premolar - (-3.21) mm, by the first molar - (-6.21) mm.

The bracket system was installed on the upper jaw (Fig. 4) with positive dynamics at the stage of treatment (Fig. 5). At the end of treatment, the normalization of occlusion, the expansion of the upper dental arch and the position of the incisorse were determined (Fig. 6).

A clinical case of the patient with severe DCT No. 3. Patient V., 16 years old, consulted a rhematologist at «Yakutsk Specialized Dental Center". He complaints of dental abnormalities, postural disorder, hypertensiveness of the skin, deformity of the chest, hypermobility of the joints. The main diagnosis: osteochondrosis of the cervical and thoracic parts, blue sclera, saddle-shaped nose, keeled chest, vegetative dystonia, mitral valve prolapse and temporomandibular joint dysfunction.

During the clinical examination, the pediatrician diagnosed severe connective tissue dysplasia. During the dental examination, the patient was diagnosed with gothic palate, mesial occlusion, reverse incisional occlusion, narrowing and shortening of the anterior part of the dental arches, shortening of the lateral parts of the upper dental arch, close position of the lower incisors, vestibulosupposition of the upper canines, macrodentium, multiple dental caries, chronic catarrhal gingivitis and temporal dysfunction mandibular joint.

The pronounced constriction of the upper and lower jaws of severe DCT were interpreted due to the index of the vault height of the hard palate - 3.1 cm, the results of measurements of the first premolar of the upper jaw - 6.02 mm, according to the first molar - (-6.54) mm, and on the lower jaw the result of measurements between the premolar - (-7.62) mm, according to the first molar - (-8.82) mm.

The braces system was installed (Fig. 7) during treatment, there was a positive ddynamics (Fig. 8). At the end of treatment, normalization of occlusion, dilation of the upper dental arch and normalization of the position of 1.3, 2.3 and frontal teeth were determined (Fig. 9).

Conclusion. The analysis of these clinical cases allows us to determine the existence of a direct relationship between the frequency increase of changes in the maxillary dentition depending on the severity of DCT. Typical local manifestations of DCT in the examined age groups of schoolchildren of the North in the form of macrodentia of incisors of the upper jaw are specific regional risk factors for the development of dentofacial anomalies. The research data of the increase of the shape abnormalities of the frontal group of teeth and constriction of the maxillary dentition depending on the severity of DCT in schoolchildren can become the basis for improving medical, preventive and rehabilitation measures.

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A CLINICAL CASE OF SUCCESSFUL APPLICATION OF VACUUM THERAPY IN THE TREATMENT OF POSTOPERATIVE STERNOMEDIASTINITIS

DOI 10.25789/YMJ.2023.81.31

УДК 617.541.1-06:616.9-085.835.14

The article presents observation of a clinical case of postoperative sternomediastinitis. The technique of using vacuum therapy in the treatment of purulent wounds is highlighted. The authors described the possibility of using vacuum therapy without performing the final reconstructive operation in a patient with severe concomitant pathology.

Keywords: sternomediastinitis, vacuum therapy, thoracic surgery, cardiac surgery.

Due to the rapid development of cardiac surgery in the late 20th century, the number of studies devoted to postoperative sternomediastinitis significantly increased [1]. At present, cardiosurgical patients are people of senile age with a significant comorbid background determining a great number of risk factors for complicated tissue healing in the surgical access area [9]. Poststernotomy mediastinitis aggravates the patient's clinical condition and increases the duration of treatment [8], while the long-term expensive treatment of postoperative complications of cardiac surgery raises the question of the cost component [10]. The cost of treatment of a deep postoperative sternal infection is excessively high doubling the cost of the overall treatment of cardiac patients [10] and reaching $500,000 in specialized centers of the United States [11], which even with an infection rate of less than 1% presents quite an impressive cost for any country.

At present, the most prevalent management of sternomediastinitis is a two-stage treatment [3, 5], including early initial surgical d-bridement of the wound [15], the process of preparing the wound for reconstruction, which is most often carried out using vacuum therapy [7] and performing reconstructive surgeries preserving the sternum tissue [14] or its complete removal [12]. The stage-by-stage approach to the treatment is determined by the severe condition of the patient, presence of concomitant pathology [2] and, clearly, by the bacterial contamination of the wound. At this, the final reconstructive surgery most frequently consisting of the extirpation of the sternum and plasty of the anterior thoracic wall defect is rather traumatic and long [5].

Widespread application of the vacuum therapy in the interstage period leads to the decrease in the percentage of cases requiring final reconstructive surgery from 42.8% to 25% as well as to reduce the rate of postoperative complications following the final chest wall reconstruction from 28.6% to 7.1% [13].

According to some observations, vacuum therapy has been sufficient for the treatment of the postoperative sternal infection with no need for further reconstructive surgery, whereas the duration of the treatment increased [6].

Vacuum therapy is an innovative method of treating wounds of various etiology accelerating the course of the wound process. For the application of a vacuum dressing, a hydrophilic polyurethane sponge, a sealing film coating, a drainage tube and a vacuum source with a container to collect the liquid are most often used. Devices for vacuum therapy are capable of creating and maintaining negative pressure in the wound for a long time in a constant or intermittent mode with a pressure in the range from 50 to 200 mm Hg. Art., with the optimal level of negative pressure in the wound being considered 125 mm Hg. [4].

A clinical case of a comorbid female patient with a severe concomitant pathology who was given vacuum therapy without reconstructive surgery can be considered as an example.

Female patient named K. aged 68 was admitted to the department of the thoracic surgery of the Regional Clinical Hospital №1 of the Ministry of Health of the Khabarovsk Region on October 11, 2010. According to her past medical history, on August 12, 2021, she underwent a simultaneous surgical intervention using a transsternal approach and laparotomy access involving tricuspid valve plasty and inferior vena cava thrombectomy. The patient had right-sided nephrectomy for the right kidney cancer, tumor thrombus of the right renal vein and inferior vena cava, recurrent renal bleeding, and acquired tricuspid valve disease. In addition, the patient had ischemic heart disease involving stable exertional angina FC II. Postinfarction cardiosclerosis was noted (lower wall myocardial infarction occurred in June, 2021). Obliterating atherosclerosis of coronary arteries was characterized by diffuse lesion of the 50% stenosed anterior descending and circumflex artery, and occlusion of the small right coronary artery (CAG, August 05, 2021). Indicators such as paroxysmal atrial fibrillation in the absence of a paroxysmal event, the risk of thromboembolic complications according to the CHA2DS-VASc score of 6, risk of bleeding according to HAS-BLED score of 2 points. EIT No. 1 360 J. was performed on August 20, 2021. The patient had Stage III hypertension, 1-st degree arterial hypertension, risk IV, CHF 2A FC II according to NYHA. Other diagnoses included CKD 3B, Type 2 Diabetes mellitus, Diabetic macro-microangiopathy, Anemia of mixed origin. Intraoperatively, during cardiac surgery, the patient had blood loss of up to 4000 ml, which required cardiopulmonary bypass and blood transfusion. In the early postoperative period, respiratory and cerebral insufficiency, acute kidney injury, metabolic disorders, hyperglycemia were present. The heart rhythm was impaired. The patient received prolonged artificial lung ventilation, sessions of renal replacement therapy and transfusion of blood components on August 20, 2021. The cardiac rhythm was restored by electropulse therapy. The patient was

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discharged on August 31, 2021. 20 days after discharge, a swelling appeared in the lower corner of the postoperative scar, later the swelling opened up on its own with the appearance of a discharge. The patient was referred to the emergency room of the Regional Clinical Hospital №1 by a general surgeon, being examined by a thoracic surgeon on October 8, 2021. Hospitalization was recommended. The patient was admitted to the emergency room on October 11, 2021. Spiral Computer Tomography (SCT) of the chest was performed. According to the SCT (performed on October 11, 2021), subcutaneous air accumulation sized 36x20 mm in the sternum manubrium area, and subcutaneous emphysema of the soft tissues of the right chest were revealed. There was a sternal bone rarefaction, diastasis of the sternum up to 15 mm along its entire length, right-sided hydrothorax (Fig. 1, 2).

After preliminary preparation, on October 21, 2021 an operation was performed - surgical treatment of the wound of the sternum (Fig. 3, 4). Opening of the wound, debridement, necrectomy. According to intraoperative picture, the sternal wound dehiscence is observed along the entire length of the sternum, the right half is practically absent, diastasis is up to 1.5 cm, along the median line an abundant amount of fibrin and purulent discharge are present, cultural flora revealed St. Epidermidis β-hemolytic strain in the amount of CFU 10^4.

Further on, within a week, the wound of the sternum was kept open during treatment, dressings were performed (Fig. 5.), all over the wound there was an abundant exudation.

From October 28, 2021 the patient received sternomediastinitis therapy using vacuum aspiration (Fig.6). Vacuum dressing was changed in the operation room every three days with the removal of necrotized tissues, defibrinization and control of the wound for bacteria. Culture of the wound discharge taken on November 5, 2021 showed the decrease in the microbial wound load (St. Epidermidis β-hemolytic strain in the amount of CFU 10^2).

Considering the patient's comorbid background and contraindications for reconstructive surgery, a decision was made to continue conservative treatment of the patient using vacuum therapy without extirpation of the sternum and thoracoplasty. A standard mode vacuum therapy was continued. For the application of a vacuum dressing, a hydrophilic polyurethane sponge, a sealing film adhesive coating, a drainage tube and a vacuum

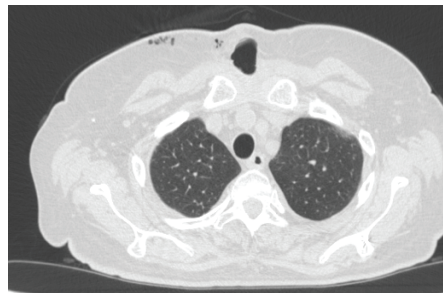

Fig. 1. Sagittal plane of the thoracic cage. Accumulation of gas is determined in the prethoracic area

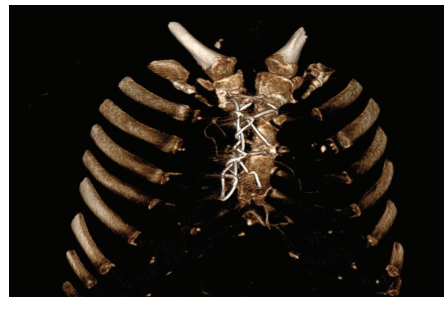

Fig. 2. 3D reconstruction of the chest, diastasis of the sternum is determined, the right part of the sternum is practically absent

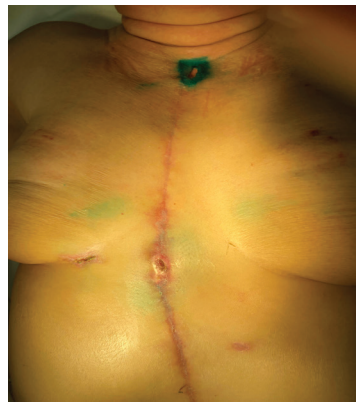

Fig. 3. Postoperative wound before surgical interference

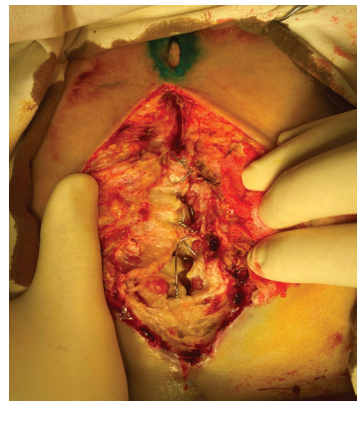

Fig. 4. The wound after opening (the sternal tissues are edematous, bone lesion is determined)

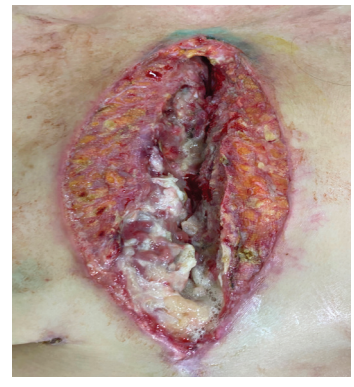

Fig. 5. Wound appearance before using vacuum aspiration.

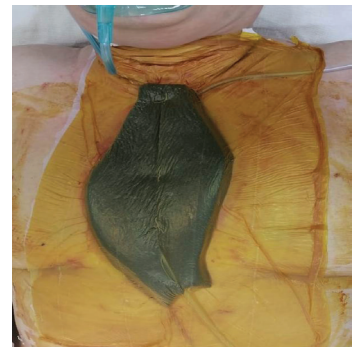

Fig. 6. The appearance of the patient with a vacuum aspiration system applied to the chest (October 28, 2021r.)

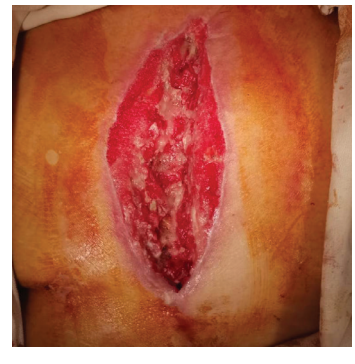

Fig. 7. Wound appearance (November 5, 2021): exudation stage cessation

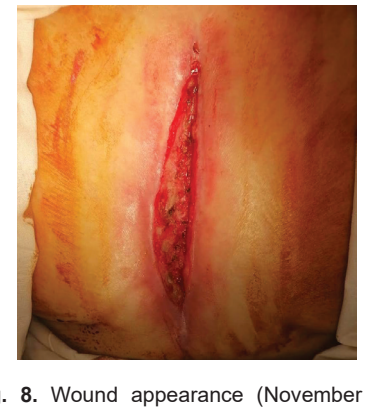

Fig. 8. Wound appearance (November 28, 2021): healing phase

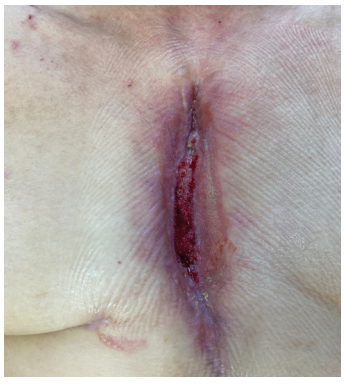

Fig. 9. Wound appearance (December 16, 2021).

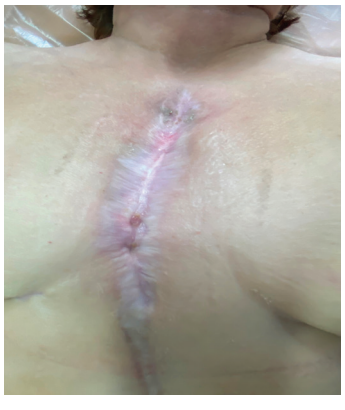

Fig. 10. Wound appearance on completion of the treatment (February 28, 2021): complete scarring of the thoracic wall wound.

source with a container for collecting fluid are most often used. Devices for vacuum therapy are capable of creating a negative pressure in the wound of 100 mm hg. crt. in continuous mode. Against the background of the therapy, the patient showed positive dynamics, she became more active, the thoracic wall wound cleared of fibrin having significantly decreased in size. On November 28, 2021 vacuum aspiration system was replaced (Fig.8). On November 29, 2021 the patient was discharged from the hospital with recommendations to continue vacuum therapy at the outpatient department.

The system of vacuum aspiration continued to be used in conditions of an outpatient department till the slit-shaped superficial wound of the thoracic wall was formed which is essentially only a skin defect (Fig. 9).

Afterwards, the wound was managed openly with ointment-based dressings until the wound defect was fully epithelialized. The appearance of the wound at the end of treatment is shown in Fig. 10.

Conclusion: Based on the clinical observation presented, we can conclude that in cases where the highly traumatic reconstructive stage of surgical treatment is contraindicated due to concomitant pathology, it is appropriate to use the possibility of vacuum therapy to achieve the healing and complete scarring of the chest wall wound.

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DOI 10.25789/YMJ.2023.81.32

УДК 616.514.4-07

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A RARE CASE OF THE CUTANEOUS FORM OF MASTOCYTOSIS IN A SAKHA CHILD

The article is devoted to a rare disease which is mastocytosis in a child. In Russia, this disease is registered with a frequency of 0.12–1 case per 1000. At the moment, the etiology and the pathogenesis of the disease are not fully understood. Mastocytosis is a rare disease with a favorable prognosis in children. Due to the small occurrence, diagnosis and treatment are often difficult. All children diagnosed with mastocytosis should be registered at a dispensary due to the possibility of transition to a systemic option at an older age. The article presents a clinical case of mastocytosis in a Sakha child.

Keywords: mastocytosis, rare disease, dispensary registration, allergy, urticaria, consultation, observation.

Introduction. Mastocytosis is a heterogeneous group of rare diseases of myeloproliferative nature in which there is excessive accumulation and proliferation of mast cells in tissues and organs. In Russia, the incidence is 0.12-1 case per 1,000 patients [5,6]. Despite the heterogeneity of the clinical picture, the leading link in the pathogenesis of mastocytosis is occupied by molecular and genetic mechanisms [4]. According to the opinions of a number of authors, mastocytosis in children is a temporary manifestation of mast cell hyperresponsiveness, which debuts at an early age, proceeds as skin forms and spontaneously regresses when the child reaches pubertal age [1,2,3,5]. Despite the available specific criteria and recommendations, the diagnosis of mastocytosis in Russia is fraught with certain difficulties. Considering the peculiarities of the course of the disease, namely its tendency to spontaneous self-resolution in children, patients with mastocytosis require consultative care first, and then medication [5,6]. Timely consultation of the patient about the disease avoids delaying treatment, as well as worsening the course of the disease, which has a positive impact on the quality of life of patients.

The clinical example: The boy named I., of Sakha origin, was born as the first child in a family in one of the Arctic regions of Yakutia. The weight at birth was 3200, the height was 50 cm. The pregnancy proceeded smoothly. The childbirth was on time and independent. The Apgar score was 8/9. Neonatal jaundice up to 1 month was registered during his neonatal period. He grew and developed during the first year according to his age. He was breastfed until the age of 1 year. Hereditary history was not aggravated. Mother had atopic dermatitis.

At the age of 9 months, the child had a rash on the face, back and chest: elements of 0.5-0.7 cm in diameter, irregularly shaped, scarlet-red color, prone to merging, rising above the skin surface. The rashes periodically reddened and swelled when rubbing, bathing in warm water. The pediatrician considered these rashes to be hemangiomas.

In August 2022, on the recommendation of the district pediatrician, the child was referred for examination to the Pediatric Center of the Republican Hospital No. 1-NCM.

The child was examined by an allergologist-immunologist. His condition was estimated as satisfactory. His well-being was not impaired. On examination of the face, chest and back, there were multiple brownish-colored itchy spots or papules of oval or circular shape, merging with each other in some places. Regional lymph nodes were not palpated. There was no fever. Pharynx was calm. Peripheral lymph nodes were not enlarged. Nasal breathing was not obstructed, no discharge. In the lungs breathing was conducted in all parts, vesicular, no rales were audible. Respiratory rate (BFR) was up to 22-24 per minute. Heart tones were clear, rhythmic, no murmurs were audible. Heart rate was 118-122 beats per minute. The abdomen was soft and painless. The liver and spleen were not enlarged. Stool and diuresis were not disturbed.

A preliminary diagnosis was made: Mastocytosis. Cutaneous form.

Paraclinically: in the general blood count: hemoglobin (HGB) - 122 g/l (RI: 120-160 g/l); red blood cells (RBC) - 4,5x10^{12}/l (RI: 4.1-5.2x 10^{12}/l); platelets (PLT) - 250x 10^{9}/l (RI: 150 - 450x 10^{9}/l); white blood cells (WBC) - 4.8x 10^{9}/l (RI: 4.5 - 13x 10^{9}/l); lymphocytes (LYMF) - 37% (RI 38-72%); monocytes - 1% (RI:2-10%); stab neutrophils - 2% (RI: 1-5%); segmented neutrophils - 48% (RI: 43-60%); eosinophils - 12% (RI: 0-5%); determining the ESR by the Panchenkov method -10 mm/h (RI: 1-15 mm/h). According to the general blood analysis, eosinophils increased.

Biochemical blood count: total protein 60g/l (56-75 g/l); albumin, 40 g/l (37-55 g/l); alanine aminotransferase (ALT), 43.43 U/L (less than 40 U/L); aspartate aminotransferase (AST), 36.4 U/L (less than 40 U/L); blood glucose, - 4 mmol/L (3.3-6.1 mmol/L); total bilirubin, - 5.2 mol/L (3.4-17.1 μmol/L), creatinine, - 38.5 μmol/L (35-110 μmol/L), urea, - 3.5 mmol/L (4.3-7.3 mmol/L). Immunogram results: immunoglobulin A - 2.3 g/L (RI: 0.21-2.82g/L); immunoglobulin M - 0.68 mg/mL (RI: 0.47-2.40 mg/mL); immunoglobulin G - 12.7 mg/mL (RI: 4.83-12.26 mg/mL); immunoglobulin E - 150 U/mL (RI: 0-60 U/mL); CD3+ - 65.00%(RI: 62.0-69.0%); CD4+ - 35. 00% (RI: 28.1-65.0%); CD8+ - 29.00% (RI: 26.0-68.0%). Conclusion: There was a sharp increase in the content of immunoglobulin E.

The level of total immunoglobulin E was 340 IU/ml.

Antibodies to giardia and Helicobacter pylori were not detected.

Allergoscreen: milk - 1.5 IU/ml.

Abdominal ultrasound examination: slight increase in the size of the spleen (56mmx21mm), which was in line with age standards.

Determination of the level of total tryptase in blood serum in the laboratory "Hemotest" ImmunoCAP - 20 ng/ml. Conclusion: the level of total tryptase was elevated, which may indicate cutaneous mastocytosis.

Dermatologist consultation: there were numerous yellow-pink, urtic, itchy

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spots and papules on the skin of the trunk and extremities. On the skin, when rubbing the elements, their reddening and blistering were noted, which indicated a positive Darier-Unna symptom.

The diagnosis was made on the basis of major criteria: characteristic clinical picture of the rash and positive Darier-Unne's symptom, as well as on the basis of laboratory tests: increase of total tryptase (20 ng/ml) in blood serum and data of instrumental methods of examination: increase of spleen. No manifestations of systemic mastocytosis were revealed. Clinical diagnosis: Mastocytosis, cutaneous form. Pigmented urticaria.

The child was prescribed antihistamines (zirtek drops 5 drops 2 times a day for 30 days), external treatment (emollients and root protectors - emolium cream, atopik, locobase ripea).

Recommendations: Dispensary observation by a pediatrician, allergologist-immunologist and dermatologist. Hypoallergenic diet: exclude cottage cheese, cheese, beef. Medical therapy: antihistamines for up to 1 month. The treatment with emollients and root protectors.

Conclusion: Cutaneous mastocytosis is a rare disease with a relatively favorable prognosis in children. Due to low incidence, diagnosis is often difficult. Therefore, we present a clinical case of a child with the cutaneous form of mastocytosis. All children with mastocytosis should be registered by a pediatrician, an allergist and a dermatologist because of the possibility of transition to the systemic form at an older age.

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DOI 10.25789/YMJ.2023.81.33

УДК 616.858-008.6

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PARKINSON DISEASE AND ISCHEMIC STROKE (CLINICAL CASE)

Parkinson's disease (PD) – is a chronic neurodegenerative disease that ranks 2nd in prevalence in the world and has a steadily progressive course. It is clinically manifested by motor disorders in the form of hypokinesia, muscle rigidity and/or rest tremor. In addition, patients have non-motor symptoms, some of which may occur long before the development of typical motor manifestations. Cerebrovascular diseases are in the first place in terms of mortality and disability. A number of studies have revealed that PD reduces vascular risk factors due to low activity of the sympathetic part of the autonomic nervous system, disorders of the hypothalamic-pituitary-adrenal axis, as well as due to treatment with dopaminergic drugs. Contradictory results are expressed by a number of other authors, according to which PD is associated, on the contrary, with an increase in the risk of stroke. This article presents a clinical case of a patient with an established diagnosis of PD who has developed an ischemic stroke. The possible mechanisms of the combination of two diseases, the influence of the neurodegenerative process on the recovery processes and the timing of hospitalization are discussed.

Keywords: Parkinson disease, ischemic stroke, non-motor symptoms, cognitive impairment.

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Introduction. Parkinson's disease (PD) – is a chronic neurodegenerative disease associated with the loss of dopaminergic neurons in the striatum with the accumulation of Levi bodies [1, 2, 5]. Several factors play a role in the development of PD, including genetic predisposition and environmental factors [1, 2]. PD ranks 2nd in prevalence among neurodegenerative diseases after Alzheimer's disease, reaching 1% in the group of people over 60 years old and up to 4% in people over 75 years old [10]. It is clinically manifested by motor disorders (hypokinesia, muscle rigidity, rest tremor) and a wide range of non-motor symptoms (affective, cognitive, sensory, vegetative, and others) [6, 8].

PD can occur in combination with other neurodegenerative and/or vascular diseases of the brain, including acute disorders of cerebral circulation (ONMC) [4, 9]. Stroke is the most important medical and social problem of the elderly, is in the first place in terms of prevalence, mortality and disability [3, 11]. The combination of PD and cerebrovascular diseases can vary from 8.6% to 12% [4]. There is a lot of contradictory data about the relationship between BP and ONMC. It has been established that PD reduces the activity of the sym-

pathetic part of the autonomic nervous system, thereby reducing the frequency of vascular risk factors, especially when treated with dopaminergic drugs [7]. According to G. Scigliano and colleagues (2006), the reduction in the risk of vascular catastrophes in patients with PD is explained by a violation of the hypothalamic-pituitary-adrenal axis [17]. In 2009, Scigliano Ji, Ronchetti and co-authors in a retrospective study revealed that taking levodopa drugs is associated with a decrease in vascular risk factors [18]. On the contrary, a 2013 study by the authors of Hong Kong University showed that PD is associated with an increased risk of ischemic stroke and higher stroke-related mortality [16]. Scientists from the UK came to similar results in 2020 [13]. Thus, both the risks of developing ONMC and the very course of stroke in patients with PD probably have their own characteristics. In this article, we present our own clinical observation of a patient suffering from PD for a long time, who developed an ischemic stroke.

Clinical case: Patient N., 78 years old, has been suffering from Parkinson's disease for the last 4 years. The disease began with a tremor of rest of the right hand, hypokinesia on the right. Considering the age of onset of the disease (after 70 years) drug therapy was started with levodopa preparations (levodopa / carbidopa) with titration up to 750 mg / 75 mg per day in three doses with a positive effect in the form of a significant reduction in tremor, hypokinesia. However, despite the therapy, the disease had a progressive course with the addition of symptoms on the left side.

On September 11, 2022, there was acute weakness and numbness in the left extremities. Within 3 hours after the onset of the disease, she was hospitalized in the Regional Vascular Center of the Republican Hospital №2 – the Emergency Medical Center of Yakutsk with ischemic stroke in the basin of the right middle cerebral artery.

Chronic diseases: a long-lasting persistent form of fibrillation-atrial flutter, tachysystolic variant. Sinus node weakness syndrome. Condition after implantation of an electrocardiostimulator (2017). Coronary heart disease. Angina pectoris of tension 2 FC. Hypertension stage 3. Arterial hypertension of the 3rd degree. Risk of MTR 4. Atherosclerosis of the arteries of the lower extremities.

Constant medication: rivaroxaban 20 mg, bisoprolol 2.5 mg, levodopa / carbidopa 750 mg / 75 mg per day in three doses.

Neurological status upon admission: Clear consciousness. The contact is complete. The behavior is calm. The eye slits are equal, the pupils are equal. The movement of the eyeballs in full, there is no nystagmus. The exit points of the branches of the trigeminal nerve are painless. Sensitivity on the face is reduced on the left. The nasolabial fold on the left has been smoothed. The pharyngeal reflex is alive. The tongue deviates to the left. Left-sided hemiplegia. Muscle tone is higher on the left, increased on the right by the type of "gear wheel", oligobradykinesia, rest tremor on the right by the type of "coin counting". Tendon reflexes are alive, D>S. Babinsky's symptom on the left. The rigidity of the occipital muscles is negative, Kernig's symptom is 90 on both sides. Hyperkinesis is absent. Coordination tests on the left does not perform due to paresis, on the right performs well. Left-sided hemihypesthesia. Mild dysarthria.

NIHSS – 13 points, Com Glasgow scale – 15 b, Rankin scale – 4 points, Rivermead scale – 0 b.

Neuropsychological status: MoCA – 11/30 (decrease due to the test of drawing hours, visual-constructive skills, decreased attention, phonetic activity of speech, abstraction, memorization of 5 words). The drawing test of the clock is 1/3 points, non-abstract (a symptom of ignoring the left side). According to the hospital Anxiety and Depression Scale (HADS) test, the anxiety level is 8 points, the depression level is 8 points. The Epworth sleepiness scale is 0 points. The scale of non–motor symptoms of BP NMSQuest is 10 points (unexplained pain, unexplained weight fluctuations, decreased attention, memory, feeling of sadness, anxiety, falls, intense dreams, talking, unpleasant sensation in the legs).

Computed tomography of the brain at the arrival of signs of hemorrhage, ischemic changes were not revealed.

In the general blood test: leukocytes – 9,3x10^9/l, erythrocytes – 3,6x10^{12}/l, hemoglobin – 110 g/l, platelets – 152 10^9/l, ESR – 34 mm/h.

Lipid profile: cholesterol – 5.9 mmol/l; HDL – 1.49 mmol/ll; LDL – 3.96 mmol/l; TG – 0.95 mmol/l.

Ultrasound of the brachiocephalic arteries revealed thrombosis in the bifurcation area of the common carotid and internal carotid arteries on the right. Stenosis in the bifurcation area of the common carotid artery on the left up to 27%, at the mouth of the internal carotid artery on the left up to 45%, at the mouth of the external carotid artery on the left up to 30%.

Taking into account the anamnesis of the disease, the clinic, computed tomography data, thrombosis in the area of bifurcation of the common carotid artery, an ischemic stroke was diagnosed in the basin of the right middle cerebral artery from 11.09.2022, of unknown etiology according to TOAST.

Thrombolysis was not performed due to coagulogram parameters (APTT is higher than the reference values of 53.7 sec).

The patient had an attempt of thromboextraction.

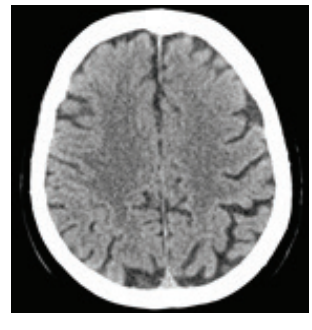
The next day after hospitalization (12.09.2022) there is no positive dynamics, left-sided hemiplegia persists.

On the CT scan of the brain from 12.09.2022, the appearance of a hypodensive area in the frontal-parietal region on the right is noted (Figure).

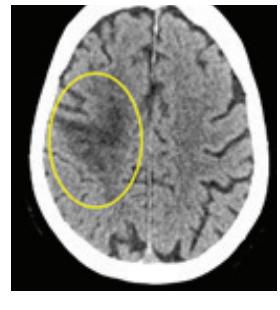
During the stay in the hospital, minimal dynamics is noted: minimal movements appeared in the left extremities. There were 23 bed days in the department. Taking into account the minimal positive dynamics, the increase in strength in the left extremities to 1.5 points was directed to the 2nd stage of rehabilitation. During his stay in the hospital, antiparkinsonian therapy was continued.

Discussion. A clinical case of a patient with ischemic stroke suffering from PD for a long time is presented. The cause of the stroke could be both car-

A



B



CT scan of the head of patient L.: A – structural changes are not detected in the images from 11.09.2022 (3 hours after the onset of symptoms); B – in the images from 12.09.2022, the zone of acute ischemia in the frontal-parietal region on the right is revealed

diogenic embolism and stenosis-thrombosis of the brachiocephalic arteries. In addition to increasing the length of hospitalization of patients with PD and stroke, they may have more frequent development of systemic complications and post-stroke cognitive impairment.

Given the increasing numbers of PD and stroke, meta-analyses were conducted to identify the relationship. The authors report that PD and stroke have common pathogenetic connections, as evidenced by age dependence, general mitochondrial dysfunction, and the fact that after a stroke, Parkinsonism syndrome occurs, as well as the disease itself [12]. In another cohort study, the authors found that PD affects the length of hospital stay, increases the risk of developing pneumonia, sepsis, reduces the risk of death in the short term, but leads to an increased risk 1 and 3 months after a stroke [15]. In a randomized large-scale study, it was revealed that PD has a significant causal relationship with ischemic stroke, namely with two types of stroke: cardioembolic and atherothrombotic variants [14].

Many aspects of the combination of PD and cerebrovascular diseases still remain open. For example, there is no consensus on the impact of PD on stroke risks, what are the exact mechanisms of the relationship between the two diseases. However, there is no doubt that in patients with PD, recovery will be worse and slower.

The authors declare that there is no conflict of interest.

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