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

2(82) `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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Address:
677000, Yakutsk, Yaroslavsky, 6/3,
Phone: (4112) 31-9394,
e-mail: yscredactor@mail.ru
ymj-red@mail.ru
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DOI 10.25789/YMJ.2023.82.01

УДК 57.084.1

MODELING OF LIVER HYPOXIA IN AN *IN VIVO* EXPERIMENT

We studied an effect of the portal triad blood flow reduction on liver enzymes, the organ status and survival of laboratory mice. The study included Balb/c mice divided into groups (n=6 for each group): group 1 – controls, blood and liver were collected; group 2 – liver blood flow reduction for 20 minutes, blood and liver were collected 2 hours after the procedure; group 3 – liver blood flow reduction for 20 minutes, blood and liver were collected 24 hours after the procedure; group 4 – liver blood flow reduction for 20 minutes, and the follow-up during the next 14 days to assess the survival. The blood flow was reduced by occlusion of the portal triad: the animals underwent laparotomy, then the portal triad was isolated and a needle with suture material was brought under it; weights were attached to the ends of the suture material, and the vessels were occluded due to the tension of the ligature. The levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in both groups with reduced blood flow were statistically significantly higher than in the control group ($p<0.05$), and also statistically significantly higher in the group with blood sampling 24 hours after reperfusion, compared to the group with blood sampling 2 hours after reperfusion ($p<0.05$). Histological examination showed signs of ischemic damage in tissues, and an increase in the number of vessels in liver samples of animals with blood flow reduction, compared to the control. The animal survival after the procedure was over 80%, which was satisfactory, but nevertheless indicated the need for such a number of animals that will allow statistical processing of the results even if some animals die. The results of the study demonstrated the model as an important tool for the study of ischemic and hypoxic-associated pathological liver states.

Keywords: hypoxia, oxygenation, liver, liver diseases, *in vivo* models.

Introduction. Insufficient supply of tissues with oxygen and the resulting hypoxia promote many diseases, and in some cases they are the main factors in the development of some pathological conditions [3, 7, 10]. Inadequate tissue oxygenation leads to oxidative stress, inflammatory processes, and dysfunction of key units in lipid and protein metabolism [6, 9]. Recent studies have demonstrated the importance of hypoxia in the development of liver diseases, such as steatosis, fibrosis, cirrhosis, and hepatocellular carcinoma [9]. In addition, cancer studies revealed the key role of the hypoxic microenvironment in the formation of a metastatic niche, epithelial-mesenchymal transition, resistance to therapy, and a more malignant phenotype of tumor cells [8].

National Medical Research Centre for Oncology: **GONCHAROVA Anna Sergeevna** – PhD in Biology, Head of Experimental Laboratory Center, fateyeva_a_s@list.ru; **GUROVA Sofia Valerievna** – junior researcher, gurova.sophie@gmail.com; **KECHERYUKOVA Takhmina Mazhitovna** – Physician in roentgen endovascular diagnostics and treatment, tkecheryukova@mail.ru; **MAKSIMOV Aleksey Yurievich** – Deputy General Director for Prospective Scientific Research, D.Med.Sci., m.d.n.maksimov@yandex.ru; **LAPTEVA Tatiana Olegovna** – Head of Pathoanatomical Department, lto-96@yandex.ru; **ROMANOVA Maria Vadimovna** – junior researcher, m.v.mindar@gmail.com; **KHODAKOVA Darya Vladislavovna** – junior researcher, khodakovaDV@yandex.ru; **ALLILUEVA Ekaterina Vladislavovna** – junior researcher, katherine_bio@mail.ru

To understand the course of various biological processes and the influence of the environment, the use of the "corresponding microenvironment – cell culture" system is the first step towards obtaining fundamental ideas about the complex interaction between the microenvironment and cells [2, 5]. 2D cell culture has been an important tool for understanding the mechanisms of cell behavior *in vivo* for more than a century; however, 2D culture fails to replicate the physiology of real tissues. Culturing of cell monolayers leads to a change in the tissue-specific architecture (forced polarized adhesion, a flattened shape), transformation of mechanical and biochemical signals, and a two-dimensional contact with neighboring cells [4]. In this regard, an *in vivo* study is required to confirm the phenomenon or mechanism observed *in vitro* [1, 4]. There are various experimental approaches to control oxygenation in *in vivo* studies of the hypoxia effects, including the use of special chambers and exposure of animals to a gas mixture with a reduced concentration of O2, temperature modification, pharmacological drugs or substances, and reduction of blood flow to study area [5]. One of the most effective methods of liver hypoxia modeling involves clamping blood vessels, namely, occlusion of the portal triad, including the hepatic artery, hepatic vein, and bile duct [11]. However, the method is technically complex; in addition, we have not found literature data on the safe duration of reduced blood flow in the liver, since a long-term study implies high survival of laboratory animals after the procedure, and the preservation of the main functions of the liver during several days to several weeks.

In this regard, the purpose of this study was to reveal the effect of portal triad occlusion on the liver state and survival of laboratory mice.

Material and methods. *Animals and their maintenance.* The study included female Balb/c mice aged 9-12 weeks with an average weight of 25 g. The animals were bred in the vivarium of the Experimental Laboratory Center, National Medical Research Centre for Oncology. Mice were kept in conventional open cages with free access to food and water. All manipulations in the study were performed in accordance with the ethical principles established by the European Convention for the Protection of Vertebrate Animals used for Experimental or other Scientific Purposes (ETSN 123, Strasbourg, March 18, 1986). The study protocol was approved by the local bioethical committee of the National Medical Research Centre for Oncology.

Technique for reducing blood supply to the liver by the portal triad occlusion. Liver blood flow was reduced using the technique proposed by Zimmerman et al. (2012) [11]. Laparotomy was performed to provide access to the liver and its blood vessels. The stomach and intestines were displaced caudally with a wet cotton swab, and the right lobe of the liver was displaced closer to the diaphragm for a clear view of the portal triad. After its visual identification, a needle with suture material (polypropylene 4/0) was brought under the portal triad, including the he-

patic artery, hepatic vein and bile duct. At each end of the suture material, a weight of 3 grams was fixed on a special holder providing reduced blood flow in the vessels of the portal triad due to the tension of the suture material. Eppendorf tubes filled with water were used as the weight. After 20 minutes, the weights were removed, and the surgical wound was sutured in layers with a continuous suture.

Euthanasia. Animals were euthanized by decapitation with the following collection of biological material (blood and liver tissue).

Distribution by groups. All animals were divided into the following groups:

group 1 (n=6) – intact controls; animals were euthanized on day 1 of the experiment, and blood and liver were collected;

group 2 (n=6) – liver blood flow reduction for 20 minutes; animals were euthanized 2 hours after the procedure, and blood and liver were collected;

group 3 (n=6) – liver blood flow reduction for 20 minutes; animals were euthanized 24 hours after the procedure, and blood and liver were collected;

group 4 (n=6) – liver blood flow reduction for 20 minutes; animals were observed during the next 14 days to assess the procedure tolerability, and then were euthanized without collecting the biological material.

Anesthesia. All surgical procedures were performed using anesthesia: veterinary preparations Xila 20 mg/kg, and Zoletil-100 50 mg/kg.

Biochemical tests. Liver enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined in blood plasma. ALT was determined using the automatic biochemical analyzer VETSCANVS2, and AST was determined using the automatic biochemical analyzer VITROS 5600.

Histological examination. The liver was fixed in 10% formalin for 24 h and embedded in paraffin; histological microsections were prepared and stained with hematoxylin and eosin by the standard method.

Statistical analysis. The data were analyzed using the STATISTICA 10.0 program. Data were presented as mean ± standard error of the mean; comparison was performed using Student's t-test; differences were considered statistically significant at $p<0.05$.

Results and discussion. During the experiment on vascular occlusion with suture material and weights, we achieved a reduction in liver blood flow, visually confirmed by a change in the color of the liver (Figure 1).

According to the literature data, a system of suspended weights is less traumatic than vascular clamps, especially when reapplying the instrument for multiple cycles of ischemia-reperfusion to achieve the effects of hypoxia-reoxygenation, since even the thinnest clamps can cause significant damage [11]. However, we identified some special aspects that must be taken into account during the procedure, namely, avoiding too deep immersion of the needle when bringing it under the portal triad due to the risk of damage to the underlying vena cava. There is also a risk of damage to the triad by the cutting edges of the needle; to prevent this, the portal triad should be preliminarily isolated and held with tweezers, while bringing the needle with the suture material under it. Ischemia and hypoxia are not synonymous conditions, since well perfused tissues sometimes are not normoxic, and poorly perfused tissues sometimes are not hypoxic [3]. In this regard, we performed histological examination of the liver tissue to confirm the development of hypoxia in response to the blood flow reduction.

Histological examination of the liver of intact animals revealed normal tissue architecture without signs of inflammation

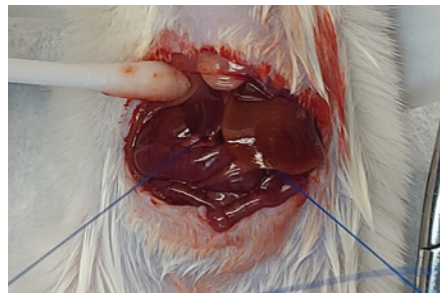

Fig. 1. Murine blood flow reduction with the portal triad occlusion

and necrosis, and without hepatocyte edema (Figure 2A). Samples obtained from animals with reduced blood flow demonstrated signs of ischemic liver damage: the tissue was characterized by an increase in the intercellular space, a noticeable edema of hepatocytes, decreased density of the cytoplasm, a large number of cells with karyolysis, and focal areas of necrosis (Figure 2B).

Also, the histological preparation obtained from an animal with induced liver hypoxia showed a much larger number of blood vessels (Figure 3B) than a corresponding preparation from an intact animal (Figure 3A), which can probably be interpreted as a phenomenon aimed at

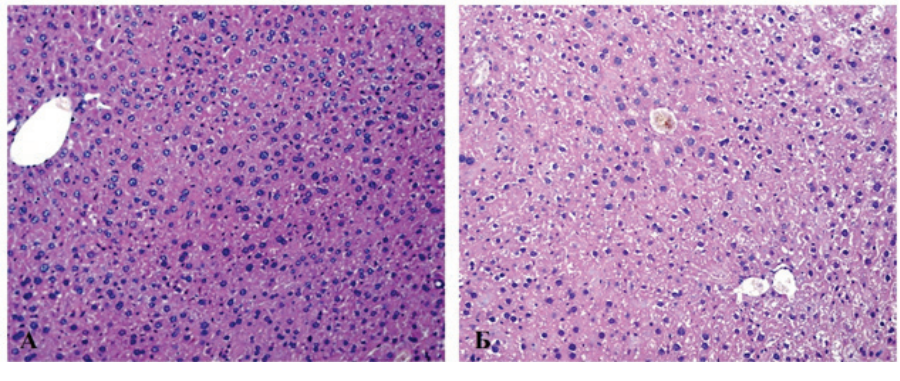

Fig. 2. Histological preparations of the Balb/c mouse liver. А – liver of an intact animal; Б – the animal's liver after a 20-minute blood flow reduction. Hematoxylin and eosin staining. Magnification: ×200

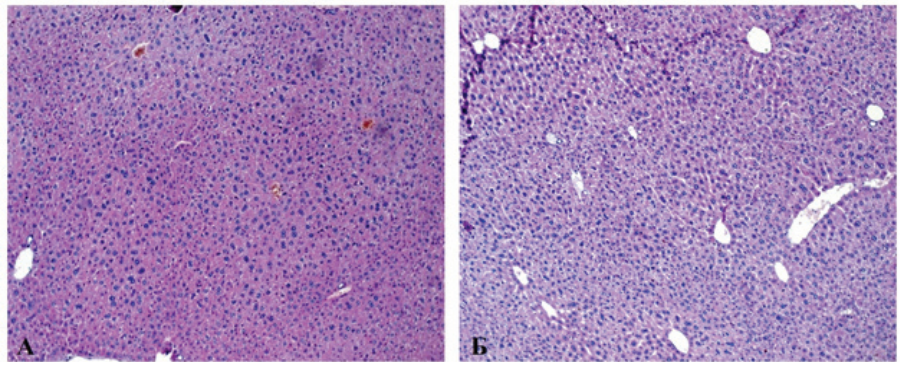

Fig. 3. Histological preparations of the Balb/c mouse liver. А – liver of an intact animal; Б – the animal's liver after a 20-minute blood flow reduction 24 hours after reperfusion. Hematoxylin and eosin staining. Magnification: ×100

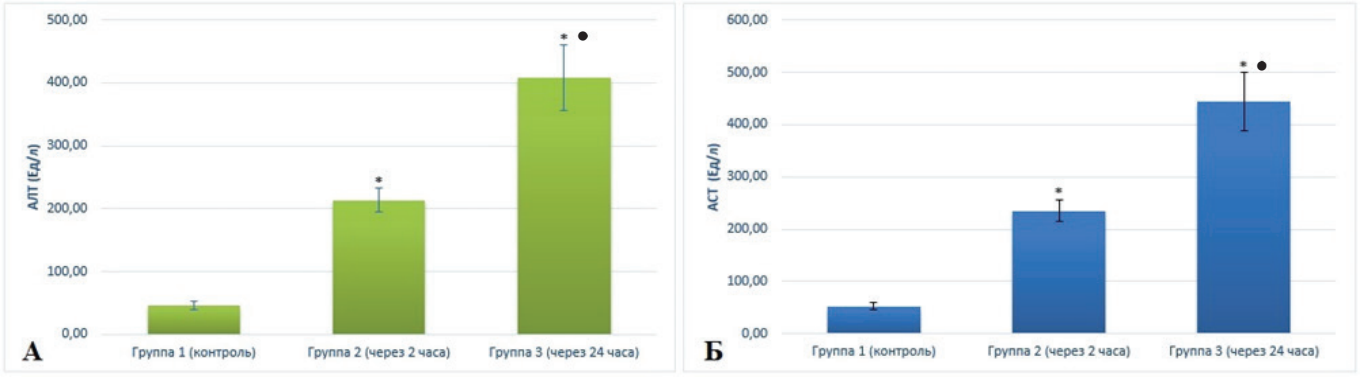

Fig. 4. Levels of liver enzymes in blood plasma in mice from groups 1-3. А – ALT levels; Б – AST levels. Note: * – statistically significant differences in enzyme levels between groups 2 and 3 and group 1 ($p<0.05$). ● – statistically significant differences in enzyme levels between group 2 and group 3 ($p<0.05$)

compensating for the resulting low tissue oxygenation.

The results of the study showed that the levels of liver enzymes in both groups with reduced blood flow were statistically significantly higher than in the control group ($p<0.05$). They were also statistically significantly higher in group 3 (24 hours after reperfusion) compared to group 2 (2 hours after reperfusion) ($p<0.05$). (Figure 4).

Determination of liver enzyme levels showed that ALT in intact mice was 40.8±6.3 U/L, in group 2 - 213.3±18.7 U/L, and in group 3 - 408.3± 52.9 U/L, which, respectively, was 5.2 and 10.0 times higher than in control animals. The level of AST in intact mice was 52.4±6.6 U/L, in group 2 - 235.3±20.7 U/L, and in group 3 - 443.8±56.4 U/L, which, respectively, was 4.5 and 8.4 times higher than in the control group. Elevated levels of liver enzymes in blood plasma were associated with ischemic cell damage; in addition, the amount of ALT and AST in the blood plasma of experimental animals increased during the day.

We also evaluated the survival rate of animals after the portal triad occlusion. One animal out of six (group 4) died the next day after the procedure, while the rest did not show signs of deterioration in health throughout the entire observation period. On the one hand, the demonstrated survival rate of more than 80% is a satisfactory result, but on the other hand, it indicated that careful planning of the number of animals is required in further experiments with technique in order to avoid losses that may preclude statistical processing of the results.

Conclusion. The results demonstrated that a 20-minute reduction of the portal triad blood flow contributes to severe hypoxia of the liver tissues, but at the same time, it is characterized by high survival rates of animals, which allows considering this method as an indispensable tool for liver pathology research.

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M.L. Polina, L.M. Mikhaleva, I.I Vityazeva, M.B. Khamoshina, I.M. Ordiyants, M.G. Lebedeva, L.A. Shelenina, P.N. Zakharova, N.I. Douglas

THE RELATIONSHIP BETWEEN THE MICROBIOTA TYPE AND IMMUNE RESOURCES OF THE ENDOMETRIUM AMONG INFERTILE WOMEN IN THE IMPLANTATION WINDOW PHASE

DOI 10.25789/YMJ.2023.82.02

УДК 618.14

The features of the microbiota and the immune profile of the endometrium of women with infertility of different genesis during the "implantation window" period have been studied. Endometrial phenotypes different in immune profile and microbiota profile within each group were identified (with infertility of unclear genesis, tube-peritoneal genesis, chronic endometritis, "thin" endometrium): "normal," dysplastic, chronic inflammation. The phenotype of chronic endometritis is revealed a significant predominance of cytokines of the pro-inflammatory Th1/Th1 profile in the presence of a dysbiotic microbiota type. The features of the dysplastic endometrial phenotype consist in a "poor" immune response (cytokines, chemokines, growth factors) against the background of pronounced fibrotic transformation. Ideas about the endometrial phenotype (normal, dysplastic, chronic inflammation) are a criterion for readiness for blastocyst implantation.

Keywords: infertility, the period of the "implantation window", chronic endometritis, immunohistochemical study, molecular phenotype, lactobacillar and dysbiotic types of microbiota.

POLINA Miroslava L. – PhD, assistant of the Department of Obstetrics and Gynaecology with a course in Perinatology of the Medical Institute of the Federal State Autonomous State Educational Institution of Higher Education People's Friendship University of Russia, Moscow, corresponding author, e-mail: polina.ml@mail.ru; **MIKHALEVA Lyudmila M.** – Corresponding Member of the Russian Academy of Sciences, Doctor of Medicine, Professor, Director of the Federal Research Institute of Human Morphology, Head of Clinical Morphology Laboratory, Moscow, Russia; **VITYAZEVA Irina I.** – MD, Head of the Department of Assisted Reproductive Technologies of the Federal State Budgetary Institution National Medical Research Center of Endocrinology of the Ministry of Health of the Russian Federation; **KHAMOSHINA Marina B.** – MD, Professor of the Department of Obstetrics and Gynaecology with the course in Perinatology of the Medical Institute FSAEI HE People's Friendship University of Russia; **ORDIYANTS I.M.** – MD, Professor of the Department of Obstetrics and Gynaecology with the course in Perinatology of the Medical Institute of the Federal State Autonomous State Educational Institution of Higher Education People's Friendship University of Russia; **LEBEDEVA Marina G.** – PhD, Associate Professor of the Department of Obstetrics and Gynaecology with the course in Perinatology of the Medical Institute, FSAEI HE People's Friendship University of Russia; **SHELENINA Lyudmila A.** – doctor of the gynecological department of F.I. Inozemtsev City Clinical Hospital, Department of Health of the city of Moscow; **DOUGLAS Natalya I.** – MD, Head of the Department of Obstetrics and Gynecology, Medical Institute of North-Eastern Federal University, Yakutsk, Russia; **ZAKHAROVA Praskovya N.** – Clinical Resident of the Department of Obstetrics and Gynecology, Medical Institute M.K. Ammosov North-Eastern Federal University, Yakutsk, Russia

From the standpoint of molecular mechanisms of embryo susceptibility formation, the endometrium, with all the scope of scientific papers, remains the most unexplored tissue of the female body. The microenvironment optimal for embryo localization, adhesion, invasion and implantation develops with differentiation of endometrial stromal cells into decidual cells and changes in the number and functional activity of immune cells [6,14]. The "subtle" mechanisms of implantation failures are associated with the difficulties of autocrine, paracrine and endocrine signaling, including sex steroids, cytokines, chemokines, growth factors and intracellular communication [16]. It is reported that more than 80% of repeated implantation losses occur on the background of an abnormal cytokine profile, however, complex molecular biological events associated with the violation of blastocyst and endometrial interactions have not been properly studied [13].

Changing ideas about the "sterility" of the endometrium to the possible presence of commensals and pathogens suggested their influence on local immune factors, but such mechanisms in the "uterine factor" of infertility require a convincing evidence base [2,5]. Shifts in bacterial balance with a decrease in the proportion of the supposedly "beneficial" microorganisms Firmicutes, Bacteroidetes, Proteobacteria and Actinobacteria are associated with impaired modulation of decidualization and implantation, activation of proinflammatory signaling pathways [2].

CE in 34-57.0% of women with repeated implantation failures is considered as a potential factor of changes in the immune environment on the background of infection with strains of gram-negative bacteria Escherichia coli and Gardnerella vaginalis, Ureaplasma urealyticum and Mycoplasma species [3,11]. The immune characteristics of the endometrium, the peculiarities of regulation of the main biological effects associated with the realization of fertility of women with CE, the influence of the composition of the microbiota on the components of the cytokine network have not been sufficiently studied. There are contradictory ideas about the molecular biological abilities of the "thin" endometrium, the effect on its "fertile resource" of the activity of immune cell populations and the microbiota during the "implantation window".

The purpose of the research: to study the features of the microbiota and the immune profile of the endometrium of women with uterine infertility in the phase of the "implantation window".

Material and methods of the research: A prospective examination of 101 infertile women of reproductive age was performed, including those after ineffective attempts of in vitro fertilization. The selection and examination of women was carried out at the bases of the Medical Center for Women's Health, the gynecological department of the 36 City Clinical Hospital of Moscow, the Department of Assisted reproductive Technologies of the Federal State Budgetary Institution "National Medical Research Center of

Endocrinology" of the Ministry of Health of the Russian Federation in Moscow, the emergency medical center of the Republican Hospital No. 2 in Yakutsk. The contingent is divided into groups: with unexplained infertility (n=11); with CE (n=22); tubal-peritoneal infertility (TPI) (n=50); with a "thin" endometrium (n=8).

The control group consisted of 10 healthy fertile women.

At the second stage, according to the results of a comprehensive examination (hysteroscopy, morphological and immunohistochemical), groups of women with different endometrial phenotypes were formed: chronic inflammation (n=32), dysplastic (n=47), norm (n=22).

Criteria for inclusion in the research: age from 25 to 40 years; infertile women with verified diseases: chronic endometritis (CE) (morphologically or immunohistochemically); TPI (obstruction of the fallopian tubes according to hysterosalpingography or chromotubation); with infertility on the EGE background; the absence of the male factor of infertility; the absence of infertility or fertility disorders of any other genesis; a voluntary informed consent to conduct the study.

Exclusion criteria: somatic diseases in the decompensation stage, acute inflammatory diseases of the pelvic organs and infectious diseases (tuberculosis, syphilis, HIV infection, viral hepatitis, acute genital herpes), autoimmune, mental diseases, the use of an intrauterine device at the time of the study, the absence of antibiotic therapy at least a month before inclusion in the study.

The examination of infertile women was carried out in accordance with the order of the Ministry of Health of the Russian Federation dated August 30, 2012 No. 107n "On the procedure for the use of assisted reproductive technologies, contraindications and restrictions to their use" (ed. dated 11.06.2015 and 01.02.2018). All patients signed an informed consent to participate in the study.

The examination of infertile women included assessment of complaints, anamnesis, general and gynecological examination, standard laboratory examination.

With sonographic CE signs on the 7-9 day of M.C., hysteroscopy was performed with biopsy sampling for morphological examination, confirmed by the detection of plasma cells labeled CD 138+.

Aspiration pipelle biopsy of the endometrium was performed in all patients during the "implantation window" (on the 20th-24th day of the menstrual cycle, 6-8 days after the peak of ovulation.

Pathomorphological and immunohistochemical examination of the endometrium was performed according to the standard methodology on the basis of the Research Institute of Human Morphology (Director of the Institute – CM of Russian Academy of Sciences, MD, Professor L.M. Mikhaleva).

The obtained biopsies were fixed with a 10% buffered formalin solution for 24 hours, followed by standard histological wiring and embedding into paraffin blocks. Histological sections 4 μm thick were made using Sacura rotary microscopes and stained with hematoxylin and eosin. The study of the preparations was carried out using a light microscope with an increase from x50 to x1000.

The interpretation of the results was carried out taking into account the stage and phase of M.C.

Immunohistochemical (IHC) examination of the endometrium was performed in the phase of the "implantation window" (luteinizing hormone LH+7) to assess the expression of cytokines, growth factors: in the glandular epithelium and stromal cells (TNF-α, IL-10, NRF2, GM-CSF and CXCL16), in the glandular epithelium – BCA1, in the stroma – TGF-β.

The analysis of the results of the IHC study was carried out taking into account the number of stained cells and the intensity of their coloring, the Histo-score (HS) was calculated according to the formula: $HS=\sum (Pi \times i)$, where Pi is the percentage of stained cells for each intensity (from 0% to 100%), i is the intensity of staining with the value 0 (absence), 1 – weak (light brown), 2 - moderate (brown) and 3 – strong (dark brown). The maximum score is 300. The analysis of the results of the IHC study with antibodies to TGF-β1 was carried out only in the endometrial stroma by a semi-quantitative method by estimating the number of positive cells regardless of the intensity of staining.

Data interpretation: 0 (the absence of positive stromal cells), 1+ (the number of cells up to 24%), 2+ (the number of cells from 25% to 49%) and 3+ (the number of cells from 50%).

The preparations were studied using a Leica DMLB light microscope with a standard set of optics. Microphotography was performed on a Leica DMLB universal biological microscope with a DFC420 color digital camera using the standard Leica Application Suite v. 3.7.0.

The reference data for the analysis of the results of immunohistochemical studies were the data of healthy fertile women (control group, n=10).

The sampling of material from the uterine cavity for microbiological examination was carried out with a double-cavity catheter for embryo transfer under aseptic conditions.

Endometrial samples were examined by real-time polymerase chain reaction (PCR) RT (Femoflor 16 tests, Scientific Production Association 50 DNA Technology LLC (Russia)) to assess the content of lactobacilli, opportunistic and pathogenic microorganisms (chlamydia, gonococci, Mycoplasma genitalium) in genome-equivalent units (GE/ml) on the IQ5 Multicolor Real-Time PCR Detection System of BIO-RAD (USA).

Samples with a bacterial titer sufficient for identification are presented (two samples from the group with a proliferative phenotype did not meet the condition, therefore 38 samples are given for analysis). The protocol for patients' monitoring and the examination program were approved by the local Ethics Committee of the Medical Institute of the Peoples' Friendship University of Russia, the study was carried out in accordance with the principles of the Helsinki Declaration of the World Association "Ethical Principles of Scientific and Medical research with human participation".

Statistical data analysis is performed in the IBM SPSS STATISTICS 22 package.

The normality of the parameter distribution was checked using the Shapiro–Wilk test. Qualitative variables were analyzed by constructing conjugacy tables using Pearson's chi-squared (χ^2) agreement criterion, with a small number of observations (less than 5), the exact Fisher test was used. The differences were considered statistically significant at $p< 0.05$. Quantitative features are presented in the form of median (Me) and upper and lower quartiles (25th and 75th percentiles).

The Mann-Whitney U-test was used for the analysis of quantitative features, and the Bonferroni correction was used for multiple comparisons (the level of statistical significance $p<0.017$).

Results and discussion. According to the results of a comprehensive study of the expression of cytokines, chemokines and growth factors in the glandular epithelium and endometrial stroma cells during the "implantation window" in groups of women with infertility of various genesis, phenotypes of normal endometrium, chronic inflammation and a dysplastic one were identified.

Molecular characteristics of the endometrium within the groups were the following: with CE – true inflammation (n=12); dysplastic (n=10); "thin" endometrium (dysplastic) (n=8); with unexplained

infertility – dysplastic (n=11); with TPI – CE (n=20), normal variant (n=12), identical in terms of indicators in the control group; dysplastic (n=18).

The conclusion about the dysplastic phenotype of the endometrium during hysteroscopy was made on the basis of pallor and thinning, pathomorphological conclusions about dystrophic-atrophic changes. Visual CE signs during hysteroscopy (focal hyperemia, stroma edema, micropolypes) were confirmed by histological (inflammatory infiltration of the stroma by lymphocytes, plasmocytes, macrophages, in most cases diffusely, around blood vessels and glands, less often – focally), expression of the CD138+ marker.

Variants of the endometrial microbiota of women with different molecular phenotypes are presented in Figure 1.

There were no significant differences in the assessment of the total bacterial mass (TBM) criterion in the groups, despite the range of values of 10^3-10^7 GE/ml. When analyzing the composition and number of microorganisms, the types of microbiota were identified: lactobacillar (the proportion of more than 90% of the total bacterial mass) and non–lactobacillar ("mixed" - with a proportion of lactobacilli less than 90.0% and a low titer of opportunistic microorganisms; dysbiotic – in the presence of only opportunistic pathogenic flora).

The lactobacillar type of microbiota was detected in women with the phenotype of the "norm" of the endometrium, half with the dysplastic one and a third with CE. The non-lactobacillar type of microbiota in the phenotype of chronic inflammation was somewhat more common than in the dysplastic one, but without significant differences. The dominance of the lactobacillar type over the non-lactobacillar type (a decrease in the titer of lactobacilli on the background of the moderate growth of opportunistic flora) took place not only with the "normal" phenotype of the endometrium, but also with the dysplastic one (ratio index – 1:0; 1.1, respectively).

In the dysplastic phenotype of the endometrium, a mixed type of microbiota prevailed – 2.5 times more often than with CE, however, no intergroup differences were found. In the group with CE, endometrial dysbiosis was detected statistically significantly more often than in the dysplastic type ($p<0.001$, $\chi^2=14.1$), due to the predominance of Gardnerella vaginalis, Ureaplasma spp. and mixtures of Atopobium vaginae/Enterobacteriaceae in high titers (10^5-10^7 GE/ml), other microorganisms in a titer of 10^3-^{4} GE/ml. A similar microbial profile in CE was noted by other authors [7].

The molecular phenotype of the "normal" endometrium is represented by a balanced production of pro-inflammatory and anti-inflammatory cytokines, chemokines and growth factors necessary for implantation (Figure 2). A moderate increase in TNF-a is necessary for the proper differentiation and development of trophoblast cells, the formation of embryo-maternal immune tolerance, extracellular trophoblast invasion and remodeling of spiral arteries [9]. We believe it is possible to assert the formation of a receptive endometrium in the presence of a eubiotic microbiota profile and the balanced expression of subtypes of Th1-proinflammatory cells and T-regulatory (Treg) cells. Our data are consistent with the statements about the favorable role of the predominance of Lactobacillus spp. on the frequency of pregnancy [12].

The leveling of the molecular biological effects of the proinflammatory cytokine "microenvironment" is associated with a favorable effect on perfusion and receptivity of the endometrium of moderate expression of cytokine IL-10 [20].

The inflammatory type of immunoregulation in CE (Figure 3) was determined by the excess expression of proinflammatory cytokines in the glandular epithelium of the endometrium in comparison with anti-inflammatory ones (an increase in TNF– α - by 1.1 times (p=0,00), GM–CSF (p=0,00), CXCL16 (p=0,00), BCA1 (p=0,00) - by 1.2 times, a decrease in IL-10 – by 2 times (p=0,00)). In endometrial stroma cells, the expression level of GM-CSF (p=0,00), TNF-α (p=0,00) and CXCL16 was significantly higher than in the control – 1.2 times (p=0,00), IL-10 – 1.8 times lower (p=0,00), *NRF2* – somewhat lower (p=0,01), TGF-α was the lowest in comparison with other groups (1 point) (p=0,01). The indicator of the TNF-α /IL-10 immunoregulatory index in glandular cells is 2.5 (with a norm of 1.1 in glandular cells) indicates molecular disturbances in the interaction of immunocompetent cells. We believe that it is the analysis of immune "networks" that will clarify the mechanisms of infertility development in the presence of a dysbiotic endometrial profile.

The formation of a microenvironment unfavorable for blastocyst implantation is due to the induction by microorganisms of a dysbiotic type of overexpression of proinflammatory cytokines of the Th1/Th17 profile, impaired tissue remodeling and trophoblast invasion.

The data obtained allow us to state that the abnormal immune microenvironment during the "implantation window" in the CE phenotype is due to an increase in the GM-CSF inflammatory response due to increased TNF-α production and impaired transmission of its signals [10]. An increase in the level of chemokine CXCL13 (BCA1) is believed to cause the recruitment of plasma cells into the endometrial stroma, the labeling of which CD138+ confirms chronic inflammation [20]. The low level of TGF-β and IL-10 expression in CE reflects the quantitative or functional deficiency of the anti-inflammatory clone of Treg cells on the background of angiogenesis disorders and fibrotic transformation of the endometrium [17]. The realization of molecular biological effects in the "inflamed" endometrium occurs on the background of a decrease in NRF2-mediated antioxidant protection in Treg cells [8].

Obviously, a change in the immune profile of the endometrium with a dysbiotic microbiota profile disrupts decidualization and architectonics, determines

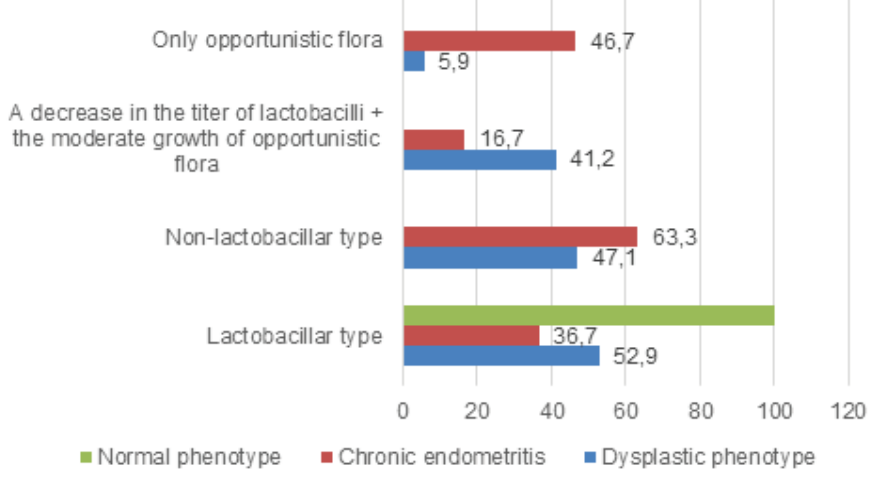
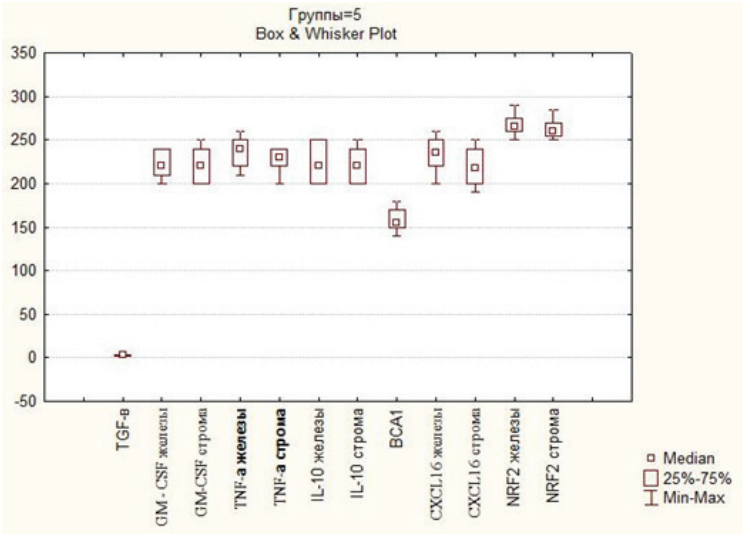


Fig. 1. Microbiota types at different phenotypes and variants at non-lactobacillary profile

a



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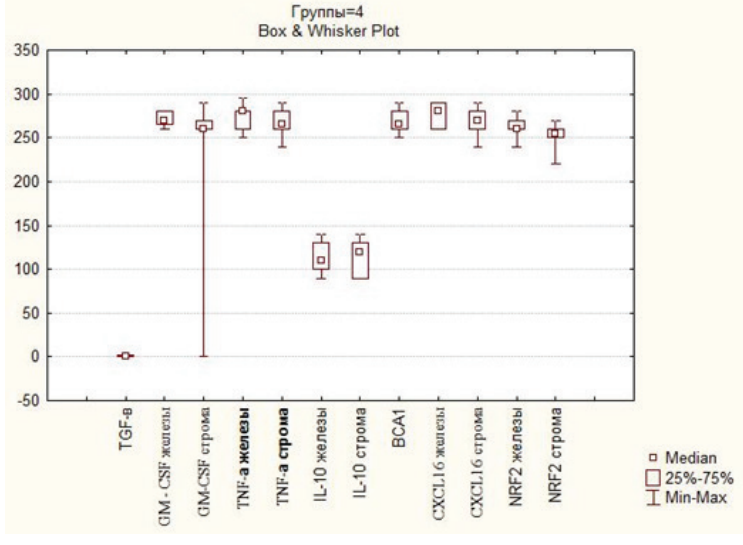

c

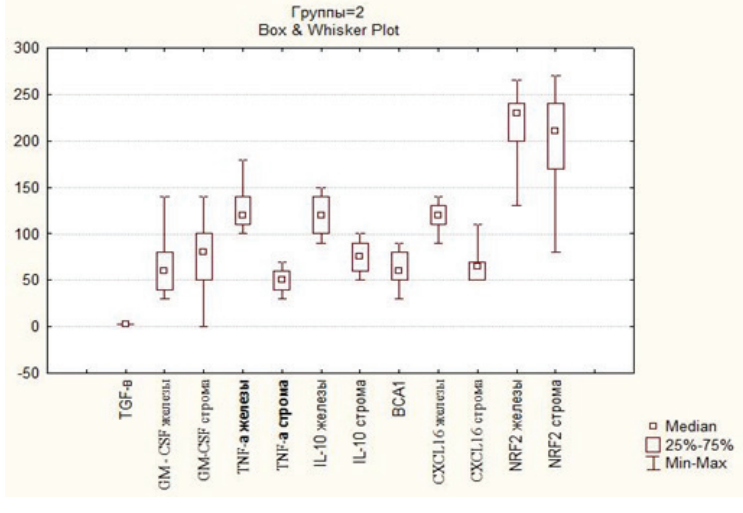

Fig. 2. Immune profile in the phenotype of normal endometrium (a), chronic endometritis (b), dysplastic phenotype (c)

abnormal expression of sex hormone receptors and an inadequate microenvironment for implantation.

We believe that in the development of molecular phenotypes of the endometrium, it is not the type of microbiota that is paramount (especially when mixed), but the immune reactions induced by microbial ligands that affect the difference in the expression of pro-inflammatory and anti-inflammatory factors, chemokines and antimicrobial metabolites.

The pathogenesis of the phenotype of chronic endometrial inflammation is based on violations of the molecular interactions of immunocompetent cells, with the predominance of the pro-inflammatory Th1 subtype. Our data suggest that the dysbiotic profile of the endometrium inevitably changes the composition of local immunocompetent cells, which, with factors recruited into the stroma, disrupt the expression of steroid hormone receptors. Overexpression of proinflammatory markers (cytokines, chemokines, and growth factors) in CE is associated with the risk of implantation failures [19].

Dystrophic-atrophic changes forming the morphological basis of the dysplastic phenotype of the endometrium were combined with a mixed type of microbiota in 41.2% (a decrease in the titer of lactobacilli <90.0% on the background of an increase in opportunistic microorganisms).

The molecular profile of the dysplastic phenotype (Figure 4) was represented by a decrease in the expression of all markers in glandular cells in comparison with the control: GM-CSF – by 3.7 times (p=0,00), TNF-α – by two times (p=0,00), IL-10 – by 1.8 times (p=0,00), CXCL16 – by 1.9 times (p=0,00), BCA1 – by 2.6 times (p=0,00), NRF2 – by 1.2 times (p=0,00). The TNF-α/IL-10 index was 1.0. In endometrial stromal cells, the expression of GM-CSF was reduced by 2.7 times (p=0,00), TNF–α by 4.6 times (p=0,00), IL-10 by 2.9 times (p=0,00), and CXCL16 by 3.4 times (p=0,00), along with the maximum TGF-β index (3 points) (p=0,01) in comparison with other phenotypes. The TNF-α/IL-10 ratio was 0.7.

The nature of the immune microenvironment of the dysplastic phenotype during the "implantation window" indicates an inhibition of the metabolic activity of the endometrium, a change in protein synthesis, and a decrease in antioxidant potential. We believe that endometrial susceptibility disorder caused by fibrotic transformation as a result of multiple intrauterine interventions is of interest from the standpoint of the "aging" of the local immune system. It is reported that

the cause of implantation failures may be premature aging of the endometrium due to local immune stresses and inflammatory damage [1]. The increase in "aging" decidual cells on the background of inability to restore proliferative activity leads to an excess of TGF-β along with suppression of the induction of differentiation of CD4 +T cells to Th17 and a decrease in the level of Treg cells [15].

The violation of local immune surveillance during the "implantation window" is likely to be connected with the activation of molecular signaling cascades associated with implantation disorders. Inhibition of NRF2 expression should be considered as the cause of a decrease in antioxidant enzymes and the development of chronic endometrial hypoxia [17]. A decrease in the expression of immune factors during the "implantation window" suggests defects in the mechanisms of stroma decidualization and secretory potential of glands, genes for controlling migration, proliferation, adhesion and cellular metabolism. Obviously, the molecular biological profiling most asynchronous to the implantation period in endometrial dysplastic phenotype was revealed in the presence of a non-lactobacillar (mixed) type of microbiota.

Heterogeneity of microbiota types in a metabolically "non-resource" endometrium probably correlates with the degree of preservation of the cellular layer and the sensitivity of receptors to steroid hormones, as well as violations of intracellular metabolism.

Conclusion. The phenotypes of the endometrium of infertile women are presented, mainly due to various immune microenvironment during the "implantation window". The heterogeneity of endometrial immune infiltration (cytokines, chemokines, growth factors) and the type of microbiota (lactobacillar, mixed and dysbiotic types) detected in various phenotypes (normal, dysplastic, chronic inflammation) determine the "consistency" of the molecular environment for embryo implantation. In the CE phenotype, defective decidualization of the endometrium is associated with a violation of the immune "landscape" during implantation and the predominance of a pro-inflammatory cytokine response. Implantation violation in CE is caused by the generation of an excessive inflammatory reaction and impaired recruitment of immune cells necessary for the formation of immune tolerance in the presence of pathogenic bacterial flora (non-lactobacillar type of microbiota). The features of the dysplastic phenotype of the endometrium, connected with plastic deficiency, are revealed in a violation of the organized interaction of immune cells (cytokines, chemokines, growth factors) on the background of severe fibrotic transformation.

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DOI 10.25789/YMJ.2023.82.03

УДК 616.716.4-005.273-07

O.A. Guryevskaya, I.N. Usmanova, I.A. Lakman, M.M. Tuigunov, R.F. Khusnarizanova, I.D. Ushnitsky, J.L. Bortsova, V.O. Senina.

SOME FEATURES OF THE MICROBIOTA COMPOSITION OF WICKHAM'S STRIAE SURFACE AND EROSIVE- ULCERATIVE ELEMENTS IN PATIENTS WITH ORAL LICHEN PLANUS

A comparative study of the microbiota profile of the Wickham mesh surfaces (a typical form of lichen planus (LP) (L43.80) and erosive-ulcerative elements in the erosive-ulcerative form (L43.82) of LP with localization on the buccal mucosa was carried out. In the studied microbiota of the surface of erosive and ulcerative elements, significant differences were revealed from the composition of the microbiota of the surface of the Wickham mesh, which is associated with clinical manifestations of the oral cavity mucous lining (LP OCML). The data on the quantitative and qualitative composition of microorganisms in the erosive-ulcerative form of LP were significantly different from the typical form of LP, which indicates the need for further studies of the microbiota in other forms of LP OCML. The revealed changes in the composition of the microbiota in the typical form of LP OCML can serve as a potential prognostic criterion for the conditional norm, and in the erosive-ulcerative form, as a criterion indicating the progression and aggravation of the clinical picture.

Keywords: lichen planus, typical form, erosive-ulcerative form, Wickham mesh, erosions, ulcers, microbiota, Candida spp.

GURYEVSKAYA Olga Anatolyevna – postgraduate student of the Department of Therapeutic Dentistry, Bashkir State Medical University, drguryevskaya@inbox.ru SPIN - 5562-7134, ORCID:0000-0003-4580-9826; **USMANOVA Irina Nikolaevna** – MD, PhD, professor, Department of Therapeutic Dentistry, Bashkir State Medical University, Ufa, irinausma@mail.ru SPIN - 1978-9470, ORCID:0000-0002-1781-0291; **LAKMAN Irina Aleksandrovna** – Ph.D., Candidate of Technical Sciences, Associate Professor, Department of Biomedical Engineering, Head of the Scientific Laboratory for the Study of Social and Economic Problems, Ufa University of Science and Technology, Ufa, Lackmania@mail.ru SPIN - 4521-9097 ORCID:0000-0001-9876-9202; **TUIGUNOV Marsel Maratovich** – MD, Professor, Head of the Department of Microbiology and Virology, Bashkir State Medical University, Ufa, tuygunov@mail.ru SPIN - 2506-9633, ORCID:0000-0002-5473-2034; **KHUSNARIZANOVA Rauza Fazilovna** – PhD in Biology, Associate Professor, Department of Microbiology and Virology, Bashkir State Medical University, Ufa, roza.khusna@mail.ru SPIN - 6490-5085 ORCID:0000-0002-1001-9587; **USHNITSKY Innokentiy Dmitrievich** – MD, Professor, Head of the Department of Therapeutic, Surgical, Orthopaedic Dentistry and Childhood Dentistry of the Medical Institute of M.K.Ammosov North-Eastern Federal University, Yakutsk, E-mail: incadim@mail.ru, SPIN 9424-3807 ORCID 0000-0002-4044-3004; **BORTSOVA Julia Lvovna** – PhD in Biology, Associate Professor, Department of Fundamental and Applied Microbiology, Bashkir State Medical University, Ufa, e-mail: juliabaim@yandex.ru, SPIN-2728-3379, ORCID:0000-0002-5839-9793; **SENINA Valeria Olegovna** – a postgraduate student of the Department of Therapeutic Dentistry, Bashkir State Medical University, Ufa, lera.senina2012@yandex.ru SPIN - 7669-5320, ORCID:0000-0002-5593-085X

Introduction. The presence of a microbial factor in the development of lichen planus involves qualitative and quantitative changes in the composition of gram positive and negative bacilli and cocci depending on the form of oral mucosal lichen planus (OLP). According to numerous studies, the oral microbial community in patients with OLP OCML undergoes dysbiosis in a significant number of cases. Signaling pathways involved in cellular processes such as keratinization, inflammation, and T-cell responses are triggered in the pathogenesis of the development and aggravation of OLP erosive forms [1-5, 7-9, 12-16, 18 - 21].

Numerous foreign studies present data on the composition of the oral microbial flora in rubella mucosa, the presence of significant amounts of Capnocytophaga sputigena, Eikenella corrodens, Lactobacillus crispatus, Mobiluncus curtisii, Neisseria mucosa, Prevotella bivia, Prevotella intermedia and S. Agalactiae, Bacteroides ureolyticus, Dialister species, Staphylococcus haemolyticus and Streptococcus agalactiae [6]. An increase number of Neisseria, Haemophilus, Fusobacterium, Porphyromonas, Rothia, Actinomyces and Capnocytophaga in oral mucosal smears was observed in patients with OLP by 16S sequencing [9, 10], as well as evidence of changes in the overall structure of the salivary microbiome in OLP in the form of high values of Porphyromonas and Solobacterium and low Haemophilus, Corynebacterium, Cellulosimicrobium and Campylobacter, Streptococcus comprising in erosive-ulcerative form [16].

Oral microbiocenosis disorder in patients with OLP is characterized by high levels of yeast-like fungi of Candida and Aspergillus, Alternaria and Sclerotiniaceae unidentified, Bovista, Erysiphe, Psathyrella, etc. Aspergillus has been identified as a "OLP-associated" fungus because of its detection with higher frequency in the clinical course of OLP compared to healthy patients [17].

A number of researchers provide evidence of the relationship between the clinical OLP OCML manifestations and the persistence of infection in the oral tissues, while the role of fungal-bacterial interactions in the OLP pathogenesis remain virtually unstudied, which led to the purpose and relevance of our investigation.

The aim of this research is a comparative study of cheek mucosa microbiota profile in patients with the typical OLP form (L43.80) and erosive-ulcer OLP form (L43.82).

Material and methods. The comparative microbiological research of some representatives of microbiota surface of erosive-ulcerative elements and Wickham's striae was carried out during complex clinical investigation of patients (n=111) with ICD-10 diagnosis (L43.82) of erosive-ulcerative and typical forms of OLP (L43.80).

We examined smears obtained from the surface of Wickham's striae and erosive-ulcerative elements localized on

the cheek mucosa. Swabs were taken before individual oral hygiene and food intake, delivered to the laboratory within 2 hours. The isolation of individual representatives of the microbiota was carried out by seeding on traditional, special and differential diagnostic media, followed by biochemical identification using HiCrome and Lachema media series. Due to a small number of species diversity, some representatives of microbiota were not identified to species.

An electronic database was created, which allowed statistical analysis of independent sample data in R Studio environment using nonparametric Mann-Whitney criterion. Differences in the frequency of occurrence or absence of a trait were determined using the χ2 criterion, including Yates' correction. The null hypothesis of no difference was rejected in favor of the alternative hypothesis if the p-value did not exceed 0.05 in all criteria.

Results and their discussion. The results of the microbiological study showed that representatives of Gram-positive and Gram-negative facultative anaerobic cocci and bacilli, as well as Candida yeast-like fungi in the average quantity from 2 to 6 Lg CFU/unit were found in almost all examined patients, regardless of the OLP form, in the microbiota of the Wickham's striae surface or erosion surface.

Nevertheless, a comparative analysis of microbiota representatives showed a significant difference in their quantitative and qualitative content. The preponderance of representatives of genera Fusobacterium (P < 0.01) and Candida (P < 0.001) was marked in patients with erosive-ulcer OLP form (L43.82), that gave the grounds to define them in the basic clinical group (n=86), other patients with typical OLP form (L43.80) formed a comparison group (n=25) (Fig. 1).

Representatives of 10 genera were studied of all the variety of qualitative and quantitative composition of Wickham's striae microbiota and erosive-ulcerative elements. A significant predominance of Candida spp. (P < 0,001) was observed in erosive-ulcerative form L43.82, this contributed to the possibility of further division of the main clinical group into two clinical subgroups - I main clinical group with erosive-ulcerative OLP form L43.82 (n=46) with 2nd degree dysbiosis manifestations, and II main clinical group with erosive-ulcerative OLP form L43.82 (n=40) with 3rd degree dysbiosis manifestations.

The detection rate of Gram-positive and Gram-negative facultatively anaerobic cocci Streptococcus spp. and Staphylococcus spp. was 84.0% and 76.0% of cases, Gram-positive and Gram-negative facultatively anaerobic bacilli respectively in 77.3% and 39.9% cases, yeast-like fungi of the Candida genus in 4% cases in the typical OLP form (L43.80).

The ratio between Gram-positive and Gram-negative cocci was 1:0.9, between streptococci to staphylococci 1:1.10, Gram-positive bacilli and facultatively - anaerobic cocci Lactobacillus spp.and Streptococcus spp.was 0.81:1. in the microbiota studied.

Lactobacillus spp. was detected in 68.0% of the microbiological investigations with a quantitative value of 6.5 Lg CFU/unit, in 4.0% of the cases Candida spp. constituted the limit of normal values from 2.0 to 2.9 Lg CFU/unit. The content of individual representatives of Gram-positive and Gram-negative facultative anaerobic cocci and bacilli, Staphylococcus spp., Neisseria spp., Streptococcus spp. and Lactobacillus spp. ranged from 3.4 to 6.2 lg CFU/mL (Table 1).

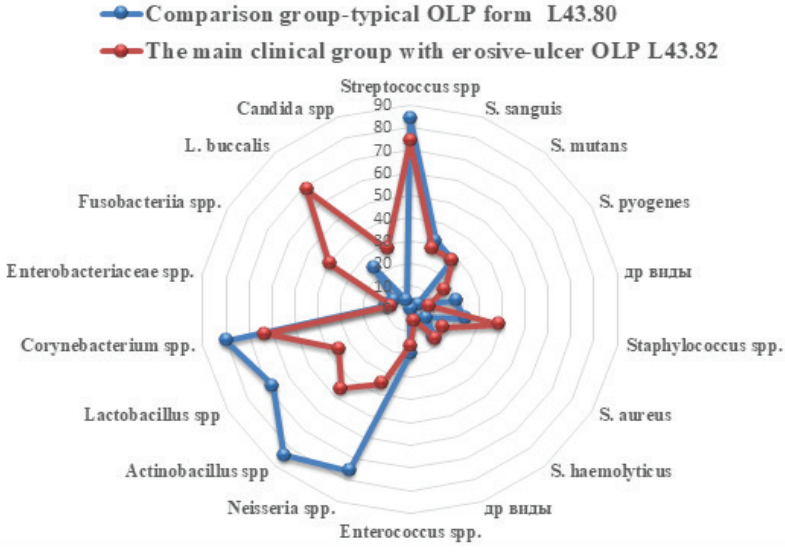
The frequency of detection of Gram-positive facultatively anaerobic cocci (Streptococcus spp.) amounted to 97.83% and 92.5% of cases respectively; Gram-positive and Gram-negative facultative-anaerobic bacilli - 47.82% and 47.5% of cases, in the main clinical groups with erosive-ulcerative OLP forms (L43.82) in the surface microbiota of erosive-ulcerative elements. The detection rate of Gram-positive and Gram-negative facultative-anaerobic bacilli was 59, 41%, 65.05%, 92.75%, 49.17% of cases, respectively, yeast-like fungi in 26.08% from 3 to 5 Lg CFU/unit and in 47.5% at 5 to 7 Lg CFU/unit, which was statistically different at p<0.001 (Table 1).

The ratio between Gram-positive and Gram-negative cocci was practically equal - 1:2,04 and 1:1,95, the ratio of Streptococci to Staphylococci respectively 1:1,87 and 1:1,94, the ratio of Lactobacillus spp. to Streptococcus spp. on the average was 0,47:1 and 0,49:1 in the composition of the studied microbiota of the surface of erosive-ulcerative elements. In 45.33% of cases, lactic acid species forming the family Lactobacillus spp. were detected at a quantitative value of 5.0 Lg CFU/unit. (lower than the comparison group at 1.5 Lg CFU/unit), differences at p<0.05 were determined only in the quantitative value of yeast-like fungi of Candida spp. genus (Table 1).

Yeast-like fungi of the genus Candida spp in the amount from 3,0 to 5,0 Lg CFU/ed (3,0-4,0 times more than in the comparison group),were revealed in the microbiota of the erosive-ulcerative surface elements of the patients of the first main clinical group, in the second main clinical group the quantity of yeast-like fungi from 4,0-6,0 Lg CFU/ed was 26,08% (2,0-3,0 times more than in the comparison group) (Table 1).

Depending on the form of OLP OCML and the place of obtaining material for microbiological study, the species diversity among streptococci and staphylococci was represented by more than 3 species of microorganisms.

As follows from Table 1, the main significant microorganisms detected from the surface of Wickham's striae and erosive-ulcerative elements were representatives of opportunistic pathogens in the form of yeast-like fungi of the genus Candida (26.08%, 47.5%), and Fusobacteri-



Degree of incidence (%) of some microbiota representatives of the Wickham striae surface and erosive ulcerative elements in patients with typical and erosive ulcerative forms of the Lichen Planus in the oral cavity mucous lining (LP OCML)

ia spp among Gram-negative facultative anaerobic bacilli (52.17%, 50.0%).

The quantitative content of the main inhabitants of the microbiota in the form of oral streptococci, regardless of the form of lichen planus and place of collection of material for microbiological study, was within the limit of 105 to 109 Lg CFU/unit.

When analyzing the composition of the microbiota from the Wickham striae surface (comparison group), representatives of the Streptococcus spp were found in the amount of 4.9 Lg CFU/ed in 84.0% of studies, the species ratio of Str. sanguis:Str. mutans was 1:1.14, with the presence of β-hemolytic streptococcus found in 4.0% of cases.

Streptococcus spp. was detected in the examined microbiota from the surface of erosive-ulcerative elements of the cheek mucosa in 97.83 and 92.5% of cases in the amount of 3.6 lg CFU/unit and 3.2 lg CFU/unit, respectively in the I and II main clinical groups of patients with erosive-ulcerative OLP (L43.82) (1.36 and 1.53 times lower than in the comparison group). The species ratio of S. sanguis:S. mutans was 1:1.38 and 1:1.3, S. Pyogenes was detected in 21.73% and 22.5% of microbiological studies, which was significantly different from the comparison group L43.80 at p<0.05 according to χ^2 - criterion.

The patients of the comparison group (L43.80) had a staphylococcal prevalence of 24.0% at 4.7 lg CFU/unit in smears obtained from the surface of the cheek mucosa at the Wickham striae location, the species ratio of S. aureus:S. haemolyticus was 0.5:1.

The clinical groups I and II with erosive-ulcerative OLP (L43.82) had the prevalence of staphylococci 2.17 and 1.97 times higher than in the comparison group in the studied microbiota from the surface of erosive-ulcerative elements, the number varied from 3.4 to 3.6 lg CFU/unit, the species ratio of S. aureus:S. haemolyticus 0.8:1 and 1.12:1, which was distinguishable from the comparison group L43.80 (values are significant at p<0.05 for the main clinical group I). (Table 1).

We revealed differences in the composition of some representatives forming the microbiota of the Wickham's striae surface and the surface of erosive and ulcerative elements, and also presented heterogeneity of their distribution among patients with the erosive and ulcerative form of oral lichen planus (L43.82).

Conclusion. There are changes of associative intermicrobial relationships accompanied by a significant decrease

Performance indicators of the species composition for microbiota of the reticular grid surface and erosive ulcerative elements in patients with typical and erosive ulcerative forms of the Lichen Planus in the oral cavity mucous lining (LP OCML)

Microorganism	Clinical group n=111						Comparative analysis of intergroup differences between the main group and the comparison groups Statistics χ^2	
	Comparison group Typical OLP form L43.80 (n=25)		I clinical main group with erosive-ulcerative OLP L43.82 (n=46)		II clinical main group with erosive-ulcerative OLP L43.82 (n=40)		p-level	
	abs	%	abs	%	abs	%	I	II
Gram-positive facultative anaerobic cocci								
Streptococcus spp.	21	84.0	45	97.83	37	92.5	**$\chi2$=2.854.** p=0.092	**$\chi2$=0.441.** p=0.507
			$\chi2$ =0.431. p=0.512					
S. sanguis	8	32.0	18	39.13	13	32.5	$\chi2$ =0.355. p=0.552	$\chi2$ =0.002. p=0.967
			$\chi2$ =0.408. p=0.523					
S. mutans	7	28.0	13	28.26	10	25.0	$\chi2$ =0.001. p=0.982	$\chi2$ =0.072. p=0.789
			$\chi2$ =0.116. p=0.734					
S. pyogenes	1	4.0	10	21.73	9	22.5	$\chi2$ =3.893*. p=0.049	$\chi2$ =4.205*. p=0.041
			$\chi2$ =0.007. p=0.933					
Other types	5	20.0	4	8.69	5	12.5	$\chi2$ =1.870. p=0.172	$\chi2$ =0.665. p=0.416
			$\chi2$ =0.330. p=0.566					
Staphylococcus spp.	6	24.0	24	52.17	19	47.5	$\chi2$ =5.269*. p=0.022	$\chi2$ =3.590. p=0.059
			$\chi2$ =0.187. p=0.666					
S. aureus	2	8.0	9	19.57	9	22.5	**$\chi2$ =0.889**. p=0.346	**$\chi2$ =1.385**. p=0.240
			$\chi2$ =0.111. p=0.739					
S. haemolyticus	4	16.0	11	23.91	8	20.0	$\chi2$ =0.663. p=0.436	$\chi2$ =0.164. p=0.686
			$\chi2$ ²=0.190. p=0.663					
Other types	0	0	4	8.69	2	5.0	**$\chi2$ =0.958**; p=0.328	**$\chi2$ =0.158**. p=0.692
			$\chi2$ =0.061. p=0.806					
Enterococcus spp.	5	20.0	10	19.56	8	20.0	$\chi2$ =0.029. p=0.864	$\chi2$ =0.000. p=1.000
			$\chi2$ =0.039. p=0.844					
Gram-negative facultative anaerobic cocci								
Neisseria spp.	19	76.0	22	47.82	17	45.5	$\chi2$ =5.269*. p=0.022	$\chi2$ =6.987**. p=0.009
			$\chi2$ =0.245. p=0.621					
Gram-positive facultative anaerobic bacilli								
Actinobacillus spp	21	84.0	24	52.17	27	67.5	$\chi2$ =7.069**. p=0.008	$\chi2$ =2.169. p=0.141
			$\chi2$ =2.082. p=0.150					
Lactobacillus spp.	17	68.0	21	45.65	18	45.0	$\chi2$ =3.252. p=0.072	$\chi2$ =3.275. p=0.071
			$\chi2$ =0.004. p=0.952					
Corynebacterium spp.	20	80.0	37	80.43	33	82.5	$\chi2$ =1.749. p=0.186	$\chi2$ =1.220. p=0.270
			$\chi2$ =0.060. p=0.807					
Gram-negative facultative anaerobic bacilli								
Enterobacteriaceae spp.	3	10.87	5	10.87	4	10.0	$\chi2$ =0.021. p=0.886	$\chi2$ =0.064. p=0.801
			$\chi2$ =0.017. p=0.896					
Fusobacteriia spp.	2	8.0	24	52.17	20	50.0	$\chi2$ =11.781***. p<0.001	$\chi2$ =10.317**. p=0.002
			$\chi2$ =0.348. p=0.555					
L. buccalis	23	92.0	41	89.13	35	87.5	$\chi2$ =0.150. p=0.699	$\chi2$ =0.324. p=0.570
			$\chi2$ =0.055. p=0.815					
Дрожжеподобные грибы								
Candida spp.	1	4.0	12	26.08	19	47.5	**$\chi2$ =3.909***. p=0.049	**$\chi2$ =11.700***** p<0.001
			$\chi2$ =4.255*. p=0.040					

Note: Bold typeface indicates $\chi2$ statistics calculated with Yates correction.
*. **. *** - differences are statistically significant at p<0.05. p<0.01 and p<0.001. respectively

in the total number of representatives of lactic acid microorganisms Lactobacillus spp., strict anaerobes Actinobacillus spp., against some increase in titers of Gram-negative facultative-anaerobic bacilli Fusobacteriia spp. and L. Buccalis, as well as conditionally pathogenic yeast-like fungi Candida spp. in the composition of the studied microbiota obtained from the surface of erosive ulcerative elements

There was significant prevalence of opportunistic yeast-like fungus Candida spp. which on the average exceeded parameters of comparison group with typical OLP form (L43.80) almost 6,52 and 11,9 times ($p<0,05$) in the composition of investigated microbiota of erosive-ulcerous surface elements in patients of I and II main clinical groups with erosive-ulcerous OLP forms (L43.82). At the same time, the reliability of differences between the second main clinical group and the first main clinical group was $p<0.001$.

Thus, the identified features of the composition of the studied microbiota of the surface of erosive-ulcerative elements and Wickham's striae make it possible to substantiate the risks of aggravation of the pathological process on the oral mucosa depending on the form of oral mucosal lichen planus.

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N.Yu. Potseluev, A.S. Nagornyak, O.V. Zhukova, K.V. Shults, A.S. Kazyzaeva, A.V. Agabekyan, A.P. Pashkov

CYTOLOGICAL INDICATORS OF BUCCAL EPITHELIUM CELLS REGARDING PHYSICAL ACTIVITY AND COMPLIANCE WITH THE DRINKING REGIME OF CERTAIN GROUPS OF YOUNG PEOPLE

DOI 10.25789/YMJ.2023.82.04

УДК 613.31:616-018

The study of the buccal epithelium also plays an important role in modern approaches to preventive medicine, in assessing the risk of various pathologies, including pathological conditions associated with the drinking regimen. The purpose of the study was to determine the cytogenetic features of buccal epithelium cells in athletes of strength sports (sambo) and team sports (volleyball) in the period between competitions with a normal training regimen, to compare with a control group of young people who do not go in for sports, and to study water consumption regimes. in the studied groups and assess the relationship with cytological and morphometric parameters. 3 homogeneous groups were formed for a comparative assessment of the cytogenetic parameters of the buccal epithelium. In total, 27 athletes of power sports (judo), 25 athletes of team sports (volleyball) and 45 representatives of the control group took part in the study. Evaluation of cytogenetic parameters revealed statistically significant patterns: Cells containing micronuclei were found 3.85 times more often in the power group and 4.3 times more often in the game group than in the control group. The frequency of cells with protrusions generally corresponded to the trend towards the absence of statistically significant differences between strength and game sports and the presence of significant differences compared to the control group.

Keywords: buccal epithelium, water consumption regimen, athletes, power sports, team sports, cytogenetic changes.

POTSELUEV Nikolai Yuryevich – Associate Professor, Candidate of Medical Sciences, Associate Professor of the Department of Hygiene and Fundamentals of Ecology, Federal State Budgetary Educational Institution of Higher Education "Altai State Medical University", Ministry of Health of Russia; Barnaul, pocelueff@gmail.com; **NAGORNYAK Aleksey Sergeevich** – Senior Lecturer, Department of Hygiene and Fundamentals of Ecology, Altai State Medical University, Ministry of Health of Russia; Barnaul, tezaurismosis@gmail.com; **ZHUKOVA Olga Viktorovna** – Associate Professor, Candidate of Medical Sciences, Head of the Department of Hygiene and Fundamentals of Ecology, Federal State Budgetary Educational Institution of Higher Education "Altai State Medical University" of the Ministry of Health of Russia; Barnaul; oov-@mail.ru; **SHULTS Ksenia Viktorovna** – Senior Lecturer, Department of Hygiene and Fundamentals of Ecology, Altai State Medical University, Ministry of Health of Russia; Barnaul; tezaurismosis@gmail.com; **KAZYZAEVA Anna Sergeevna** – PhD, Associate Professor of the Department of Hygiene and Fundamentals of Ecology, Altai State Medical University of the Ministry of Health of Russia; Barnaul; kazyzaeva@rambler.ru; **AGABEKYAN Avetik Vaginakovich** – Senior Lecturer, Department of Physical Education and Healthy Lifestyle, Federal State Budgetary Educational Institution of Higher Education "Altai State Medical University" of the Ministry of Health of Russia; Barnaul; avetik.62@mail.ru; **PASHKOV Artyom Petrovich** – Associate Professor, Candidate of Medical Sciences, Head of the Department of Medical Knowledge and Life Safety, Altai State Pedagogical University; Barnaul; pashkart@mail.ru

Introduction. One of the urgent tasks of medicine is the use of non-invasive methods for the early diagnosis of diseases. Buccal epithelium is also used as a material for non-invasive methods. According to modern data, the buccal epithelium is a sensitive "mirror" that allows you to identify many factors that affect human health. The study of the buccal epithelium also plays an important role in modern approaches to preventive medicine, in assessing the risk of various pathologies, including pathological conditions associated with the drinking regimen [1, 4].

A correlation was determined between laboratory parameters: cholesterol in obese children, total protein in healthy children and the number of binuclear cells. There is a 10x increase in the number of cells with condensed chromatin in obese children [1].

A number of studies have shown differences in the frequency of occurrence of various cytogenetic disorders in healthy individuals of different nationalities, gender, and age. To a greater extent, there is a different ratio of the number of cells with micronuclei, protrusions of the "broken egg" type, nuclei with perinuclear vacuoles, protrusions of the "notched" type, protrusions of the "tongue" type, and other disorders in the studied samples. Apparently, this is due to the adaptive abilities of the body to new environmental conditions, the individual characteristics of the body, hormonal activity, general physical fitness, the state of the nervous system, and the processes of humoral regulation [3, 4, 5].

Various ecotoxicants, smoking, alcohol and negative factors of the labor process have a significant impact on the state of the nuclei of buccal epithelium cells. Thus, pesticides cause activation of the processes of proliferation and destruction [6], almost always there is a statistically significant increase in the number of micronuclei in workers in hazardous industries and residents of regions with chemical industries [7, 8, 9].

The dynamics of changes in buccal epithelium cells in power sports athletes at various stages of the competitive process was studied. Correlations between the level of various types of aggression and other indicators of the psychophysiological state of athletes have been revealed, and complex models for the regulation of these changes have been proposed [10]. The results obtained can be considered markers of the effectiveness of preparation for competitions, and the low invasiveness of the micronucleus test allows you to control these processes in dynamics.

Based on the above trends, we set the goal of the study - to determine the cytogenetic features of buccal epithelium cells in athletes of strength sports (sambo) and team sports (volleyball) in the period between competitions with a nor-

mal training regimen, to compare with the control group of young people, non-athletes, as well as to study the water consumption regimes in the studied groups and evaluate the relationship with cytological and morphometric parameters.

Materials and methods. 3 homogeneous groups were formed for a comparative assessment of the cytogenetic parameters of the buccal epithelium. In total, 27 athletes of power sports (judo), 25 athletes of team sports (volleyball) and 45 representatives of the control group took part in the study.

All groups included males aged 19 to 25 years. Before the start of the study, everyone completed a questionnaire. All respondents claimed that they did not have bad habits, chronic diseases, complaints from the dentoalveolar system. All respondents also claimed to have been in Barnaul for the past year or more. All respondents stated that they train in the period between competitions in the usual way, which includes 3 workouts per week, lasting 2 astronomical hours each.

Representatives of the control group met the indicated criteria, but did not go in for professional sports and did not attend the gym on a regular basis, however, as students of a higher educational institution, they attended a general physical training class once a week as part of the main health group (2 academic hours).

The screening questionnaire about the drinking regimen included questions about the characteristics of water consumption (“ tap ” , “ bottled ”), knowledge about the drinking regimen (“ I know and observe ” , “ I don’t know ” , “ I know and do not observe ”), signs of dehydration (“ I feel thirsty in the morning constantly ” , “ I feel thirsty in the morning periodically ” , “ I don’t feel thirsty in the morning ”) Further comparison of water consumption regimens was carried out between athletes in general and the control group.

The selection of the buccal epithelium was carried out with a wooden spatula, from the mucous surface of the oral cavity and applied to a glass slide with further fixation over the flame of an alcohol burner and stained with methylene blue. Visual microscopy was performed at a magnification of 100x10, digital microscopy and morphometry using a ToupCam 3.2 megapixel video camera, at least 1000 cells were viewed in each preparation.

Based on the sample sizes, the Shapiro-Wilk test was used to assess the compliance with the normality of the data distribution. To detect the absence or presence of differences between the indicators in the studied groups, the Kruskal-Wallis test was used; if statistically significant differences were found, post-hoc intergroup comparisons were carried out using the Mann-Whitney test. In a simple paired comparison, the significance level was taken equal to $p = 0.05$; in cases of paired comparisons between three groups, the critical significance level was taken equal to $p = 1 - 0.95^{1/3} = 0.017$. In the text and tables, data are presented as the median and the values of the first and third quartiles in parentheses. The calculations were carried out in IBM SPSS Statistics 23.0.

Results. At the first stage of the study, a screening assessment of the drinking regime was carried out on the basis of a questionnaire. It turned out that in the group of athletes 71% use bottled water and only 29% tap water, in the control group these values correspond to 57% and 43%, respectively. To the question about compliance with the drinking regime, the answers were distributed as follows: athletes - “ I know I comply ” - 89%, “ I don’t know ” - 2%, “ I know I do not comply ” - 9%, the control group - “ I know I comply ” - 11%, “ I don't know " - 56%, " I know I don't comply " - 33%. In the group of athletes, when asked about the signs of dehydration, the answers were distributed as follows - “ I feel thirsty in the morning all the time ” - 12%, “ I feel thirsty in the morning periodically ” - 31%, “ I don’t feel thirsty in the morning ” - 57%; in the control group, respectively - " I feel thirsty in the morning all the time " - 22%, " I feel thirsty in the morning periodically " - 40%, " I do not feel thirsty in the morning " - 38%.

Next, we compared the values of the area of the cytoplasm and the nucleus in the cells of the buccal epithelium of athletes of all categories and the control group. Morphometry in the group of athletes showed that the median area of the cytoplasm corresponds to 3621.3 (2911.2-4887.3) μm^2, and the area of the nucleus is 61.3 (56.8-66.6) μm^2. In the control group, the median area of the cytoplasm corresponds to 3017.8 (2613.7-4113.3) μm^2, and the area of the nucleus is 58.9 (53.1-68.3) μm^2. It was statistically reliably established that the median of the cytoplasm area in the group of athletes is greater than in the control group, which may be due to better conditions for observing the drinking regimen. There were no statistically significant differences between the medians of the core area.

The obtained results testify to the positive effect of the pedagogical work of the coaching staff, counting on high achievements, including through the fight against dehydration and timely replenishment of microelement deficiencies, however, to clarify the role of bottled water, additional research is required on the composition and additives used, such as zinc, selenium, iodine, fluorine, etc.

The data in the control group indicate the need to intensify sanitary and educational work among different groups of young people not associated with sports, organize lectures, schools for a healthy drinking regime.

Earlier studies supplemented the results organically, so in [11, 14] it was shown that men's total daily water intake was about 0.25 l less than the recommended adequate intake, while women's intake was about the same as the adequate intake. On average, men and women aged 60 years and older, non-Hispanic black men and women, Hispanic men and women, men and women with low physical activity, and men with moderate physical activity consumed less than the adequate daily norm, which also indicates an indirect positive effect of physical activity on the maintenance of internal environmental constancy in athletes. Also of interest for practical implementation are studies [12, 13, 15], where it was found that an increase in water consumption can contribute to the prevention of overweight. It can be said that a balance between adequate physical activity, a full drinking regime and lifestyle is an effective mechanism of health saving in all age groups.

At the second stage of the study, before conducting intergroup comparisons, we assessed the type of distribution for each of the studied parameters, since the sample did not exceed 50 cases for each of the groups, the Shapiro-Wilk test was used, for all values except for the frequency of cells with an atypical nucleus ($p=0.057$) and the frequency of cells with protrusions of the “ tongue ” type ($p=0.066$), it took values of $p<0.05$, which indicates non-normal types of distribution in the studied samples. Thus, further, we used nonparametric criteria to establish differences between the studied groups.

Evaluation of cytogenetic parameters revealed statistically significant patterns: Cells containing micronuclei were found 3.85 times more often in the power group and 4.3 times more often in the game group than in the control group. The frequency of cells with protrusions generally corresponded to the trend towards the absence of statistically significant differences between strength and game sports and the presence of significant differences compared to the control group. Within this indicator, a number of features stand out, so the frequency of cells with protrusions of the " bubble " type is more

common in the power group than in the game and control groups. The frequency of cells with protrusions of the " broken egg " type is 2 times more common in the power group and 2.4 times more common in the game group than in the control group. When pairwise comparing the frequency of cells with an atypical nucleus, it turned out that such changes are more typical for representatives of team sports (7.64 times more often than in the control group and 2.37 times more often than in the game group). The integral indicator of the cytogenetic effect corresponded to the general trend and generally showed that statistically significant differences were found between the control group and the strength and play groups (see Table 1). It can also be noted that in the group of athletes of both power and game groups, rare anomalies such as " many micronuclei " (see Fig. 1) and " atypically shaped core " (see Fig. 2) were more common, statistically significant differences compared to the control group could not be established due to the low frequency of observation of these changes. The results obtained are generally consistent with existing studies and are primarily due to reactive processes in the body - a systematic increase in the level of cortisol, inflammatory mediators, changes in circadian rhythms due to participation in competitions in different time zones and climatic zones, however, rather high values of the frequency of occurrence micronuclei in all groups, including the control group, require an additional assessment of environmental factors and an assessment of the role of nutrition, drinking regimen, and environmental factors.

Grade features indicators of proliferation of cells of the buccal epithelium made it possible to determine that the main differences were represented (see Table 2) by an increase in the frequency of occurrence of cells with two or more nuclei in the game group, cells with double nuclei and circular notches in the power group (2.01 times more often than in the control group and 1.84 times more often than in the game group), which ultimately led to statistically significant differences between all the groups studied, so the integral proliferation index is highest in the game group, the second place is the power group and the last is the control group. These changes may reflect the constant processes occurring in the body of athletes - increased testosterone levels, chronic stress, some of the changes are also due to the background level of environmental factors.

Evaluation of indicators of early and late destruction of the cell nucleus (see Table 3) did not reveal significant differences, only the frequency of cells with perinuclear vacuole in the game group was statistically significantly higher in the game group than in the power and control groups (5.56 times), these changes can be explained by active preparation for participation in competitions at the regional level.

Evaluating the results obtained, it seems relevant to identify the role of individual trace elements such as potassium, calcium, magnesium in the formation of indicators of mental and physical performance, correction of cytogenetic, proliferative apoptotic changes in the nuclei of buccal epithelial cells. Ways to additionally compensate for micronutrient deficiencies may include both changes in dietary behavior and the consumption of specialized bottled waters enriched with essential substances.

Table 1

Cytogenetic parameters, ‰

Indicators	Power, group 1 (n=27)	Gaming, group 2 (n=25)	Control group, group 3 (n=45)
Cells with micronuclei	7.36 (4.5-7.9)	8.22 (6-10)	1.91*** (0-2.75)
cells with protrusions	2.82 (1-3)	2.22 (1-4)	1.73*** (1-2)
Cells with vesicular protrusions	2.09** (0-3)	1.44 (0-3)	1.18 (1-2)
Cells with " broken egg " protrusions	0.18 (0-1)	0.22 (0-1)	0.09*** (0-1)
Cells with tongue protrusions	0.55 (0-1)	0.56 (0-1)	0.45 (0-1)
Cells with an atypical nucleus	1.45* (1-2)	3.44* (2-4)	0.45* (0-1)
Integral indicator of cytogenetic action	10.18 (5.5-12.5)	10.44 (7-11)	3.64*** (1-6)

Note: * $p < 0.017$ changes are significant between all groups in a pairwise comparison (1-2, 2-3, 1-3), ** $p < 0.017$ changes are reliable in pairs of groups 1-2 and 1-3, *** $p < 0.017$ changes are reliable in pairs of groups 1-3 and 2-3

Conclusions:

1) The features of water consumption of athletes in game and power sports were determined in comparison with the control group and related changes in cytological parameters;

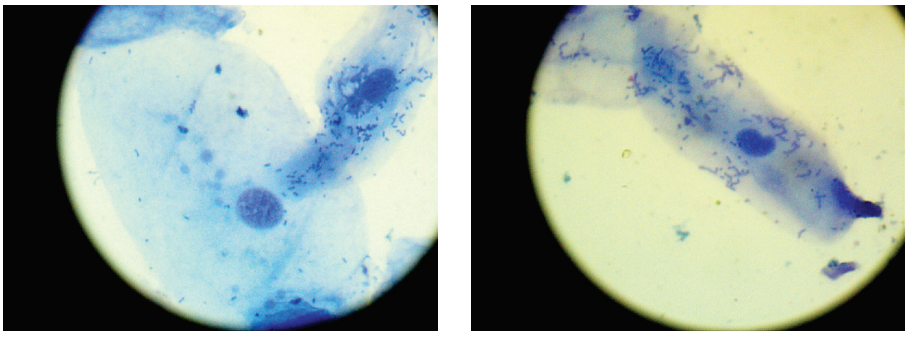
2) Athletes in the conditions of preparation for competitions are under stress and the risk of increasing the level of genome instability, determined by cytogenetic and proliferative indicators;

3) The control of cytological parameters of the buccal epithelium seems to be a promising method for assessing the effectiveness of training;

4) An assessment of the cytological landscape of the representatives of the control group is necessary to clarify the contribution of other risk factors for genomic instability due to regional characteristics;

5) It is necessary to conduct ad-

a b



Microscopic picture of anomalies of the buccal epithelium of the type "many micronuclei" (a), type "atypical nucleus" (b)

Table 2

Proliferation indices, ‰

Клетки	Power, group 1 (n=27)	Gaming, group 2 (n=25)	Control group, group 3 (n=45)
Cells with two or more nuclei	2.00 (1-2.5)	3.67** (2-5)	1.82 (0.5-2)
Cells with double nuclei and circular notches	2.00*** (0-3.5)	1.44 (0-3)	1.18 (0-1.5)
Integral proliferation index	4.00* (2-6)	5.11* (3-8)	3.00* (1-4.5)

Note: * $p < 0.017$ changes are significant between all groups in a pairwise comparison (1-2, 2-3, 1-3), ** $p < 0.017$ changes are reliable in pairs of groups 1-3 and 2-3, *** $p < 0.017$ changes are reliable in pairs of groups 1-2 and 1-3

Table 3

Indicators of early and late destruction of the cell nucleus, ‰

Indicators	Power, group 1 (n=27)	Gaming, group 2 (n=25)	Control group, group 3 (n=45)
Cells with perinuclear vacuole	0.36 (0-0.5)	2.00** (0-3)	0.36 (0-0.5)
Cells with chromatin condensation	4.64* (1-7)	19.00* (2-33)	1.73* (1-2.5)
Cells with nuclear vacuolization and onset of karyolysis	7.64 (4.5-10)	8.89 (3.25-9.33)	4.00 (1.5-6)
Cells with karyopyknosis	4.73 (2-9)	5.89 (5-8)	2.55 (1-4.5)
Cells with karyorrhexis	3.36 (1.5-4)	4.22 (1-4.5)	2.36 (0.5-4)
Cells with complete karyolysis	60.73 (38.5-75)	68.44 (41-134.5)	51.73 (21-63.75)
Apoptotic index	73.82 (51-83)	99.56 (35-154.5)	58.73 (25.5-71)

Note: * $p < 0.017$ changes are significant between all groups in a pairwise comparison (1-2, 2-3, 1-3), ** $p < 0.017$ changes are reliable in pairs of groups 1-2 and 2-3

ditional studies on large sample sizes to build predictive models such as "environmental risk factors - social risk factors - lifestyle - cytogenetic changes" .

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V.A. Dorovskikh, N.V. Simonova, M.A. Shtarberg, S.V. Panfilov, M.I. Arkhipova, V.A. Zatvornitsky, M.O. Sharapova

COMPARATIVE ASSESSMENT OF THE INTENSITY OF OXIDATIVE STRESS IN VARIOUS EXPERIMENTAL MODELS

DOI 10.25789/YMJ.2023.82.05

УДК 616-092.9:59.085

The possibilities of modeling oxidative stress in vivo include a fairly wide range of effects from the introduction of xenobiotics to the use of a temperature factor, irradiation, etc. On the basis of the Amur Medical Academy, pharmacologists summarized many years of experience in using various models of the formation of oxidative stress in a warm-blooded organism - the processes of peroxidation lipids were induced by exposure to low and high temperatures, ultraviolet irradiation, and a low-frequency alternating magnetic field. The intensity of lipid peroxidation processes in various models was assessed by the degree of accumulation of diene conjugates, lipid hydroperoxides, malondialdehyde and the level of ceruloplasmin, vitamin E, and catalase activity in the blood of laboratory animals on days 7, 14, and 21 of the experiments. The results of a comparative assessment of the intensity of oxidative stress in various experimental models showed that the most pronounced changes in the antioxidant status are caused by the cooling of animals and exposure to ultraviolet rays, and the latter model triggers a shift in the balance to the prooxidant side by the end of the first week of the experiment, which is confirmed by the accumulation of lipid peroxidation products by 48 -61% and a decrease in the activity of the components of the antioxidant system by 31-33% compared with the control. Thermal exposure to rats and the effect of a low-frequency alternating magnetic field causes less pronounced, but more stable changes in the dynamics from 7 to 21 days in the state of the prooxidant/antioxidant system, which, similarly to models using hypothermia and ultraviolet light, allows us to ascertain the formation of oxidative stress.

Keywords: experimental models, hypothermia, hyperthermia, ultraviolet irradiation, low-frequency variable magnetic field, oxidative stress, lipid peroxidation products, antioxidant system, rats.

Today, there is no doubt about the dominant role of oxidative stress in the pathogenesis of many diseases and pathological conditions, and therefore, since the end of the last century, pharmacologists have been actively engaged in the search for effective drugs that prevent and/or level the consequences of excessive intensity of lipid peroxidation processes [3, 5, 6, 11, 12]. Naturally, when testing pharmacocorrectors at the preclinical stage, the question arises of how to model oxidative stress [1, 4, 8]. Various models of stress are known with the use of xenobiotics, temperature effects, ionizing and ultraviolet radiation, etc. [2, 7, 9, 10]. Due to the variety of existing models of oxidative stress, it is quite logical that the researcher faces the question of choosing an adequate model. For several decades, the Department of Pharmacology of the Amur State Medical Academy of the Ministry of Health of Russia has successfully used models of oxidative stress induced by cold exposure (since the 80s of the last century), thermal exposure (since the 2000s), ultraviolet irradiation (since 2007) , alternating magnetic field of low frequency (since 2020). The accumulated experience of the effectiveness of these experimental models became the reason for presenting in this paper the results that reflect the comparative aspects of the induction of lipid peroxidation (LPO) processes in vivo in dynamics, in order to facilitate the problem of choosing a model for novice researchers.

The purpose of the study is a comparative assessment of the intensity of oxidative stress in various experimental models.

Material and methods. The experiments were carried out on outbred male rats weighing 200–250 g, obtained from the nursery of the Central Scientific Research Laboratory of the AGMA, Blagoveshchensk. The animals were kept in a vivarium under natural light under conditions of controlled temperature (22 ± 2) 0C and humidity (65 ± 10)% of the air with free access to water and standard food. The experiments were carried out in accordance with the National Standard of the Russian Federation GOST R 53434 - 2009 "Principles of Good Laboratory Practice", Order of the Ministry of Health and Social Development of the Russian Federation of August 23, 2010 No. 708n "On Approval of the Rules of Laboratory Practice". All conducted studies are approved by the Local Ethics Committee of the Amur State Medical Academy and comply with the regulatory requirements for conducting preclinical experimental studies.

Oxidative stress in laboratory animals was modeled by the following actions:

1. Cold exposure - daily cooling of rats (exposure duration - 3 hours) in the conditions of the Fentron climate chamber (Germany) at a temperature regime of -150C for 21 days;

2. Thermal exposure - daily overheating of rats (exposure duration - 45 min) under the conditions of an air laboratory thermostat TVL-K (St. Petersburg) at a temperature regime of +40±1-2°C for 21 days;

DOROVSKIKH Vladimir Anatolyevich – MD, Professor, https://orcid.org/0000-0002-5418-2466; **SIMONOVA Natalya Vladimirovna** – MD in Biology, Professor, Professor of the Department of Hospital Therapy with a Course of Pharmacology, Amur State Medical Academy of the Ministry of Health of Russia, e-mail: simonova.agma@yandex.ru, https://orcid.org/0000-0001-6805-2577; **SHTARBERG Mikhail Anatolyevich** – PhD, Senior Researcher, Central Scientific Research Laboratory, Amur State Medical Academy, Amur Region, Blagoveshchensk, https://orcid.org/0000-0002-4656-638X; **PANFILOV Stepan Vladimirovich** – post-graduate student of the Department of Hospital Therapy with the Course of Pharmacology, Amur State Medical Academy of the Ministry of Health of Russia; e-mail: panfilstep59@gmail.com; **ARKHIPOVA Maria Igorevna** – Assistant of the Department of Nervous Diseases, Psychiatry and Narcology, Amur State Medical Academy of the Ministry of Health of Russia; e-mail: i_love_grinch@mail.ru; **ZATVORNITSKY Vitaly Alekseevich** – assistant of the Department of Physical Education with a course of physiotherapy exercises, Amur State Medical Academy of the Ministry of Health of Russia; e-mail: amursmadk21@mail.ru; **SHARAPOVA Marina Olegovna** – 2nd year student of the Faculty of Medicine, Amur State Medical Academy of the Ministry of Health of Russia; e-mail: amursmadk21@mail.ru

3. Ultraviolet irradiation - daily irradiation of rats (exposure time - 3 min) in an ultraviolet chamber [1] for 21 days;

4. Low frequency alternating magnetic field (LF LF) - daily exposure of rats to LF MF (exposure duration - 3 hours), created by a system of Helmholtz rings (diameter 1 meter), powered by an alternating current source with a frequency of 50 Hz, with a magnetic field induction of 0,4 mT for 21 days. The exposure time for each experimental exposure was tested by multiple studies at the preliminary stage in order to select the optimal exposure duration that induces a shift in the equilibrium in the LPO/AOS system to the prooxidant side with the formation of oxidative stress. All exposures to animals were carried out under adequate conditions of humidity and ventilation. The controls in each experimental model were intact animals under standard vivarium conditions. Animals were slaughtered by decapitation on the 7th, 14th, and 21st days of the experiments, 10–12 rats from the control and experimental groups. After decapitation of the animals, the blood was collected into cooled tubes with heparin, centrifuged at 3000 rpm for 15 min, the obtained blood serum was stored at –20 0C until the moment of the study. The intensity of lipid peroxidation processes was assessed by examining the content of diene conjugates (DC), lipid hydroperoxides (HL) according to the methods developed by I.D. V.G. Flask [3], vitamin E according to the method of R.Zh. Kiselevich, catalase according to the method modified by E.A. Borodin in the blood of rats. These techniques are reflected in our previously published works [5, 7, 9, 10]. The following instruments were used in the work: a KFK-2mp spectrophotometer, a UNICO spectrophotometer, a Solar PV 1251 C photoelectric colorimeter. Statistical processing of the results was carried out using the Student's test (t) using the Statistica v.6.0 program, differences were considered significant at $p < 0.05$. When presenting the data, the results obtained in the control groups (intact rats) are conditionally taken as one (100%), the graphical representation of the dynamics of the LPO/AOS components in the experimental animals (subjected to various influences) reflects the percentage deviation from the control.

Results and discussion. Cold exposure to rats induces an increase in the activity of lipid peroxidation processes with the accumulation of LPO products and a decrease in the activity of AOS components in the blood of cooled animals: the content of DC increases by

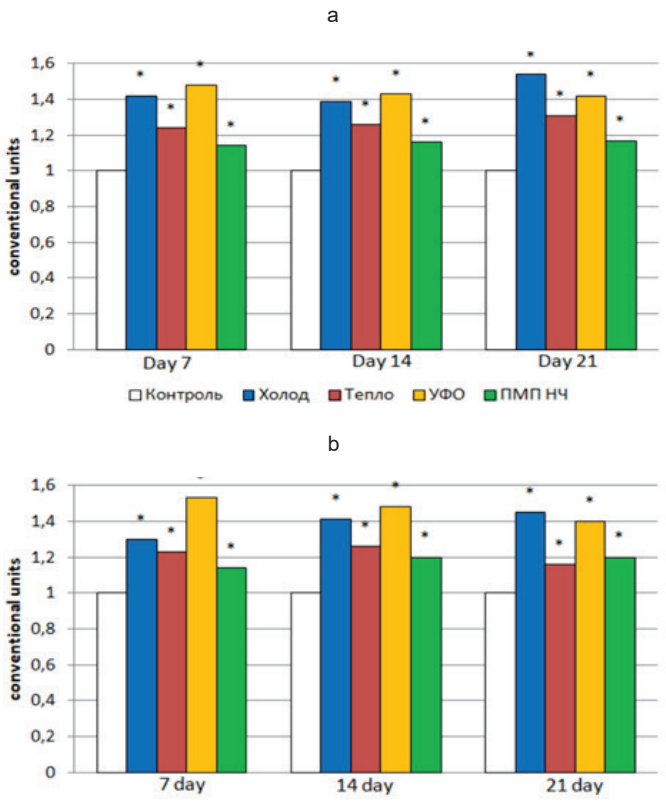

Fig. 1. Dynamics of diene conjugates (a) and lipid hydroperoxides (b) in intact (control) and exposed laboratory animals. In Fig. 1-3: * - significance of differences in relation to control at $p < 0.05$

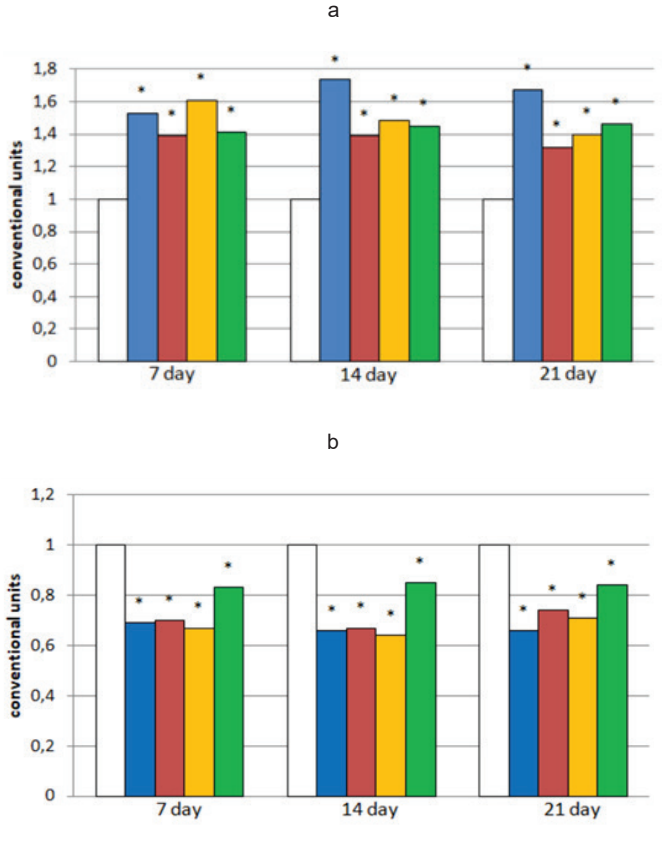

Fig. 2. Dynamics of malondialdehyde (a) and ceruloplasmin (b) in intact (control) and exposed laboratory animals

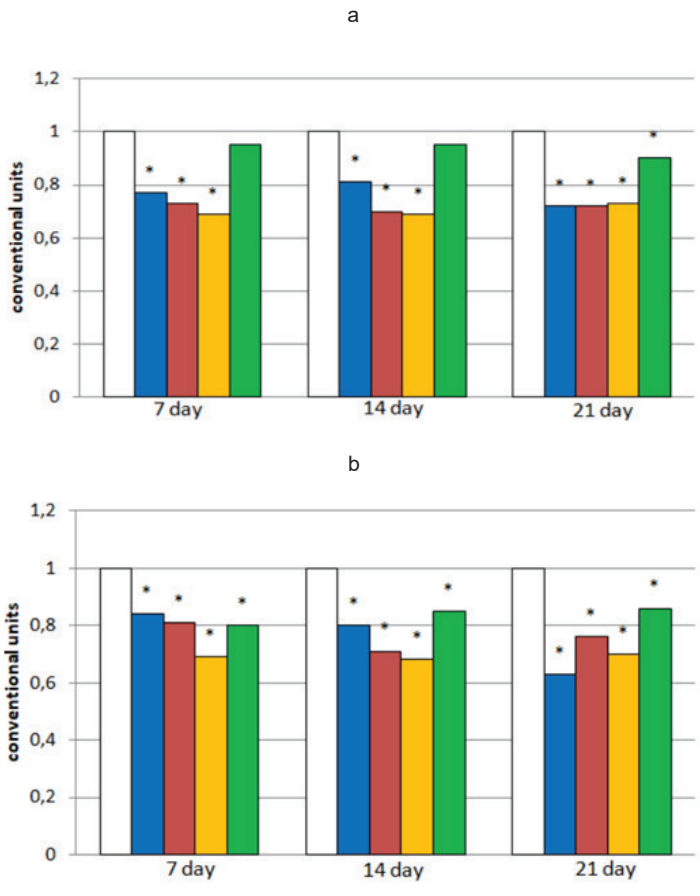

Fig. 3. Dynamics of vitamin E (a) and catalase activity (b) in intact (control) and exposed to various effects of laboratory animals

42%, 39%, 54% by the end of the first, second and third weeks of the experiment, respectively, relative to intact rats (Fig. 1), GL - by 30%, 41%, 45%, respectively (Fig. 2), MDA - by 53%, 74%, 67% (Fig. 3); against this background, the concentration of ceruloplasmin decreases by 31%, 34%, 34%, respectively (Fig. 4), vitamin E - by 23%, 19%, 28% (Fig. 5), catalase - by 16%, 20%, 37 % (Fig. 6). When using the thermal model of the experiment, the growth of lipid peroxidation products relative to the control (intact animals) was 24% (day 7), 26% (day 14), 31% (day 21) in relation to DC (Fig. 1), 23%, 26%, 16%, respectively, in relation to GL (Fig. 2), 39%, 39%, 32% in relation to MDA (Fig. 3), which was accompanied by a decrease in the level of ceruloplasmin by 30%, 33%, 26%, respectively (Fig. 4), vitamin E - by 27%, 30%, 28% (Fig. 5), catalase - by 19%, 29%, 24% (Fig. 6). Ultraviolet irradiation (UVR) of laboratory animals leads to the accumulation of DC by 48%, 43%, 42% by the end of the first, second and third weeks of the experiment (Fig. 1), GL - by 53%, 48%, 40%, respectively (Fig. 2), MDA - by 61%, 48%, 40% (Fig. 3); under these conditions, the content of ceruloplasmin decreases by 33%, 36%, 29%, respectively (Fig. 4), vitamin E - by 31%, 31%, 27% (Fig. 5), catalase - by 31%, 32%, 30 % (Fig. 6) in comparison with similar parameters in animals of the control group. Exposure to PMF NPs in rats is accompanied by an increase in the content of DC by 14%, 16%, 17% by the end of the first, second and third weeks of the experiment, respectively, relative to intact rats (Fig. 1), GL - by 14%, 14%, 20%, respectively (Fig. 2), MDA - by 46%, 45%, 46% (Fig. 3); the decrease in the content of ceruloplasmin was 17%, 15%, 16% on days 7, 14, 21 of the experiment (Fig. 4), vitamin E - by 10% by the end of the experiment (only a downward trend was recorded on days 7 and 14) (Fig. 5), catalase - by 20%, 15%, 14% (Fig. 6). As a result of a comparative assessment of various models of the formation of oxidative stress in laboratory animals, we can state an earlier response (already by the end of the first week of the experiment) of the LPO/AOS system with a shift to the prooxidant side when exposed to ultraviolet rays on a warm-blooded organism, which, in our opinion, is due to - firstly, with the mechanism of action of ultraviolet rays and the formation of free radicals from valence-saturated lipid molecules in biological systems at the initial stage of chain nucleation under UV conditions; secondly, with the genus of laboratory animals (rats), for which exposure to ultraviolet radiation is the most pronounced stress factor in comparison with other effects studied by us. In turn, cold exposure leads to a stable accumulation of lipid peroxidation products against the background of a decrease in AOS activity by the end of the third week of the experiment, which exceeds the previous model in parameter values and can be used in experiments of sufficient duration, for example, when studying the antioxidant activity of phytopreparations, a lasting effect from the use of which develops, as a rule, after 3-4 weeks. It is important to note the absence of significant fluctuations from the 7th to the 21st day of all determined indicators when using the thermal model, the range of which was from 0 to 10%, and the PMF LF, where the changes were in the range from 0 to 6%, which indicates stable and unidirectional processes occurring in vivo, however, the concentration of LPO products/AOS components during hyperthermia was 1.5-2 times higher than similar parameters under magnetic induction conditions.

Thus, depending on the purpose of modeling oxidative stress in a warm-blooded organism, we recommend ultraviolet irradiation of laboratory animals if it is necessary to create an experimental model in a shorter time; stable changes in the LPO/AOS system in models of hyperthermia and magnetic induction are more adequate when testing different doses of new antioxidants or registered drugs tested for the presence of antioxidant activity.

Conclusions.

1. Modeling oxidative stress by exposure to ultraviolet rays on laboratory animals allows, by the end of the first week of the experiment, to induce an increase in the intensity of lipid peroxidation processes with the accumulation of lipid peroxidation products by 48-61% and a decrease in the activity of AOS components by 31-33% in comparison with the control, which exceeds similar parameters on day 7 in models of hypo-, hyperthermia and magnetic induction.

2. Cold exposure in rats is accompanied by a more pronounced shift in the LPO/AOS system towards the prooxidant side by the end of the third week, which is confirmed by an increase in the concentration of lipid peroxidation products by 45-67% and a decrease in the level of AOS components

by 28-37% relative to intact animals.

3. Induction of LPO processes under in vivo conditions by hyperthermia and PMF LF leads to a more stable state of the LPO/AOS system in dynamics from 7 to 21 days of the experiment, according to which, when assessing the values of the main parameters, it is possible to ascertain the formed oxidative stress.

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M.V. Shubina, S.Yu. Tereshchenko, N.N. Gorbacheva, L.V. Lapteva

PREVALENCE OF FUNCTIONAL GASTROINTESTINAL DISEASES IN SCHOOLCHILDREN OF KRASNOYARSK BY ROME IV CRITERIA

DOI 10.25789/YMJ.2023.82.06

УДК 616.3-008.1

Federal Research Center "Krasnoyarsk Scientific Center of the Siberian Branch of the Russian Academy of Sciences (FRC KSC SB RAS), Research Institute for Medical Problems in the North (RIMPN), Krasnoyarsk, Russia: **SHUBINA Margarita Valerievna** – junior research associate, Clinical Research Unit of Somatic and Mental Health of Children, RIMPN, FRC KSC SB RAS, Krasnoyarsk, Russia, e-mail: marg-shubina@mail.ru; **TERESHCHENKO Sergey Yurievich** – MD, Professor, Head of Clinical Research Unit of Somatic and Mental Health of Children, RIMPN, FRC KSC SB RAS, Krasnoyarsk, Russia, e -mail: legise@mail.ru; **GORBACHEVA Nina Nikolaevna** – senior research associate, Clinical Research Unit of Somatic and Mental Health of Children, RIMPN, FRC KSC SB RAS, Krasnoyarsk, Russia, e-mail: n-n-gorbacheva@yandex.ru; **LAPTEVA Lyudmila Vladimirovna** – PhD, leading research associate, Clinical Research Unit of Somatic and Mental Health of Children, RIMPN, FRC KSC SB RAS, Krasnoyarsk, Russia, e- mail:yara3011@yandex.ru

The prevalence of functional gastrointestinal diseases (FGDs) in adolescents of 11-18 years old in school (500 individuals) and in a specialized gastroenterology unit (141 individuals), identified by questioning the Russian-language version of the QPGS - RIV questionnaire (Questionnaire on Pediatric Gastrointestinal Symptoms, Rome IV Version) was analyzed. As a result, the prevalence of FGDs in school was as follows: functional dyspepsia (FD) - 5.3%, irritable bowel syndrome (IBS) - 0.6%, abdominal migraine (AM) - 1.0%, functional abdominal pain syndrome (FAPS) - 0.2%, functional constipation (FC) - 5.3%. In the study profile of recurrent abdominal pain (RAP) among children in hospital, FD was 73.6%, IBS - 22.6%, FAPS - 3.8%, 17% of children had both FD and IBS. Compared to the previous version, according to the new criteria, instead of IBS, the FD diagnosis prevailed (due to a decrease of the criterion for the prevalence of pain syndrome, as well as the inclusion of postprandial distress syndrome (PDS) for diagnosis), and the IBS incidence rate decreased threefold (due to the new limiting criteria).

Keywords: adolescents, recurrent abdominal pain, functional gastrointestinal diseases, prevalence, ROME IV.

Introduction: The new Rome criteria of FGDs for revision IV (ROMEIV) were introduced in May 2016 (Table 1).

Significant changes mainly affected functional dyspepsia (FD) and irritable bowel syndrome (IBS). Currently, FD is divided into two independent forms: postprandial distress syndrome (PDS) and epigastric pain syndrome (EPS) both in adults and in children. The criteria for diagnosing IBS have been corrected, i.e. in the previous version, along with abdominal pain, two more conditions should have occurred: amelioration after bowel move-

ments and a change in stool frequency or consistency, now just one of them is enough. However, there appeared to be new IBS criteria, which didn't occur in the previous version, namely the absence of association with food (i.e. pain in IBS should not occur during meals or immediately after eating) and lack or infrequent relief from constipation medication, and lack of association with menstruation for girls.

Taking into account all the changes added to the Rome criteria for FGDs, as well as a lack of current specific epidemiological data on FGDs in Russian children (in the official statistical reports of most hospitals, the diagnoses such as chronic gastritis and gastroduodenitis are in the first place [1]). The purpose of our study was: to determine the prevalence of FGDs in schoolchildren, according to the new Rome IV revision, and also to find out the profile of RAP in children admitted to inpatient care in the gastroenterology unit.

Materials and methods: To establish the prevalence of FGDs in children, according to ROMEIV, 500 schoolchildren (217 boys and 283 girls) aged 11-18 years were examined in secondary school № 153 in Krasnoyarsk. All children filled out screening questionnaires to identify complaints from the gastrointestinal tract over the past month, i.e upper abdominal pain, heartburn, discomfort after eating a normal-sized meal, inability to finish the usual portion of food, pain in the lower abdomen and around the navel, intense pain around the navel area for the last year, nausea, belching and stool frequency forcing to interrupt all activity. If a complaint was defined with incidence of 4 or more days a month, the child was asked to fill out the Russian version of QPGS-RIV to determine the specific FGDs. To clarify nosological structure of RAP (in accordance with ROMEIV), 141 children (60 boys and 81 girls) aged 11-18 years were examined in children in hospital, who were admitted for in-patient care in the gastroenterology unit of the Hospital of RIMPN in Krasnoyarsk. The study results of qualitative parameters in the experimental groups are presented as P (CI)%, where P is incidence, CI — 95% (confidence interval). The statistical significance of differences in qualitative traits was analyzed using the Difference test between two proportions.

Results and discussion. According to the classical criteria identified by J. Apley and N. Naish, clinically significant RAP should be understood as "3 or more episodes of abdominal pain in the last 3 months, disrupting the child's daily activity." On the other hand, in the protocol of the expert meeting on FGDs (ROME IV criteria, 2016, [7]) there is only a sign of RAP incidence ("at least once a week for the last 2 months"), and the subjective pain severity is not included in diagnosing FD, IBS and FAPS.

According the data we obtained in the school sample, only 9.7 (6.5-14.4) % of children matched the J. Apley and N. Naish criteria. There was sure to be more such children in hospital - 29.1 (22.2-37.1) %. Even less number of children in school sample had RAP according to the ROME IV criteria, i.e. "More often than once a week for 2 months" - 4.3% (2.8-6.5) %. As to the hospital, on the contrary, there were slightly more individuals than those who met the J. Apley and N. Naish criteria - 35.7 (28.3-44.0) % (Table 2). Thus, we can conclude that the RAP incidence in the population of Russian schoolchildren does not differ from the median of RAP prevalence in children in other countries, determined by the results of numerous foreign population studies (8.4 (5.7-11.8) %) [5]. Girls can also be noted to have 2 times more often clinically significant RAP, whatever how it was assessed. Similar data indicating a higher prevalence of RAP in adolescent girls, compared to boys, were obtained by other researchers [4].

The analysis results of the nosological structure in children sample with RAP, as well as the general prevalence of FD, IBS, and FAPS, in accordance with the ROME IV criteria, are given in Table 3.

As can be seen from the data obtained, in school-age children, the incidence of clinical signs of FGDs is ranged from 0.4 (0.1-1.5) % for FAPS to 5.3 (3.7-7.7) % for FD. As expected, complaints more

Table 1

Diagnostic criteria for the main nosological causes of recurrent abdominal pain (RAP) in children. All criteria were developed for children aged 4-18 years. It is based on the pediatric section of the "Rome IV Criteria"

Functional dyspepsia	Irritable bowel syndrome	Functional abdominal pain	Abdominal migraine
• Symptoms should bother at least once a week for the last 2 months			• 2 episodes or more in the last 6 months
1. Postprandial distress syndrome (PDS): • Discomfort, feeling of full stomach, nausea, or bloating after eating a normal-sized meal • Inability to finish the usual portion of food due to a feeling of fullness in the stomach ***2. Epigastric pain syndrome (EPS):*** • Pain above the navel and/or a burning sensation behind the breastbone • Lack of association with defecation (alleviation, onset or aggravation of symptoms, as well as association with a change in stool frequency or consistency	• Abdominal pain followed by one (or more) signs in at least 25% of cases when it occurs: • a) association with defecation (alleviation, onset or aggravation of symptoms); • b) association with stool frequency; • c) association with a change in stool frequency or consistency; • lack of association with only food intake or menstruation; • rare (25% or less) pain relief after taking laxatives for constipation.	• Abdominal pain • Lack of association with defecation (alleviation, onset or aggravation of symptoms, as well as association with a change in stool frequency or consistency • The patient does not meet the criteria for other GIT disorders	• Paroxysmal episodes of intense acute umbilical pain that lasts an hour or more • Attack-free intervals of a normal health condition last from several weeks to several months • Painful episodes accompanied by disruption of the normal child's activity (cannot play, do daily activity); • Pain attacks accompanied by at least two of the following features: a) anorexia b) nausea c) vomiting d) headache e) photophobia f) pallor
• Symptoms, after appropriate medical assessment, cannot be attributed to another health condition.			

*All criteria are developed for the age group of 4-18 years on the basis of the pediatric section of the Roman Criteria IV.

often correspond to a specific diagnosis according to the ROME IV criteria in children sent to a gastroenterological hospital. It is noteworthy that the dominant nosological unit, both in school and in inpatient sample, is FD. This contradicts the results of our previous study [3], as well as similar studies of foreign authors using the previous version of the Roman criteria ROME III for the nosological structure of FGDs to be assessed, whereby IBS was the leading cause of RAP in children [2]. That is mainly due to two reasons. The first one is that the diagnostic criteria for FD have been expanded (by adding PDS and lowering the threshold for pain incidence), and the second one is that the diagnostic criteria for IBS have been reduced (due to the emergence of new more differential requirements for the diagnosis). Indeed, the increase in FD was mainly due to the PDS, with the prevalence being 4.5 (3.0-6.7) % in school and 36.4 (28.9-44.7) % in hospital, while EPS was at the same low level both in school (1.2 (0.6-2.6)%) and in hospital (3.6 (1.6-8.1)%). Along with that, the requirements for IBS have increased, in particular, excluding the concept of discomfort from the definition of IBS, adding new conditions for the lack of association with food intake and menstruation, as well as the lack of relief after taking laxatives. Such findings were also noted in the first population study in English-speaking countries, where the ROMEIV criteria were used, with the prevalence of IBS in adults being halved compared to the ROME III criteria: from 11% to 6.1% in the United States; from 11.7% to 5.8% in Canada and from 10.6% to 5.5% in Great Britain [10].

The prevalence of FAPS in schoolchildren in accordance with the ROME IV criteria should be expected to decrease from 2.9 (2.0-4.2) % in 2008 [3] to 0.2 (0.05-1.1) %, and in hospital from 2.3 (0.8-6.6)% [3] to 1.4 (0.4-5.0)%, given that both for FAPS and IBS, additional conditions of lack of association with food intake or menstruation were added in the ROME IV criteria. Moreover, children who met the EAPS criteria according to the previous version of the questionnaire are likely to be in the FD group.

The prevalence of FD with switching to the ROMEIV criteria, on the contrary, increased from 4.5 (2.9-6.7) % to 5.3 (3.6-7.7) % in school and from 12.1 (7.7 -18.6) % to 22.1 (16.1-29.7) % in hospital. This appeared to be influenced by the fact that some of the children with IBS-like symptoms, with a gain after taking laxatives, have currently moved into this group. According to foreign studies, functional

Table 2

Prevalence of RAP in Krasnoyarsk adolescents according to J. Apley and N. Naish. and ROMEIV criteria. % (95% CI)

РБЖ	Total sample	Boys	Girls	P	Age 11-14 years old	Age15-18 years old	P
School sample							
By J. Apley and N.Naish criteria *	N=216	N=91	N=125		N=125	N=91	
	9.7 (6.5-14.4)	6.6 (3.1-13.7)	12.0 (7.4-18.9)	0.187	8.8 (0.5-15.1)	11.0 (6.1-19.1)	
By ROME IV criteria	N=491	N=214	N=277		N=300	N=189	
	4.3 (2.8-6.5)	1.4 (0.5-4.0)	6.5 (4.2-10.0)	0.036	5.7 (3.6-8.9)	2.1 (0.9-5.3)	0.056
Inpatient sample							
	N=141	N=60	N=81		N=64	N=77	
By J. Apley and N.Naish criteria *	29.1 (22.2-37.1)	15.0 (8.2-26.2)	39.5 (29.6-50.4)	0.002	31.3 (21.2-43.4)	27.3 (18.6-38.2)	
By ROME IV criteria	35.7 (28.3-44.0)	23.3 (14.5-35.5)	44.4 (34.1-55.3)	0.011	29.7 (19.9-41.8)	40.3 (30.0-51.5)	

Note: * - To diagnose RAP according to J. Apley and N. Naish was slightly changed (pain with incidence of 1 or more times a month limiting daily activity, instead of 3 episodes in the last 3 month limiting daily activity, i.e. excluding the time factor, was taken into account).

Table 3

The general prevalence of FGDs and nosological structure of RAP in school and inpatient samples, according to ROME IV criteria, % (95% CI)

	School sample		Inpatient sample	
FGDs	General school sample	RAP children by ROMEIV criteria	General inpatient sample	RAP children by ROMEIV criteria
	N=500	N=27	N=140	N=53
FD	5.3 (3.7-7.7)	59.2 (40.6-75.5)	38.6 (30.9-46.9)	73.6 (60.3-83.5)
IBS	0.6 (0.6-1.8)	11.1 (4.0-28.2)	8.6 (5.0-14.4)	22.6 (13.5-35.6)
FAPS	0.2 (0.05-1.1)	0	1.4 (0.4-5.0)	3.8 (1.2-12.7)
FD+IBS	0.4 (0.1-1.5)	7.4 (2.3-23.5)	6.4 (3.5-11.8)	17.0 (9.3-29.3)

disorders are detected in almost every fourth child, i.e. 15.3% [6], 24.9% [8], 0.7-29.6% [9].

The prevalence of AM in schoolchildren, determined by ROME IV, when compared with the results obtained by ROME III, remained at the same level and amounted to 1.0%, predominantly in girls, being consistent with the results of foreign studies [6]. However, in hospital, AM significantly decreased from 8.1% by the ROME III criteria to 2.9 (1.2-7.1) % by the ROME IV, which can be explained by a 2-fold increase in attack frequency required for the AM diagnosis according to the new version of ROME IV, compared to ROME III (earlier 2 episodes in a year, today 2 episodes in half a year).

Conclusion:

1. The incidence of RAP in the population of Russian schoolchildren (11-18 years old) does not differ from the median of the RAP prevalence, determined by the results of foreign population studies, and ranges from 4.3%, according to the ROME IV criteria, to 9.7%, according to J Apley and N. Naish, predominantly in girls.

2. The overall prevalence of FD, IBS, FAPS, AM and FC according to the ROME IV criteria in children of the unbiased school sample was 5.3%, 0.6%, 0.2%, 1.0% and 5.3%, respectively.

3. In most cases the complaints correspond to the diagnosis of FD (73.6%), IBS (22.6%) according to the ROME IV criteria in children sent to inpatient examination and treatment for RAP. A combination of FD and IBS symptoms occurred in 17.0% of children in inpatient sample with non-cyclic RAP.

4. According to the ROMEIV criteria, as contrasted with the previous version, the nosological structure of RAP has sig-

nificantly changed, with the FD diagnosis prevailing (due to the inclusion of PDS for diagnosis), and the IBS incidence rate decreasing threefold (due to the new limiting criteria).

5. Particular attention should be paid to the high prevalence of PDS among children observed in almost half of children with RAP in-school sample (44.4 (27.5-62.8) and in 66.7 (53.3-77.8)% of cases of RAP in hospital, since it is the PDS that indicates an evacuation disorder from the stomach, congestion in the intestine, thereby encouraging the development of peptic ulcer disease and GERD.

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E.A. Balashova, M.Yu. Gavryushin, I.L. Shadrina

PHYSICAL AND SEXUAL DEVELOPMENT OF BOYS WITH LATENT IRON DEFICIENCY WITHOUT ANEMIA

DOI 10.25789/YMJ.2023.82.07

УДК: 616.155.194.8:616-095

The aim of the study was to analyze the possible impact of iron deficiency (ID) without anemia on physical and sexual development of adolescent boys

Materials and methods. 46 adolescent boys with ID without anemia (serum ferritin (SF) level <15 μg/l in the absence of inflammation defined by normal level of C-reactive protein) and 99 healthy peers were recruited in the study. All the participants were without underlining chronic illness. Average age was 14.8±0.9 years.

Results. Physical development of adolescent boys with ID without anemia did not differ from that of their healthy peers. We found no correlation between anthropometric indicators and SF level. The SF level was significantly higher in overweight or obese children as determined by bioelectrical impedance then in normal weight children (35.15 (20.8; 48.6) vs. 18.8 (16,4; 20,0), p<0.001) and more so in obese children (40.8 (19.4; 56.3) vs. 18.8 (16.4; 20.0), p=0.012). Body fat (rs = 0.210 p = 0.013), visceral fat (rs = 0.208 p = 0.014) and body fat percentage (rs = 0.239 p = 0.005) correlated with SF level. Sexual development of boys with ID without anemia was within the age norm, but it was generally on the earlier stage then in the control group and correlated with the level of SF: for pubic hair rs = 0.186, p = 0.028 and for genitalia development rs = 0.224, p = 0.008.

Conclusion. ID without anemia did not altered physical development of adolescent boys. Obesity or excess weight is associated with a higher level of SF, which should be considered when diagnosing ID. ID is associated with sower sexual development in boys.

Keywords: bioelectrical impedance, iron deficiency, adolescents, sexual development, physical development

Introduction. Iron deficiency (ID) is one of the most common metabolic disorders. Results of the meta-analysis have shown a global rate of 16.42% of iron deficiency anemia and 17.95% of iron deficiency without anemia among children under the age of five, who are at the highest risk of this condition [15]. The frequency of iron deficiency (ID) in adolescents is significantly lower and subjected to gender differences. For example, according to Zakharova I.N., iron deficiency in adolescent girls occurs 1.6 times more often than in boys [8]. Another study in the Stavropol Region has shown that in the age group of 12-18 years, 70% of patients who received inpatient treatment for IDA were girls [7]. However, some studies suggest a relatively high incidence of ID without anemia in adolescent boys. For example, according to Sharuko G.V., the frequency of ID without anemia is 20.6% in boys under 14 years and increases to 32.1% in adolescence [2].

ID developing during the first 1000 days of life, that is, from conception and up to 2 years of age, has multiple detrimental effects on children's health, including changes in immunological reactivity and increased infectious morbidity [19], delayed cognitive [12] and psychomotor development [26], as well as a delay in linear growth and body weight [10]. At the same time, the effects of ID, especially without anemia, developing later in life

BALASHOVA Elena A. – Samara State Medical University, department of hospital pediatrics, Russia, Samara, e.a.balashova@samsmu.ru ORCID 0000-0001-5766-6741; **GAVRYUSHIN Michail Yu.** – Samara State Medical University, Institute of Preventive Medicine, Russia, Samara, m.yu.gavryushin@samsmu.ru ORCID 0000-0002-0897-7700; **SHADRINA Inna L.** – Samara City Hospital #7, Russia, Samara, Samara State Medical University, department of hospital pediatrics. 7gbshadrina@mail.ru ORCID 0000-0002-6000-1959

during adolescence, are less understood. The impact of ID on reproductive health has been studied quite comprehensively in girls. Even ID without anemia promotes a decrease in ovarian reserve [4], and up to 43% of women with infertility suffer from ID [6]. Studies of sexual development and reproductive function in males are predominantly focused on iron overload but not ID [16, 22].

The aim of this study was to analyze the possible impact of ID without anemia on the physical and sexual development of adolescent boys.

Methods. The study was held out in Samara Cadet Corps as a part of a routine annual examination.

Inclusion criteria: age at the time of the study from 12 to 15 full years, consent of parents or legal representatives to participate in the study.

Exclusion criteria: refusal to participate in the study, acute infectious diseases with hyperthermia during the entire study period, diarrhea within 7 days before the laboratory analysis, routine medication of any kind, severe swelling of the extremities, failed complete blood count and/or biochemical blood test at the recruitment (clot formation, hemolysis, insufficient volume of material), significant limbs' edema.

Anthropometric measurements (head, chest, hips, and shoulder circumference) were measured with a soft centimeter tape [16]. Standing height was measured by a stadiometer and weight - by electronic floor scales with an accuracy of 100 grams. Skin-fat folds thickness at 4 points was measured with a plastic caliper and the results were rounded to the nearest 0.5 cm [17]. Overweight was defined as body mass index (BMI) exceeding 1 SD, obesity - > +2SD, and underweight < -2 SD. Bioelectrical impedance analysis was carried out in the morning 2.5-3 hours after breakfast using ABC-02 "Medass" (Russia) analyzer at a probing current frequency of 50 kHz according to the eight-pole circuit [17]. The fat mass percentage was evaluated per body fat reference curves by McCarthy H. et al. [18], the excess adipose tissue was defined as above the 85th centile for the corresponding age, obesity - above the 95th centile, adipose tissue deficiency - below the 2nd centile. Sexual development was assessed by the Tanner method by a trained pediatric endocrinologist [19].

Laboratory assays were performed as follows: automated complete blood examination (Sysmex XT-2000i, Sysmex, Japan), serum ferritin and C-reactive protein (fluorescent flow cytometry, Integra 400 plus, Roche, Switzerland). Anemia was defined as hemoglobin level below 120 g/l, ID was defined as serum ferritin level less than 15 μg/l in the absence of inflammation (C-reactive protein <5mg/L) [2]. IDA was diagnosed when anemia and ID were concomitant, latent ID or ID without anemia - in the presence of iron deficiency and the absence of anemia.

Statistic analysis. Accumulation, correction, systematization of initial information, and visualization of the obtained results were carried out in Microsoft Office Excel 2016 spreadsheets. Statistical analysis was carried out using the STATISTICA 13.3 program (StatSoft. Inc). Quantitative indicators were evaluated for distribution using the Kolmogorov-Smirnov criterion, as well as indicators of asymmetry and kurtosis. Quantitative indicators with a normal distribution were presented as mean (M) and standard deviation (SD), and quantitative indicators with a distribution that differs from normal were described as median (Me) and the lower and upper quartiles (Q1;Q3). Comparison of quantitative data was done by calculating Student's t-test for normally distributed data and The Mann-Whitney U-test for data whose distribution differed from normal. The relationship between quantitative data was assessed by the calculation of the Spearman rank correlation coefficient.

Results and discussion. Due to the limited number of children in the cadet corps and the low ID rate identified during the initial examination, we enrolled adolescents in the study in two steps: from 18.03.2021 to 30.04.2021 and from 08.11.2022 to 16.12.2022 by continuous sampling as part of a routine examination. We assessed iron storage levels in 265 adolescents. 51 (19.2% of examined) boys had ID, 5 of them - in the form of IDA. Due to the small number of children with IDA, we excluded them from further analysis. The ID group consisted of 46 adolescents with ID without anemia. The healthy control group included 99 adolescents with adequate iron stores and normal values of complete blood count. The average age of adolescents

Table 1

Anthropometric indicators in the comparison groups

Indicator	ID, n=46	Healthy control, n=99	p
*Height, cm	166.4 (8.0)	166.3 (9.4)	0.978
*BMI	21.6 (2.1)	21.3 (3.1)	0.464
*Weight, kg	59.6 (8.0)	59.2 (11.0)	0.781
**Waist circumference, cm	71.0 (70.0; 76.0)	71.0 (65.0; 75.0)	0.196
*Hip circumference, cm	90.5 (4.9)	89.7 (6.3)	0.389
**Wrist circumference, cm	16.4 (16.0; 17.0)	16.0 (15.5; 16.5)	0.128
**Chest circumference at maximum exhalation, cm	89.2 (7.4)	87.9 (7.4)	0.331
*Chest circumference at maximum inhalation, cm	82.1 (6.2)	81.3 (6.9)	0.485
*Shoulder circumference at maximum muscle tension, cm	28.7 (3.1)	27.9 (3.2)	0.200
* Shoulder circumference in a relaxed state, cm	25.7 (2.3)	25.4 (2.7)	0.373
*Head circumference, cm	55.5 (1.5)	55.4 (1.6)	0.829

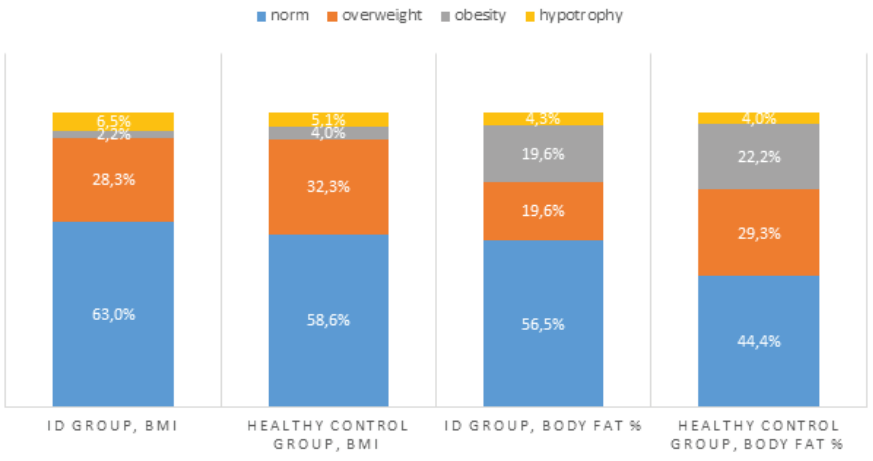
* - normal distribution, M (m). Welch's t-test
** - non-normal distribution, Me (Q1; Q3). Mann-Whitney U-test

Table 2

Results of anthropometry in the comparison groups

Fold thickness, mm	ID, n=46	Healthy control, n=99	p
over the biceps	8.0 (4.0; 9.0)	7.0 (4.0; 9.0)	0.625
over the triceps	10.0 (8.0; 12.0)	10.0 (6.0; 11.0)	0.703
over the scapula angle	10.0 (8.0; 12.0)	10.0 (8.0; 12.0)	0.885
over the inguinal fold	8.0 (7.0; 10.0)	8.0 (7.0; 10.0)	0.409

non-normal distribution, Me (Q1; Q3). Mann-Whitney U-test



Body weight assessment in the comparison groups by BMI and body fat percentage

was 14.8 (0.9) years, age in the comparison groups did not differ: 14.6 (0.9) years in the main group and 14.8 (0.7) years in the control group (p=0.278).

Main anthropometric indicators in the comparison groups did not differ (Table 1).

BMI of most adolescents was within the normal range: 63.0% (29) in the ID group and 58.6% (58) in the control group (p=0.609). The percentage of overweight children was 28.3% (13) and 32.3% (32), respectively. One adolescent in the ID group (2.2%) and four in the healthy control group (4.0%) were obese. Additionally, we found that 6.5% (3) in the ID group and 5.1% (5) in the healthy control group were underweight.

The thickness of the subcutaneous fat fold as measured by a caliper also did not differ between the groups (Table 2): the mean value of skinfold thickness was 7.5 mm (6; 9.5) in the ID group and 9.0 mm (6.75; 10.5) in the healthy control group (p=0.228).

Results of biological impedance have shown that the average body fat, body fat %, and visceral fat in the groups did not differ. The body fat in the ID group was 12.7 (3.4), while in the healthy control group - 13.0 (4.8) (p=0.675), body fat percentage 21.4 (5.4) and 21.5 (5.8), respectively (p=0.874).

The proportion of children diagnosed as overweight and obese by BMI significantly changes when using body fat percentage determined by bioelectrical impedance analysis (Figure). The differences between groups remain insignificant (p=0.534).

Other parameters of bioelectrical impedance analysis, such as active cell mass and skeletal muscle mass in comparison groups, did not differ (Table 3).

The SF level is significantly higher in children who are overweight and, especially, obese as determined by body fat percentage.

Data on the relationship between obesity and ID is inconclusive. Some studies did not reveal any association between these conditions [23,24]. On the other hand, a meta-analysis has found an association between IDA and obesity in children (OR 2.1, 95% CI 1.4-3.2) [25]. The possible mechanism of association of ID and obesity is through chronic inflammation and subsequent modulation of hepcidin level and reduction of iron absorption in the intestine [18]. Coexisting inflammation could mask ID. Therefore, the use of inflammation-independent parameters of iron stores, such as soluble transferrin receptors, or SF cut-off val-

Table 3

Comparison of bioelectrical impedance analysis parameters in the comparison groups

Parameter	ID, n=46	Healthy control, n=99	p
Active cell mass	25.4 (4.8)	25.2 (5.3)	0.826
Active cell mass percentage	53.9 (3.2)	54.4 (3.2)	0.415
Skeletal muscle mass	27.3 (4.1)	26.8 (4.1)	0.494
Skeletal muscle mass percentage	58.0 (1.9)	58.3 (4.2)	0.619

normal distribution, M (m)

Table 4

Serum ferritin level and body mass correlation

Obesity and overweight as defined by BMI			
	Obesity and overweight, n=50	Normal body weight, n=87	p
Serum ferritin, µg/l	30.7 (19.3; 45.7)	28.7 (17.2; 43.3)	0.617
	Obesity. n=5	Normal body weight. n=72	p
Serum ferritin, µg/l	40.6 (36.7; 50.0)	28.7 (17.2; 43.3)	0.247
Obesity and overweight as defined by body fat percentage			
	Obesity and overweight. n=69	Normal body weight. n=70	p
Serum ferritin, µg/l	35.15 (20.8; 48.6)	18.8 (16.4; 20.0)	<0.001
	Obesity. n=31	Normal body weight. n=53	p
Serum ferritin, µg/l	40.8 (19.4; 56.3)	18.8 (16.4; 20.0)	0.012

Примечание. Распределение, отличное от нормального, Me (Q1; Q3). U критерий Манна-Уитни

Table 5

Correlation of the level of SF, mcg/l, with body weight and individual bioimpedance parameters characterizing fat metabolism

Body weight, kg	BMI	Fat mass	Visceral fat	% of fat mass	% skeletal muscle mass
$r_s = 0.118$ $p = 0.165$	$r_s = 0.037$ $p = 0.665$	$r_s = 0.210$ $p = 0.013$	$r_s = 0.208$ $p = 0.014$	$r_s = 0.239$ $p = 0.005$	$r_s = -0.136$ $p = 0.111$

Note. Spearman's Rho coefficient, significant differences at $p < 0.05$.

ues corrected for inflammation, can give more reliable results [23]. Bioelectrical impedance analysis is a preferable method in overweight and obesity diagnostics.

Further analysis showed a correlation between SF and adiposity indicators of bioelectrical impedance but not between SF and BMI (Table 5).

Our results are similar to other studies, which also did not reveal a relationship between BMI or anthropometric parameters and ID [17].

The sexual development of all boys was within the age norm, which is partially explained by the wide age limits for puberty onset in boys: from 9 to 14 years [3]. Nevertheless, in the ID group, sexual development was slower than in the control group. Pubic hair development in the ID group was rated at 2.5 (2; 3), while in the healthy control group - at 3 (3; 4) points ($p = 0.009$), and genital development at 3 (2; 4) and 4 (3; 5) scores, respectively ($p=0.022$). There were no significant differences in axillary hair growth ($p=0.296$). We also found a correlation between the sexual development level of adolescent boys and SF: for pubic hair growth $rs = 0.186$, p (2-tailed) $= 0.028$, for genitals development $rs = 0.224$, p (2-tailed) $= 0.008$.

Existing studies of iron storage and sexual development relationship are primarily focused on severe iron overload. The negative impact of infusion-dependent thalassemia and hereditary hemochromatosis on sexual development and reproductive function is known [14]. At the same time, iron plays a critical role in spermatogenesis and sperm motility [13, 20], and testosterone is a natural regulator of iron metabolism through hepcidin synthesis inhibition [14]. Thus, ID may be a result of puberty delay in boys and the absence of testosterone effect on iron absorption.

Conclusion. The physical development of children with ID, assessed by anthropometric measurements, does not differ from healthy controls and does not correlate with SF level. Overweight and obesity determined by body fat percentage are associated with higher SF levels, probably due to mild chronic inflammation. In cases of visceral obesity, higher cut-off values of 30 µg/l for SF can improve the accuracy of diagnosing ID. Further studies of iron metabolism in obese children and adolescents are necessary to develop practical guidelines for their management. The association of ID with slower sexual development in boys determines the need for ID screening and subsequent prophylactics in pubescent boys.

Financing information. The study was carried out with the financial support of the Samara Region Government grant in science and technology.

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

Informed consent. Informed consent was obtained from patients or their parents or legal representatives.

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DIAGNOSTIC AND TREATMENT METHODS

S.E. Avetisov, A.K. Dzamikhova, T.Y. Shilova

RESULTS OF CLINICAL APPLICATION OF DYNAMIC PNEUMOAPPLANATION METHODS OF THE CORNEA IN MYOPIA

DOI 10.25789/YMJ.2023.82.08

УДК 617.753.2

The introduction into clinical practice of research methods based on dynamic corneal pneumoapplanation significantly expanded the possibilities of clinical (in vivo) assessment of the "biomechanics" of eye structures in myopia. This review summarizes the results of using pneumoapplanation methods to assess biomechanical indices in initial myopia and after its laser correction.

Keywords: cornea, biomechanical properties, pneumoapplanation methods, myopia.

The size and shape of the fibrous (corneoscleral) envelope of the eye are the main components of refractive disorders formation. Myopic defocus can be associated primarily with an increase in the anteroposterior axis (APA) as well as an increase in corneal refraction. According to the three-factor theory of myopia pathogenesis by E.S. Avetisov, APA instability with a tendency to increase due to impaired mechanical properties of the sclera is an anatomical cause of progressive myopia [1]. On this basis, the main focus of biomechanical studies of the ocular fibrous membrane in myopia is related to the evaluation of various sclera parameters. On the basis of a set of studies (in vitro mechanical tests, measurement of eyeball stiffness, determination of the deformation coefficient and acoustic density of the sclera, ophthalmomechanography), it was established "that the range of elastic deformations of the sclera decreases and the contribution of the viscous component ..... increases with progressing myopia, resulting in irreversible stretching of the sclera and in an increase in the OPC" [2]. In high myopia, the sclera is characterized by a decrease in strength and elastic modulus predominantly in the equatorial and posterior regions, with these changes occurring first in the equatorial zone, followed by changes in the posterior part of the sclera.

The introduction into clinical practice of research methods based on dynamic corneal pneumoapplanation significantly expanded the possibilities of clinical (in vivo) assessment of the "biomechanics" of eye structures in myopia. This review summarizes the results of pneumoapplanation techniques application for biomechanical evaluation in initial myopia and after its laser correction.

Modern techniques of bidirectional pneumoapplanation of the cornea. The first device to use the effect of an air jet for dynamic corneal deformation was the ORA (Ocluar Response Analyzer, USA). Biomechanical parameters generated by standard ORA software are corneal hysteresis (CH) and corneal resistance factor (CRF). CH is a conventional value reflecting visco-elastic properties of the cornea, while CRF characterizes the resistance of corneal tissue itself which would exist at zero ophthalmotonus [6, 39, 45-46].

Corvis ST technology (Oculus, Germany) belongs to an alternative method of biomechanical characteristics measurement using corneal pneumoapplanation. This device uses high-speed Scheimpflug camera to fix transverse section of cornea (4330 frames per second) during deformation in real time followed by program analysis to obtain different biomechanical indices, the most used ones according to literature data are as follows [2, 4, 17, 22-23, 35, 40, 42]:

- Applanation-1 Time (A1T), ms - time of the first applanation;
- Applanation-2 Time (A2T), ms - time of the second applanation;
- Applanation-1 Lenght (A1L), mm - diameter of the "flattened" corneal area during the first applanation;
- Applanation-2 Length (A2L), mm - diameter of the "flattened" cornea during the second applanation;
- Applanation-1 Velocity (A1V), m/s - inner corneal velocity during the first applanation (it indirectly reflects corneal viscosity);
- Applanation-2 Velocity (A2V), m/s - speed of corneal outward movement to the initial position at the second applanation (the higher the value, the higher the degree of corneal elasticity);
- Highest Concavity Peak Distance (HCPD), mm - diameter of maximum concavity, i.e. the distance between the two highest points of the cornea at its greatest concavity (it indirectly reflects corneal stiffness);
- Highest Concavity Radius (HCR), mm - radius of curvature of the concavity of the cornea at the greatest deformation;
- Deformation amplitude (DA), mm - amplitude of deformation, the value of displacement of the corneal apex at its maximum "indentation" relative to the original shape (MDA - maximum amplitude of deformation);
- Central corneal thickness (CCT), μm - thickness of the cornea in the central zone;
- Deformation Amplitude Ratio (DA Ratio) - ratio between the deformation amplitude at the corneal apex and the

AVETISOV S.E. – PhD, MD, Professor, Academician of Russian Academy of Science, M.M. Krasnov Research Institute of Eye Diseases, Director on Science, I.M. Sechenov First Moscow State Medical University, chief of the Department of Ophthalmology, https://orcid.org/0000-0001-7115-4275; **DZAMIKHOVA A.K.** – post-graduate student, M.M. Krasnov Research Institute of Eye Diseases, https://orcid.org/0000-0002-2245-6346; **SHILOVA T.Y.** – PhD, MD, Professor, Clinic of Modern Ophthalmology, https://orcid.org/0000-0002-6164-5393

deformation amplitude in the paraoptical zone with a radius of 2 mm.

According to theoretical studies, the following changes in the indicated indices may indicate a decrease in corneal stiffness [27, 51]:

- decrease in the time (A1T) and length of the first applanation (A1L);
- increase in the first applanation velocity (A1V) and deformation amplitude (DA) during the first applanation;
- increase in amplitude of deformation (DA) and maximum amplitude of deformation (MDA);
- short peak distance (HCPD) and increased concavity radius (HCR);
- increased second applanation time (A2T), decreased second applanation diameter (A2L) and second applanation velocity (A2V);
- decrease in the amplitude of deformation (DA) during the second applanation.

It has been noted that the radius of greatest concavity (HCR), the rate of second aplanta (A2V) and its diameter (A2L) have large differences in terms of coefficient of variation, while the maximum amplitude of deformation (MDA) is a stable index [5, 49].

It should be noted that in addition to biomechanical parameters, both of the above devices allow determining a number of indices reflecting the level of intraocular pressure.

Results of dynamic corneal pneumoapplanation in baseline myopia. In a series of studies using ORA, a clear correlation between a significant decrease in CH and CRF and an increase in ROP (i.e., degree of myopia) was shown [6-7, 10-11, 28, 36]. At the same time, the difference in CH correlated with the interocular difference in the POC value between the two eyes of each patient [13]. On this basis, it was suggested that eyes with a lower CH value and more easily deformable fibrous membrane are at greater risk of ROP elongation. Most authors explain the decrease in CH value by the fact that the development of myopia is associated with a decrease in sclera and extracellular matrix thickness, an increase in the enzyme matrix metalloproteinase that destroys collagen. In addition, a decrease in collagen fibril diameter and proteoglycansynthesis content was found in the development of myopia, which leads to an additional decrease in sclera thickness and scleral tissue stretching. Similar changes can occur in the cornea during the development of myopia, with the corneal "biomechanics" reflecting its viscoelastic properties and mechanical strength of stromal collagen fibrils interacting with the extracellular proteoglycan matrix [36, 52].

At the same time, other studies did not reveal the above correlation, which may be related to the characteristics of the clinical material (age range, degree of myopia, ethnicity). Thus, with an average myopia of 2.35±2.49 D, no dependence of CH on refractive index was detected. The mean CH and CRF were 11.78 ± 1.55 (range, 6.93-16.53) and 11.81 ± 1.71 (range, 7.83-16.83) mmHg, respectively. These values did not differ significantly by age, gender, or race (the study included individuals from India, Singapore, and China) [24]. In another study with myopia ranging from (-) 9.00 to (-) 19.00 dpts, no correlation was found between biomechanical indices of bidirectional corneal pneumoapplanation and the degree of myopia. The mean values differed between women and men: CRF, 10.326 and 9.810 mmHg (P=0.0266); CH, 10.421 and 9.727 mmHg (P=0.0031), respectively. In addition, there was a negative correlation between biomechanical indices and age and a positive correlation with corneal thickness in the central zone [39].

In a comparative study using the Corvis ST device, 94 patients with myopia between (-) 0.5 and (-) 17.5 dptr aged 29 to 74 years and 25 "emmetropes" aged 19 to 75 years were examined [6]. In high degree myopia, there was an increase in outward applanation velocity (A2V) and peak distance (PD) (-0.398 ± 0.014 m/s and 2.48 ± 0.04 mm) compared to those in moderate degree myopia (-0.352 ± 0.009 m/s and 2.37 ± 0.03 mm) and emmetropia (-0.347 ± 0.012 m/s and 2.36 ± 0.06 mm). In addition, a positive correlation was found between the amplitude of deformation (DA) and the magnitude of APA and a negative corneal greatest concavity (HCR) index with the mean keratometry and APA data.

When comparing the results obtained using Corvis ST and ORA in 172 patients with different degrees of myopia, the dependence of the decrease in the index of the greatest corneal concavity (HCR) on the degree of myopia was revealed. Corneal hysteresis (CH) also tended to decrease with increasing degree of myopia [21].

In a study of 266 Indians with myopia between 19 and 36 years of age, 23 of 32 Corvis ST values were independent of the degree of myopia and only 9 were significantly different in high degree myopia [43]. In another study, the time required for the second applanation (A2T) and the amplitude of deformation (DA) were significantly lower for the second applanation, while the amplitude of deformation (DA) for the first applanation and the radius of deformation (DA Ratio) were higher for high degree myopia [38]. It should be noted that the deformation amplitude index (DA) is an indicator of corneal biomechanical properties and a decrease in corneal thickness is accompanied by an increase in its deformation potential.

In high myopia, we observed a decrease in the radius of greatest concavity (HCR), an increase in the maximum amplitude of deformation (MDA), a higher rate of second aplasia (A2V) and a decrease in its diameter (A2L), which, according to the study authors, indicates that the cornea is more deformable when the anteroposterior axis is increased [19]. Similar results were obtained in other comparative studies [6, 50].

Results of dynamic corneal pneumoapplanation after laser correction of myopia. Modern laser technologies of keratorefractive surgery used for myopia involve a change in corneal curvature (flattening) as a result of a varying degree of corneal thickness reduction due to the so-called ablation. In the currently most used methods of laser correction this is technologically realized on the basis of surface laser influence on the cornea without flap formation (PRK), preliminary flap formation (LASIK) and intrastromal removal of so called lenticule through a small incision (SMILE) [9, 44, 47-48, 50]. The necessity of clinical researches including methods of corneal pneumoapplanation is dictated by potential changes of initial "biomechanics" of a cornea due to its thickness reduction. Considering the known variability of pneumoapplanation technique indices, this review presents only studies in which postoperative changes in corneal "biomechanics" were compared with the initial data.

After LASIK, a decrease in preoperative CH and CRF values (from 11.52 +/- 1.28 to 9.48 +/- 1.24 and from 11.68 +/- 1.40 to 8.47 +/- 1.53 mmHg, respectively) and correlation of the degree of decrease with refractive effect was noted [18]. In other studies, after LASIK, CH and CRF decreased from 10.44 to 9.3 mmHg and from 10.07 to 8.13 mmHg. [26] and from 9.5 +/- 1.9 to 6.7+/-1.7 and from 9.7 +/- 1.8 to 8.0 +/- 1.6 mmHg, respectively [15]. In one study, the Delta score was used to characterize the reduction of CH and CRF after LASIK. The correlation with ablation depth was stronger for DeltaCRF (r=0.457) than for DeltaCH (r=0.271) [14]. When analyzing results after LASIK and its modification (LASEK), with a mean baseline CH of 10.8 +/- 1.5 mmHg, the mean postoperative ones de-

creased statistically significantly to 9.0 ± 1.3 and 8.6 ± 2.1 mmHg, respectively.

In a series of studies, a comparative assessment of changes in biomechanical parameters after different laser correction techniques was performed. The retrospective study presented changes in ORA and Corvis ST values after LASIK and SMILE (mean baseline myopia 5.16±1.42 and 5.43±1.17 Dpts, respectively). ORA data one month after the interventions showed a greater decrease in CH and CRF after LASIK (8.46±1.76 and 7.45±2.39; 9.99±1.76 and 9.43±1.55 mmHg after LASIK and SMILE, respectively) [16]. At the same time, a more pronounced decrease in the first aplasia time (A1T), greatest concavity (HC Time) and second aplasia time (A2T) was noted after SMILE, which, according to the authors, may reflect the preservation of greater corneal stiffness after the "flapless" procedure. At the same time, the increase in flattened corneal diameter at the second applanation (A2L), concave corneal radius of curvature (HCR) and maximum concavity diameter (HCPD) after LASIK, suggesting stronger corneal deformation to the inside during the air pulse.

More pronounced changes in CH and CRF after LASIK were also noted in other studies in high myopia correction, which presumably, in addition to an increase in ablation volume, could be due to the necessity of corneal flap formation during this intervention [29, 41]. The authors of other studies are of the same opinion [18, 25, 30, 34]. The results of comparative assessment of changes in biomechanical indices of ORA after LASIK and PRK (which does not imply formation of a superficial corneal flap) are indirectly in favor of this assumption [31]. The decrease of CH and CRF was more pronounced after LASIK (by 0.6 and 0.7 mmHg on average, respectively, compared to those after PRK). Moreover, irrespective of the correction technique, there was a high correlation between the initial myopia value and postoperative changes in biomechanical indices.

The effect of flap formation on corneal "biomechanics" after LASIK may be related to corneal delamination exactly in the surface layers of stroma. It has been revealed experimentally that anterior part of corneal stroma (from 100 to 120 microns) is the most rigid due to tightly interwoven anterior collagen plates. This physiological corneal property was confirmed in a study in which lower CH and CRF values after LASIK were found only in a subgroup of patients with high myopia, i.e. with increased ablation affecting these stromal layers [32].

The potential effect of stratification on corneal "biomechanics" is indirectly confirmed by the data of analysis of PRK and SMILE results, in which this technical element is absent. The average decrease of CH (by 1.9 and 2.5 mmHg) and CRF (by 3.4 and 3.2 mmHg), respectively, was found to be close in values [33].

From the position of excluding the possible influence of initial corneal biomechanical properties on postoperative results of pneumoapplanation it is worth mentioning an original study from the methodological point of view in which the so-called "paired-eyed" design was used: in a group of 30 patients with medium degree myopia LASIK was performed in one eye and SMILE in the other eye [29]. The results obtained are in a certain contradiction with the above studies: 6 months after LASIK and SMILE the CH and CRF values were 9.02±1.27 and 8.07±1.26; 8.95±1.47 and 7.77±1.37 mm Hg respectively, i.e. there was no tendency for a more pronounced decrease of biomechanical indices after LASIK. It may have been due to limitations in the degree of initial myopia.

In conclusion of this section, the main conclusion of two foreign literature reviews concerning the results of pneumoapplanation after various techniques of laser myopia correction is that SMILE "flapless" ("non-flap") technology, involving preservation of corneal surface layers, has less effect on changes in biomechanical indices [37,53].

Conclusion. The research results presented in the review indicate that in myopia the clinical application of corneal pneumoapplanation techniques to determine biomechanical indices may address two main objectives:

1. evaluation of biomechanical changes of the fibrous membrane with increasing anteroposterior axis value and, as a consequence, myopia;

2. to analyze the dependence of corneal "biomechanics" changes due to corneal thickness reduction on technological peculiarities of laser refractive interventions.

In general, the results obtained while solving the above-mentioned tasks are of expected character. Both a significant increase in PPO axis in high myopia and a decrease in corneal thickness as a result of laser surgery are accompanied by a certain decrease in various biomechanical indices determined by bi-directional corneal pneumoapplanation. According to the majority of sources, flap formation and increased ablation volume make a "decisive contribution" to the change of biomechanical indices during laser correction.

In spite of the zone of application of mechanical influence of pnevoapplanation methods (cornea!), it is necessary to consider that anatomic integrity of sclera and cornea as components of fibrous membrane to some extent complicates selective evaluation of their biomechanical properties, because the applanation "response" under influence on cornea most likely depends on fibrous membrane condition in general. Nevertheless, considering the "causality" of biomechanical changes, we can conventionally consider that at initial myopia they can be connected with sclera structure disturbances, and after laser refractive surgery - with cornea.

In perspective, from a practical point of view, the solution of the first problem can contribute to improving the monitoring algorithm for progressive myopia and the second one - to assess reliably the intraocular pressure level after laser myopia correction using aplanar tonometry techniques.

It should be emphasized once again that at initial myopia and analysis of pneumoapplanometry data one should consider the potential influence of not only corneal condition but also the known biomechanical changes in the sclera as a component of the fibrous membrane increased in size to various degrees on the findings. Biomechanical "response" to targeted pneumoapplanation of only cornea does not exclude "participation" in its formation of the sclera as well. Proceeding from it, researches in this direction can be focused on experimental biomechanical tests which algorithm will demand the decision of questions connected with reception of isolated samples of a cornea and a choice of testing technique.

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I.A.Yusubov

THE ROLE OF RELAPAROSCOPY IN THE DIAGNOSIS AND TREATMENT OF POSTOPERATIVE BILE LEAKS

DOI 10.25789/YMJ.2023.82.09

УДК 616.367-002-02:616.367-003.7-072.1-089.85

The effectiveness of relaparoscopy in the diagnosis and treatment of patients who developed bile leakage after surgery on the gallbladder and bile ducts was studied. It was revealed that the use of minimally invasive endoscopic technologies in the early diagnosis of postoperative bile leakage of the gallbladder and biliary tract makes it possible to determine the nature of this complication, the optimal method of elimination, justify the transition to conversion, and avoid inappropriate relapses.

Keywords: bile discharge, laparoscopic cholecystectomy, postoperative complications.

Introduction. Cholecystectomy (CE) is the most common operation, and the number of cholecystectomies performed annually exceeds 500 thousand [5, 8].

One of the leading places in the structure of early postoperative complications after cholecystectomy is the outflow of bile, which should be considered as an independent problem. Bile outflow after cholecystectomy is observed in about 0.5% of cases [3, 6, 7, 14].

This indicator increases to 1-1.2% when using laparoscopic techniques to remove the gallbladder, as well as in cases of conversion, when difficulties and complications arise during surgery [1, 2, 11, 12].

After open cholecystectomy, bile leakage is observed in 5-15% of cases, after mini-access cholecystectomy, in 3.6%, after laparoscopic cholecystectomy, in 2-5% of cases [3, 4, 9, 10, 13].

About 750,000 cholecystectomies are performed each year in the United States, most of which are performed laparoscopically. Complications after cholecystectomy are not uncommon and lead to an increase in morbidity and financial burden. Some of the most common complications of laparoscopic cholecystectomy include damage to the biliary tract (0.08%-0.5%), bile leakage (0.42%-1.1%), stones in the common bile ducts (0.8%-5.7%), postcholecystectomy syndrome (10%-15%) and diarrhea after cholecystectomy (5%-12%).). [10, 14] Endoscopy plays an important role in the diagnosis and treatment of biliary complications and in many cases can provide the final treatment. There is no consensus on the best therapeutic approach to biliary complications. [10]

Ultrasound (ultrasound) - diapeutics and laparoscopy, used in surgical pathologies of the abdominal cavity, open up wide opportunities for optimizing early diagnosis and treatment tactics of postoperative bile leakage.

The aim of the study was to evaluate the effectiveness of laparoscopy in the diagnosis and treatment of bile leaks that occur after cholecystectomy and operations on extrahepatic bile ducts.

Materials and methods of research. The research work was carried out during 2010-2021 at the clinical bases of two departments of general Surgery of the Azerbaijan State Institute of Advanced Medical Training named after A.Aliyev. Based on the results of diagnosis and treatment of patients who developed bile leaks in the postoperative period, operations were performed on the gallbladder and bile ducts.

The main group included patients (n=567) who, in surgical tactics and for the treatment of complications, were given preference to the active use of endovideosurgery in the development of complications after surgical interventions in the bile ducts.

The control group included patients (n=148) who used "traditional" methods of surgical correction of complications that arose after similar surgical interventions.

The criterion for inclusion in the study is the occurrence of intra–abdominal complications in the early period after surgical interventions on the bile ducts.

The criterion for exclusion from the study is the critical severity of the patients' condition.

Complications in the early postoperative period were clinically diagnosed in 160 out of 567 patients (28.2%) in the developed main group and in 41 out of 148 patients (27.7%) in the control group. In the main group, bile leaks was detected in 88 patients after laparoscopic cholecystectomy, in 16 patients after mini-laparotomic cholecystectomy and in 56 patients after traditional cholecystectomy.

In 15 clinical cases (9.4%), when performing minimally invasive interventions with damage to the extrahepatic bile ducts is clearly impractical, relaparotomy was performed according to the indications. Laparoscopy was performed in 145 (90.6%) patients of the main group according to indications that were confirmed on the basis of bile drainage, peritoneal signs and ultrasound signs of fluid in the abdominal cavity.

The control group included 41 patients with postoperative bile leaks, but without the use of minimally invasive technologies.

To assess postoperative bile leaks, we used a modified classification by Morgensten L. (2006) [15], in which not only the daily output of bile flowing from the abdominal drainage tube, but also the volume of a limited liquid derivative in the projection of the gallbladder bed, as well as ultrasound data mainly took into account the presence of free fluid in the abdominal cavity and its localization.

YUSUBOV Ilgar Alyasa oglu – MD, Chief Physician of the Khazar Clinic, assistant of the Department of General Surgery, Azerbaijan State Institute of A. Aliyev Advanced Medical Education, Baku, Azerbaijan https://orcid.org/0000-0002-6854-6363, med_avtor@mail.ru

We started an instrumental examination of all patients with ultrasound in order to identify free fluid or its limited accumulation in the abdominal cavity when bile leaks from the drainage in the abdominal cavity. In addition, we also evaluated the diameter of the extrahepatic bile ducts during ultrasound as one of the important characteristics of impaired bile outflow with the main bile ducts. Percutaneous puncture was also performed under ultrasound control in 18 (11.3%) patients in order to obtain additional information about the nature of the liquid derivative in the abdominal cavity.

Statistical processing of numerical indicators obtained during the study was carried out on the basis of nonparametric (Mann-Whitney) criteria and the Pearson correlation coefficient.

Results and discussion. During the study, grade I bile leaks (excretion of up to 100 ml of bile during the day from the drainage in the abdominal cavity or the presence of a limited amount of liquid derivatives up to 5 cm in diameter in the projection of the gallbladder bed during ultrasound) was observed in 38 (23.8%) patients, grade II bile leaks (excretion of 100-500 ml of bile during ultrasound days from drainage in the abdominal cavity or the presence of free fluid in the suprahepatic or subhepatic region during ultrasound) in 108 (67.5%) patients, Grade III bile leaks (removal of more than 500 ml of bile during the day from the drainage in the abdominal cavity or the presence of free fluid in 3 or more areas of the abdominal cavity during ultrasound) was detected in 14 (8.7%) patients.

Bile leaks after mini-laparotomy cholecystectomy was 3.4 and 4.7 times more frequent compared to laparoscopic and traditional cholecystectomy, respectively. The difference between the incidence of postoperative bile leaks found in the groups was statistically significant ($p<0.05$). There were 2 times more cases of bile leaks after emergency operations compared to planned operations.

The tactics of observation with active dynamics were applied under the condition of absolute ultrasound control in case of grade I bile leaks, estimated by the amount of bile released from the drainage or the presence of a limited liquid derivative, the size of which does not exceed 5 cm according to ultrasound data in the projection of the gallbladder bed, insufficient condition of the patient, absence of peritoneal symptoms, changes in blood. In such cases, as a rule, invasive interventions are not required. The bile leaks stopped by itself 2-5 days after the operation.

Fistulography was performed to determine the source of bile leaks in patients whose cholecystectomy ended with drainage of the gallbladder. The diagnosis of iatrogenic lesions of the extrahepatic bile ducts was confirmed in 5 patients during cholangiography.

We did not consider it appropriate to use laparoscopy in cases of obvious impossibility of correcting bile leaks at the preoperative stage using less invasive technology-in the presence of clinical, ultrasound, radiological signs of damage to the intrahepatic bile ducts, insufficiency of hepatocholedog sutures, biliodigestive anastomoses. A total of 15 patients with suspected grade III bile leaks and damage to the intrahepatic bile ducts were recommended recurrent laparotomy without diagnostic laparoscopy.

We considered the following indications for therapeutic and sanitization laparoscopy in patients with postoperative bile leaks:

1. Bile leaks of II-III degree (bile leaks of more than 100 ml after operations on the bile ducts) (n=93);
2. The appearance of peritoneal symptoms along with ultrasound signs of the presence of free fluid in the abdominal cavity (n=45);
3. Impossibility or ineffectiveness of percutaneous drainage under ultrasound control when a limited liquid derivative is detected in the abdominal cavity (n=7).

The main cause of bile leaks in most patients was additional bile flows in the gallbladder bed, as well as iatrogenic lesions of the bile ducts (Table 1).

Contraindications to laparoscopy, we found out the following:

1. Excessively severe condition of the patient (n=3);
2. Hemodynamic instability in patients (blood pressure below 100 mmHg, pulse rate more than 120 beats per minute) (n=2);

In such clinical situations, therapeutic and rehabilitation laparoscopy was performed after the patient's condition was stabilized with the help of intensive care measures.

With observations in 73.1% (n=106) during repeated laparoscopy, it was possible to determine the source of bile leaks after surgery.

Grade I bile leaks (n=48) was associated with additional outflows of bile in the gallbladder bed in 33.3% of cases (n=16) (in 7 patients - outflow of Lyushka, in 9 patients - additional outflow of the square lobe of the liver), in 16.7% (n=8) cases - with insufficiency of bile ducts pathways, and in 12.5% of cases (n=6) - with iatrogenic lesions of the extrahepatic bile ducts. In 37.5% of observations (n=18), it was not possible to determine the source of bile leaks.

Grade II bile leaks (n=93) in 34.4% of cases (n=32) is associated with additional outflows of bile in the gallbladder bed (in 14 patients - outflow of the Lushka, in 18 patients - additional outflow of the square lobe of the liver), in 22.6% (n=21) cases - with iatrogenic lesions of extrahepatic bile ducts, and in 20.4% of cases (n= 19) - with insufficient outflow of the gallbladder. In 22.6% of observations (n=21), it was not possible to determine the source of bile leaks.

All observations with grade III bile leaks (n=4) were associated with iatrogenic damage to the main bile ducts (Table 2).

In patients of the main group with clinical signs of bile leaks , laparoscopic surgery was performed on 3.5 ± 2.2 days of the subsequent period, and in patients of the control group - on 6.1±4.6 days of relapse. Repeated intervention in the first three days was performed in 56.8% of the observations in the main group and in 37% in the control group.

Table 1

Sources of bile leaks after surgery according to laparoscopy

Group of patients (method of initial surgery)	Source of bile leaks					Total
	The presence of additional secretions in the bed of the gallbladder	Defeat of the bile ducts	Absence of gallbladder outflow	Spontaneous drainage of the gallbladder	The source is not specified	
Laparoscopic cholecystectomy	31	16	15	---	23	85 (58.6)
Minimally invasive cholecystectomy	8	---	4	---	2	14 (9.6)
Traditional cholecystectomy	9	15	6	2	14	46 (31.8)

Note. HE – cholecystectomy.

Table 2

Dependence of the degree of postoperative bile leaks on the source

Degree of bile leaks	Source of bile leaks					Total	Pearson correlation coefficient, r
	The presence of additional secretions in the bed of the gallbladder	Damage to the bile ducts	Absence of gallbladder outflow	Spontaneous drainage of the gallbladder	The source is not specified		
I degree	16	6	8	---	18	48	0.797
II degree	32	21	17	2	21	93	0.861
III degree	---	4	---	---	---	44	0.629
Total	48 (33.1)	31 (21.4)	25 (17.2)	2 (1.4)	39 (26.9)	145 (100)	

A correlation interaction between the degree of bile leaks and its source (R<0.01) was established during statistical analysis.

The amount of bile in the abdominal cavity ranged from 70 ml to 200 ml when bile leaks occurred from the duct of the Club. Local accumulation in the subhepatic region was observed in 14 patients (limited in 1 patient), and bile outflow to the right subhepatic region was observed in 7 patients. After the sanitation of the abdominal cavity, the bed of the gallbladder was carefully examined. We determined the leaks of bile from the bun ducts in the form of a section in the bed of the gallbladder with a diameter of up to 3 mm, through which bile is excreted in drops. In such cases, we performed additional sanitation of the abdominal cavity after stopping the outflow of bile by inserting 1-2 clamps into the aberrant flow, and at the end-drainage into the bed of the gallbladder. Laparoscopy duration averaged 35±7.9 minutes. The postoperative period in all patients was calm. Drainage is removed from the abdominal cavity on the 2nd day. The average duration of treatment in the clinic was 12±3.4 bed days. No fatal outcome was recorded.

When performing therapeutic and prophylactic laparoscopy associated with bile leaks from the gallbladder (n=27), from 100 ml to 150 ml of bile was detected in the abdominal cavity, with one observation, a diagnosis of disseminated bile peritonitis was established. After the abdominal cavity is sanitized, the operating area is examined and the insufficiency of the direction of the outflow of the gallbladder is detected, the latter is tied with a clamp. The operation ended with drainage of the subcutaneous area. The average laparoscopy duration was 63.6±3.2 minutes. The period after the second intervention passed without complications. The average duration of treatment was 10.3±2.6 bed days.

Since in 3 of our observations it was not possible to eliminate bile leaks by clamping the damaged intrahepatic outflow of bile, retrograde cholangiography was performed, followed by endoscopic papillosphincterotomy and endoprosthetics of the damaged outflow with plastic stents.

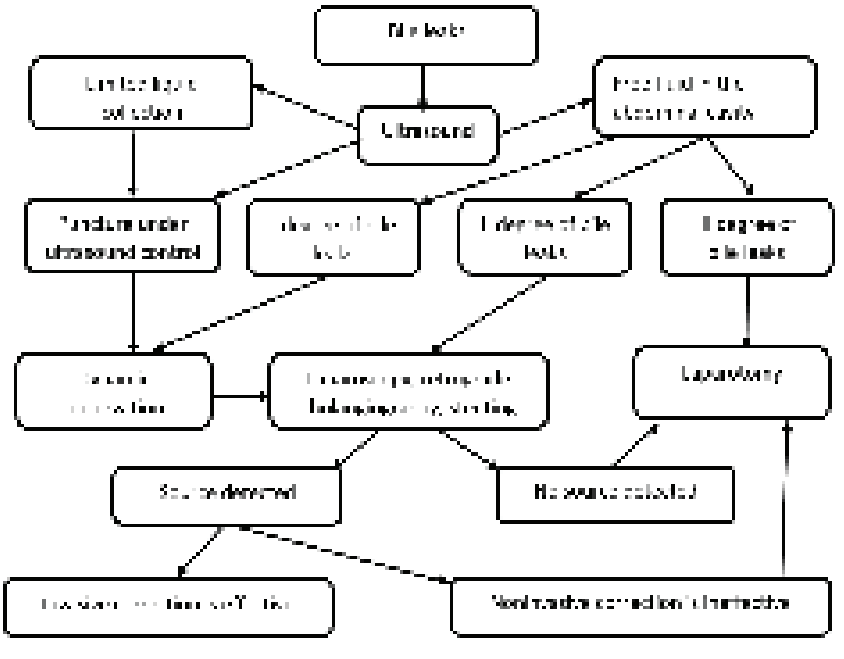
In 39 of our observations, when examining the surgical intervention area, it was not possible to detect the source of bile leaks . The criterion for refusing recurrent laparotomy in 24 clinical situations, we considered the absence of bile leakage during an adequate examination of the operating area and sanitation manipulation (while in 18 there was no accumulation of bile in the abdominal cavity during laparoscopy, despite leakage of up to 100 ml of bile with drainage during the day).

11 patients who had iatrogenic injuries of the extrahepatic bile ducts during laparoscopy, as well as 4 patients underwent relaparotomy due to active bile leakage during the rehabilitation event and the inability to visualize this source. 3 patients died, 1 due to pulmonary embolism and 2 due to cardiovascular insufficiency after relapse due to iatrogenic damage of extrahepatic bile ducts.

In cases where it is impossible to adequately stop bile leakage during laparoscopy (n=8), as well as when bile leakage occurs after recurrent laparoscopy and it is impossible to visualize its source during repeated laparoscopy with relapse performed on day 2-4 (n= 11), conversion to recurrent laparotomy is performed.

Thus, at the diagnostic stage of relaparoscopy, complications were excluded in 12.4% of our observations (N=18), relaparotomy was recommended in 10.3% (N=15), and conversion was recommended in 13.1% (N=19) due to the ineffectiveness of minimally invasive technologies.

In 64.2% of our observations (n=93) effective minimally invasive interventions were performed, and in 23.4% (n=34) relaparotomy was performed. The duration of surgery in the main group averaged 30.2±8.9 minutes, postoperative compli-



Diagnostic and therapeutic algorithm for postoperative bile leaks

cations 4.8% (n=7), the duration of inpatient treatment was 12.8±5.6 days. The mortality rate was 2.1% (n=3).

The duration of surgery in the control group was 64.3±10.7 minutes, the number of complications after surgery was 34.1% (n=14), the duration of inpatient treatment was 28.8±8.4 days. The mortality rate was 26.8% (n=11).

The use of our proposed algorithm for the diagnosis and treatment of postoperative bile leaks based on minimally invasive technologies significantly reduces the postoperative mortality rates, which is explained by the earlier detection and recurrence of the developing complication, timely operations and a lower degree of surgical aggression. (Picture)

Thus, we considered the indications for the conversion of laparoscopy into laparotomy:

1. Preservation of signs of bile discharge after sanitation and the inability to detect the source of bile discharge due to clearly visible inflammatory-infiltrative and adhesive processes;

2. Impossibility of endosurgical removal of bile discharge.

Conclusion

1. The use of minimally invasive endoscopic technologies in the early diagnosis of postoperative bile leaks of the gallbladder and biliary tract makes it possible to determine the nature of this complication, the optimal method of elimination, justify the transition to conversion, and also avoid inappropriate relapses.

2. The use of diagnostic and therapeutic algorithm for postoperative bile leaks with the help of minimally invasive technology allowed to reduce the duration of the operation by about 2 times (30.2 ± 8.9 min and 64.3 ± 10.7 min, respectively), to reduce postoperative complications from 34.1% to 4.8%, and the duration of inpatient treatment can be reduced from 28.8 ± 8.4 days to 12.8 ±5.6 days with accuracy ($p<0.05$).

Reference

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DIAGNOSIS AND TREATMENT OF HEART INJURIES

DOI 10.25789/YMJ.2023.82.10

УДК 616.12-001.4-072-089

The main objective of this work is to develop optimal tactics for early diagnosis of heart injuries for the most rapid decision-making on the need to perform therapeutic manipulations. We studied the clinical symptoms found in patients upon admission the most frequent clinical symptoms of heart injuries with ranking them as reliable and probable. Conclusions were drawn about the optimal tactics of managing emergency surgical patients admitted with suspected heart injury. It was determined that the most effective method of treating patients with penetrating heart trauma is emergency surgical treatment.

Keywords: heart injury, surgical treatment of heart injury, additional research methods, videothoracoscopy, thoracotomy, heart wound suturing.

Introduction. The problems associated with heart injuries are currently relevant, since the issues of choosing the optimal tactics for early diagnosis and treatment of these injuries are extremely serious in surgery. Terminal conditions develop very quickly, with traumatic injury to the heart. A combination of factors (cardiac tamponade, damage to the coronary arteries and intracoronary structures, bleeding, combined injuries) increase the likelihood of death in patients with traumatic heart injury, along with this, the progression of terminal states and shock phenomena often contribute to errors in treatment and diagnostic tactics [3,6,10,11]. Mortality among patients with heart injury during hospitalization according to the last ten-year data was 31.25%, which corresponds to similar mortality data for the previous 30 years [6,11].

The aim of the study was to develop an algorithm for choosing therapeutic measures to improve early diagnosis and the most rapid application of the necessary surgical intervention in patients with penetrating heart injuries.

Material and methods. The study used data on 268 patients admitted to the surgical departments of the Regional Clinical Emergency Hospital, Barnaul, the clinical base of the Department of General Surgery, Operative Surgery and Topographic Anatomy of the Altai State Medical University with penetrating heart injuries since 1990 by 2020. There were 234 men and 34 women. Age ranged from 18 to 69 years. Patients were admitted to the hospital at various time intervals, the largest number within 5-40 minutes from the moment of injury (205 patients). Diagnosis included clinical symptoms and instrumental data (electrocardiographic (ECG), echocardiographic (ECHO-KG), radiography (RG), computed tomography (CT), videothoracoscopy).

In the structure of heart injuries, their different nature was noted (Fig. 1 and Table 1).

Most often, single injuries of the heart occurred in 254 (94.8%) patients, while injuries of the ventricles were most often observed in 230 (86.8%) patients.

Most often, the left ventricle was damaged in 129 (48.1%) patients, less often - the right ventricle in 89 (33.2%) patients, damage to the left atrium was noted in 9 (3.4%) patients, damage to the right atrium - in 26 (9 .7%), both ventricles - in 15 (4.8%).

At the same time, in 21 cases, simultaneous damage to two chambers of the heart was noted, of which 6 patients underwent surgical treatment due to gunshot wounds, and 15 patients - multiple stab wounds. In relation to the cavities of the heart, penetrating wounds were most often observed (Fig. 2).

At the same time, in patients with non-penetrating wounds of the heart during thoracotomy, various depths of myocardial damage (from 0.1 to 0.3 cm) were determined, which had no connection with the heart cavities.

Results and discussion. Heart injuries were diagnosed on the basis of the analysis of the symptom complex and indicators of additional studies. The clinical signs were based on a number of symptoms (Table 2).

So, in patients, the symptom complex consisted of various combinations of clinical symptoms. In more cases, there was a visible wound in the area of the anatomical projection of the heart in 258 (96.3%) patients, in 6 (2.2%) patients, the wounds were located in the subscapular region and in 4 (1.5%) - in the axillary region.

Among the various clinical manifestations of heart injuries, reliable and probable ones were identified (Table 3).

The diagnosis was made in accor-6dance with the clinical picture in each

Table 1

The nature of the wounds of the heart

Heart wounds	Abs. number	%
isolated	179	66.8
combined:	89	33.2
with lung injury	51	19
thoracoabdominal. of which:	38	14
with liver injury	12	4.5
with spleen injury	12	4.5
with stomach injury	8	2.9
with intestinal injury	6	2

ZEIMAKH Evgeniy Aleksandrovich – MD, Professor, Head of the Department of General Surgery, Operative Surgery and Topographic Anatomy, Federal State Budgetary Educational Institution of Higher Education Altai State Medical University MH RF, e-mail: yea220257@mail.ru; **BULDAKOV Pavel Nikolaevich** – surgeon of the surgical department No. 2, Regional State Budgetary Healthcare Institution 'Regional Clinical Emergency Hospital, Barnaul', **MAKIN Andrey Anatolyevich** – chief physician of the Regional State Budgetary Healthcare Institution Regional Clinical Emergency Hospital, Barnaul; **RUCHEYKIN Nikolay Yurievich** – Assistant of the Department of General Surgery, Operative Surgery and Topographic Anatomy, Federal State Budgetary Educational Institution of Higher Education Altai State Medical University MH RF, e-mail: leo920721@mail.ru; **KARKAVIN Vadim Mikhailovich** – surgeon of the Surgical Department No. 2, Regional State Budgetary Healthcare Institution Regional Clinical Emergency Hospital, Barnaul; **DOROKHINA Svetlana Alekseevna** – surgeon of the surgical department No. 2, Regional State Budgetary Healthcare Institution Regional Clinical Emergency Hospital, Barnaul; **AVERKINA Anna Alekseevna** – surgeon of the surgical department No. 2, Regional State Budgetary Healthcare Institution Regional Clinical Emergency Hospital, Barnaul.

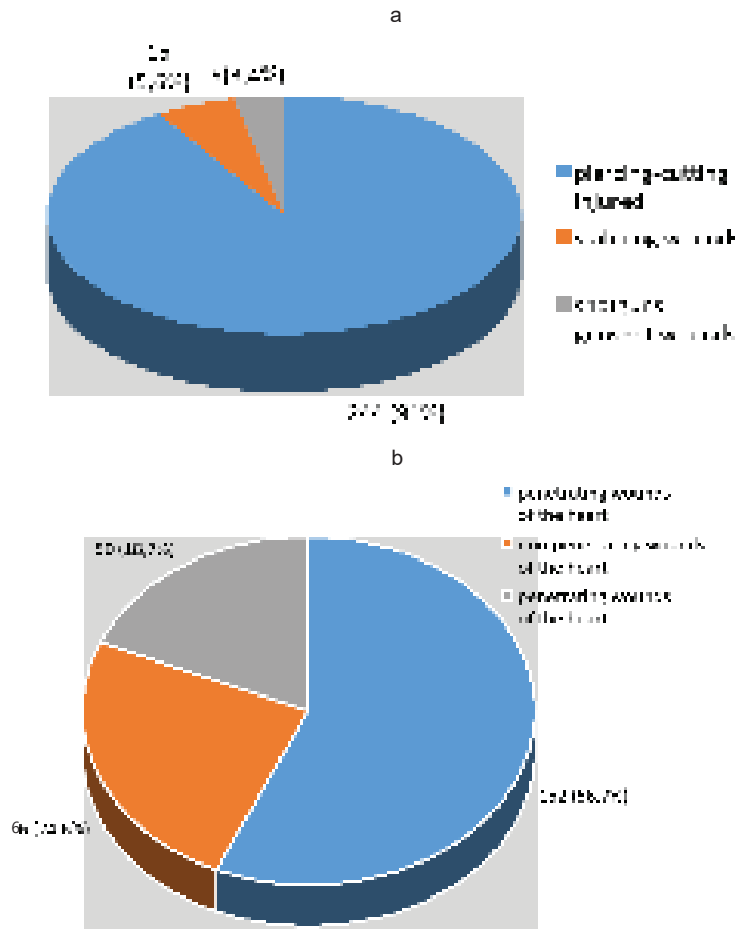

Fig. 1. The nature of heart injuries by the type of damaging agent (a) and in relation to the cavities (b)

individual patient. Thus, it was possible to establish the diagnosis of heart injury in time in 259 (96.6%) patients, which made it possible to perform surgical treatment in 228 (88.0%) patients as soon as possible (10-20 minutes from the moment of admission to the hospital).

Taking into account the experience of treating patients with penetrating heart injuries in our clinic, an algorithm for choosing the method of surgical treatment was compiled (Fig. 3).

In 18 (6.7%) patients there was an error in diagnosis. Such patients underwent surgery 1 to 4 hours after admission to the clinic with a diagnosis of lung injury in 12 (4.5%) patients and heart injury in 6 (2.2%). This group was characterized by a satisfactory general condition of patients, no changes in hemodynamics, the presence of wounds with a deep wound channel in the anatomical region of the heart. At the same time, moderate hemothorax was noted in 10 (3.7%) patients, minor ECG changes (sinus tachycardia) in 12 (4.5%) patients. Surgical intervention was necessary, since all patients were diagnosed with a heart injury of a different nature. In most cases, there was a wound of the heart with penetration into the ventricle on the left or the atrium on the right.

Of course, surgical treatment, performed on an emergency basis, is the correct treatment tactic that allows you to save the life of the victim. Emergency thoracotomy was performed with reliable

Table 2

Frequency of clinical symptoms in heart injury

Clinical sign	Absolute number	%
External or intrapleural bleeding	210	78.4±2.51
Cardiac tamponade syndrome	42	15.7±2.22
Pulse: - normal - tachycardia - bradycardia - absent	 28 187 32 21	 10.5±1.87 69.8±2.81 11.9±1.98 7.8±1.64
Systolic blood pressure: - normal - 90-70 mm Hg - below 70 mm Hg - absent	 65 75 91 37	 24.3±2.62 28.0±2.74 34.0±2.89 13.7±2.10
PExpanding the boundaries of cardiac dullness	33	12.2±2.0
Deafness of heart sounds	186	69.4±2.82

Table 3

Clinical symptoms in heart injury

Credible	Probable
The presence of a wound in the chest (preferably in the anatomical region of the heart) with massive bleeding (external or intrapleural)	The presence of a wound with a deep wound channel in the anatomical localization of the heart without massive bleeding
A significant decrease in blood pressure, or its absence	Moderate decrease in blood pressure and muffled heart sounds
Signs of cardiac tamponade are determined	Signs of cardiac tamponade are not determined
According to the results of the ECG, damage or ischemia of the heart muscle is determined	ECG results show sinus tachycardia
According to the results of X-ray or ECHO-KG, hemopericardium is determined	According to the results of X-ray or ECHO-KG, hemopericardium is determined
	According to the results of fluoroscopy, a decrease in the pulsation of the projection of the shadow of the heart is determined

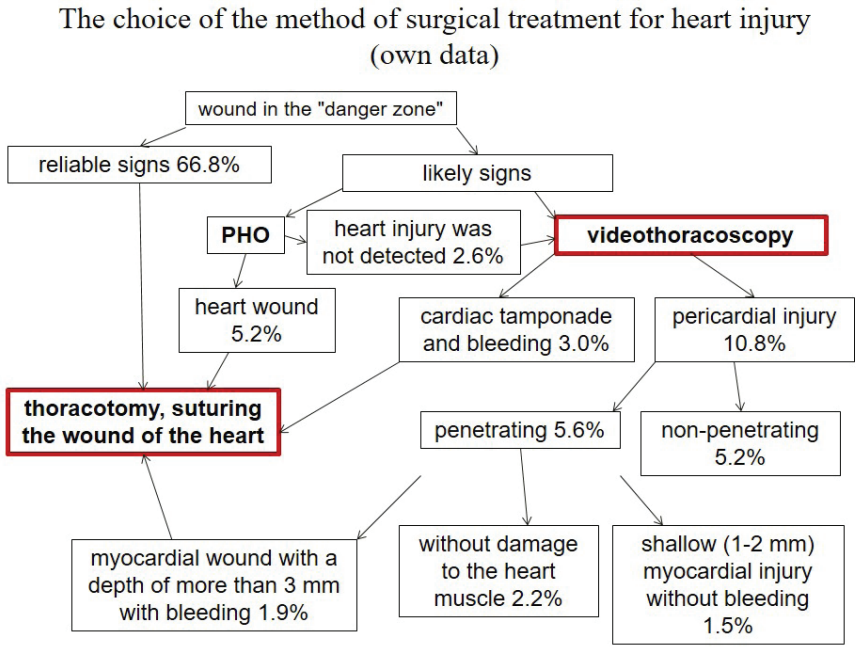

Fig. 3. Algorithm for choosing the method of surgical treatment

signs of heart damage in 210 (66.8%) patients: hypotension - 144 (53.7%); pericardial tamponade syndrome - 42 (15.7%); myocardial damage according to ECG - 186 (69.4%); X-ray and echocardiographic signs of hemopericardium - 164 (61.2%). Primary surgical debridement was performed in 21 (7.8%) patients. The diagnosis of heart injury was confirmed in 14 (5.2%) patients and they underwent thoracotomy. In 7 (2.6%) cases, during the revision of the wound, no convincing evidence for a heart injury was obtained, given the stable hemodynamics in these patients, they underwent videothoracoscopy. Videothoracoscopy in case of suspected heart injury was performed in 37 (13.8%) patients: in 8 (3.0%) cases, cardiac tamponade or intrapleural bleeding was detected during surgery, these patients underwent emergency thoracotomy; in 29 (10.8%) patients, damage to the pericardium was found (of which 14 (5.2%) had non-penetrating into the cavity of the heart bag and 15 (5.6%) had penetrating, which required pericardiotomy). Of 15 (5.6%) patients, thoracoscopic revision of the pericardium revealed: 4 (1.5%) patients had tangential ("lateral") damage to the heart muscle 1-2 mm deep without ongoing bleeding; in 6 (2.2%) - no damage to the heart muscle was detected; 5 (1.9%) patients had myocardial wounds more than 3 mm deep with ongoing bleeding, which required emergency thoracotomy with suturing of the heart muscle. In 24 (9.0%) cases (out of 268 patients), videothoracoscopy allowed to refrain from thoracotomy, ending the operation with drainage of the pleural cavity.

U-shaped sutures were placed on the wound of the heart in 59 (22.0%) patients, interrupted in 209 (78.0%). Suturing was performed with atraumatic suture material, without endocardial suturing. The method of closing the wound defect must be chosen according to the nature and localization of the wound. Thus, interrupted sutures should be used to close penetrating wounds of the ventricles and small wounds of the atria, or non-penetrating wounds. With these wounds, this type of seam creates the desired tightness. Large-sized atrial wounds should be sutured with U-shaped sutures, since this type of suture is preferable for a thinned atrial wall or flabby myocardium. Most patients (240 patients (89.6%) were discharged from the hospital for outpatient observation, 11 patients (4.1%) were transferred to the cardiology department for treatment, 17 (6.3%) died.

Death during surgery or a few hours after it was observed in 17 (6.3%) patients due to injuries incompatible with life or acute cardiovascular failure due to acute blood loss and hemorrhagic shock, and in 3 (1.1%) patients - due to acute renal failure in the early postoperative period with combined damage to internal organs (heart, lungs, liver). At the same time, extracorporeal hemodialysis did not bring a positive result (after 9–12 days, the patients died).

Analyzing the long-term effects of treatment (period from 1 to 10 years) of patients (38 people) with heart injuries (25 - penetrating and 13 - non-penetrating), who were discharged for outpatient observation in a satisfactory condition, it was noted that the ability to perform heavy physical activity was preserved in 19 people, and the presence of cicatricial changes in the heart muscle according to ECG in 9 people.

Conclusions:

1. Injuries to the heart remain an extremely serious problem in surgery. Very quickly, the victims develop terminal conditions, which increases the likelihood of death. At the same time, timely diagnosis of heart injuries is difficult in some cases.

2. A complex of symptoms, consisting of reliable signs (the presence of a wound in the chest area with profuse bleeding, a pronounced drop in blood pressure, up to its absence, signs of cardiac tamponade, damage or ischemia of the heart muscle, hemopericardium) are determined in most patients. Videothoracoscopy allows to detect the presence of damage to the heart in cases of difficulty in diagnosis and determine its nature in the shortest possible time.

3. Emergency surgery is the only effective treatment for patients with heart injuries. Interrupted sutures should be used to close penetrating wounds of the ventricles and small wounds of the atria, or non-penetrating wounds. With these wounds, this type of seam creates the desired tightness. Large-sized atrial wounds should be sutured with U-shaped sutures, since this type of suture is preferable with a thinned wall or flabby myocardium.

4. The algorithm presented in the paper using videothoracoscopy allows you to choose the optimal tactics for early diagnosis of heart injuries for an early decision on surgical treatment of the patient.

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A.K. Okoneshnikova, P.V. Nikiforov, A.G. Fedorov, T.I. Nikolaeva, M.I. Tikhonova

DOI 10.25789/YMJ.2023.82.11

УДК 617-089

EVALUATION OF THE INFORMATIVE VALUE OF TRANSTHORACIC TREPAN LUNG BIOPSY UNDER THE CONTROL OF MULTISLICE COMPUTED TOMOGRAPHY OF INTRATHORACIC FORMATIONS IN THE DIAGNOSIS OF LUNG FORMATIONS

As of today, minimally invasive interventional diagnostic and therapeutic methods play a crucial role in modern medicine. Percutaneous transthoracic biopsy (TTB) is a minimally invasive and highly effective procedure that allows for histological verification of intrathoracic neoplasms. The aim of the study was to evaluate the information content of the method of percutaneous transthoracic trephine lung biopsy under the control of multislice computed tomography in the diagnosis of intrathoracic lesions in patients with suspected malignant neoplasm of the lung. The study included 155 patients who underwent TTB of intrathoracic formations under MSCT guidance between 2021 and 2022 at the Oncology and Radiology Hospital of the Republic of Sakha (Yakutia). Of these, 94 (60.65%) were male and 61 (39.35%) were female. The average patient age was 65.4 years (range 44-89). As a result of the study, malignancy was detected in 118 patients (76.13%), with primary lung malignancies identified in 102 (65.81%) patients, secondary lung malignancies in 16 (10.32%), and benign lung neoplasms in 27 (17.42%). Non-informative material (scarce material) was obtained in 10 (6.45%) cases. Perioperative complications were identified and included pneumothorax requiring Bülau pleural cavity drainage in 25 patients (16.67%), which occurred in patients with concurrent upper respiratory tract pathology such as bullous emphysema, interstitial changes, giant bullae, and hemoptysis developed in 16 patients (10.67%). Thus, it was determined that the application of percutaneous TTB of the lung under multislice computed tomography guidance in lung cancer has significant diagnostic value.

Keywords: percutaneous transthoracic trephine biopsy, lung trephine biopsy, computed tomography, biopsy under MSCT guidance, lung biopsy, non-small cell lung cancer.

OKONESHNIKOVA Alyona Konstantinovna – PhD, associate professor of the Department of Surgery and Dentistry of the NEFU Medical Institute, surgeon of the Department of Radio-Surgical Methods of Diagnosis and Treatment (RSMDT) at YROD, alena-okoneshnikova@mail.ru, http://orcid.org/0000-0003-1556-3095; **NIKIFOROV Petr Vladimirovich** –deputy chief physician for Outpatient Issues of the State Budgetary Institution of the Sakha Republic's YROD, surgeon, sncologist, nicciforof@mail.ru, http://orcid.org/0000-0002-2758-155X; **FEDOROV Aisen Germanovich** – head of YROD's RSMDT, doctor for X-Ray Endovascular Diagnostics and Treatment, surgeon, mailto:altdeleteman@mail.ru, **NIKOLAEVA Tatyana Ivanovna** – PhD, associate professor of the Department of Oncology at the NEFU's Medical Institute, chief physician at YROD, NTI_Nika@mail.ru; **TIKHONOVA Maya Ivanovna** – head of the Center for Radiation Diagnostics at YROD, http://orcid.org/0000-0002-7954-4507

Introduction. Currently, according to statistical data in the Russian Federation for 2020, lung cancer ranks first among malignant neoplasms in men and first in mortality among both men and women in Russia and globally [2]. This problem is also prevalent in the oncology service in the Sakha Republic. It has been established that in the period from 2013 to 2022, there has been an increase in lung cancer incidence among the population by 22.1% [2].

Considering the growing trend in detecting new focal lung neoplasms in patients, one of the most pressing tasks in thoracic surgery and oncology is the timely and safest diagnosis of intrathoracic lung neoplasms [1, 11, 13]. Numerous methods for obtaining morphological materials from intrathoracic tumors have been described in domestic and foreign literature [3, 4, 12].

For tumors located in the trachea and bronchi, bronchoscopy is used, which allows obtaining material from exophytic bronchial tumors. For central lung tumors, the endobronchial ultrasonography method is used [6, 7]. The aforementioned methods do not allow obtaining material from peripherally located lung tissue neoplasms. To diagnose these formations, clinicians often resort to performing diagnostic invasive operations, such as diagnostic thoracotomy, video thoracoscopy, and mediastinoscopy [6, 8, 14]. The application of these methods implies hospitalization, significant intraoperative trauma, and considering that the highest percentage of oncology patients are elderly and senile individu-

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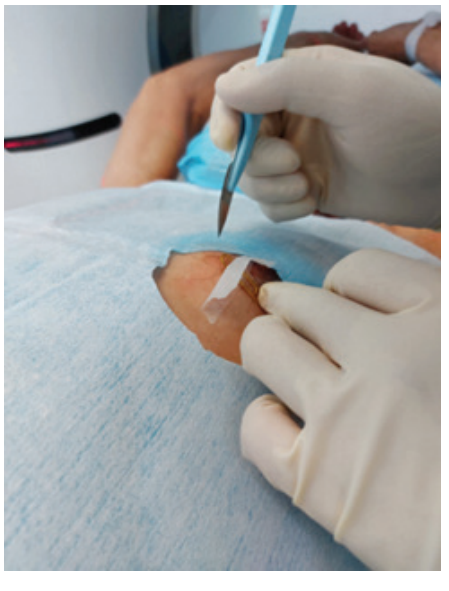

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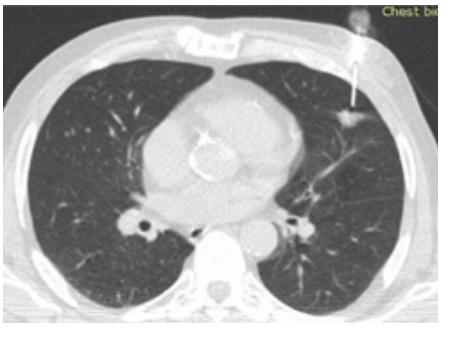

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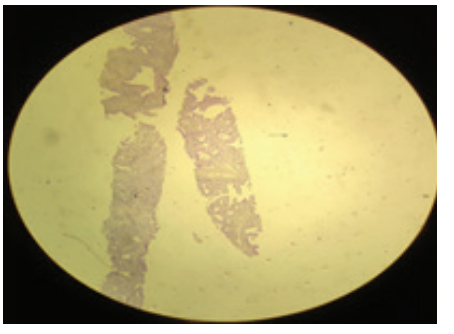

Intraoperative photos of the transthoracic trepanobiopsy of the left lung formation under MSCT navigation: a - 128-PHILIPS X-ray computed tomography with fluoroscopy, b - MAGNUM pistol with a single 18 - gauge biopsy needle G 150 mm long, c - layered anesthesia of the skin with a local anesthetic, d - incision of the skin at the puncture site, e - installation of the coaxial system to the formation of the left lung, f - MSCT control of the placement of the coaxial needle over the tumor, g, h - obtaining histological material, i - morphological picture, stained with hematoxylin and eosin, increased 200. In the material of the structure of the lung adenocarcinoma with the immunophenotype: TTF1(+), Napsin(+), CK7(+), CK20(-)

als with multiple comorbidities of varying degrees of compensation, the use of the aforementioned surgical interventions is associated with a high risk of anesthesiologic assistance and the development of unfavorable postoperative outcomes (American Society of Anesthesiologists), as well as an increased duration for patients to receive specialized treatment [10, 14].

Currently, minimally invasive interventional diagnostic methods, such as percutaneous transthoracic core biopsy (TCB) [3-5], are widely used in the diagnosis of intrathoracic neoplasms. This method is a highly effective procedure that allows for histological verification of intrathoracic neoplasms [4-6, 14].

We previously conducted a retrospective analysis of the results of percutaneous TCB under the control of multislice computed tomography (MSCT) in 156 patients, in which 76.28% of cases were verified as malignant processes.

Aim of the Research: To assess the information content of the method of percutaneous transthoracic trephine lung biopsy under the control of multislice computed tomography in the diagnosis of intrathoracic lesions in patients with suspected malignant neoplasm of the lung.

Materials and Methods: From January 2020 to December 2022, 270 transthoracic biopsies (TTBs) were performed at the Department of Radiosurgical Diag-

nostic and Treatment Methods (DRDTM) of the Sakha Republic's Yakutsk Republican Oncological Dispensary in Yakutsk, among which 155 patients underwent percutaneous TTB of lung neoplasms under MSCT control. Of these, 94 were men (60.65%) and 61 were women (39.35%). The average age of the patients was 65.4 years (44; 89). Absolute contraindications for percutaneous TTB included neoplasm localization in a single lung, anticoagulant intake, blood disorders accompanied by increased bleeding, pronounced respiratory insufficiency, and written patient refusal of the procedure.

All biopsies were performed using the MAGNUM automatic gun with a disposable 18 G biopsy needle of 150 mm length. Navigation was provided by a 32-slice BodyTom MSCT and a 128-slice PHILIPS X-ray computed tomography with fluoroscopy (Fig.1-A, E). Patients were examined in supine, lateral, or prone positions depending on the neoplasm localization. Scanning was conducted with a slice thickness of 1 mm. The procedure was performed under local infiltration anesthesia with a 20 mg/ml Lidocaine solution after triple antiseptic treatment of the operative field (Fig.1-B, C, D). Biopsies were performed using the MAGNUM automatic gun with a disposable 18 G biopsy needle of 150 mm length (Fig.1-B). Three tissue columns were collected and fixed in a 10% formalin solution (Fig.1-F, G, H). The puncture site was covered with an aseptic dressing. Patients were then routed to a hospital ward for dynamic observation of vital functions. The average procedure duration was 33±12 minutes. Chest radiography was performed at 2-4 hours and the following morning (12-24 hours), with immediate radiography in case of pneumothorax symptoms or signs of respiratory insufficiency.

The average total radiation dose during the biopsy ranged from 2.67 to 10.84 mSv. To reduce radiation exposure to medical personnel, all biopsies were conducted wearing radioprotective aprons with collars and protective glasses.

The diagnostic procedure stages are shown in Fig. 1.

Statistical analysis of the research results was performed using Microsoft Excel and Statistica-8 software.

Results and Discussion: The registered anthropometric data are presented in Table 1. The analysis revealed that the majority of patients were male and elderly (60-74 years old).

As a result of the study, malignant characteristics were detected in 118 patients (76.13%): malignant lung neoplasms were found in 102 patients (65.81%), secondary malignant lung neoplasms in 16 patients (10.32%), and benign lung neoplasms in 27 patients (17.42%). Non-informative material (scarce material) was observed in 10 cases (6.45%).

Upon evaluating the lung tumor size and histological type, it was determined that the tumor size at the time of TTB was statistically significantly larger in patients with non-small cell lung cancer (NSCLC) ($p < 0.05$). However, the tumor size was not dependent on the histological type of NSCLC ($p > 0.05$). These data are presented in Table 2.

During the analysis of perioperative complications, the following were identified: development of pneumothorax requiring drainage of the pleural cavity using Bülau's method in 25 patients (16.67%). Pneumothorax predominantly occurred in patients of older age groups with concomitant upper respiratory tract pathology, such as bullous emphysema, interstitial changes, giant bullae, and hemoptysis developed in 16 patients (10.67%). All the aforementioned complications were resolved.

Transthoracic core needle biopsy (TTB) of intrathoracic lesions under CT guidance, according to various authors, varies from 89% to 96%. In a study by Kim D.Y. et al., they analyzed the outcomes of TTB under CT guidance in 70 patients with suspected lung cancer and found that the overall diagnostic sensitivity of the selected method was 85.7%, with non-informative material obtained in 18.6% of cases, and the overall complication rate was 35.7%. The authors concluded that a long transpulmonary needle path was a factor for developing pneumothorax ($p=0.007$) [9]. Cristina Borelli et al. evaluated the effectiveness of TTB under CT guidance in 183 patients. The authors divided the results into diagnostic and non-diagnostic. In 150 cases, diagnostic results (informative material) were obtained. Among these, 87.3% had malignant lung processes verified, and 12.7% had benign processes. Of the 33 non-diagnostic results (non-informative material), 66.7% underwent repeat biopsy and authors reported malignant processes in these cases, while 33.3% had benign processes. Multivariate analysis

Table 1

Gender and Age Characteristics of Patients, n (%)

Gender	Age Group				p
	18-44	45-59	60-74	75-90	
Total Number (n=155)	5 (3.2)	29 (18.7)	94 (60.6)	27 (17.4)	
Men (n=94)	4 (4.3)	16 (17.0)	62 (66.0)	12 (12.8)	0.156
Women (n=61)	1 (1.6)	13 (21.3)	32 (52.5)	15 (24.6)	

Note. In Tables 1-2, p is the achieved level of significance (Pearson's criterion $\chi 2$).

Table 2

Dimensions and Morphological Type of Lung Formations. Subjected to TTB Biopsy Under MSCT Control

Type		Total	Tumor Size				
			05-10	11-20	21-40	41-60	more than 60
Small Cell		3 (1.9)	0 (0)	1 (33.3)	0 (0)	1 (33.3)	1 (33.3)
NSCLC	Adeno NSCLC	51 (32.9)	1 (2.0)	6 (11.8)	30 (58.8)	8 (15.7)	6 (11.8)
	Squamous NSCLC	33 (21.3)	0 (0)	3 (9.1)	15 (45.5)	7 (21.2)	8 (24.2)
Neuroendocrine Lung Tumors		9 (5.8)	0 (0)	1 (11.1)	3 (33.3)	4 (44.4)	1 (11.1)
Other		6 (3.9)	1 (16.7)	2 (33.3)	3 (50.0)	0 (0))	0 (0)
Tuberculosis		3 (1.9)	0 (0)	3 (100)	0 (0)	0 (0)	0 (0)
Hamartoma		2 (1.3)	0 (0)	2 (100)	0 (0)	0 (0)	0 (0)
Other		22 (14.2)	1 (4.5)	4 (18.2)	11 (50)	5 (22.7)	1 (4.5)
Non-Informative		10 (6.5)	2 (20.0)	3 (30.0)	5 (50.0)	0 (0)	0 (0)
Metastasis		16 (10.3)	4 (25.0)	1 (6.3)	5 (31.3)	6 (37.5)	0 (0)
p			0.001				

of diagnostic failures revealed that when the lesion size was ≤ 20 mm (p = 0.006), the proportion of non-informative material was higher [5].

A meta-analysis assessing the complication rate of TTB under CT control found that, among 8133 TTB of the lung, the overall complication rate was 38.8%, and the rate of serious complications was 5.7% (extensive pneumothorax, hemoptysis with aspiration). The authors reported that the number of complications increased in 38.8% of cases with larger needle diameters. Other factors contributing to complications included a long transpulmonary needle path and lesion diameter ≤ 20 mm [8]. The effectiveness of TTB under CT guidance was evaluated by Yarynich K.V. et al., who assessed the procedure's effectiveness in 133 patients. Lesion size ranged from 5x8 mm to 10 cm, and tumor depth was 0-45 mm from the parietal pleura. The authors reported that 80 cases (59.3%) had malignant processes, 35 (25.8%) had benign processes, 8 (5.9%) had non-informative material, and 5 (3.8%) had false-negative results. Complication assessment revealed that 12 patients developed partial pneumothorax in 8.9%, and no pulmonary hemorrhages were detected. Similar results were obtained by Perepelevsky A.N. et al., who evaluated the outcomes of TTB under CT guidance in 63 patients. The study reported high informativeness of TTB in 96.6%. Complications were observed in 5 (7.6%) patients, pneumothorax developed in 4 (6.1%), and hemoptysis in 1 (1.5%) [3].

In our clinical study assessing the effectiveness of transthoracic biopsy (TTB) under CT guidance, we found that the effectiveness of the method was 93.59%. A systematic review of specialized literature allowed us to compare our results with global data [4-6, 8-13].

Conclusion: The use of percutaneous transthoracic core needle biopsy under multi-slice computed tomography (MSCT) control for lung cancer has significant diagnostic value in the histological verification of lung lesions. This method is simple to use and safe for the patient.

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V.P. Shubin, D.Yu. Pikunov, A.N. Loginova, A.A. Barinov, Yu.A. Shelygin, A.S. Tsukanov

THE ROLE OF MOLECULAR-GENETIC RESEARCH IN THE DIAGNOSIS OF HEREDITARY POLYPOSIS SYNDROME

DOI 10.25789/YMJ.2023.82.12

УДК 616–006.66

To determine the molecular genetic cause of the disease in a patient with colon oligopolyposis, whole exome sequencing was performed. The c.333+5G/C variant was detected in the BMPR1A gene. The functional significance of the found variant was elucidated, which demonstrated exon elongation at the mRNA level. This made it possible to confirm the diagnosis of juvenile polyposis in the patient.

Keywords: oligopolyposis, NGS, BMPR1A gene, mRNA, cDNA, reverse transcription.

Introduction. About 3-5% of all cases of colorectal cancer are caused by hereditary oncological syndromes, including Lynch syndrome (OMIM:120435) [12], familial adenomatous polyposis (OMIM:175100) [14], MutYH-associated polyposis (OMIM:608456) [16], Peutz-Jeghers syndrome (OMIM:175200) [15] and juvenile polyposis (OMIM:174900) [3,13]. These syndromes have different clinical manifestations, the course of the disease, as well as in molecular genetic characteristics [11]. In this regard, the clinical picture often helps in the initial choice of a target for molecular genetic investigation – a gene with possible pathogenic variant causing the disease. However, in some cases, the clinical manifestations of the disease are unclear, so they may correspond to several hereditary syndromes simultaneously. At the same time, if molecular diagnostics of genes in which pathogenic variants are most often found is ineffective, next-generation sequencing becomes the option of choice, allowing for the study of the whole exome or genome [4]. A feature of whole exome or whole genome sequencing is the identification of a wide variety of variants, while difficulties arise in interpreting previously undescribed variants and it is not always possible to verify them as pathogenic (in such cases they are called variants of unknown significance) [6]. Only use of additional advanced molecular genetic analysis allows finding out the functional significance of such variants.

Patients and Methods. A male 28-years old patient with periodic blood mixture in stools was checked up by the proctologist at the local hospital. During colonoscopy, numerous polyps were revealed in the bowel. The patient was directed to the federal hospital with diagnosis “familial adenomatous polyposis” and there he received genetic counseling. According to the family history, mother of the patient at the age of 45 was diagnosed with the infiltrative ulcerative stomach cancer, which caused her death; besides there were some cases of oncological diseases in maternal relatives, but without accurate data on tumor site and the time of their occurrence, and the degree of kinship. Colonoscopy revealed 19 polyps throughout the bowel with different sizes and shapes (some on wide bases, and some on a long thin stalks) from 0.3 to 3.5 cm in diameter, while only 6 of them were more than 1 cm in diameter. The endoscopic picture corresponded to adenomatous polyps; the biopsy from the largest polyps revealed fragments of tubular and villous adenomatous structures with low-grade epithelial dysplasia. The patient underwent endoscopic removal of the largest polyps (>1 cm in diameter); the histological examination of the removed polyps revealed tubular adenomas with low-grade epithelial dysplasia, villous adenoma with low-grade epithelial dysplasia, as well as 2 polyps with juvenile structure. Thus, the clinical picture of the patient's disease could correspond to both adenomatous polyposis syndrome (an attenuated form of familial adenomatous polyposis or *MutYH*-associated polyposis) and “mixed” polyposis, in which both adenomatous and juvenile polyps could be detected in the bowel [19]. To verify the diagnosis, a molecular genetic study was conducted, with venous blood as a material. The patient was under medical supervision in accordance with the observation protocols and gave written informed consent for the study. This study corresponded to the ethical principles of the Helsinki Declaration and was approved by the local Ethics Committee of the NMRC of Coloproctology of the Health Ministry of Russia, Moscow, the Russian Federation.

DNA extraction. The samples of patient's DNA were extracted from the venous blood using an automatic MagNaPure Compact station (Roche, Switzerland), using the MagNa Pure Compact Nucleic Acid Isolation Kit I (Roche, Switzerland), according to the manufacturer's protocol.

PCR and Sanger Sequencing. Polymerase chain reaction and sequencing by the Sanger method of *APC* and *MutYH* genes were carried out according to the method presented in previously published papers [2,18].

MLPA Analysis. The detection of extended rearrangements was carried out by the multiplex test-dependent ligase reaction with subsequent amplification (MLPA) using reagent kits: 1. SALSA MLPA P043-APC v.D1; 2. SALSA MLPA Probemix P158 JPS; SALSA MLPA Probemix P378 MUTYH (MRC-Holland, Netherlands) according to the manufacturer's protocol.

SHUBIN Vitaly P. – PhD, senior researcher department laboratory of genetics, A.N. Ryzhikh National Medical Research Center of Coloproctology, shwit@mail.ru; **PIKUNOV Dmitry Yu.** – PhD, senior researcher department of oncoproctology, A.N. Ryzhikh National Medical Research Center of Coloproctology, pikunov.gnck@mail.ru; **LOGINOVA Anna N.** – PhD, researcher department laboratory of genetics, A.N. Ryzhikh National Medical Research Center of Coloproctology, anloginova@mail.ru; **BARINOV Alexey A.** – junior researcher department laboratory of genetics, A.N. Ryzhikh National Medical Research Center of Coloproctology, gerbarinov@gmail.com, **SHELYGIN Yury A.** – scientific director, academician of RAS, professor, A.N. Ryzhikh National Medical Research Center of Coloproctology, Russian Medical Academy of Continuous Professional Education, info@gnck.ru; **TSUKANOV Alexey S.** –– MD, leading researcher department laboratory of genetics, A.N. Ryzhikh National Medical Research Center of Coloproctology, tsukanov81@rambler.ru.

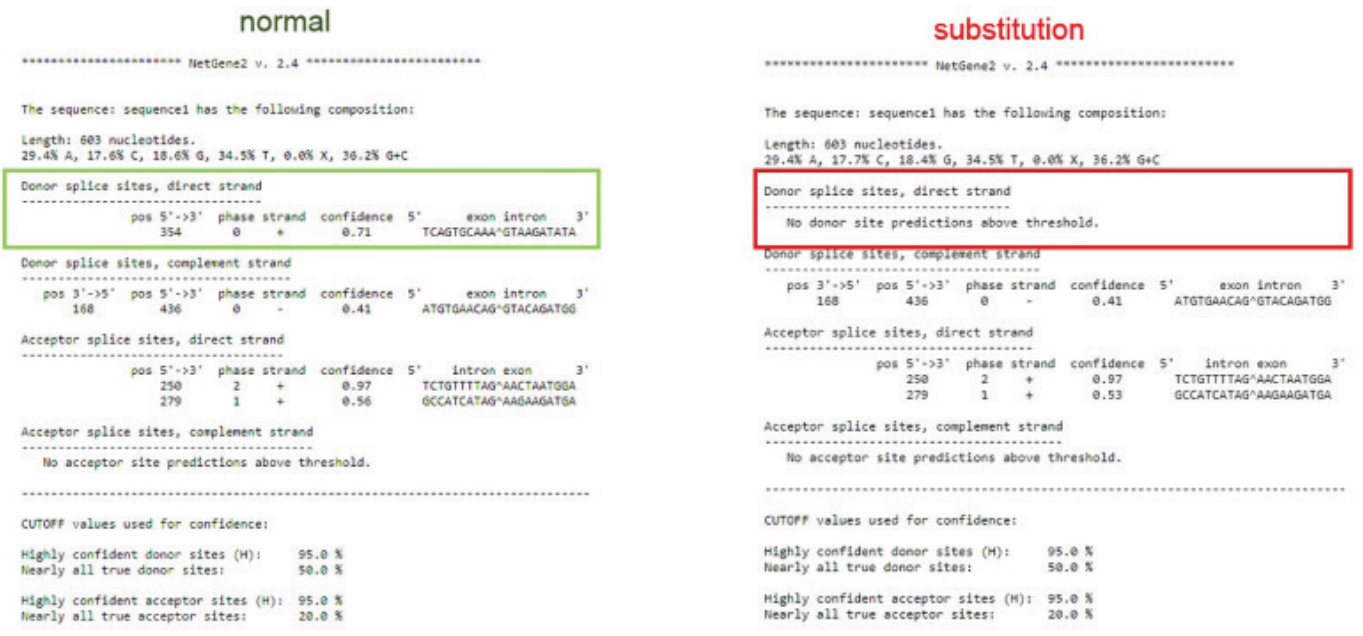

Fig. 1. The result of the analysis of possible outcomes in the case of variant c.333+5G>C using the NetGene2 splicing site prediction program: prediction of variant c.333+5G is a green frame; prediction of variant c.333+5C is a red frame

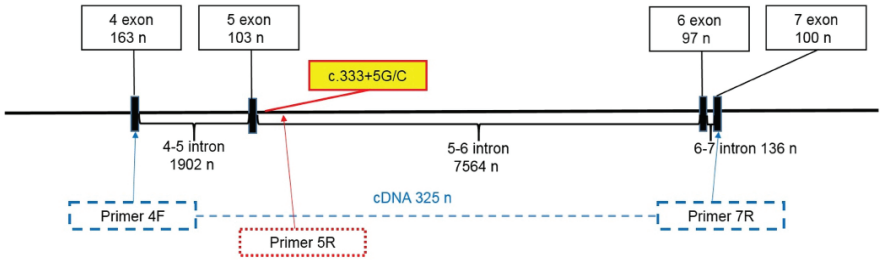

Fig. 2. Exon-intron region of the site from 4 to 7 exons of the BMPR1A gene. A yellow rectangle with a red frame is the name and location of the variant c.333+5G>C. The blue dotted frames indicate the location of the primers on the cDNA. The red dot frame shows the location of the primer in the intron after replacing c.333+5G>C.

Whole exome sequencing. We used 100 ng of total genomic DNA. The stages of sample preparation were carried out, followed by enrichment of exome regions using the Illumina Truseq Exome protocol and sequencing on the NextSeq550 platform with a reading length of 2*75 nctd (Illumina, USA).

mRNA extraction. mRNA was extracted from the patient's blood using the MagNa Pure Compact RNA Nucleic Acid Isolation Kit (Roche, Switzerland), using an automatic MagNaPure Compact station (Roche, Switzerland).

Reverse Transcription. The reverse transcription reaction was performed using the Thermo Scientific RevertAid Reverse Transcriptase kit (Thermo Scientific, Latvia).

PCR and Sanger Sequencing. DNA fragments were amplified by polymerase chain reaction (PCR). Primers of the *BMPR1A* gene were selected using the Primer3Plus program (https://www.primer3plus.com /): 4F – AGCACCAGAGGATACCTTGC; 7R – AATGAGCAAAACCAGCCATC; 5F – aacatgctagctacaattattgtga 5R – ggtgtacacatcgctgtatgttc (large caps – exon, small caps – intron).

Results. A sequential molecular genetic study of the *APC* and *MutYH* genes, including the search for extended deletions/insertions, did not reveal the presence of pathogenic variants. As a further diagnostic search, we decided to conduct a whole-exome sequencing. As a result, a variant c.333+5G>C, located in the intron (between exon 5 and 6) of the *BMPR1A* NM_004329.3 gene, was identified and then confirmed by Sanger sequencing.

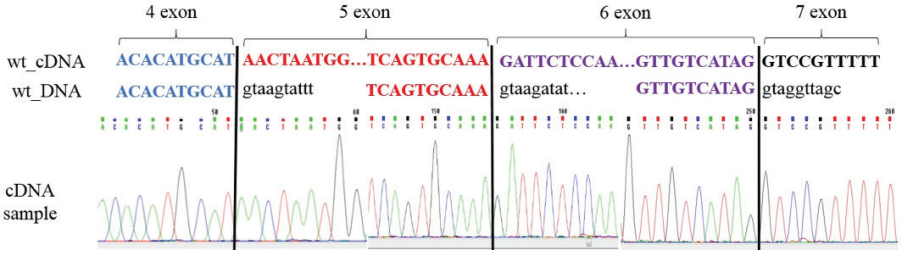

Рис. 3. Электрофореграмма участка кДНК пациента с вариантом c.333+5G/C в гене *BMPR1A*. В рис.3-4: wt_кДНК – референсный фрагмент кДНК гена *BMPR1A*; wt_ДНК – референсный фрагмент ДНК гена *BMPR1A* (ensembl.org)

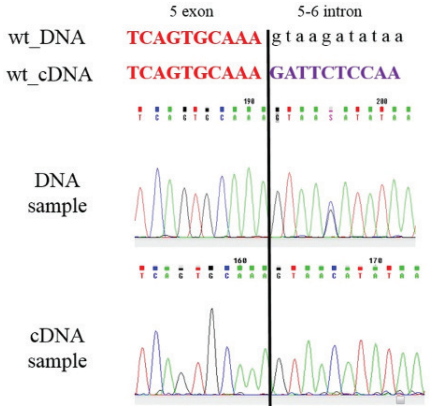

Рис. 4. Электрофореграммы участков ДНК и кДНК пациента с вариантом c.333+5G/C в гене *BMPR1A*

To find out the functional significance, an *in silico* analysis was performed using the NetGene2 splicing site prediction program (http://www.cbs.dtu.dk/services/NetGene2). It was found that variant c.333+5G>C leads to the loss of the donor splicing site and, accordingly, the variant may be functionally significant (Figure 1).

As the second stage, mRNA (with concentration of 15 ng/μL) was extracted from the patient's blood, then the reverse transcription reaction was performed, and the amplification of the cDNA fragment from exons 4 to 7 of the *BMPR1A* gene was carried out (Figure 2).

According to our first hypothesis, in the case of the functional significance of the found variant, the loss of an entire exon could be possible and this change should have been registered during sequencing of this site. According to the results of the analysis of the obtained sequencing fragment, there were no changes in the cDNA, and the fragment corresponded to the cDNA reference site (Figure 3).

Another hypothesis suggested that the replacement of c.333+5G>C could lead to the embedding of the intron site located between the 5th and 6th exons, which led to the amplification of the cDNA fragment only from the wild–type allele, since the intron had a large size – 7564 nucleotides. For confirmation of the fact of intron embedding, we amplified a cDNA fragment from the 4th exon to the 5th intron, while the reverse intron primer located further than the replacement of c.333+5G>C site by 82 nucleotides, and the length of the amplified fragment would consist of 348 nucleotides.

It should be noted that in the case of justified our hypothesis the fragment should look like a section of cDNA with an attached intron and a monoallelic variant of c.333+5C. Finally, the study confirmed this hypothesis (Figure 4).

Thus, even despite the lack of data on the number of embedded nucleotides from the intron, it can be stated with full confidence that variant c.333+5G>C of the *BMPR1A* gene leads to the formation of a premature stop-codon, being a pathogenic mutation of the splicing site.

Discussion. Oligopolyposis is a patient's condition with 10 to 99 polyps in the bowel [8]. Depending on the number of the predominant type of polyps, oligopolyposis can be classified into: adenomatous, hamartomic, serrated, and others. In some cases, it is difficult to understand which type of polyps dominate and, accordingly, it is difficult to make a correct diagnosis with further treatment tactics.

We have produced an advanced molecular genetic investigation in a patient who had mainly adenomatous polyps and only 2 juvenile ones. Formally, the patient does not fit the Russian clinical guidelines “Adenomatous polyposis syndrome” developed by Shelygin, Yu. A. et al. [1]. In addition, we could not suspect juvenile polyposis since our patient did not fit the clinical selection criteria described in the clinical guidelines authored by Richard Boland et al. [9]. According to these criteria, a genetic study for the determination of juvenile polyposis should be carried out for:

- *Patients with 5 or more juvenile-polyps in the colon and rectum;*
- *Patients with 2 or more juvenile polyps located in other parts of the gastrointestinal tract, except the large intestine;*
- *Patients with any number of juvenile polyps having 1 or more first-line relatives with juvenile polyposis.*

In addition, single juvenile polyps can occur in 2-3% of children and adolescents [7].

Despite the fact that the clinical manifestation of our patient did not match the Russian criteria for the attenuated form of familial adenomatous polyposis (the presence of 20 to 100 polyps in the bowel, predominantly in the proximal parts) and *MutYH*-associated polyposis (the presence of 20 to 100 polyps), we decided to investigate the *APC* and *MutYH* genes, as the patient's mother had malignant tumor of the stomach at the age of 45, but no information was about the presence or absence of polyps in her bowel. An extended study of these genes, including the MLPA method, revealed no mutations. It is worth to note that the set for the study of large rearrangements of the *APC* gene includes diagnostics of the duplication of the *GREM1* gene (15q13.3) which is associated with mixed polyposis syndrome [10]. For the first time, mixed polyposis syndrome was described in an Ashkenazi Jewish family whose members had polyps of more than one histological type: adenomas, hyperplastic and juvenile [10].

Whole exome sequencing revealed an intronic variant of c.333+5G>C in the *BMPR1A* gene. This variant was not found in the gnomAD population databases (0/250910 alleles). However, it was previously encountered in the study of the Invitae group (rs1554888331), but it was characterized as a variant of unknown significance. It is worth noting that we founded the pathogenic variant IVS3+5G→C in the HGMD (The Human Gene Mutation Database) database [17]. It could be assumed as a similar option. However, a specific name (according to the HGVS nomenclature) is not given in the specified database, therefore, it is not possible to confirm the identity of this variant and c.333+5G>C. In this situation, we performed a study for proving the pathogenicity of a previously unknown variant according to the algorithm of our early investigation [5]. In present work, we also proved the pathogenicity of the unknown variant, because its presence in the *BMPR1A* gene leads to the loss of the splicing site, elongation of the exon and the formation of a premature stop-codon (TAA).

Conclusion. As a result of a complex molecular genetic analysis, it was proved that the variant c.333+5G>C of the *BMPR1A* gene was pathogenic. This gave us ability to confirm a genetically verified clinical diagnosis of juvenile polyposis in a patient with 17 adenomatous and 2 juvenile polyps in the bowel.

The work was carried out within the framework of the state assignment: “Diagnostic of hereditary forms of colorectal cancer by whole exome sequencing and MLPA (multiplex probe-dependent ligase reaction with subsequent amplification) methods.

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HEALTHY LIFESTYLE. PREVENTION

S.K. Kononova, V.M. Nikolaev, D.M. Vinokurova, O.G. Sidorova, A.N. Romanova

THE ATTITUDE OF YOUNG PEOPLE OF REPRODUCTIVE AGE TO ETHICALLY CONTROVERSIAL ISSUES OF THE PRENATAL DIAGNOSIS OF HEREDITARY DISEASES

DOI 10.25789/YMJ.2023.82.13

УДК 616-056.7-053.81

The article discusses the results of a survey conducted among young people in the City of Yakutsk regarding ethically difficult issues of prenatal DNA-testing for hereditary diseases. Most respondents (74%) consider prenatal diagnosis a necessary procedure. Analysis of attitude of young people to morally ambiguous issues of prenatal diagnosis shows that the decision to terminate pregnancy after prenatal diagnosis is affected by the severity of damage to fetus. Compared to similar survey results in other countries, young reproductive age people in Yakutsk show lower values on the issue of pregnancy termination when confronted with Down's syndrome (49%) and an ethically ambiguous issue of pregnancy termination in case of a deaf child (19%). There is no connection between the opinions of respondents on prenatal diagnosis being a necessary or an unnecessary procedure, and their own desires to terminate pregnancy in case of a disorder.

Keywords: prenatal diagnosis, bioethics, survey, young people, hereditary diseases.

Introduction. Prenatal diagnosis (PND) is a modern means of diagnosing the state of a fetus and detecting possible disorders during pregnancy at different stages of gestation. Various diagnostic methods and their combinations are employed, such as ultrasound, biochemical, cytogenetic, molecular-genetic testing, including invasive and non-invasive methods of fetal examination [2].

According to European guidelines, the objective of PND is providing prenatal diagnostic testing services (for genetic conditions) that enable families to make informed choices consistent with their individual needs and values and which support them in dealing with the outcome of such testing [20].

When conducting a PND for hereditary monogenic disorders, fetal samples obtained via chorionic villus sampling at early stages of pregnancy are processed to extract DNA from cells, after which a molecular-genetic analysis is performed to detect damage (mutations) to genes. There are many different methods of genetic testing available today, from direct PCR diagnosis to detect mutations, to analysis of full genome sequencing of an individual's DNA. Modern genetic testing technologies can detect mutated gene variants and variations of genetic markers, which are connected to disorders based mostly on calculations of probability of disease manifestation. PND is a complicated and expensive procedure which often comes with moral and ethical dilemmas, both for geneticists and families that undergo PND and make a difficult decision to be tested [22]. Main bioethical issues include informed consent for PND, individual autonomy, right to reproductive choice [4]. In case of a risk of severe fetal disorder that makes it non-viable, or high probability of congenital genetic disease, families make emotionally difficult decisions to terminate pregnancy, and it is known that 80 to 90% of families decide to terminate [6,10,18]. The remaining families decide to continue pregnancy with an affected fetus due to their moral values or religious beliefs [13].

Yakut Science Center of Complex Medical Problems: **KONONOVA Sardana Kononovna** – PhD, Head of Scientific Research, konsard@rambler.ru, https://orcid.org/0000-0002-2143-0021; **NIKOLAEV Vyacheslav Mikhailovich** – PhD in Biology, senior researcher, nikolaev1126@mail.ru; **SIDOROVA Oksana Gavrilevna** – research associate, https://orcid.org/0000-0001-7089-6736; **ROMANOVA Anna Nikolaevna** – MD, director, https://orcid.org/0000-0002-4817-5315; M.K. Ammosov North-Eastern Federal University: **VINOKUROVA Dekabrina Mikhailovna** – PhD in Social Sciences, Associate Professor of the Institute of Psychology, https://orcid.org/0000-0003-3259-3907

There are many difficult and contentious ethical issues relating to prenatal diagnosis of fetal disorders that do not affect its viability. For example, is termination of pregnancy justified in case of disorders with variants of a gene (penetrance), when the detected mutation does not allow for definitive conclusion on development of a disease, or is it justified in cases of mutations with late onset, when the individual will develop a disease in adulthood [1].

The study of public opinion on genetic technologies in practical medicine of the Sakha Republic (Yakutia) is conducted in the form of sociological surveys. The study evaluates the perception and thoughts of the population, which is important for identifying a number of fundamental issues relating to regulation of human genome studies.

The objective of this article is to analyze the results of survey on contentious ethical issues of PND of hereditary diseases, and the attitude of young reproductive age people living in Yakutsk to PND.

Methods. The survey was conducted in Yakutsk using a standard method of sample survey that was done distantly. The number of respondents was 300 people. The objective of the sociological survey was to study the attitude of residents of Yakutsk to DNA-testing for hereditary diseases as a new method used in the practical medicine of the Sakha Republic (Yakutia). The questionnaire consisted of 24 different types of questions: multiple choice, binary (yes, no), matrix (questions in the form of a table where the necessary response should be marked with a tick). There were also a number of open questions, such as: "If you think that prenatal diagnosis should not be performed, could you explain why? (please write down your own answer)".

Questionnaire results were processed using IBM SPSS Statistics 22 software.

Confidence coefficient is 95%.

Confidence interval is (±%) 5.66.

When comparing groups by answers of respondents $\chi 2$ criterion and Fisher's criterion (F) for small samples were used.

Results of analysis of attitude of young people to DNA-testing (first part of the questionnaire) were published by us in International Journal of Circumpolar Health (2020) [12].

This article discusses the results of a survey conducted among young reproductive age people in Yakutsk regarding a number of issues related to prenatal DNA testing for hereditary diseases. Social and demographic profiles of respondents are shown in Table 1.

Most of the respondents (75%) were representatives of the Sakha people with an age of 29 years.

Results and discussion. The survey results are presented in Table 2. For respondents who answered "No" we gave an opportunity to voice their opinion using an open question "Why do you think prenatal diagnosis should not be performed?" This led to a number of different answers, with most respondents citing possible risks for the fetus, some saying "better not to know", or "I'm worried for my spouse", or "everything is in God's hands".

Most young people (74%) of Yakutsk consider prenatal diagnosis a needed and necessary procedure. Our data corresponds to Julian-Reynier (1993) study results, where reproductive age women from Italy also voiced the usefulness of PND for trisomy 21 syndrome (Down' syndrome), 78% of respondents would like to be tested even if there was a 1% chance of trisomy 21 syndrome [11]. One can imagine that most people would consider it a good thing to prevent birth of children with disabilities, however there are movements for rights of disabled people in our society that think that life with disabled traits should not necessarily damage the ability of "special" people or their families to have a decent life [15].

After a PND and getting information on severe disorder affecting a fetus, a pregnant woman or a couple is forced to make a difficult decision to terminate or to continue the pregnancy [16]. This is a very stressful process that requires additional information for parents and support from specially trained medical staff or clinical psychologists [6,9,19,21]. A survey of 207 married couples carried out by Quadrelli R (2007) with the objective of finding out the decisions of parents after a PND that identified chromosomal anomalies had the following results: in case of Down's syndrome or fetal aneuploidy with severe prognosis, 89% and 96% of patients respectively would terminate the pregnancy, while in case of chromosomal disorders with low risk of an anomalous clinical phenotype up to 90% of patients would continue the pregnancy [17]. In our survey, in case of Down's syndrome prognosis, 49% of respondents would terminate the pregnancy (figure 1).

A difficult and contentious ethical issue comes to the forefront when deciding to terminate pregnancy (based on PND results) in case of disorders which are not life-threatening (deafness, blindness), or in case of anomalous phenotypes such as short stature, short limbs, facial dysmorphism, etc. [5]. An example of a difficult bioethical issue is the possibility of PND for 3M syndrome or Yakut short stature syndrome (YSS), widespread in the Sakha population (12.72:100000). According to Maksimova et al., 2007, all affected by YSS have characteristic clinical features and phenotype: postnatal growth and physical development retardation, large head, facial dysmorphism, short and wide thorax, enlarged abdomen, lumbar lordosis, muscle hypo-

Table 1

Socio - demographic characteristics of respondents

characteristics of respondents	values (n=300)	%
Sex:		
Female	146	48.7
Male	154	51.3
Age:		
average value	29.7	
median	23	
moda	22	
Marital status:		
not married	170	56.7
married	84	28.0
Education:		
Higher	214	71.3
college	80	26.7
Having children:		
without children	197	65.7
One or more children	103	34.3
type of activity:		
Student	180	60.0
Working in various fields	143	47.7
Nationality:		
Sakha (Yakuts)	225	75.0
Other nationalities	75	25.0

tonia and others, but with no motor and sexual development retardation, normal intelligence and no mental deficiency [14]. In cases of prenatal diagnosis of 3M syndrome parents are informed of risks of having a child with this disorder and possible difficulties in the child's social life due to short stature. According to Gotovtseva (2014) there were 40 PND procedures for 3M syndrome performed in 5 years with 11 fetuses identified as carriers of CUL7 mutation [3]. There is no data on the number of terminated or continued pregnancies as the bioethical issues of this monogenic disorder have not been studied and there are no ethical rules for PND and DNA-testing for YSS.

Fu et al. (2016) studied the ethically ambiguous issues of DNA-testing and prenatal diagnosis for recessive forms of hereditary deafness by surveying college students in Shanghai. After a brief written information was presented to them with an example of GJB2, the most widespread recessive gene of deafness, 67.7% of respondents voiced their interest in undergoing genetic testing to find out if they were carriers of GJB2 mutation. In hypothetical scenario of carrying GJB2 recessive mutation, 86.9% of respondents would ask their partners to also take the test. If both partners were carriers, 88.7% would consider prenatal diagnosis, and 80.7% would consider terminating pregnancy [8]. In another study, Deng et al. (2018) conjecture that PND and genetic consultation protocol contain detailed information that can help couples from high risk families to prepare for childbirth and future family planning. For mutation carrying newborn, PND and genetic consultation would facilitate the implementation of strategy of "early screening, early diagnosis, early intervention" [7].

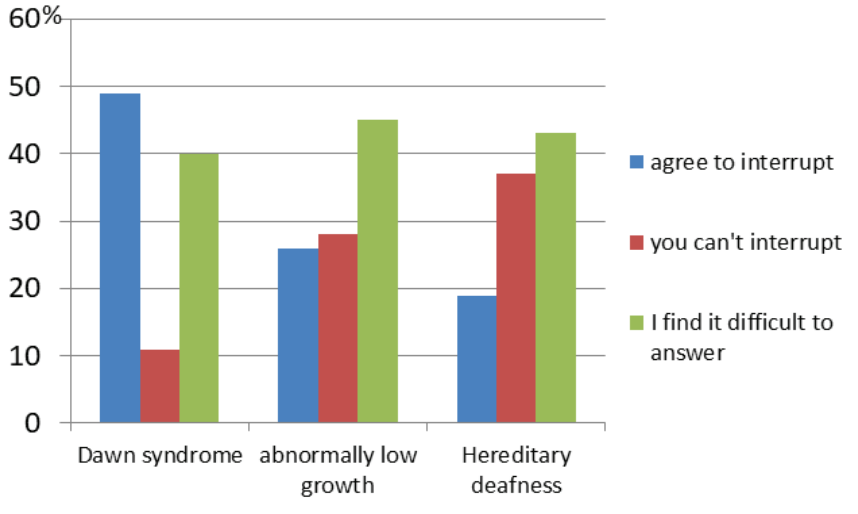
Using questionnaires we studied the attitude of young people in Yakutsk to morally ambiguous issues of terminating pregnancy as a result of PND. A hypothetical question "How justified do you think is the termination of pregnancy as a result of prenatal diagnosis?" in the form of a table was divided into more specific parts: Down's syndrome, the most well-known and severe disorder, and morally ambiguous reasons for terminating pregnancy: anomalously short stature (dwarfism), as well as possible hereditary deafness.

The results of our survey show that the decision to terminate pregnancy after a PND is affected by the severity of a fetal disorder. 49% of young reproductive age respondents think that pregnancy termination is justified if there is a risk of Down's syndrome; 26% think it is justified if there is a risk of anomalously short stature (dwarfism). By contrast to high numbers of Chinese young people who think that termination of pregnancy in case of possible deafness is justified (80.7%), only 19% of our respondents consider such a possibility in similar circumstances. Another distinctive result of our survey is that in all three cases (Down's syndrome – 40%, dwarfism – 45%, deaf-

Table 2

Respondents' answers to the question: "How do you feel about prenatal DNA diagnostics?

answer options	number of people	%
I consider it useful and necessary	222	74
I think that it does not need to be done	30	10
I find it difficult to answer	43	14.3
Other answers	5	1.7
Total	300	100



Respondents' answers to the question: "How do you think termination of pregnancy is justified based on the results of prenatal diagnosis?"

Table 3

Comparison of the opinions of two groups of respondents on termination of pregnancy according to the results of PD

How do you feel about prenatal DNA diagnostics?	I consider PD useful and necessary				I think that PD does not need to be done				X2	p	F(p)
How do you think termination of pregnancy based on the results of prenatal diagnosis is justified?	I agree to terminate the pregnancy		You can't interrupt		I agree to terminate the pregnancy		You can't interrupt				
number of respondents	n	%	n	%	n	%	n	%			
Down syndrome	119.000	83.217	24.000	16.783	13.000	72.222	5.000	27.778	0.607	0.435	0.608
Abnormally low growth	70.000	52.632	63.000	47.368	9.000	52.941	8.000	47.059	0.000	0.980	1.000
Hereditary deafness	46.000	34.586	87.000	65.414	7.000	43.750	9.000	56.250	0.520	0.470	0.581

ness – 42%) many respondents had no answers to morally difficult questions of terminating pregnancy if hypothetical disorders were present. In our opinion this once again confirms the conclusion that all children have special value and significance for the Sakha people as part of the ethnic mindset, considering that 75% of respondents were representatives of the Sakha people (table 1). We divided the respondents into two groups by positive and negative answers to the question of "What is your opinion on prenatal DNA-testing?" and compared them by their answers to the questions on terminating pregnancy in case of Down's syndrome, dwarfism, and hereditary deafness (table 3). We did not find any statistically significant differences when comparing these groups, which shows that there is no connection between the respondents thoughts on prenatal diagnosis being a necessary or an unnecessary procedure, and their own desires to terminate pregnancy in case of a disorder.

Conclusion. Analysis of attitude of young people in Yakutsk to morally ambiguous issues of prenatal diagnosis shows that the decision to terminate pregnancy after PND is affected by the severity of damage to fetus. Compared to similar survey results in other countries, young reproductive age people in Yakutsk show lower values on the issue of pregnancy termination when confronted with Down's syndrome (96% in Italy, 49% in Yakutsk) and an ethically ambiguous issue of pregnancy termination in case of a deaf child (80.7% in Shanghai, 19% in Yakutsk).

Conclusion on the special value and significance placed on any child among the Sakha people as part of an ethnic mindset was confirmed, as 75% of respondents of representatives of the Sakha people.

There is no connection between the respondents opinions on prenatal diagnosis being a necessary or an unnecessary procedure, and their own desires to terminate pregnancy in case of a disorder.

Development and practical application of genetic technologies, such as using prenatal diagnosis for "selecting" healthy fetuses and, in the future, editing genomes to change the genes of embryos, leads to discussions on the ethics of applying advances in genetics. Is there a line where we should stop applying the advances in genetic technology and where should it be drawn? Which kind of genetic testing is useful and necessary, and which should be declared ethically unacceptable? In order to find answers to these questions we need to study public opinion.

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ORGANIZATION OF HEALTHCARE, MEDICAL SCIENCE AND EDUCATION

S.A. Bogachevskaya, S.N. Kiselev, N.A. Kapitonenko, A.N.Bogachevskiy

THE STATE OF THE SYSTEM OF CONTINUOUS MEDICAL EDUCATION ACCORDING TO THE RESULTS OF SURVEYS OF HEALTHCARE PROFESSIONALS IN THE FAR EASTERN FEDERAL DISTRICT

DOI 10.25789/YMJ.2023.82.14

УДК 614.252.1:379.046.4:303.442

The aim of the research is a sociological survey of doctors of various specialties to identify the dynamics and potential for the development of changes in the system of medical education. This survey was conducted in two time periods: at the initial stage of the practical implementation of the system in the years 2013–2015 (341 respondents) and 7-9 years later in the year 2022 (93 respondents). The data were evaluated by calculating relative values and errors of relative values, as well as comparative analysis. According to the results of the study, it was revealed that more negative attitudes towards the system of continuing medical education prevailed at the initial stage, due to both low information of potential users and defects in the organization of the system (functioning of the Internet platform, defects in the legislative framework, organizational problems). At the second stage of the research, a definitely positive trend was revealed, in addition, new problems of applying this education system and possible ways to solve them were discussed. In the initial phase of reforming medical education, the main part of the target audience remained unprepared. The reasons for the rejection of the new system were the increased level of distrust of specialists in the actions of the Ministry of Health of Russia, as well as their low motivation to change the education system. In relation to the introduction of the standard of continuing medical education, positive changes were observed: 47.3±5.2% of positive reviews in the year 2022 against 13.3±1.8% in the years 2013-2015. It was expected that the regulatory documents over the course of observation should streamline the process and increase the effectiveness of the new model of postgraduate education. Improvement in reviews towards the system of continuing medical education among medical workers implies a higher interest in acquiring professional skills as well as the level of medical care. However, gaps in legislation that have not been resolved reduce the effectiveness of this new system. Thus, over the course of observation, a streamlining of the process of including healthcare professionals in the model of continuous medical education and carrying out appropriate activities within the new system of postgraduate education was observed. Further active interaction of control and regulatory systems with direct participants in the executive processes of medical education is necessary to increase the overall satisfaction of citizens with the work of the Russian healthcare system.

Keywords: continuing medical education, sociological research, educational modules

Introduction. The aim of state policy regarding additional professional training of medical and pharmaceutical specialists is an effective management of the quality of medical care in the Russian Federation. The development of continuing professional education in the field of healthcare is carried out within the framework of federal laws and orders of Healthcare Ministry [4,6,7,8], which provide for the transition from traditional postgraduate advanced training once every 5 years to new educational practices: continuous medical education (CME) and accreditation during a five-year training cycle.

The organizational and motivational problems were indicated on at the initial stage. Expected that regulatory documents over time should streamline the process of conducting and increase the effectiveness of the new model of postgraduate education.

Materials and methods. The object of observation were high medical education specialists from nine subjects of the Far Eastern Federal District (FEFD). To obtain scientific information, a sociological research method was used. The units of observation were determined by the method of simple random sampling. The observation periods were 2.5 years (2013–2015) and 1 year (2022). The study involved doctors of various specialties, including healthcare organizers (341 respondents in the first observation period and 93 respondents in the second observation period). The collection of primary information was carried out at both stages by means of a questionnaire survey. The questionnaire, developed by the authors in accordance with domestic and foreign guidelines for medical and sociological monitoring [13,15], consisted of 30 questions. The questionnaires touched upon the main provisions of the reform of education of healthcare professionals, respondents' assessments of the availability and quality of medical care, individual awareness of specialists in the field of vocational education using closed and semi-closed questions. The data obtained were evaluated by calculating relative values and errors of relative values, as well as comparative analysis.

Results. Of the total number of respondents in the first stage, 89 were men (26%), 252 were women (74%), in the second stage 21 men (23%) and 72 women (77%) participated. Respondents aged 51–60 years formed the largest groups at both stages of observation (33% at the first stage and 29% at the second stage).

It should be noted that by the second

BOGACHEVSKAYA Svetlana Anatolyevna – PhD, Doctor of functional and ultrasound Diagnostics of the Federal Center for Cardiovascular Surgery of the Ministry of Health of the Russian Federation (Khabarovsk); Associate Professor of the Institute of Continuous Prof. education and accreditation of the Far Eastern State Medical University, bogachevskayasa@gmail.com, ORCAD 0000-0001-7150-2620; **KISELEV Sergey Nikolaevich** – MD, Head of the Department, Vice-rector for Educational Work, Professor, Far Eastern State Medical University, ORCID 0000-0003-2047-9824; **KAPITONENKO Nikolay Alekseevich** – MD, Professor, Far Eastern State Medical University; **BOGACHEVSKY Alexander Nikolaevich** – cardiovascular surgeon, head of department. Sakhalin Regional Clinical Hospital, ORCHID 0000-0002-5319-027X. Federal Center for Cardiovascular Surgery, Far Eastern State Medical University of the Ministry of Health of Russia, Sakhalin Regional Clinical Hospital.

stage of the study, each of the surveyed medical workers (100%) was already familiar with this system: 87 out of 93 specialists actively participated in CME. Positive dynamics on the main issues of additional professional education (APE) among the respondents is presented in the table 1.

As in the first stage of the study, there was a need for new information in the work in the specialty and, in particular, in raising the level of knowledge in the section of cardiovascular pathology. The technical ability to use the Internet increased and amounted to 93.5% compared to 89.5% [2]. Of the 6 people in the 2022 survey who do not use the Internet at work, 5 planned to complete work in their specialty by the time of personal periodic accreditation.

Half of all respondents at the first stage of the study reacted negatively to the introduction of the CME standard, and a third found it difficult to answer the question. The second survey showed a significant improvement in the perception of the CME system (positive feedback already from 47.3% (44) of the respondents, mostly those who passed the stage of periodic accreditation, against 13.3±1.8% of the respondents of the first stage).

At the same time, the subjective explanation of the negative assessment of the transformations by the respondents did not change significantly: the system "is still not fully developed", there is not enough available information on a given topic.

In the 2013-2015 survey 23.0 ± 3.2% of respondents noted the possibility of mastering new practical skills, subject to mandatory separation from work, as an undoubted positive aspect of the new education system. During the second survey in 2022, it became obvious that the employer often has the opportunity to organize training on the job, which more than 35% (35.5±5.0%) of respondents already noted as a negative factor.

Discussion. The obligatoriness of changing the existing education system in health care has not lost its relevance: according to experts, knowledge is completely updated every 6 years (by 15% per year) [16].

Negative attitude of physicians towards the CME system at the first stage of the study (2013-2015) was determined by an increased level of distrust of the innovations of the Healthcare Ministry of Russia and its ability to implement the tasks assigned to it.

It is obvious that the attitude of physicians towards the new CME system has improved over time. On the one hand, due to the fact that the number of APE programs and their participants is growing dynamically due to the inclusion of CME in the process of periodic accreditation of specialists. On the other hand, more positive reviews were received taking into account the dynamic amendments to the legislation from the beginning of the practical use (not a pilot project) of the new education system [11]. However, the total number of positive respondents is still only close to 50%.

A superficial look of NMO and unwillingness to perceive it as a whole was revealed in 36.4% of the respondents [2]. 28.7% of respondents (98) did not know the main provisions of CME, 55.4% (189) complained about the lack of information about CME [2]. By 2022, less than a third of respondents, 30.1%, found it difficult to answer questions about the organization of CME (28).

At the first stage of the study, none of the respondents paid attention to the fact that in the new system the number of required training hours increased significantly over 5 years: from 144 to 250, which additionally testified to the low awareness of health workers about the CME system. The second stage of the survey of respondents showed that the reduction in the number of study hours that would be required to increase the level of knowledge to 144 hours had a positive response from 26% (24) of the respondents.

The system of educational loans came to us from abroad, where it is quite common [17]. Merging into domestic conditions, the system began to acquire its own characteristics, often not in favor of itself.

If at the first stage of the study, the respondents justified their negative attitude towards the CME system being introduced mainly by the fact that by 2015 the necessary conditions for its implementation and functioning of the system had not been created, then at the moment, under the new conditions, it became possible to formally study and obtain relevant documents. This became possible due to the fact that some of the educational organizations of the APE have the ability to circumvent the requirements. Having nothing to do with medical education, without having the resources and teaching staff to implement medical training programs, these organizations receive educational licenses and conduct training without restrictions in specialties, including medical ones. This is facilitated by the growth of interested parties and shortcomings in the current regulatory documents.

Interested persons are physicians (especially the more experienced generation), who do not consider it necessary to improve their own qualifications in cycles of additional university programs (training cycles), simply having the opportunity not to spend time on education due to low interest in their own professional growth, or considering the knowledge gained in this way obsolete. In fact, a competent system is being created that allows even motivated specialists to circumvent the requirements of the APE without engaging in advanced training in additional programs.

Dynamic survey of healthcare professionals on the state of the system of additional professional education

Questions	Answers 2013-2015 гг. (p±m), % n-341 (*n-174)			Answers 2022 г. (p±m), % n-93		
	Positive	Negative	Difficulty answer	Positive	Negative	Difficulty answer
How do you feel about the implementation of the CME standard?	13.3±1.8	50.5±2.7	36.4±2.6	47.3±5.2	32±4.8	20.7±4.2
Do you think that the specialist himself should pay for his education?	7±1.4	82.6±2.1	10.0±1.6	10±3.1	72±4.7	18±4.0
Do you feel the need for new information in your specialty?*	85.1±2.7	8.0±2.1	6.9±1.9	82±4.0	13±3.5	5±2.3
Do you feel the need for new information on modern methods of diagnosis and treatment of cardiovascular diseases?*	85±2.4	7.5±2.0	4.0±1.5	78±4.3	6.5±2.6	5.5±2.4
Do you use medical information from the Internet in your work?*	86.2±2.6	11.5±2.4	2.3±1.1	92±2.8	6.5±2.6	-

The continuing problem of staff shortages (especially in small towns and rural areas), combined with the emerging opportunities to "bypass" mandatory full-time forms of education, provokes heads of medical institutions to formal full-time training in reality on the job, even against the background of a decrease in the number of hours (points) gained from 250 to 144 [8,11].

As a result, on a paid basis, a specialist can formally undergo training in any specialty without conflicts at the place of work.

One of the three main components of acquiring points in the CME system - various events (conferences, congresses, etc.) in the face-to-face format are still not available to most specialists, and the implemented on-line format, with all attempts to "bind" event participants to drying of reports has the possibility of formal presence, participation is often carried out in parallel with the work process (work on the days of events "no one canceled").

Electronic educational modules are not sufficient for the development of a five-year program, their quantity and quality should be regulated. In addition, there are no uniform requirements for the semantic content of the modules.

To increase the motivation of specialists for learning, the issue of distance learning is being actively addressed. The problem of training at a convenient time for doctors is more complicated [1].

Time restrictions on on-line events for certain regions of the country, which has 9 time zones, are at best solved by transferring records of events off-line, and often they are not solved at all and become problems for the student.

The results of a survey of 4276 respondents on the educational platform of the Healthcare Ministry of showed that 56% of specialists with less than 5 years of experience do not support the use of Internet content in the CME system, while 41% of those with longer experience expressed the opposite opinion [3].

Under these conditions, probably, young specialists experience not only a lack of knowledge, but also a lack of live communication with more experienced colleagues. The problem can be partially solved by including practice-oriented cycles in the content. At the same time, the problem of practice orientation remains relevant in the context of short training cycles in the CME system.

At the second stage of the study, 36.6% (34) of respondents independently noted a positive effect from the offset of points scored in related specialties. This algorithm, indeed, greatly facilitated the work in the system for doctors with several specialties.

The practical use of the CME system has shown that with all the advantages of re-crediting points in related specialties, there are also disadvantages. A transfer option that has a positive effect on experience in the specialty: cycles for clinical specialties in various diagnostic methods and, conversely, for diagnostic specialists, in clinical aspects of pathology. An application that does not affect skills in the main specialty: cycles in general education programs (for example, on topical infectious diseases, blood transfusion, pre-trip examinations, etc., which are also mandatory. As a result, the situation is real, when over a five-year period of study a specialist may not complete educational cycles directly in the specialty. Interdisciplinary training actively introduced in this way should be more harmoniously integrated into the main process of a five-year continuous education of a specialist.

As a result, a more optimal option for a five-year training cycle seems to be a mandatory combination of full-time cycles mainly in the specialty (2 out of 5), correspondence courses mainly in related specialties (2 out of 5) and practical skills (simulation centers - 1 cycle). However, it should be understood that simulation training cannot replace clinical experience, but is only its valuable addition [9,17].

Continuing medical education remains subject to state regulation. However, gaps in legislation that have not yet been resolved reduce the effectiveness of the system being implemented. The growth of a formal attitude to education, which was assumed at the first stage of the study [2], received additional prerequisites under these conditions. In order to overcome the problems of CME, special organizations and associations have been created, for example, the association "Rosmedobr" (association of teachers of medicine, https://www.rosmedobr.ru/), but today their efforts are not enough in the fight against unresolved gaps in the legislation.

Until now, more than half of the citizens are not satisfied with the work of the Russian healthcare system (53% in 2008, 59% in 2014, 58% in 2019) and more and more citizens are pessimistic about the prospects for the development of domestic healthcare in the next decade (2008 - 18%, 2014 - 24%, 2019 - 30%) [16].

In addition to the previously recommended events to improve the efficiency of legal regulation in the field of further vocational education and the development of professional communities [12], it makes sense, as part of the development of high-quality educational events for CMEs, to more actively develop and improve practice-oriented technologies and simulation courses, as well as conduct targeted training of qualified personnel. reserves of the teaching staff of universities.

The organization of the educational process is based on the methodology, where the basis is the competence-based approach [16]. This approach should be supplemented with an adaptive approach, which will allow, based on feedback, to offer students an individual educational plan.

The medical university, as the main organizer of the healthcare workforce, must undeniably undergo innovative changes in the system of organizing continuous professional postgraduate training of specialists. Various forms of education are being widely introduced using interactive methods and distance learning technologies [14]. The possibilities of the university should be used as widely as possible.

Conclusions. Thus, over the course of observation, there is an ordering in the perception of the CME model by healthcare professionals and the implementation of appropriate activities in the new system of postgraduate education. The improvement in attitudes towards the CME system among medical professionals implies a higher interest in acquiring professional skills, as well as an increase in the level of acquiring professional skills and, accordingly, the level of medical care. To counteract unscrupulous educational organizations, it is necessary to take additional legal decisions. Further active interaction of control systems and regulatory bodies with direct participants in the executive processes in the field of medical education is necessary to increase the overall satisfaction of citizens with the work of the Russian healthcare system.

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HYGIENE, SANITATION, EPIDEMIOLOGY AND MEDICAL ECOLOGY

S. V. Pryanichnikov

FEATURES OF STRESS-IMPLEMENTING AND REGULATORY SYSTEMS OF THE BODY OF MEDICAL STUDENTS IN HIGH LATITUDES

DOI 10.25789/YMJ.2023.82.15

УДК 159.91,612.172.2

The aim of the study was to study the influence of the level of situational (SA), personal anxiety (PA) on the psycho-emotional state of the body and the cardiovascular system (CS) in high latitudes. The study involved 65 people (girls, age 18.67± 3.75 years) who filled out questionnaires of differentiated self-assessment of functional status (WAM), the methodology for determining the level of situational anxiety and personal anxiety by C. D. Spielberger and Y. L. Khanin. The heart rate variability (HRV) indicators were recorded using the Omega-M hardware diagnostic medical complex. To quantify the concentration of cortisol in blood serum, the method of solid-phase enzyme immunoassay was used. To analyze the prevalence of vitamin D deficiency, an enzyme immunoassay was used to quantify 25-OH in serum and plasma. It is shown that with a change in the level of anxiety, the subjective assessment of well-being, activity, and mood decreases. At the functional level of the work of the cardiovascular system, significant differences were found in the form of changes in the temporal and frequency characteristics of the HRV, in which the influence of the mechanisms of sympathetic modulation of the heart rhythm prevails. The functional state of the hypothalamic-pituitary-adrenal system (HPAS), estimated as the quantitative content of cortisol in the blood and does not change depending on the level of anxiety. Significant differences in the level of vitamin D in the blood and its general deficiency in the study participants were shown.

Keywords: well-being, activity, mood, situational and personal anxiety, cortisol, vitamin D, heart rate variability.

PRYANICHNIKOV Sergey Vasilyevich – Researcher at the Scientific Department of Biomedical Problems of Human Adaptation in the Arctic of the Kola Scientific Center of the Russian Academy of Sciences, ORCID 0000-0002-8321-6805; e-mail: Pryanichnikov@medknc.ru

Introduction. The Geneva Conference of 1964 defined the territories lying north of 66°33 north latitude to be designated by the term "high latitudes" [1].Living here is determined by the influence of a number of external factors, among which the cold factor, contrast photoperiodics, heliogeomagnetic effects, color

deprivation, the peculiarity of the diet, etc. stand out. Currently, it is considered that the effects on the human body of non-runaway climatic stimuli are integrative in nature [2-4]. The process of adaptation to living conditions in "high latitudes" has certain features, including hemodynamic, vasomotor, psychoemotional and others [5-7]. It is important to note that adaptation processes are not only physiological in nature, but also manifest themselves through psychological and social features. "Severeral climatic and geophysical conditions of the northern latitudes cause the development of northern stress in humans ("polar stress syndrome")"[8]. One of the manifestations of this syndrome is free-floating anxiety of varying severity, as well as physiological deviations of regulatory systems, manifested by the dominance of sympathicotonic vegetative reactions. With prolonged exposure, the polar stress syndrome can lead to the development of various psychosomatic forms of pathology.

Understanding the impact of all of the above factors and, in particular, establishing a link between the state of regulatory systems and stress markers contributes to solving the issues of human adaptation to unchangeable environmental factors. The study of regional peculiarities and mechanisms of the formation of the health of children and adolescents living in various territories of the Russian Federation is of great importance for the preservation of the health of the younger generation [9, 10].

Methodology. Comparative results of a cross-sectional study of the psychophysiological state (PPHS) of 1st-year students of the Kola Medical College (KMC) in Apatity, Murmansk region in the number of 65 people (girls, average age 18.67±3.75 years) are presented. The study was conducted in the autumn period (october), before the session, after school, in the evening. At the time of the study, the selection criterion was the absence of complaints and diseases in the acute stage of the course. Anonymous questioning allowed to obtain information about the phase of the ovarian-menstrual cycle (OMC) and differentiate the subjects into groups. The participants were familiarized with the purpose, conditions and methods of the study with the provision of written consent to the processing of personal data. The possibility of conducting the study was confirmed by the local ethics committee, Protocol No. 11 of 19.12.2016.

The psychophysiological state was assessed by the following methods: C. D. Spielberger – Y. L. Khanin, situational (SA) and personal (PA) anxiety, at the

Table 1

Indicators of HRV, well-being, activity, mood, vitamin D and cortisol levels at low and medium levels of anxiety

	Situational anxiety		p-уров.	Personal anxiety		p-уров.
Level	<=30 (n=9)	31-44 (n=37)		<=30 (n=9)	31-44 (n=37)	
HR (b/pm)	70.22±1.92	70.83±1.86	0.977	59.1±4.45	72.62±2.12	0.052
RRNN (ms)	858.39±23.86	864.49±22.26	0.977	1032.17±85.48*	847.24±24.15*	0.045*
R-R min (ms)	664.67±21.3	696.39±17.11	0.383	699.83±20.74	688.81±17.74	0.715
R-R max (ms)	1061.39±47.44	1043.59±33.42	0.718	1269±65.9*	1026.5±38.96*	0.038*
SI	107.78±39	119.87±22.69	0.657	22.3±1.37	138.47±28.04	0.059
RMSSD (ms)	82.29±18.96	78.48±10.87	0.579	146.17±35.73*	69.29±10.5*	0.038*
pNN50 (%)	41.52±9.77	37.48±4.28	0.677	74±4.5*	32.68±4.55*	0.012*
SDNN (ms)	75.36±13.48	69.45±7.09	0.618	113±20.04	65.27±7.25	0.068
CV (%)	8.6±1.36	7.81±0.69	0.454	11.3±2.47	7.42±0.66	0.114
AMo (%)	33.58±5.49	37.27±2.58	0.390	24.1±3.88	38.78±2.89	0.078
TP (ms^2)	5055.06±1300.33	4557.48±697.15	0.781	7884.17±1255.53	4613.56±811.69	0.145
HF (ms^2)	2155.78±770.55	2446.13±514.72	0.955	4502.5±1434.43	2415.58±600.83	0.089
LF (ms^2)	1384.22±452.61	1096.54±158.52	0.677	1485.5±425.81	1193.77±211.32	0.331
VLF (ms^2)	1514.94±571.7	1014.67±157.19	0.824	1896±1211.18	1004.13±157.28	0.395
%HF	42.52±7.67	43.95±3.45	0.739	62.23±18.26	40.33±3.59	0.224
%LF	28.01±5.22	26.95±1.54	0.824	16.23±5.52	29.08±1.75	0.052
%VLF	29.53±7.18	29.13±2.67	0.835	21.6±12.99	30.63±2.74	0.362
LF/HF	0.92±0.21	1.06±0.18	0.657	0.4±0.25	1.22±0.2	0.114
Index of vegetative equilibrium	132.43±45.92	165.25±25.68	0.406	45.23±8.05	177.51±29.64	0.078
Vegetative rhythm indicator	2.89±0.99	3.59±0.51	0.438	1.9±0.2	4.18±0.58	0.202
An indicator of the adequacy of regulatory processes	41.5±7.31	46.08±4.09	0.406	24.17±0.97	48.73±4.79	0.073
Well-being	5.76±0.26*	4.77±0.17*	0.016*	6±0.64	5.02±0.19	0.181
Activity	5.09±0.32	4.41±0.13	0.056	5.47±0.47	4.57±0.17	0.162
Mood	6.37±0.13*	5.42±0.16*	0.007*	6.33±0.18	5.65±0.16	0.171
Vitamin D	17.34±3.78*	9.32±1.49*	0.037*	22.27±5.38	9.9±1.6	0.067
Cortisol	535.03±23.8	569.43±13.86	0.689	524.81±25.46	565.33±27.72	0.942

*– significantly significant differences.

level of 30 and less points – low, 31-45 – average, 46 and more – high [11];, at the level of 30 and less points – low, 30-50 – average, 50 and more – high indicators [12], the "Individual minute" test according to the Halberg method (1969) with indicators of 30 seconds. and less – mental instability, 30-40 – high anxiety, 40-55 – mild anxiety and 55-65 – optimal condition.

The physiological state of the body was assessed by indicators of heart rate variability (HRV). The conditions for the registration of HRV indicators were carried out in standard leads, in a lying position, at rest, for 5 minutes using the Omega-M hardware diagnostic medical complex (research and production company «Dynamics», St. Petersburg) in accordance with the standards adopted in 1996. standards of measurement, physiological interpretation and clinical use of HRV indicators [13].HRV assessment included the following data: time indicators of the cardiorhythmogram: "R–R (ms) is the average interval, SDNN (ms) is the standard deviation of NN intervals, RMSSD (ms) is the square root of the sum of the squares of the difference in the values of consecutive pairs of intervals NN, <...> the voltage index of regulatory systems (Si, u. e.) – the state of the central regulation circuit; <...> spectral analysis by nonparametric fast Fourier transform method: high–frequency range (HF, ms^2)–0.4–0.15 Hz, low–frequency range (LF, ms^2)–0.15–0.04 Hz, very low-frequency range (VLF, ms^2) - 0.04-0.003 Hz and total power of the spectrum (TP, ms^2)" [14]; analysis of the structure of wave power contributions (HF,%, LF,%, VLF,%), LF/HF balance – the ratio of sympathetic, parasympathetic effects and secondary indicators of variational pulsometry according to RM. To Bayevsky: index of vegetative equilibrium, vegetative indicator of rhythm, indicator of the adequacy of regulatory processes [14].

To assess the functional state of the hypothalamic-pituitary-adrenal system (HPAS), quantitative determination of the concentration of cortisol in human blood serum by solid-phase enzyme immunoassay using a set of reagents "Steroid ELISA-cortisol" ("Alkor–bio", St. Petersburg) was used. A range of reference values from the sets was used as normative indicators.

To analyze the prevalence of vitamin D deficiency, an enzyme immunoassay for

Table 2

Indicators of HRV. well-being. activity. mood. vitamin D and cortisol levels at low and high levels of anxiety

	Situational anxiety		p- level	Personal anxiety		p- level
Level	<=30 (n=9)	>45 (n=19)		<=30 (n=9)	>45 (n=19)	
HR (b/pm)	70.22±1.92	78.29±2.44	0.052	59.1±4.45*	74.79±1.75*	0.007*
RRNN (ms)	858.39±23.86	799.44±23.67	0.140	1032.17±85.48	822.17±16.6	0.011*
R-R min (ms)	664.67±21.3	677.79±17.45	0.921	699.83±20.74	681.79±16.55	0.638
R-R max (ms)	1061.39±47.44	955.53±33.37	0.115	1269±65.9*	989.76±23.8*	0.009*
SI	107.78±39	150.48±29.94	0.192	22.3±1.37*	128.34±19.89*	0.011*
RMSSD (ms)	82.29±18.96	50.96±6.88	0.140	146.17±35.73*	65.27±9.71*	0.026*
pNN50 (%)	41.52±9.77	27.65±5.27	0.237	74±4.5*	33.29±4.55*	0.013*
SDNN (ms)	75.36±13.48	52.17±5.67	0.153	113±20.04*	60.71±6.51*	0.026*
CV (%)	8.6±1.36	6.39±0.57	0.184	11.3±2.47	7.25±0.69	0.097
AMo (%)	33.58±5.49	39.98±3.68	0.218	24.1±3.88	37.99±2.9	0.141
TP (ms^2)	5055.06±1300.33	2919.91±635.34	0.218	7884.17±1255.53*	3353.92±542.84*	0.048*
HF (ms^2)	2155.78±770.55	1210.01±328.26	0.279	4502.5±1434.43*	1463.35±281.92*	0.026*
LF (ms^2)	1384.22±452.61	874.65±215.91	0.301	1485.5±425.81	895.89±156.2	0.124
VLF (ms^2)	1514.94±571.7	835.04±167.45	0.622	1896±1211.18	994.47±198.73	0.363
%HF	42.52±7.67	36.17±3.79	0.605	62.23±18.26	41.1±3.43	0.178
%LF	28.01±5.22	30.41±2.35	0.712	16.23±5.52	27.33±1.8	0.085
%VLF	29.53±7.18	33.48±3.83	0.431	21.6±12.99	31.61±3.39	0.273
LF/HF	0.92±0.21	1.24±0.27	0.749	0.4±0.25	1±0.18	0.149
Index of vegetative equilibrium	132.43±45.92	195.33±32.68	0.184	45.23±8.05*	176.1±25.12*	0.026*
Vegetative rhythm indicator	2.89±0.99	4.14±0.8	0.506	1.9±0.2	3.41±0.59	0.638
An indicator of the adequacy of regulatory processes	41.5±7.31	53.32±5.86	0.375	24.17±0.97*	49.27±4.22*	0.022*
Well-being	5.76±0.26*	3.81±0.24*	0.0003*	6±0.64*	4.06±0.18*	0.033*
Activity	5.09±0.32*	3.71±0.17*	0.001*	5.47±0.47*	3.89±0.13*	0.009*
Mood	6.37±0.13*	4.27±0.27	0.0001*	6.33±0.18*	4.64±0.23*	0.03*
Vitamin D	17.34±3.78	9±1.86	0.057	22.27±5.38*	9.2±1.64*	0.049*
Cortisol	519.73±34.42	569.43±13.86	0.805	543.5±25.3	565.33±27.72	0.933

*– significantly significant differences.

the quantitative determination of 25-OH vitamin D in serum and plasma "25-OH Vitamin D (total) ELISA" ("DRG Instruments", Marburg, Germany) was used.

Statistical processing of the results was performed using the software package "Microsoft Excel 2007" (Microsoft company), software "Statistica 10.0" (TIBCO company) and is represented by the arithmetic mean (M), standard error (± m). The difference indicators were calculated using the Mann-Whitney U–test and were considered statistically significant at the $p<0.05$ level.

Results and discussion. A comparative analysis of the indicators of the psychophysiological state of the body of students was carried out by ranking the results of the study by the level of anxiety and OMC using an anonymous questionnaire, selected techniques and cardiohemodynamics (HRV) data. To do this, the students were divided into groups: low SA and PA (group 1), medium SA and PA (group 2) and high SA and PA (group 3) and mixed type (low SA and high PA; high SA and low PA), as well as by FMC: group 1–follicular phase (FF), group 2 –luteal phase (LF). Since no significant differences were found in the groups with mixed SA and PA indicators, they were excluded for further comparative analysis.

A posteriori comparisons of intergroup indicators of HRV and psycho-emotional state showed the following results. Significant differences between groups 1 and 2 in the SA category are noted only by the WAM method, in the categories of well-being ($U = 79.5$, $p = 0.016$) and mood ($U = 69.5$, $p = 0.007$). This suggests that in group 1 with low SA indicators, subjective feelings of well-being and mood, which is characterized by unaccountability and low severity, the comfort of the physiological and psychological state was assessed higher than in group 2 [15]. Also, significant differences between these groups were found in the level of vitamin D ($U = 49.0$, $p = 0.037$), higher data were noted in group 1. This suggests that in group 2, the subjects have a predisposition to a potentially significant risk factor for the development of diseases associated with a reduced concentration of vitamin D in the blood [16-21]. Statistical analysis of HRV indicators, when compared in the

Table 3

Indicators of HRV, well-being, activity, mood, vitamin D and cortisol levels at medium and high levels of anxiety

	Situational anxiety		p-уров.	Personal anxiety		p-уров.
Level	31-44 (n=37)	>45 (n=19)		31-44 (n=37)	>45 (n=19)	
HR (b/pm)	70,83±1,86*	78,29±2,44*	0,02*	72,62±2,12	74,79±1,75	0,363
RRNN (ms)	864,49±22,26	799,44±23,67	0,079	847,24±24,15	822,17±16,6	0,506
R-R min (ms)	696,39±17,11	677,79±17,45	0,359	688,81±17,74	681,79±16,55	1,000
R-R max (ms)	1043,59±33,42	955,53±33,37	0,103	1026,5±38,96	989,76±23,8	0,568
SI	119,87±22,69	150,48±29,94	0,249	138,47±28,04	128,34±19,89	0,563
RMSSD (ms)	78,48±10,87	50,96±6,88	0,166	69,29±10,5	65,27±9,71	0,953
pNN50 (%)	37,48±4,28	27,65±5,27	0,194	32,68±4,55	33,29±4,55	0,953
SDNN (ms)	69,45±7,09	52,17±5,67	0,179	65,27±7,25	60,71±6,51	0,723
CV (%)	7,81±0,69	6,39±0,57	0,311	7,42±0,66	7,25±0,69	0,817
AMo (%)	37,27±2,58	39,98±3,68	0,597	38,78±2,89	37,99±2,9	0,670
TP (ms^2)	4557,48±697,15	2919,91±635,34	0,161	4613,56±811,69	3353,92±542,84	0,493
HF (ms^2)	2446,13±514,72	1210,01±328,26	0,150	2415,58±600,83	1463,35±281,92	0,659
LF (ms^2)	1096,54±158,52	874,65±215,91	0,291	1193,77±211,32	895,89±156,2	0,502
VLF (ms^2)	1014,67±157,19	835,04±167,45	0,533	1004,13±157,28	994,47±198,73	0,598
%HF	43,95±3,45	36,17±3,79	0,158	40,33±3,59	41,1±3,43	0,761
%LF	26,95±1,54	30,41±2,35	0,291	29,08±1,75	27,33±1,8	0,323
%VLF	29,13±2,67	33,48±3,83	0,341	30,63±2,74	31,61±3,39	1,000
LF/HF	1,06±0,18	1,24±0,27	0,203	1,22±0,2	1±0,18	0,497
Index of vegetative equilibrium	165,25±25,68	195,33±32,68	0,283	177,51±29,64	176,1±25,12	0,723
Vegetative rhythm indicator	3,59±0,51	4,14±0,8	0,659	4,18±0,58	3,41±0,59	0,220
An indicator of the adequacy of regulatory processes	46,08±4,09	53,32±5,86	0,279	48,73±4,79	49,27±4,22	0,795
Well-being	4,77±0,17*	3,81±0,24*	0,002*	5,02±0,19*	4,06±0,18*	0,0003*
Activity	4,41±0,13*	3,71±0,17*	0,002*	4,57±0,17*	3,89±0,13*	0,002*
Mood	5,42±0,16*	4,27±0,27*	0,0007*	5,65±0,16*	4,64±0,23*	0,001*
Vitamin D	9,32±1,49	9±1,86	0,611	9,9±1,6	9,2±1,64	0,641
Cortisol	519,73±34,42	535,03±23,8	0,673	524,81±25,46	543,5±25,3	0,534

*Статистически значимые различия.

PA category, by changes in the duration of consecutive intervals showed significant differences in the following temporal characteristics of heart rate: RRNN (ms) (U = 13.0, p = 0.045), R-R max (ms) (U = 12.0, p = 0.038), RMSSD (ms) (U = 12.0, p = 0.038) and pNN50 (%) (U = 5.0, p = 0.012), where higher rates were noted in group 1 (see Table 1). This suggests that in group 1, the modulation of the heart rate is determined by high-frequency fluctuations of the heart rhythm with the predominant influence of the parasympathetic division of the autonomic nervous system (ANS) [22].

When comparing the indicators between groups 1 and 3, significant differences in the SA category are noted in the WAM test: well-being (U=11.5, p=0.0003), activity (U=20.0, p=0.001), mood (U=6.5, p=0.0001), where the indicators are higher in group 1. This suggests that in the group with low SA, the qualitative characteristic of the subjective state is indicated to be more comfortable both psychologically and physiologically. Statistical analysis of HRV data, when compared in the PA category, by changes in the duration of consecutive intervals showed significant differences in the following heart rate characteristics (by time indicators): HR (beats/min) (U=2.00, p=0.007) with the reverse sign, RRNN (ms) (U=4.00, p=0.001), R-R max (ms) (U=3.00, p=0.009), SI (U=4.00,p=0.001), RMSSD (ms) (U=9.00,p=0.026), pNN50 (%) (U=5.00, p=0.013), SDNN (ms) (U=9.00, p=0.026); (by frequency characteristics): TR (ms2) (U=13.00, p=0.048), HF (ms2) (U=9.00, p=0.026); (according to secondary indicators of variational heart rate monitoring): Hebrew (U=9.00, p=0.026) and PAPR (U=8.00, p=0.022) with the opposite sign. HRV data show that in group 1, the temporal characteristics of the heart rate are mainly due to the influence of the parasympathetic department of the ANS, which in turn indicates a more energy-efficient way of heart rate modulation. The voltage index of regulatory systems is more pronounced in group 2, which indicates the predominant activity of sympathetic regulation mechanisms and a more energy-consuming behavior of the state of the central circuit [22]. Frequency analysis of HRV showed that the data of the total power spectrum (TP) and high-frequency oscillations (HF) of the wave structure of heart rate variability predominate in group 1, which suggests that the total neurohumoral activity is influenced by the activation of vagal heart rate control with a predominant predominance of the parasympathetic department of the ANS. Secondary indicators of variational heart rate monitoring are more pronounced in the group with high PA (group 2), which also indicates the modulating effect of the sympathetic department of the ANS on heart rate indicators [22]. In the PA category, during the intergroup comparison, significant differences are also shown in the results according to the WAM method: well-being (U=10.5, p=0.033), activity (U=3.00, p=0.009), mood (U=10.00, p=0.030). These data show that the subjective assessment of the functional state in group 1 is higher than in group 3, which indicates a more comfortable state in the group with low LT indicators. Significant differences between these groups were also found in serum vitamin D levels (U=9.00, p=0.049), higher data were also found in group 1 (see Table 2). Low vitamin D levels in the group with high PA indicate short-term or long-term possibilities for the development of infectious diseases, diseases with metabolic disorders and others [23-25].

Analysis of HRV data in comparison between groups 2 and 3 showed significant differences in HR rhythm characteristics (beats/min.) (U=217.00, p=0.020). Although the heart rate values in the studied groups are in the zone of reference values for their age period, nevertheless, in group 3, the indicators are close to the upper limit of the norm. Significant differences in the WAM technique are also shown, both in the SA category: well-being (U=179.50, p=0.002), activity (U=177.50, p=0.002), mood (U=157.00, p=0.0007) and in the PA category: well-being (U=217.00, p=0.0003), activity (U=258.50, p=0.002), mood (U=240.00, p=0.001) (see Table 3). This indicates that in group 2, the subjective assessment of the functional state exceeds the indicators of group 3 in both SA and PA and corresponds to a more comfortable psychological and physiological state of the subjects.

The state of the HPAS, depending on the level of anxiety, was assessed by the concentration of cortisol in the blood. The average cortisol level in the study groups was (535.25±16.9 nmol/L). Cortisol indices did not exceed the reference values for SA in group 1 (519.73±34.42nmol/L), group 2 (535.03±23.8 nmol/L) and in group 3 (569.43±13.86 nmol/L), for LT in group 1 (543.5±25.3 nmol/L), group 2 (524.81±25.46 nmol/L) and in group 3 (565.33±27.72 nmol/L). No significant differences in the background concentration of the hormone were found. In an intergroup comparison, depending on the stage of the ovarian-menstrual cycle (OMC), significant differences were revealed. In the group of subjects in the follicular phase of OMC, the cortisol level is significantly higher (589.30±6.77 nmol/L) than in the group of girls in the luteal phase of OMC (369.61±34.31 nmol/L), which is confirmed by literature data [26-30]. Thus, at the time of the study, the level of cortisol is reflected by the HPAS only depending on the stage of OMC.

The prevalence of vitamin D deficiency, depending on the level of anxiety, was determined by a quantitative determination test in serum and plasma. The average value of vitamin D in the study groups was 10.33±1.18ng/ml. The indicators are significantly lower than the reference values, which indicates a significant deficiency of vitamin D in all the study groups. At the same time, in group 1, the vitamin D index was (17.34±3.78ng/ml), in group 2 (9.32±1.49ng/ml) and in group 3 (9±1.86ng/ml), respectively. Significant differences in the concentration of vitamin D in the blood were found between group 1 and 3. This suggests that vitamin D deficiency may indirectly affect the level of situational and personal anxiety [31,32]. During the intergroup comparison, depending on the stage of the ovarian-menstrual cycle (OMC), significant differences were also revealed. In the group of subjects in the follicular phase of OMC, the vitamin D level is significantly higher (11.47±1.35 ng/ml) than in the group of girls in the luteal phase of OMC (5.48±1.74 nmol/L), which corresponds to ambiguous literature data and requires further study [33-35].

Conclusions. A differentiated analysis of the psychophysiological state of the body of students in the "high latitudes" revealed a number of features characteristic of certain levels of anxiety. According to SA indicators, the subjective assessment of well-being, activity and mood varies depending on the level of anxiety. With an increase in SA, the emotional background, psychological comfort, the volume of interaction with the physical and social environment decreases. No changes were found at the level of regulation of the cardiovascular system. According to PA indicators, changes were found at the level of functioning of the cardiovascular system, manifested in changes in the mechanisms of regulation of heart rhythm. With an increase in the level of anxiety, the temporal and frequency characteristics of HRV change, the influence of the mechanisms of the sympathetic nervous system in the modulation of the heart rhythm and the energy-consuming state of the central regulation circuit prevail. The level of SA and PA does not have a significant impact on the

performance of the HPAS, while it is reflected in the stages of the ovarian-menstrual cycle. A significant deficiency of vitamin D is present in all study groups and requires correction in accordance with individual and practical recommendations of medical specialists.

Ethical standards. All studies were conducted in accordance with the principles of biomedical ethics formulated in the Helsinki Declaration of 1964 and its subsequent updates, and approved by the local Ethics Committee at the Research Center for Biomedical Problems of Human Adaptation in the Arctic - a branch of the Federal State Budgetary Institution of Science of the Federal Research Center "Kola Scientific Center of the Russian Academy of Sciences" (Apatity).

Informed consent. Each participant of the study submitted a voluntary written informed consent signed by him after explaining to him the potential risks and benefits, as well as the nature of the upcoming study.

Financing of work. The work was carried out in accordance with the topic of research No. 122022200516-5 "Studying the features of the territorial morbidity of the population of reproductive age in the Arctic zone of the Russian Federation with the identification of factors affecting the main functional systems of the body and the development of complex methods to reduce the negative impact of extreme environmental conditions"

Conflict of interests. The author declares the absence of obvious and potential conflicts of interest associated with the publication of this article.

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N.A. Danilov, A.L. Sukhomyasova, M.E. Nikiforova, Ya.A. Munkhalova, T.M. Klimova, V.G. Chasnyk, T.E. Burtseva

EPIDEMIOLOGY OF CONGENITAL DYSFUNCTION OF THE ADRENAL CORTEX IN THE REPUBLIC OF SAKHA (YAKUTIA)

DOI 10.25789/YMJ.2023.82.16

УДК 159.91, 612.172.2

In the article the results of the long-term monitoring of the incidence of new cases and prevalence of congenital adrenal cortical hyperplasia in children of the Republic of Sakha (Yakutia) are presented. The Republic of Sakha (Yakutia) is one of those model regions where epidemiological studies of the incidence of hereditary diseases can be carried out in view of the relative constancy of the population and low migration. The results of the neonatal screening and the register of the endocrinology department of the Pediatric Center of the Republican Hospital No.1-NCM showed an increased incidence and prevalence of congenital hyperplasia of the adrenal cortex in children of the Republic of Sakha (Yakutia). Epidemiological studies should become the basis for conducting in-depth molecular genetic studies in view of the high prevalence of severe forms of congenital hyperplasia of the adrenal cortex in children of the Republic of Sakha (Yakutia).

Keywords: Congenital hyperplasia of the adrenal cortex, adrenogenital syndrome, congenital adrenal hyperplasia, Yakutia, epidemiology, frequency, prevalence.

Introduction. Congenital adrenal hyperplasia (CAH) (adrenogenital syndrome, congenital adrenal hyperplasia) is a group of diseases with autosomal recessive type of inheritance, caused by mutations in the genes encoding enzymes involved in cortisol biosynthesis. Depending on the type and severity of the enzyme deficiency, this can lead to impaired biosynthesis of glucocorticoids, mineralocorticoids and sex hormone production [1, 2, 5, 6]. The most common disorder is 21-hydroxylase deficiency, which occurs in up to 95% of cases as a result of mutations or deletions in the CYP21A2 gene. Enzyme deficiency leads to impaired production of cortisol, aldosterone, and an excess of androgens [5, 7].

Congenital adrenal hyperplasia is one of the most common autosomal recessive diseases. Active introduction of neonatal screening has revealed the frequency of congenital adrenal cortical hyperplasia in various populations [1,2]. According to findings of Pang S. Y. et al., the incidence of the classic form of CAH due to 21-hydroxylase deficiency was 1 case per 14199 live births for homozygous subjects and 1 in 60 for heterozygous subjects, respectively, between 1980 and 1988 [8].

A systematic review including results from 58 studies from 31 countries (for the period 1969-2017) showed an average incidence of CAH of 1:9498 (95% confidence interval: 1:9089- 1:9945). The highest incidence was detected in Eastern Mediterranean and Southeast Asian countries; the lowest incidence was registered in Asia-Pacific countries [7]. According to the results of neonatal screening in the Russian Federation, the incidence of classical forms of 21-hydroxylase deficiency is 1 case per 9638 live births [1]. The incidence is highest in the Ural Federal District (1:6749) and lowest in the Northwestern Federal District (1:14876) [1].

Timely diagnosis and prescription of adequate treatment of CAH are urgent tasks of modern endocrinology and pediatrics. Despite a number of profound scientific and clinical achievements in recent years, the data on the frequency, prevalence and molecular genetic features of the disease in different populations require updating.

The aim of the study is to estimate the incidence of new cases and prevalence of congenital adrenal cortical hyperplasia in children in the Republic of Sakha (Yakutia).

Materials and Methods. The data from neonatal screening in the period 2006-2020 were analyzed to estimate the incidence of new cases of congenital adrenal hyperplasia in the Republic of Sakha (Yakutia). To estimate the prevalence of CAH, a registry of children with congenital hyperplasia of the adrenal cortex was compiled according to the endocrinology department of the Pediatric Center of the State Budgetary Institution of the Republic of Sakha (Yakutia), the Republican Hospital No.1-NCM, as the head institution for diagnosing this disease.

The study protocol was approved by the Ethical Committee of the Federal State Budgetary Institution Yakutian Re-

DANILOV Nikolay Andreevich – endocrinologist, Pediatric Gastroenterology and Endocrinology Department, Pediatric Center, Republican Hospital No.1-NCM, postgraduate student of the Medical Institute M.K. Ammosov North Eastern Federal University; **SUKHOMYSOVA Aitalina Lukichna** – PhD, Head of the Medical Genetic Center of State Budgetary Institution of the Republic of Sakha (Yakutia), Republican Hospital No.1-NCM, **NIKIFOROVA Margarita Egorovna** – Head of the Department of Pediatric Gastroenterology and Endocrinology, Pediatric Center of the Republican Hospital No.1-NCM; **MUNKHALOVA Yana Afanasyevna** – PhD, Head of the Department of Pediatrics and Pediatric Surgery, MI NEFU; **KLIMOVA Tatiana Mikhaylovna** - PhD, associate professor, Department of Pharmacology and Pharmacy, MI NEFU, Associate Professor, YSC CMP, e-mail: biomedykt@mail.ru; **CHASNYK Vyacheslav Grigorievich** – Doctor of Medical Sciences, Professor, Head of the Department of Hospital Pediatrics, Federal State Budget Educational Institution of Higher Education Saint Petersburg State Pediatric Medical University; **BURTSEVA Tatiana Egorovna** – MD, Professor, Department of Pediatrics and Pediatric Surgery, MI NEFU, head of the laboratory, Yakut Science Centre of Complex Medical Problems, e-mail:bourtsevat@yandex.ru

Table 1

The results of the neonatal screening of newborns for adrenogenital syndrome in the Republic of Sakha (Yakutia) in 2006-2020

Year	The number of newborns in the Republic of Sakha (Yakutia) according to the Yakutian Republican Medical Information and Analytical Center	Examined for adrenogenital syndrome	Coverage. %	The number of ill ones	Frequency (1 case per total number of the surveyed ones)
2006	13623	5559	40.8	0	0
2007	15152	14931	98.5	1	14931
2008	15254	10746	70.4	2	5373
2009	15783	15468	98.0	0	0
2010	15877	15662	98.6	0	0
2006-2010	75689	62366	82.4	3	20788.7
2011	16173	16092	99.5	1	16092
2012	16922	16832	99.5	4	4208
2013	16611	16546	99.6	1	16546
2014	16964	16946	99.9	2	8473
2015	16469	16459	99.9	0	0
2011-2015	83139	82875	99.7	8	10359.4
2016	15418	15385	99.8	0	0
2017	13710	13693	99.9	0	0
2018	13472	13456	99.9	1	13456
2019	12713	7720	60.7	1	7720
2020	13034	6465	49.6	1	6465
2016-2020	68347	56719	83.0	3	18906.3
2006-2020	**227175**	**201960**	**88.9**	**14**	**14425.7**

search Center of Complex Medical Problems (Report No.52 dated January 28, 2021, Resolution No.1).

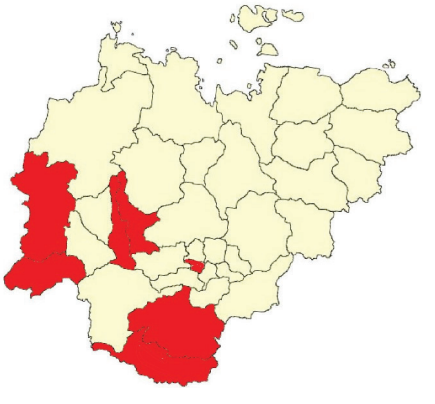
Results and Discussion. Neonatal screening for adrenogenital syndrome in the Republic of Sakha (Yakutia) has been performed since 2006 at the Medical Genetics Center of the Republic of Sakha (Yakutia), the Republican Hospital №1-NCM (head, candidate of medical sciences Sukhomyasova A.L.). A total of 201960 newborns were examined in 2006-2020 (Table 1). During this period 14 children with adrenogenital syndrome were identified. Thus, the incidence of adrenogenital syndrome for 2006-2020 in the population of newborns of the Republic of Sakha (Yakutia) according to neonatal screening was 1 case per14426 studies (14:201960). When divided into 5-year periods, it was found that the frequency of CAH detection was maximal in 2011-2015. For the most recent time interval (2016-2020), the incidence of new cases was 1 case per 18906 neonates examined. Thus, according to neonatal screening, an increase in the frequency of congenital hyperplasia of the adrenal cortex in newborns in the Republic of Sakha (Yakutia) has been observed over the 15-year period.

To assess the dynamics of CAH prevalence, a comparative analysis of the data for 2007 and 2020 (Table 2) according to the register of children with congenital adrenal hyperplasia of the endocrinology department of the Pediatric Center of the State Budgetary Institution of the Republic of Sakha (Yakutia) "Republican Hospital No.1-NCM" was performed. In 2007 there were 11 children with CAH under observation at the endocrinology department of the State Budgetary Institution of the Republic of Sakha (Yakutia), the Republican Hospital No.1-NCM, with a rate of 5.3 per 100,000 of the total child population of the republic. At the end of 2020 there were 21 children with congenital dysfunction of the adrenal cortex (7.9 per 100,000 of the child population). The increase in the prevalence of CAH also indicates an improvement in the results of treatment of the disease.

Table 2

Prevalence of classical CAH in children of the Republic of Sakha (Yakutia) according to the data of the registry

Registry		
Год	Number	Per 100 000 infant population
2007	11 cases per 207405 child population	5.3
2020	21 cases per 264141 child population	7.9



Distribution of patients with CAH by administrative districts of the Republic of Sakha (Yakutia).

Thus, the incidence of congenital dysfunction of the adrenal cortex in the Republic of Sakha (Yakutia) is comparable with the world data [8]. At the same time, studies conducted earlier in the republic have shown that the incidence of mutations associated with the disease in the child population is rather wide and varies in individual ethnic groups [3,4].

As of 2022, there were 23 children with a confirmed diagnosis of congen-

ital adrenal cortical hyperplasia in the Republic of Sakha (Yakutia). Picture 1 shows the areas of residence of patients with the disease.

Of the total number of patients, there are fourteen in Yakutsk and Zhatai, three in Neryungri District, two in Verkhnevilyuisky District, two in Aikhal and Udachny, Mirny District, and one each in the cities of Lensk and Aldan. The ethnic composition of patients with CAH is as follows: 10 children were Sakha, there were 12 Russian and one was of Evenk ethnicity. Among them there are 13 girls and 10 boys. It should be noted that there are 2 orphans among the total number of patients with CAH, half-siblings, from different fathers, living in the City Baby Home of Yakutsk.

Among the children enrolled in the registry of patients with CAH, 95.5% (or 21 in absolute numbers) of children have the solteric form of the disease. Their age ranges from 4 months to 16 years. Ethnic composition of patients with the solterian form is as follows: 11 Russians, 9 Sakha, and 1 Evenk. Gender structure - 11 girls, 10 boys.

Conclusion. The incidence of CAH among children in the Republic of Sakha (Yakutia) according to neonatal screening for the period 2006-2020 is 1 case per 14,426 newborns. Since 2006 the incidence of hereditary pathology has tended upwards (from 1 case per 20789 children in 2006-2010 to 18906 in 2016-2020 respectively). The prevalence of CAH according to the register at the beginning of 2021 was 7.9 cases per 100,000 children. In dynamics, the prevalence of CAH tends to increase, which reflects the accumulation of cases against the background of increasing frequency and improving treatment of the disease. The most common form of CAH among children in the Republic of Sakha (Yakutia) is the solenteric form, the most severe in its course. This fact indicates a high prevalence of severe mutations of groups 0 and A, leading to complete cessation of 21-hydroxylase activity. It is necessary to further investigate the frequency of mutations leading to the deficiency of enzymes involved in cortisol biosynthesis in Yakutia population.

The research was executed within the research theme "Physical development and health of the child population in the Far North (with the example of Yakutia)" (state registration number: 1021062411641-9-3.2.3), within the state assignment of Ministry of Science and Education of the Russian Federation (FSRG-2023-0003, FSRG-2020-0014).

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TOPICAL ISSUE

DOI 10.25789/YMJ.2023.82.17

УДК 616.8

E.E. Konnikova, A.I. Borisov, A.A. Tappakhov, T.Ya. Nikolaeva

COGNITIVE IMPAIRMENT AFTER COVID-19 IN YOUNG PEOPLE

Cognitive impairment is one of the frequent neurological manifestations of post-COVID syndrome. **The aim** of this study was to assess cognitive impairment in young people after a mild novel coronavirus infection (COVID-19).

Materials and methods. The main group included 50 people with mild COVID-19 at the age of 19–35 years, incl. 17 (34%) men and 33 (66%) women. The control group included 50 people without a history of COVID-19, aged 18 to 33 years. All participants underwent neuropsychological testing: the Beck depression scale, the Spielberg questionnaire for identifying personal and situational anxiety, the subjective asthenia assessment scale (MFI-20), the 12-picture memory test, the 5-word memory test, the study of phonetic speech activity, and the Schulte test.

Research results. Study participants who had COVID-19 during the acute phase of the disease complained of general weakness (90%), headaches (86%), mental exhaustion (72%), muscle and joint pain (66%), decreased attention (64%), decreased sense of smell (62%), sleep disturbance (60%), apathy (54%), shortness of breath (34%) and chest pressure (26%). All complaints regressed after recovery, and their frequency did not differ from those of complaints in the control group. Neuropsychological examination revealed a somewhat higher level of depression (10.5 vs. 6.5 points on the Beck scale), some decrease in visual memory (11 vs. 11.5 pictures) and a higher incidence of general asthenia (74% vs. 44%) in patients COVID-19 ($p \leq 0.05$). There was no correlation between the severity of cognitive impairment and the duration of COVID-19.

Conclusions. COVID-19 is mildly accompanied by the development of mild cognitive impairment in young patients.

Keywords: cognitive impairment, COVID-19, depression, anxiety, asthenia.

Introduction. The outbreak of a new coronavirus infection began in December 2019 in Wuhan, Hubei Province (China), and on February 11, 2020, the World Health Organization recognized the outbreak of a new coronavirus disease as a pandemic and assigned the official name of the infection COVID-19 [2]. In Moscow, the first case of COVID-19 was registered on March 2, 2020, and on March 18, 2020, the first patient was detected in Yakutsk. [1]

Scientific observations have shown that in 70–76% of patients after infection, long-term consequences are possible with damage to one or more organs [11, 12]. In addition, with COVID-19, the central nervous system is affected more often than with other respiratory infections [3]. Chronic hypoxia, a pathological immune response, the direct damaging effect of the virus and the neurotropism of immune complexes, endothelial dysfunction of cerebral vessels, the state of the intestinal microbiota, and complications of drug therapy are considered as probable factors in the pathogenesis of neurological post-COVID syndrome [3, 5]. A wide range of neurological manifestations of coronavirus infection has been described in the form of asthenia, headaches, anosmia, insomnia, cognitive impairment, affective disorders, depression, and increased anxiety, which manifest themselves in 34% of people who have had COVID-19 [4, 8, 9]. Long-term monitoring of the health status of 1,733 patients in China by Huang C. et al. showed that weakness (63%), sleep disorders (26%), anxiety and depression (23%) are more common in post-COVID syndrome [14]. One of the most frequent complaints of patients after COVID-19 is a condition described by patients as "brain fog" and detected in 85.1% of cases [10]. At the same time, most of the studies were carried out mainly among people aged 40–65 years who had the disease in the acute stage in moderate and severe forms.

Currently, there are very few studies aimed at studying cognitive impairment after a new coronavirus infection among young people.

The aim of this study was to assess cognitive impairment in young people after a mild novel coronavirus infection (COVID-19).

Materials and research methods. A one-time study was conducted on the basis of the Department of Neurology and Psychiatry of the Medical Institute and the Laboratory of Neuropsychophysiological Research of the M.K. Ammosov North-Eastern Federal University. All participants signed a voluntary informed consent for inclusion in the study.

Inclusion criteria: 1) age of patients from 18 to 35 years; 2) a mild novel coronavirus infection (COVID-19); 3) absence of cognitive and affective complaints prior to COVID-19 disease.

Non-inclusion criteria: 1) the presence of neurological, mental and somatic diseases, which are accompanied by cognitive impairments and/or manifestations of which prevent the full implementation of the study protocol; 2) a new coronavirus infection (COVID-19) in moderate and severe severity; 3) the presence of cognitive complaints before COVID-19 disease; 4) the presence of migraine, tension headache before the disease.

The main group included 50 people with mild COVID-19 at the age of 19–35 years (median age 21.6 [19.8; 22.8] years, mean age 22.2 ± 4.4 year), incl. 17 (34%) men and 33 (66%) women. The control group included 50 people without a history of COVID-19, aged 18 to 33 years (median age 21.4 [19.2; 22.0] years, mean age 21.7 ± 3.2 years), including 15 (30%) men and 35 (70%) women. Both groups were statistically comparable in terms of sex and age ($p > 0.05$).

Assessment of cognitive functions was carried out using the following neuropsychological tests:

1) Beck depression scale. The presence of 10 or more points was regarded as the presence of depression;

2) Spielberg questionnaire to identify personal and situational anxiety;

3) Subjective scale for assessing asthenia (MFI-20). The analysis was carried out separately on subscales: general asthenia, reduced activity, decreased motivation, physical asthenia, psychological asthenia. If the total score on the subscale was 12 points or more, this indicated the presence of a pathology;

КОННИКОВА Эдилия Эдуардовна – к.м.н., доцент Медицинского института СВФУ им. М.К. Аммосова, edilia@mail.ru; **БОРИСОВ Алексей Иннокентьевич** – студент 5 курса Медицинского института СВФУ им. М.К. Аммосова, seelru@bk.ru; **ТАППАХОВ Алексей Алексеевич** – к.м.н., доцент Медицинского института СВФУ им. М.К. Аммосова, с.н.с. Центра нейродегенеративных заболеваний ЯНЦ КМП, tappakhov@gmail.com; **НИКОЛАЕВА Татьяна Яковлевна** – д.м.н., проф., зав. кафедрой Медицинского института СВФУ им. М.К. Аммосова.

4) Visual memory was assessed using a 12-picture memory test. Delayed recall (3 minutes after the interference task) and recognition (out of 48 presented images) were assessed.

5) Auditory memory was assessed using a 5-word memory test. Delayed playback and playback with categorical cues were evaluated.

6) Study of phonetic speech activity. The participant within one minute had to name words beginning with the letter "L" (except for proper names).

7) Methodology "Schulte Tables" for assessing attention.

Statistical processing of the results of the study was carried out using SPSS Statistics 22. Quantitative data are given as a median and the 25th and 75th quantiles (Me [Q25; Q75]). To compare two independent groups, the analysis was carried out using the Mann-Whitney U-test. When comparing qualitative data, Pearson's χ2 test and Fisher's exact test were used. Correlation analysis was carried out using Spearman's test. The critical level of statistical significance for the two groups was determined at $p \leq 0.05$.

Research results. The main group during the acute phase were treated on an outpatient basis and were included in the present study in the range from 12 to 800 days after recovery from COVID-19 (median - 167.5 [52.0; 466.5] days). Thirteen patients (26%) had recurrent disease. 41 (82%) patients of the main group and 45 (90%) patients of the control group were vaccinated against COVID-19 (p=0.249). All persons of the main group during the acute phase of the disease underwent computed tomography of the lungs, according to the results of which changes characteristic of COVID-19 were not detected.

Study participants who had COVID-19 during the acute phase of the disease complained of general weakness (90%), headaches (86%), mental exhaustion (72%), muscle and joint pain (66%), decreased attention (64%), decreased sense of smell (62%), sleep disturbance (60%), apathy (54%), shortness of breath (34%) and chest pressure (26%). All complaints regressed after recovery, and their frequency did not differ from those of complaints in the control group (table 1).

Neuropsychological examination revealed a slightly higher level of depression, some visual memory loss, and a higher incidence of generalized asthenia in COVID-19 survivors ($p \leq 0.05$). Representatives of the same group had higher assessment results for situational and personal anxiety, motivation, and asthe-

Table 1

Complaints in patients with COVID-19 and in the control group

Clinical presentation	Complaints in the acute phase of COVID-19	Complaints at the time of inclusion in the study	Control group	p-level
General weakness	45 (90)	14 (28)	9 (18)	$p_{1.2}<0.001$ $p_{2.3}=0.235$
Muscle/joint pain	33 (66)	8 (18)	7 (14)	$p_{1.2}<0.001$ $p_{2.3}=0.779$
Exhaustion during mental work	36 (72)	14 (28)	12 (24)	$p_{1.2}<0.001$ $p_{2.3}=0.648$
Decreased memory	29 (58)	22 (44)	15 (30)	$p_{1.2}=0.118$ $p_{2.3}=0.147$
Pressure in the chest	13 (26)	1 (2)	1 (2)	$p_{1.2}=0.002$ $p_{2.3}=1.0$
Dyspnea	17 (34)	5 (10)	6 (12)	$p_{1.2}=0.002$ $p_{2.3}=0.749$
Headache	43 (86)	18 (36)	23 (46)	$p_{1.2}<0.001$ $p_{2.3}=0.309$
Decreased sense of smell	31 (62)	5 (10)	1 (2)	$p_{1.2}<0.001$ $p_{2.3}=0.092$
Mood swings	20 (40)	13 (26)	11 (22)	$p_{1.2}=0.143$ $p_{2.3}=0.64$
Decreased attention	32 (64)	21 (42)	19 (38)	$p_{1.2}=0.013$ $p_{2.3}=0.683$
Decreased motivation	27 (54)	13 (26)	10 (20)	$p_{1.2}<0.001$ $p_{2.3}=0.476$
Anxiety	23 (46)	17 (34)	11 (22)	$p_{1.2}=0.146$ $p_{2.3}=0.181$
Sleep disturbance	30 (60)	15 (30)	16 (32)	$p_{1.2}<0.001$ $p_{2.3}=0.829$

Table 2

Neuropsychological examination of persons of the main and control groups

Clinical presentation	Main group, n = 50	Control group, n = 50	p-level
Beck Depression Scale, points	10.5 [6.0; 18.0]	6.5 [2.0; 14.3]	0.018*
Spielberg questionnaire, situational anxiety, scores	46.0 [35.8; 51.0]	39.0 [32.8; 48.3]	0.081
Spielberg questionnaire, personal anxiety, scores	51.5 [43.5; 57.0]	47.0 [41.0; 54.3]	0.096
Delayed visual memory, word count	11.0 [10.0; 12.0]	11.5 [11.0; 12.0]	0.046*
Delayed auditory memory, word count	4.5 [4.0; 5.0]	4.5 [4.0; 5.0]	0.937
Schulte Table technique, sec	25.0 [22.0; 30.5]	28.0 [23.7; 33.2]	0.08
Phonetic speech activity, number of words	12.5 [9.8; 15.0]	11.0 [8.8; 13.3]	0.07
General asthenia	37 (74%)	22 (44%)	0.002*
Reduced activity	30 (60%)	23 (46%)	0.161
Decreased motivation	22 (44%)	17 (34%)	0.305
Physical asthenia	24 (48%)	21 (42%)	0.546
Psychological asthenia	28 (56%)	25 (50%)	0.548

* Статистически значимый уровень (p≤0,05).

Table 3

Correlation analysis between the duration of COVID-19 and the results of neuropsychological test

	Depression on the Beck scale	Spielberg questionnaire, situational anxiety	Spielberg questionnaire, personal anxiety	Short-term visual memory	Attention "according to the Schulte table"	Short-term auditory memory
Correlation coefficient	0.274	0.182	0.033	0.051	-0.106	-0.274
p-level	0.054	0.207	0.818	0.725	0.465	0.234

nia, but a statistically significant level was not reached (Table 2).

To determine the relationship between the severity of cognitive impairment and the time after recovery, we conducted a correlation analysis. However, no relationship was found between the results of neuropsychological tests and the duration of COVID-19 (Table 3).

Discussion. According to the results of our study, in young people in the acute phase with a mild course of COVID-19, complaints from the nervous system predominate (for example, headaches, decreased attention, sense of smell, sleep disturbances). On the contrary, complaints of shortness of breath, pressure in the chest occur only in a third of patients.

According to a meta-analysis by J.P. Rogers, more than 18% of patients who survived the coronavirus of the Middle East respiratory syndrome had a decrease in concentration and memory impairment for a period of 6 to 39 months [13]. Pelen A.I. et al. studied the prevalence of cognitive and autonomic disorders of the nervous system among students of the Izhevsk State Medical Academy by the method of questioning. The study involved 139 people. The study was conducted by the MoCA neuropsychological test. According to the data obtained, it was found that cognitive disorders of the nervous system are more common in young people with a coronavirus infection than in those who have not been exposed to the disease [6].

Semenov V. A. et al. investigated cognitive impairment in young adults after suffering from COVID-19. They studied 172 apparently healthy people, aged 18–27 years on the MMSE scale. According to the results of the study, it was revealed that the pre-dementia state is quite common in young people, and moderate cognitive impairment is more common in people who have had a coronavirus infection [7].

The originality of our study is that we assessed the cognitive status of young people after mild COVID-19. We have shown that even after a mild course of COVID-19, disorders such as asthenia, mild depression, and a decrease in short-term visual memory are detected.

The main limitation of our work was the wide range of recency of COVID-19. Of course, a broad study is required with the division of patients according to the terms of recovery and the inclusion of neurophysiological methods of research, for example, cognitive evoked potentials. However, we have shown a general trend that, regardless of the duration of the disease, patients will have some or other impairments in the cognitive sphere.

Work without financial support. The authors are solely responsible for the food version of the manuscript in print. All authors participated in the development of scientific articles and writing the manuscript. The final version of the manuscript was approved by all authors.

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N.A. Ilyina, S.S. Sleptsova, S.S. Sleptsov

EPIDEMIOLOGICAL CHARACTERISTICS OF THE NOVEL CORONAVIRUS INFECTION COVID-19 IN THE REPUBLIC OF SAKHA (YAKUTIA) FOR 2020-2021

DOI 10.25789/YMJ.2023.82.18

УДК 616.9-036.22(571.56) «2020/2021»

Introduction. The first confirmed case of COVID-19 in the Republic of Sakha (Yakutia) was registered on March 18, 2020, and in June the regional incidence rate reached 267.7 people/100,000 population, which was significantly higher than the national average.

Objective. Conduct an analysis of the epidemiological situation of the incidence of a new coronavirus infection (NCVI) in the RS (Y) for 2020-2021.

Materials and methods. The work used data from official statistics of the Office of Rospotrebnadzor in the Republic of Sakha (Yakutia), FGUZ "Center for Hygiene and Epidemiology in the Republic of Sakha (Yakutia)" and Rosstat.

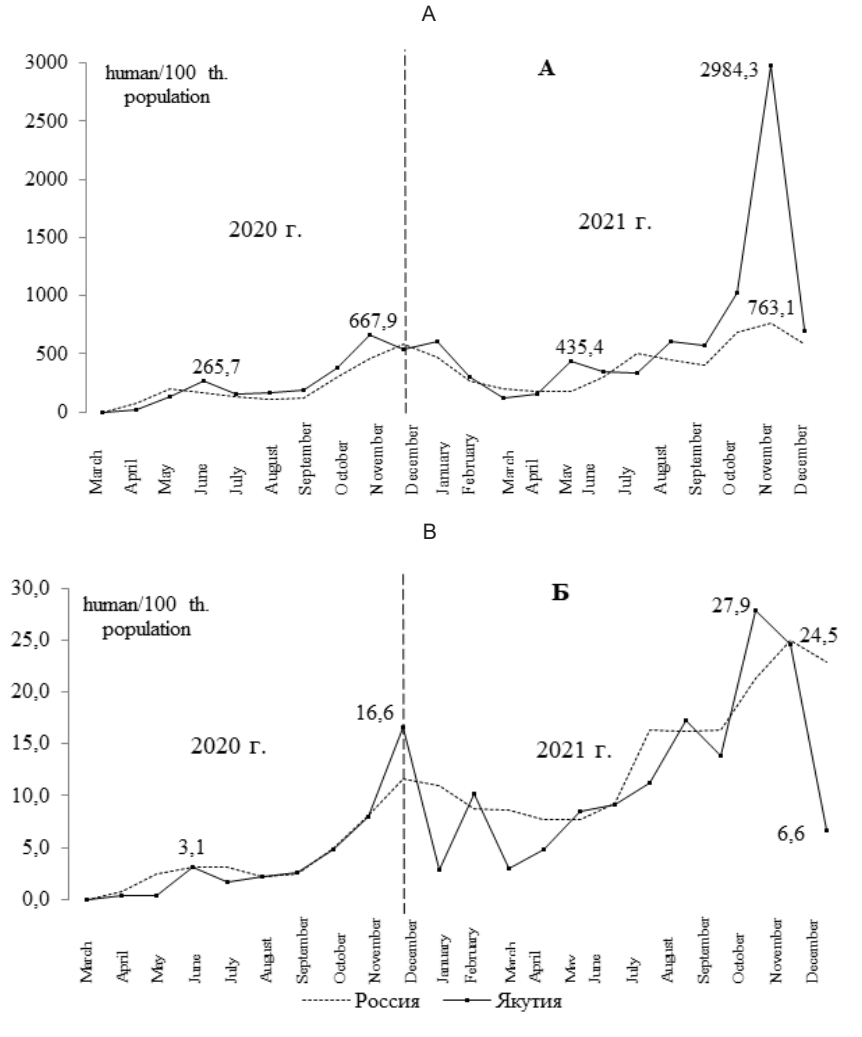
Results. Despite the regional peculiarities of the population of Yakutia, epidemic rises in the incidence of NCVI COVID-19 in the Republic of Sakha (Yakutia) corresponded to the periods of spread of the "Wuhan" strain of the virus in 2020 (2,531.0 people/100 thousand people) and the Indian strain " delta" in 2021 (8,196.9 people/100 thousand people), which was reflected in a 3.6-fold increase in the mortality rate in the Republic of Sakha (Yakutia) (from 39.0 to 141.8 people/100 thousand of us.). The incidence in the first year of the pandemic was directly related to the transport accessibility of certain territories of Yakutia and the implementation of anti-epidemic measures. The incidence of COVID-19 in all territories in 2021 was significantly higher than in 2020.

Conclusion. Morbidity and mortality rates of NCVI for 2020-2021 comparable with periods of rising morbidity and mortality in Russia.

Keywords: COVID-19, coronavirus pandemic, Yakutia, morbidity, mortality, epidemic rises.

Introduction. The new coronavirus infection COVID-19, caused by the SARS-CoV-2 virus and registered for the first time in China at the end of 2019, has spread unprecedentedly rapidly throughout the world, including Russia [1, 6, 8]. So, despite the introduction by the state of a number of preventive and anti-epidemic measures, already in the first half of 2020, this disease was noted in all regions of the country, even the most remote ones. The Republic of Sakha (Yakutia) was no exception - the first confirmed case of COVID-19 was registered on March 18, 2020. As of May 13, 2020, 652 cases of NKVI were registered in the republic, of which 452 people were infected. were on inpatient treatment, 147 people. recovered, 4 patients died. Cases of the disease have been reported in Yakutsk, Neryungri, Aldan, Suntar, Lensky, Vilyuysky, Gorny, Mirny, Oymyakonsky,

ILYINA Natalina Alexandrovna – postgraduate student of the Department of Infectious Diseases, Phthisiology and Dermatovenereology, e-mail: bnatalinush@mail.ru, ORCID 0000-0002-9458-403x; **SLEPTSOVA Snezhana Spiridonovna** – MD, associate professor, Head of the Department of Infectious Diseases, Phthisiology and Dermatovenereology, e-mail: sssleptsova@yandex.ru, ORCID 0000-0002-0103-4750; **SLEPTSOV Spiridon Spiridonovich** – PhD in Biology, associate professor, senior researcher, Laboratory of Clinical Population and Medical and Social Research, e-mail: sachaja@yandex.ru, , ORCID 0000-0002-2482-2928



Morbidity rates (A) and mortality (B) from a new coronavirus infection in the Russian Federation and the Republic of Sakha (Yakutia) by months, 2020-2021 [2, 3]

Khangalassky, Ust-Yansky and Megino-Kangalassky districts [7]. In June of the same year, the regional incidence rate reached 267.7 people/100,000 people, which was significantly higher than the national average. Of course, at first, one of the factors in the widespread spread of the infection was both the underestimation of the danger of a new infection by a significant part of the population (and often against the backdrop of the so-called infodemic), and the insufficient preparedness of the health care system for the emergence of such an unexpected epidemic scenario.

Objective. To analyze the epidemiological situation of the incidence of a new coronavirus infection in the Republic of Sakha (Yakutia) for 2020-2021.

Materials and research methods. The work used data from official statistics of the Office of Rospotrebnadzor in the Republic of Sakha (Yakutia), FGUZ "Center for Hygiene and Epidemiology in the Republic of Sakha (Yakutia)" and Rosstat. To study the features of the spread of the pandemic across the region, the medical-geographical zoning of Yakutia was used [4], according to which it is conditionally divided into 6 zones:

1. Polar zone - consists of 13 regions of Yakutia included in the Arctic zone of Russia. It is characterized by extreme natural and climatic conditions, considerable remoteness from industrial centers, transport isolation. The population density is 0.04 people/km2, as of January 1, 2021, 67.7 thousand people live.

2. Eastern Yakutia - includes 3 regions of Yakutia, including the Oymyakonsky ulus, known as the cold pole of the Northern Hemisphere. Population density - 0.09 people / km2, 27.7 thousand people live. A significant part of the population is represented by visiting citizens employed in the mining industry.

3. Western Yakutia (Vilyui group of regions) - the zone includes agricultural regions, the climate is sharply continental, but relatively mild. Population density - 0.05 people / km2, 93.7 thousand people live.

4. Southern (Verkhnelenskaya) zone - occupies the southern part of the territory of the republic, the climate is milder than in the rest of Yakutia. Population density - 0.03 people / km2, 60.8 thousand people live.

5. Central zone - characterized by a sharply continental climate. 188.6 thousand people live on the territory, of which more than 90% are the indigenous population (Yakuts).

6. The zone of large cities includes mainly residents of the cities of Yakutsk,

Table 1

COVID-19 incidence rates by medical-geographical zones of the Republic of Sakha (Yakutia)

№	Район	Incidence rate					
		2020 г.			2021 г.		
		abs., human.	share, %	human /100 th. population	abs., human.	share, %	human./100 th. population
1	Abyi	24	0.09	607.7	66	0.08	1685.4
	Anabar	88	0.36	2409.0	29	0.04	789.8
	Allaikhovsky	161	0.66	5969.6	196	0.25	7190.0
	Bulunskiy	73	0.29	857.5	426	0.54	5011.2
	Nizhnekolymsky	37	0.15	868.5	285	0.36	6740.8
	Verkhoyansk	212	0.86	1917.0	640	0.81	5824.0
	Upper Kolyma	23	0.09	574.6	262	0.33	6576.3
	Zhigansky	212	0.87	5155.6	601	0.76	14381.4
	Momsky	55	0.23	1384.0	199	0.25	4912.4
	Ust-Yansky	76	0.31	1084.5	165	0.21	2345.4
	Eveno-Bytantaisky	1	0.00	35.1	167	0.21	5800.6
	Oleneksky	191	0.78	4497.3	558	0.70	12898.8
	Srednekolymsky	80	0.33	1091.1	361	0.45	4937.1
	Total by zone	**1233**	**5.04**	**1822.6**	**3955**	**4.98**	**5833.5**
2	Oymyakonsky	222	0.91	2818.3	788	0.99	10030.5
	Tomponsky	116	0.47	926.1	850	1.07	6755.1
	Ust-Maisky	103	0.42	1377.7	405	0.51	5576.2
	Total by zone	**441**	**1.80**	**1581.8**	**2043**	**2.57**	**7374.9**
3	Verkhnevilyuisk	334	1.37	1589.0	762	0.96	3597.7
	Vilyuysky	408	1.67	1633.2	2033	2.56	8097.3
	Nyurbinsky	411	1.68	1740.6	1084	1.36	4565.6
	suntarsky	286	1.17	1221.0	1416	1.78	5979.7
	Total by zone	**1439**	**5.89**	**1546.7**	**5295**	**6.66**	**5650.4**
4	Lensky	398	1.63	1095.4	1275	1.60	3496.9
	Olekminsky	274	1.12	1118.8	1159	1.46	4751.6
	Total by zone	**672**	**2.75**	**1104.8**	**2434**	**3.06**	**3999.8**
5	Amginsky	352	1.44	2096.0	1471	1.85	8727.4
	Mountain	324	1.33	2677.0	572	0.72	4664.1
	Kobyaisky	164	0.67	1351.0	680	0.86	5631.5
	Namsky	440	1.80	1768.1	1803	2.27	7185.0
	Megino-Kangalassky	840	3.43	2718.0	3574	4.50	11382.9
	Tattinsky	330	1.35	2038.7	1357	1.71	8340.0
	Ust-Aldan	342	1.40	1671.3	1836	2.31	8949.5
	Khangalassky	330	1.35	1004.8	4562	5.74	13815.5
	Churapchinsky	280	1.15	1333.3	1160	1.46	5495.8
	Total by zone	**3402**	**13.92**	**1816.2**	**17015**	**21.41**	**9021.7**
6	Aldan	895	3.66	2286.1	3523	4.43	9076.2
	Mirninsky	853	3.49	1181.4	5790	7.28	8053.1
	Neryungri	743	3.04	1005.1	3087	3.88	4121.4
	Yakutsk	14 763	60.40	4217.8	36327	45.71	10155.9
	Total by zone	**17254**	**70.59**	**3223.3**	**48727**	**61.31**	**8968.6**
Total		**24441**	**100**	**2514.5**	**79469**	**100**	**8092.8**

Designation of zones: 1 - Zapolyarnaya; 2 - East; 3 - Western; 4 - South (Verkhnelenskaya); 5 - Central; 6 - zone of large cities.

Mirny, Neryungri and Aldan, in which more than half (543.3 thousand people) of the total population of Yakutia (981.9 thousand people) live.

Results.

The Republic of Sakha (Yakutia) is located in the northeastern part of the Russian Federation and is part of the Far Eastern Federal District. Decree of the Government of the Republic of Sakha (Yakutia) dated December 23, 2021 No. 536 "On the differentiation of settlements of the Republic of Sakha (Yakutia) by transport accessibility and remoteness to ensure the livelihoods of the population" 15 settlements of the republic are defined as limited access, 100 - remote, 208 - hard to reach. Therefore, when organizing medical care in Yakutia, regional features are always taken into account: low population density, significant distances between settlements (in some areas, the service radius of medical organizations is more than 500 km2), a weak transport scheme between settlements, and the absence of regular transport links.

Dynamics of the incidence of COVID-19 in Yakutia in 2020-2021 characterized by periods of rise and fall of varying duration. In 2020, the average annual NCVI was 2,514.5 people/100,000 people. (24,441 cases), in 2021 - 8,092.8 people/100 thousand people. (79,469 cases). In general, epidemic rises in incidence for 2020-2021 in the Russian Federation and the Republic of Sakha (Yakutia) corresponded to the periods of spread of the "Wuhan" virus strain in 2020 and the Indian "delta" strain in 2021 [2, 3, 5].

The spread of a new coronavirus infection in the Republic of Sakha (Yakutia) at first occurred due to imported cases from disadvantaged territories of the Russian Federation and from abroad (Switzerland, England, USA). By the end of 2020, the incidence of COVID-19 in the republic tended to increase, and from the beginning of 2021, to decrease, which in April was again replaced by high rates of its growth [5].

In the first half of 2020, the highest incidence rate in Yakutia was observed in May (1,284 people), in the second half of the year, a pronounced increase in the incidence began in autumn, followed by a peak in November (6,450 people). The beginning of 2021 was also characterized by a significant number of cases (5,879 cases in January). In early spring (February-March), a slight decrease in the incidence rate was noted, but since April there has been another rise with a peak in November (28,933 cases). As a rule, at the same time, a peak in mortality was observed - in the period from 10/01/2021 to 11/31/2021, 515 people died, which accounted for 37.4% of the total annual number of deaths. In general, mortality in 2021 amounted to 141.8 people/100 thousand people, while a year earlier this figure was at the level of 39.0 people/100 thousand people. A similar dynamics of mortality was observed throughout the country, which is associated with the circulation of certain strains of COVID-19 in the period 2020-2021. (Fig. 1).

It can be seen from the data in Table 1 that during the observation period, the main number of cases was registered in the urban area - 70.6% in 2020 and 61.3% in 2021. It is obvious that a slight decrease in the proportion of cases in 2021 in the area of large cities is mainly due to a wider spread of the virus in rural areas, including due to foci of coronavirus infection that have arisen in Vilyuisky, Tattinsky, Amginsky, Oymyakonsky and Zhigansky uluses.

In the structure of the diseased persons aged 18 years and older accounted for 80.4-86.5%, while the largest proportion was in patients aged 30 to 49 years (33.1-33.5%). According to intensive indicators, the most affected were persons over 65 years of age. For example, in 2021, the incidence rate in this group was 12,769.3 people/100,000 people. (Table 2).

According to the patients themselves, a significant proportion of those infected were infected by family members (43.2% in 2020, 38.3% in 2021). There were also cases of infection at work (11.5% in 2020, 4.9% in 2021) and in medical institutions (28% in 2020, 1.1% in 2021). But more than half of the patients (51-54.9%) found it difficult to answer this question.

Regardless of the year, the vast majority of people with positive test results (n = 103,910) experienced asymptomatic or mild COVID-19 (68.7-69.6%), moderate severity was recorded in 27.2-27.7%

Table 2

Age composition of patients with a new coronavirus infection in the Republic of Sakha (Yakutia), 2020-2021

Age group	Years					
	2020			2021		
	human	%	human./100 th. population	human	%	human./100 th. population
Up to 1 year	196	0,8	1 444,7	983	1,2	7 571,4
1-6 years old	959	3,9	995,6	4 733	5,9	5 073,5
7-14 years old	1457	6,0	1 249,8	7 116	9,0	5 978,4
15-17 years old	676	2,8	1 792,0	2 764	3,5	7 119,1
Total up to 17 years	**3288**	**13,5**	**1 244,5**	**6 3873**	**19,6**	**9 055,1**
18-29 years old	3 497	14,3	2 173,6	10 397	13,1	5 332,0
30-49 years old	8 093	33,1	2 854,4	26 621	33,5	9 231,4
50-64 years old	5 805	23,8	3 250,9	16 179	20,4	9 128,9
65 years and older	3 756	15,4	4 784,8	10 676	13,4	12 769,3
Total 18 years and older	**21 151**	**86,5**	**3 015,2**	**79 469**	**80,4**	**8 196,9**
Total	24 441	100,0	2 531,0	15 596	100	5 904,8

Table 3

Distribution of people who died from a new coronavirus infection by age in the Republic of Sakha (Yakutia) for 2020-2021

Age group	Год					
	2020			2021		
	human	%	human./100 th. population	human	%	human./100 th. population
18-29 years old	0	0,0	0,0	3	0,2	1,5
30-49 years old	21	5,6	7,3	52	3,8	18,0
50-64 years old	91	24,1	51,3	335	24,4	189,0
65 years and older	266	70,4	318,2	985	71,6	1178,1
Всего	378	100,0	39,0	1375	100,0	141,8

of patients. The infection was severely transferred in 2020 - 2.7%, in 2021 - 4% of patients. But at the same time, it must be pointed out that due to the increase in the incidence, the absolute number of moderate and severe COVID-19 patients increased from 7,141 people to in 2020 to 24,807 people in 2021. Of course, this not only increased the workload at all stages of medical care, but also essentially reflected in the number of deaths - from 387 cases (39.0 people / 100 thousand people) to 1375 people. (141.8 people/100 thousand people).

64.2% of the deceased had a pathology of the cardiovascular system (IHD, hypertension, angina pectoris), 12.4% had pathology of the respiratory organs (COPD, bronchial asthma), 24.2% had diseases of the endocrine system (diabetes mellitus) , oncological diseases were noted in 6.9%, HIV infection in 0.1%. More than 90% of the dead are people aged 50 and older (Table 3).

At the end of 2020, mass vaccination against COVID-19 began. As of December 31, 2021, 526,006 people were vaccinated for the first time in the region. (including 106,027 people over 60), the second vaccination - 493,018 people, i.e. 99,271 people completed the full course of immunization. In total, 90,630 people were revaccinated in the Republic of Sakha (Yakutia).

Conclusion. Dynamics of the incidence of COVID-19 in the Republic of Sakha (Yakutia) in the period 2020-2021 was characterized by periods of rise and fall of various durations, epidemic rises corresponded to the periods of spread of the "Wuhan" strain of the virus in 2020 (2,531.0 people / 100 thousand people) and the Indian strain "delta" in 2021 (8,196, 9 people/100 thousand people). This was reflected in a 3.6-fold increase in the regional mortality rate from 39.0 to 141.8 people/100,000 population, which also corresponds to the all-Russian indicators, including the dynamics of the incidence and mortality from COVID -19 by month.

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A.I. Fedorov, A.L. Sukhomyasova, A.L. Danilova, A.N. Sleptsov, V.V. Filippova, N.R. Maksimova

MEDICO-GEOGRAPHICAL ANALYSIS OF FREQUENT CONGENITAL MALFORMATIONS IN NEWBORNS IN THE PERIOD FROM 2007 TO 2020 IN THE REPUBLIC OF SAKHA (YAKUTIA)

DOI 10.25789/YMJ.2023.82.19

УДК 616-007-053.1

The article presents the results of the analysis of data from 13-year monitoring of congenital malformations (CM) in the Republic of Sakha (Yakutia) according to the Republican Genetic Register of Hereditary and Congenital Pathology of the Medical and Genetic Center of the SAI RH # 1 – NCM. The results of the application of the cartographic method in the analysis of the frequency of CM showed the geographical unevenness of its distribution on the territory of Yakutia. During the period of long-term monitoring, the highest frequency of CM was recorded in the Arctic regions of the Republic.

Keywords: congenital malformations, monitoring, medical geography, Yakutia.

Introduction. Congenital malformations (CM) are among the most serious abnormalities in children's health [4], leading to physical or mental disabilities and are the main cause of infant mortality [10, 12]. According to the World Health Organization (WHO), approximately 3.2 million children worldwide are born with CM every year, and about 300,000 newborns diagnosed with CM die within the first 28 days of life [13]. Malformations of various organ systems (cardiovascular, musculoskeletal, urinary, central nervous systems, multiple CM) make an unequal contribution to the overall indicator of the frequency of CM. The structure of malformations in the regions differs: the contribution of different organ systems to the prevalence of malformations in territorial population groups varies significantly [4].

When conducting comparative epidemiological studies of the frequency of CM in populations, it is necessary to take into account standard markers, such forms of malformations that are quite common and unambiguously diagnosed by doctors of all specialties. Long-term observation of populations by such markers makes it possible to assess the prevalence and dynamics of CM, to conduct a comparative analysis.

In many countries, CM monitoring is essentially the only effective tool for controlling the level of congenital malformations and is used to study their etiology. The main task of the monitoring system is to determine population frequencies and other epidemiological characteristics, which is of paramount importance when planning and organizing preventive measures for congenital malformations [1].

The first registers for the monitoring of CM began to work back in the 70s of the last century. Currently, two international systems of monitoring registers are successfully functioning, one of them is the European International Organization for Joint Activities in the Field of Research of Congenital Anomalies and Multiple Pregnancies (EUROCAT). EUROCAT is a joint network of 43 population registers of congenital anomalies based in 23 countries. The registries collect data on congenital anomalies that occur during live birth, late miscarriages (20-24 weeks of pregnancy), stillbirths (> 24 weeks of pregnancy) and termination of pregnancy due to fetal abnormalities [4, 9]. The second system is the International Information Center for Epidemiological Surveillance and Research in the field of birth Defects (ICBDSR), which combines surveillance programs for HPV and research projects from around the world. Currently, the organization has 42 programs from 36 countries of the world [14].

In Russia, the CM monitoring system has been operating since 1999. During all this time, the Veltischev Research and Clinical Institute for Pediatrics and Pediatric Surgery of the Pirogov Russian National Research Medical University (Department of Information Technology and Monitoring) collects and analyzes information about malformations from the regions of the Russian Federation. During this time, considerable material has been accumulated on congenital anomalies, which allows us to estimate with a high degree of reliability the frequencies of individual forms of malformations characteristic of specific regions and in total for all regions of Russia participating in monitoring [2].

In the Republic of Sakha (Yakutia), the monitoring of the CM began on the basis of the order of the Ministry of Health of 10.09.1998 No. 268 and the Order of the Ministry of Health of the RS (Ya) of 28.03.2001 No. 01-8/4-112 since 2001. A long-term study of the CM in Yakutia allowed to determine the structure, dynamics of the frequency of CM. The basic frequencies of chromosomal diseases, defects of the central nervous system and congenital heart defects by region were determined. Differences between industrial and agricultural regions of Yakutia were revealed [7]. The geographical subdivision observed in Yakutia led to further investigation of the frequency of CM by cartographic methods.

The aim of the study is a medico-geographical analysis of data on the frequency of congenital malformations in the Republic of Sakha (Yakutia).

Materials and methods. The material for the study was monitoring data from 2007 to 2020 of the Republican Genetic Register of Hereditary and Congenital

M.K. Ammosov North Eastern Federal University, Yakutsk: **FEDOROV Afanasy Ivanovich** – Ph.D., senior researcher, Medical Institute, ai.fedorov@s-vfu.ru; **SUKHOMYASOVA Aytalina Lukichna** – Ph.D., leading researcher, Medical Institute, **DANILOVA Anastasia Lukichna** – Ph.D., senior researcher Medical Institute; **SLEPTSOV Arkhip Nikolaevich** – junior researcher, Medical Institute; **MAKSIMOVA Nadezhda Romanovna** – MD, Chief Researcher, Medical Institute. **FILIPPOVA Viktoria Viktorovna** – PhD, associate professor of Institute of Nutrition. M.K. Ammosov NEFU, Senior Researcher Institute for Humanitarian Studies and Problems of Indigenous Peoples of the North SB RAS

Pathologies of the Medical Genetic Center of the SAI RH # 1 - NCM, formed on the basis of notifications of congenital malformations with updated diagnoses. The calculation of the frequency of cases of congenital malformations was carried out among 1000 births in the corresponding years of monitoring. Integral indicators of the frequency of congenital malformations for the affected body systems were calculated for the entire monitoring period with distribution over all uluses (districts) and urban districts of the Republic of Sakha (Yakutia). The frequency of congenital malformations was recorded at the place of observation of mothers.

To display the situation on the prevalence of congenital malformations among newborns in the uluses (districts) of Yakutia, a medical-geographical analysis of data on the frequency of congenital malformations among 1000 newborns was carried out using the cartographic method [3]. Malformations were grouped according to the affected body systems. The display of frequencies is carried out in five gradations of the scale of the same color: low, below average, medium, above average and high. The integral indicator of the frequency of congenital malformations for the affected organ systems is shown through a digital designation. Each digit corresponds to one of the 9 most commonly affected systems, numbered in descending order of their integral frequency. The compiled maps are aimed at the formation and extraction of spatial and territorial statistical information about the geography of the prevalence of malformations among newborns by uluses of the Republic of Sakha (Yakutia).

Results and discussion.

This study determined the frequencies of the entire spectrum of congenital malformations among newborns in the period from 2007 to 2020 in the regions of the Republic of Sakha (Yakutia). The results of the study are given in table. 1. It should be noted that during the analysis, all uluses were combined into groups - Central, Western, Arctic, Eastern and Southern [6]. These zones differ in natural and climatic conditions, levels of socio-economic development, population, ethnic composition, transport accessibility, migration processes and other parameters, which could contribute to the formation of foci of accumulation of genetic load in the population, and this, in turn, may affect the structure congenital malformations.

The results of the study showed that during the monitoring period, the weighted average frequency of the entire spectrum of congenital malformations differed from the national average, depending on the geographical and socio-economic position of the uluses. In most uluses of the Republic, with the exception of Anabarsky, Olenyoksky and Eveno-Bytantaisky, the frequency variability of the entire spectrum of congenital malformations is within the limits of statistical fluctuations, i.e. has no significant differences from the weighted average frequency for the region. At the same time, in the Anabarsky, Olenyoksky and Eveno-Bytantaysky uluses, the total frequency of congenital malformations exceeds the national average for the same period by more than 40%. This increase in frequency is statistically significant. These uluses belong to the Arctic group. As is known, the Arctic group is characterized by extreme natural and climatic and difficult socio-economic conditions, which of course negatively affects the life and general well-being of a person [5].

We also assessed the dynamics of the overall frequency of congenital malformations over the years of monitoring. During the monitoring period, two peak increases in the frequency of the entire CM

Average incidence of congenital malformations per 1000 newborns in Yakutia from 2007 to 2020

п/п	Administrative divisions	The frequency of congenital malformations per 1000 newborns
Arctic districts		
1	Abyysky	22.19
2	Allaikhovsky	32.51
3	Anabar	48.68*
4	Bulunsky	23.15
5	Verkhnekolymsky	13.89
6	Verkhoyansky	27.95
7	Zhigansky	34.11
8	Momsky	27.70
9	Nizhnekolymsky	31.00
10	Oleneksky	40.39*
11	Srednekolymsky	23.57
12	Ust-Yansky	23.79
13	Eveno-Bytantaysky	40.43*
Central districts		
14	Amginsky	18.14
15	Mountain	28.82
16	Kobyaysky	29.63
17	Megino-Kangalassky	19.06
18	Namsky	26.68
19	Tattinsky	31.70
20	Ust-Aldansky	37.18
21	Khangalassky	28.79
22	Churapchinsky	36.69
23	Yakutsk	37.69
Eastern districts		
24	Oymyakonsky	28.76
25	Thompson	26.22
26	Ust-Maysky	13.38
Western districts		
27	Verkhneviluysky	23.34
28	Vilyuysky	24.88
29	Lensky	10.47
30	Mirninsky	11.77
31	Nyurbinsky	34.91
32	Suntarsky	31.34
Southern districts		
33	Aldansky	14.99
34	Neryungrinsky	14.27
35	Olekminsky	17.28
Average for Yakutia		
36	Republic of Sakha (Yakutia)	28.47*

Note: * - statistically significant differences.

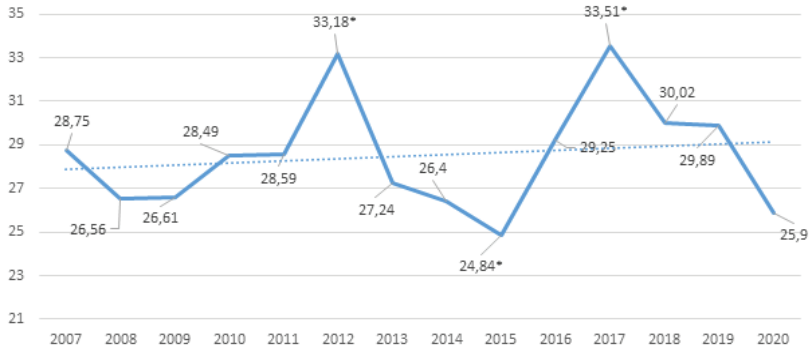

Fig. 1 - Dynamics of the overall incidence of congenital malformations per 1000 newborns 2007-2020
Note: *- statistically significant indicators

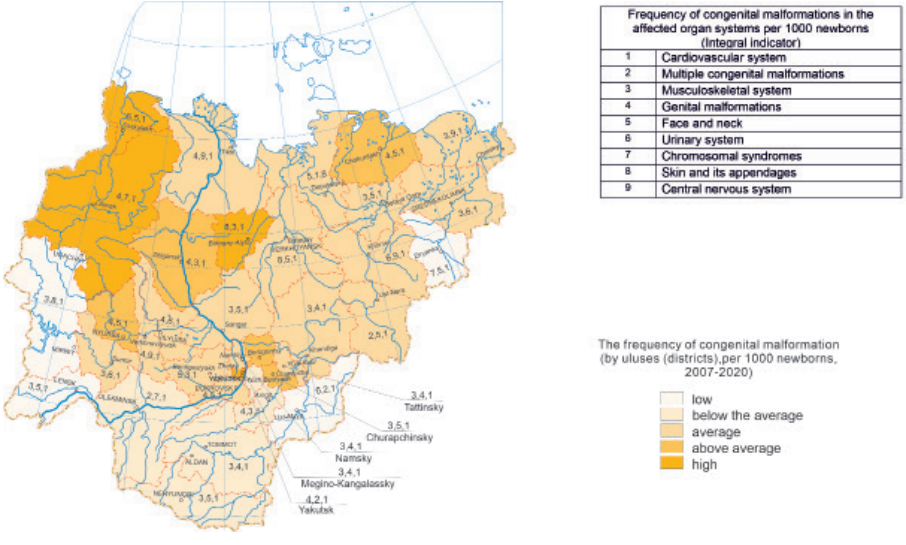

Fig. 2. The frequency of congenital malformations (by uluses (district), per 1000 newborns, 2007-2020)

spectrum were observed - in 2012 and 2017. During these years, the frequency of CM was statistically significantly higher than the weighted average frequency in Yakutia for the entire monitoring period. At the same time, in 2015 there was a significant decrease in this indicator. The dynamics of the CM frequency is illustrated in Fig.1.

Further, a medical-geographical study was carried out, the results of which are shown in Fig.2. This analysis clearly demonstrated the uneven geographical distribution of the overall frequency of congenital malformations and affected body systems. High rates of the overall frequency of congenital malformations were noted in the Arctic and Central uluses of Yakutia. The cardiovascular and musculoskeletal systems are most often affected, and multiple lesions of different systems are often recorded. A low frequency of malformations is recorded in the Western and Southern groups of uluses. At the same time, it is considered that the overall frequency of congenital malformations should be above 20 cases per 1000 births. Lower rates may be the result of insufficient detection or registration of malformations [11]. It should be emphasized that the Southern group of uluses is characterized by a low birth rate [8].

Since 2012, the republic has been implementing a program for early prenatal diagnosis of CM and chromosomal abnormalities, according to the decision of the perinatal council, the level of medical care and routing of pregnant women are determined depending on the severity of congenital malformations. At the same time, in cases of severe and uncorrectable CM, a decision is made to eliminate the fetus. The frequency of registration and the nature of CM can also be an indicator of the level and quality of prenatal diagnostics in each of the 35 administrative units of Yakutia.

Conclusion.

According to the monitoring data, the observed features of the distribution of the frequency of CM may be due to different birth rates and diagnostic capabilities of medical organizations in Yakutia. The geographical factor (remoteness, inaccessibility of territories) can still influence the formation of the population frequency of congenital malformations among certain groups of the population of the North. During the study period, there is a wave-like frequency dynamics of the entire CM spectrum with intervals of 2-3 years between peak values. This pattern may be explained by some intra-population processes that have not been studied in this work. According to the results of monitoring for 2007-2020, the weighted average frequency of congenital malformations in the Republic of Sakha (Yakutia) was 28.47 cases per 1000 newborns. Despite the significant dynamics of the overall frequency of CM in some years, there was no stable tendency to decrease or increase this indicator. The problem of high frequency of CM persists in the Arctic group of Yakutia uluses. Thus, in the Eveno-Bytantay and Olenek uluses, the total frequency of compulsory registration CM during the monitoring period exceeds the national average by 1.5-2 times.

The data obtained will make it possible to take measures to improve the organization of monitoring, periconceptional prevention and prenatal diagnosis of CM in the republic.

The work was supported by the State Assignment of the Ministry of Science and Higher Education of the Russian Federation (Project № FSRG-2020-0014 "Genomics of the Arctic: epidemiology, heredity and pathology").

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ARCTIC MEDICINE

A.A. Nikanorova, N.A. Barashkov, V.G. Pshennikova, S.S. Nakhodkin, S.A. Fedorova

ASSOCIATION OF GENE POLYMORPHISM *PTGS2* rs689466 WITH PLASMA IRISIN LEVEL IN YAKUTS

DOI 10.25789/YMJ.2023.82.20

УДК 577.17

NIKANOROVA Alena Afanasyevna – junior researcher, Laboratory of Molecular Genetics, Federal State Budgetary Scientific Institution Yakut Science Center of Complex Medical Problems, e-mail: nikanorova.alena@mail.ru, http://orcid.org/0000-0002-7129-6633; **BARASHKOV Nikolay Alekseevich** – PhD in Biology, Head of laboratory of Molecular genetics, Federal State Budgetary Scientific Institution Yakutsk Scientific Center of Complex Medical Problems, e-mail: barashkov2004@mail.ru, https://orcid.org/0000-0002-6984-7934; **PSHENNIKOVA Vera Gennadievna** – PhD in Biology, Head of laboratory of Populational Genetics, Federal State Budgetary Scientific Institution Yakutsk Scientific Center for Complex Medical Problems, e-mail: pshennikovavera@mail.ru, https://orcid.org/0000-0001-6866-9462; **NAKHODKIN Sergey Sergeyevich** – scientific researcher of the Research Laboratory of Molecular Biology Institute of Natural Sciences, Federal State Autonomous Educational Institution of Higher Education M.K. Ammosov North-Eastern Federal University; e-mail: sergnahod@mail.ru, https://orcid.org/0000-0002-6917-5760; **FEDOROVA Sardana Arkadievna** – MD in Biology, Head of the Research Laboratory of Molecular Biology Institute of Natural Sciences, Federal State Autonomous Educational Institution of Higher Education M.K. Ammosov North-Eastern Federal University, e-mail: sardaanafedorova@mail.ru, https://orcid.org/0000-0002-6952-3868.

Prostaglandin E2 may be involved in an increase in body temperature during cold stress by the type of fever. At the same time, a significant part of heat production is produced during shivering thermogenesis, due to arbitrary muscle activity, which is accompanied by the release of the hormone irisin. Prostaglandin E2 is formed from arachidonic acid by the enzyme cyclooxygenase-2, which is encoded by the *PTGS2* gene. The transcriptional activity of the *PTGS2* gene depends on its allelic variants, which can affect thermoregulation processes in different ways. In this regard, the aim of this study is to analyze the polymorphism rs689466 of the *PTGS2* gene with the level of irisin in blood plasma in a population of Yakuts living in cold climatic conditions. The study involved 183 women and 80 men (average age 19.73±1.99 years). Analysis of the association of polymorphism rs689466 of the *PTGS2* gene with irisin levels showed that in men with the TT genotype, irisin levels (8.2±1.85 μg/ml) were statistically significantly higher (U=261; p=0.005), compared with men with CT+CC genotypes (7.1±1.25 μg/ml). In addition, it was found that men with the TT genotype (63.6±6.67 kg) had a lower weight than men with the CT+CC genotypes (67.93±7.28 kg; U=279; p=0.01). The detected association of the TT rs689466 genotype of the *PTGS2* gene with elevated irisin levels and with a lower weight in men may indicate the effect of prostaglandin E2 on shivering thermogenesis during cold stress, which may play a role in human adaptation to a cold climate.

Keywords: irisin, prostaglandin E2, gene *PTGS2*, rs689466, fever, cold stress.

Introduction. One of the key mechanisms in human physiology is the ability to sense and regulate body temperature, which is crucial for survival. The body's defense reactions include fever, which is accompanied by an increase in body temperature in response to pyrogens [14] that stimulates an immune response [6]. An increase in body temperature during fever occurs due to shivering (in skeletal muscles) and nonshivering (in brown adipose tissue) thermogenesis, and a decrease in passive heat loss occurs due to vasoconstriction [8]. However, the main contribution to increased heat production in fever is made by shivering thermogenesis, which is accompanied by involuntary muscle contraction (shiver) and the release of the hormone irisin into the blood [7,15].

Prostaglandin E2 is a principal fever mediator that can also control the basal mechanisms of thermoregulation. In 2015, J. Foster and his colleagues pub-

lished a paper on a new hypothesis about the role of prostaglandin E2 in thermoregulation processes under cold stress [12]. The main propositions of the hypothesis are based on the fact that cold-sensitive neurons and EP3 neurons (prostaglandin E2 receptor) activate the same areas of the hypothalamus that are responsible for thermoregulation [12]. In this regard, involuntary thermogenic reactions to maintain body temperature during cold stress are identical to the mechanisms that increase body temperature during fever [12].

Prostaglandin E2 is formed as a result of oxygenation and cyclization of arachidonic acid by the enzyme cyclooxygenase-2 [1]. The cyclooxygenase-2 enzyme is encoded by the *PTGS2* gene [2] located on chromosomal region 1q25.2-q25.3 and containing 10 coding exons [2,3]. A large number of single-nucleotide polymorphic regions (SNPs) are known in the *PTGS2* gene, some of which are considered functionally significant [13]. These regions include SNP rs689466, which is located in the promoter region of the *PTGS2* gene [10, 13]. Analysis of mRNA in human esophageal tissues showed that the normal T allele rs689466 leads to a higher transcriptional activity of the *PTGS2* gene compared to the mutant C allele [4, 10]. Since there is variability in the transcriptional activity of *PTGS2* depending on allelic variants of the rs689466 polymorphism [4, 10], it is likely that this may affect the role of prostaglandin E2 in thermoregulation under cold stress.

In this regard, the aim of this work is to analyze the relationship of the rs689466 polymorphism of the *PTGS2* gene with the irisin level in blood plasma in Yakuts living in cold climatic conditions.

Material and Methods. *Subjects.* The study involved 263 Yakuts (183 women and 80 men), with an average age of 19.73±1.99 years. At the time of the study, none of the participants had any health complaints. The study participants filled out a questionnaire on their own, indicating their gender, ethnicity, and age. All participants gave written informed consent to participate in the study. Study was approved by the local Biomedical Ethics Committee at the Yakut Scientific Center of Complex Medical Problems, Siberian Branch of the Russian Academy Scientific of Medical Sciences, Yakutsk, Russia (Yakutsk, Protocol No. 16, and 13 December 2014).

Anthropometric parameters. Anthropometric parameters (body weight in kilograms, height in centimeters) were measured in all participants using standardized methods. Body mass index (BMI) was calculated by dividing body mass by the square of height. The sample was divided into three groups according to BMI categories [11]: underweight (≤18.49 kg/m^2), normal weight (18.5–24.99 kg/m^2), and overweight/obese (≥25 kg/m^2).

ELISA of irisin levels. Irisin levels in fasting blood plasma (μg/ml) were determined using an enzyme-linked immunosorbent assay (ELISA) "Irisin ELISA BioVendor" (BioVendor-Laboratorni medicina A..S., Czech Republic). Irisin concentration in the samples was measured at a wavelength of 450 nm on a VICTOR X5 Multilabel Plate Reader Reader (Perkin Elmer Inc., USA).

PCR-RFLP analysis of rs689466 of the PTGS2 gene. Genomic DNA was isolated from blood by phenol-chloroform extraction. Genotyping was performed using PCR-RFLP analysis. The original oligonucleotide primers were selected using the FastPCR program (http://primerdigital.com/). The following primer sequences were used for rs689466 of the *PTGS2* gene: F: 5'-ATGAGTTGTGACCATGGATCAA-3', R: 5'-AAAAACCTCCAAGTGAGTCTCTT-3'. Detection was performed using standard PCR on a T100 Thermal Cycler (Bio-Rad, Hercules, USA). The PCR conditions for rs689466 were as follows: denaturation-95°C (5 min), annealing-58°C (45 sec), elongation-72°C (7 min), a total of 30 cycles. Restriction fragment length polymorphism (RFLP) analysis was performed using endonuclease *Bst4C* I (SibEnzyme, Russia), in accordance with the manufacturer's recommendations. After incubation with *Bst4C* I, the T allele of rs689466 remains intact (432 bp), while the C allele is split into 295 bp and 137 bp. The hydrolysis products were separated in horizontal electrophoretic chambers in 2% agarose gel. Electrophoregrams were visualized using gel-video documentation systems from Bio-Rad (Hercules, USA).

Statistical analysis. The data obtained were analyzed using the statistical program Statistica 13.5 (TIBCO Software Inc., USA). Quantitative results are presented as "mean ± standard deviation". The frequency of genotypes of the rs689466 polymorphism of the *PTGS2* gene in the Yakut population (n=263) was checked for compliance with the Hardy-Weinberg equilibrium using the χ^2 criterion. To check the normality of the distribution, the Kolmogorov-Smirnov test was performed. Associations between the rs689466 genotypes of the *PTGS2* gene

Table 1

Average irisin levels (μg/ml) in men and women, taking into account BMI

BMI categories	Women	Men
Underweight	7.88±1.96 (n = 25)	8.52±2.64 (n = 11)
Normal weight	8.43±2.94 (n = 142)	7.65±1.66 (n = 60)
Overweight	8.27±1.96 (n = 16)	9.17±2.11 (n = 9)

Table 2

Associative analysis of irisin levels and anthropometric parameters with rs689466 genotypes of *PTGS2* gene in Yakut population

Parameters	Mean ± standard deviation		*U*	*p*
	TT (n=114)	CT+CC (n=88)		
Irisin, μg/ml				
W	8.47±3.05 (n=84)	8.38±2.8 (n=58)	2397	0.87
M	**8.2±1.85** (n=30)	**7.1±1.25** (n=30)	**261**	**0.005**
Weight, kg				
W	55.87±6.28 (n=84)	55.1±5.10 (n=58)	2345	0.71
M	**63.6±6.67** (n=30)	**67.93±7.28** (n=30)	**279**	**0.01**
High, cm				
W	160.76±6.3 (n=84)	161.26±5.7 (n=58)	2332	0.67
M	172.17±5.84 (n=30)	174.37±5.71 (n=30)	382	0.32
BMI, kg/m^2				
W	21.59±1.71 (n=84)	21.17±1.48 (n=58)	2086	0.15
M	21.44±1.86 (n=30)	22.31±1.86 (n=30)	331	0.08

Note: U – the Mann-Whitney criterion; p – level of statistical significance; W – women; M – men; statistically significant differences are highlighted in bold (p<0.05)

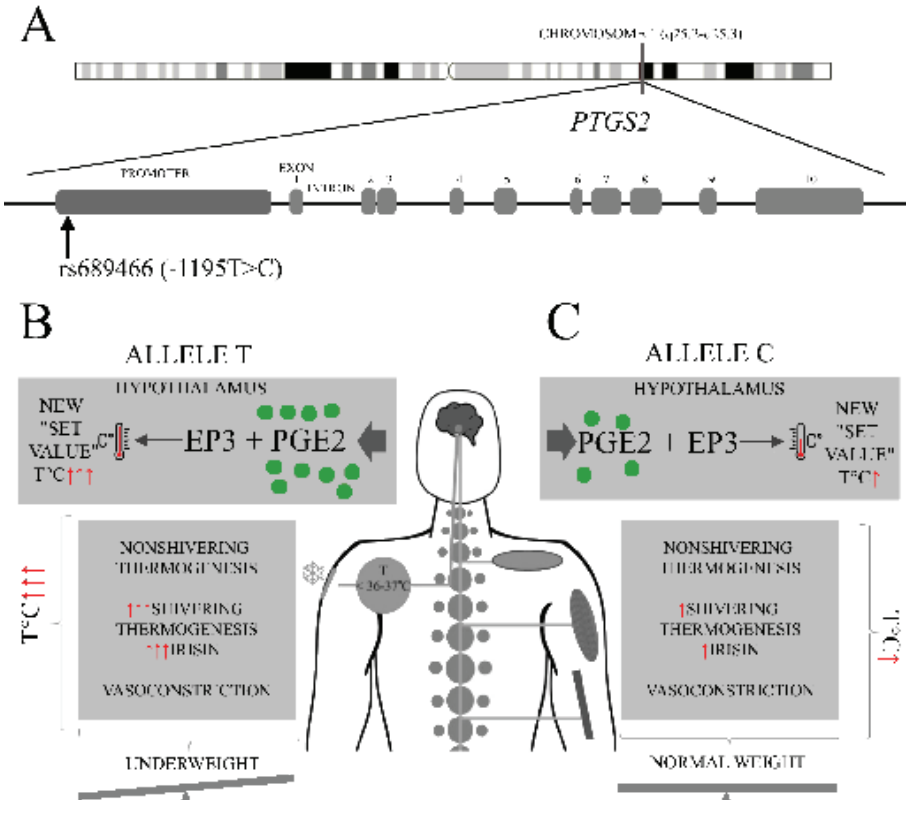

Figure. Prostaglandin E2 (PGE2) in thermoregulation mechanisms during cold stress. **A** – Localization of the *PTGS2* gene on chromosome 1 (q25.2-q25.3) and the structure of the gene indicating the location of rs689466 in the promoter region [Anyona et al., 2020]. **B** – the mechanism of PGE2 thermoregulation and its effect in carriers of the T allele rs689466 of the *PTGS2* gene. **C** – the mechanism of thermoregulation of PGE2 and its effect in carriers of the C allele rs689466 of the *PTGS2* gene.
Note: T°C is body temperature, EP3 is prostaglandin E2 receptor, ↑ is a slight increase, ↑↑↑ is a strong increase ❄ is the effect of cold on the body.

and irisin levels, weight, height, and BMI were analyzed using the Mann-Whitney *U*-test. The values of $p<0.05$ were considered statistically significant.

Results and Discussion. *Frequency distribution of alleles and genotypes of the rs689466 polymorphism of the PTGS2 gene.*

To search for a possible role of rs689466 allelic variants of the *PTGS2* gene in thermoregulation under cold stress, associative analysis of rs689466 genotypes with irisin levels in blood plasma was performed in Yakutia residents living in extremely cold climatic conditions of Eastern Siberia. The frequencies of alleles and genotypes of the rs689466 polymorphism of the *PTGS2* gene were determined in the Yakut population (n=263). The frequency of the normal T allele was 75%, and the frequency of the mutant C allele was 25%. The frequency of occurrence of the TT genotype was 55%, the heterozygous CT variant was 40%, and the CC genotype occurred with a frequency of 5%. The frequency distribution of rs689466 genotypes in the Yakut sample (n=263) corresponded to the Hardy-Weinberg equilibrium ($\chi^2=1.366$, $p=0.24$).

Irisin level depending on the genotypes rs689466 of the PTGS2 gene. Mean irisin plasma levels in women (n=183) and men (n=80), taking into account BMI, are presented in Table 1. For an associative analysis of irisin levels with rs689466 genotypes, the rare CC genotype was combined with the heterozygous CT genotype (CT+CC). The analysis was performed separately for men (n=60) and women (n=142) of normal weight. The Kolmogorov-Smirnov test revealed that irisin levels in Yakuts with normal weight (n=202) did not meet the criteria for normal distribution ($D=0.122$; $p<0.01$), so the association analysis was performed using the nonparametric Mann-Whitney *U*-test. As a result, significant associations were found in men, but not in women (Table 2). In carriers of the TT genotype, irisin levels (8.2±1.85 μg/ml) were statistically significantly higher ($U=261$, $p=0.005$) compared to the CT+CC genotypes (7.1±1.25 μg/ml) (Table 2). Additional analysis of the association of rs689466 genotypes with anthropometric parameters (weight, height, and BMI) (Table 2) showed that men with the TT genotype (63.6±6.67 kg) had a lower weight than men with the CT+CC genotypes (67.93±7.28 kg; $U=279$, $p=0.01$).

Possible mechanism of action of prostaglandin E2 in cold stress. Under conditions of thermoneutrality, for optimal life activity, the body temperature is kept in the range of 36-37°C [9]. Cold stress leads to a decrease in body temperature (<36-37°C), which in turn stimulates the synthesis of prostaglandin E2 [12]. To protect the body from hypothermia, prostaglandin E2 acts on the EP3 receptor in the preoptic region of the hypothalamus, which leads to the activation of emergency thermoregulation mechanisms similar to a fever [12].

Most likely, the higher the level of prostaglandin E2, the higher the new "set value" of body temperature will be. Since the T allele rs689466 of the *PTGS2* gene is characterized by higher transcriptional activity [4,10], we assume that carriers of the TT genotype should have higher levels of prostaglandin E2, which should lead to a higher "set value" of body temperature. As a result, carriers of the TT genotype will have a more intense or longer stage of shivering thermogenesis and, consequently, increased irisin levels in the blood than those with the CT and CC genotypes (Figure).

However, with constant exposure to cold, as in Yakutia, where winter lasts about 6 months, and the temperature of the atmospheric air during this period varies from -60°C to -20°C, the mechanism of thermoregulation by the type of febrile reaction can greatly deplete the body. Therefore, we assume that the relatively low weight of carriers of the TT genotype, compared with carriers of the CC and CT genotypes, is due to the fact that macronutrients coming from food are consumed for more intensive or prolonged shivering thermogenesis, and not for the storage function, since in cold climatic conditions the body is primarily aimed at maintaining thermal homeostasis. In turn, the adaptive role of the allele variant T rs689466 of the *PTGS2* gene is probably associated with protective mechanisms directed against extremely low atmospheric temperatures, to prevent rapid hypothermia and cold injury.

Conclusion. In the present study, the TT rs689466 genotype of the *PTGS2* gene was found to be associated with an increased irisin level and with a reduced weight in men, which may indicate the

effect of prostaglandin E2 on shivering thermogenesis under cold stress. We suggest that the increased transcriptional activity of the *PTGS2* gene in the rs689466 T allele may play a role in human adaptation to cold climates.

The work was carried out within the framework of the Yakutsk Scientific Center for Complex Medical Problems "Study of the genetic structure and burden of hereditary pathology of populations of the Republic of Sakha (Yakutia)", the State Assignment of the Ministry of Science and Higher Education of the Russian Federation (FSRG-2023-0003).

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SCIENTIFIC REVIEWS AND LECTURES

S.D. Nusugurov, A.F. Potapov, A.A. Ivanova, T.A. Grigoriev

UNINTENTIONAL INTRAOPERATIVE HYPOTHERMIA IN ONCOLOGICAL SURGERY AND MAINTAINING NORMOTHERAPY AS PREVENTION OF CARDIAC COMPLICATIONS: THE CURRENT STATE OF THE ISSUE

DOI 10.25789/YMJ.2023.82.21

УДК 616-006.36 : 616-039.71

A review is done of the latest research on unintentional intraoperative hypothermia (UIH) in oncological surgery, as well as its contribution to cardiac complications. Data are presented on the risk factors for developing UIH, the impact of surgery duration and type, as well as different anesthesia types and methods on the stage of the patient's hypothermia. Data on the relationship between the severity of UIH and the surgical profile of a patient and the patient's comorbidity were studied. It was revealed that cancer patients are at risk of developing UIH in the perioperative period, indicating the importance of preventing hypothermia during the surgery and anesthesia. The results of research on the undesirable effects of UIH, the impact of hypothermia on the development of various events, including cardiac complications, were analyzed. Data on the prevention of UIH and the methods of its prevention were systematized. The potential of maintaining the patient's normothermia for reducing the risk of developing cardiac complications in the immediate postoperative period is shown.

Keywords: unintentional intraoperative hypothermia, cardiac complications, temperature monitoring, active warming, early postoperative period.

NUSUGUROV Semyon Dmitrievich – post-graduate student of the Medical Institute of M.K. Ammosov North Eastern Federal University, anesthesiologist-resuscitator of the State Budgetary Institution of the Republic of Sakha (Yakutia) Yakut Republican Oncological Dispensary, nusugurovsemen@gmail.com; **POTAPOV Alexander Filippovich** – MD, Associate Professor, Prof. Medical Institute M.K. Ammosov NEFU, potapov-paf@mail.ru; **IVANOVA Albina Ammosovna** – MD, Associate Professor, Head of the Department of the Medical Institute of M.K. Ammosov NEFU, iaa_60@mail.ru; **GRIGORIEV Timofey Alexandrovich** – head SBI RS(Y) Yakut Republican Oncological Dispensary", grigorievtemu4in@mail.ru.

Unintentional intraoperative hypothermia (UIH) is a decrease in core temperature (CT) of the patient's body below 36°C during surgery. UIH is caused by adverse factors of surgical treatment that increase body heat loss (the operating room temperature, immobility of the patient, opening of body cavities and their

irrigation with solutions); the initial state of the patient; the severity of the underlying and concomitant pathology; as well as the effect of anesthesia on thermoregulation mechanisms [5].

This article reviews the scientific literature on UIH in oncological surgery, the factors for its development, the complications of UIH, and the methods for its prevention. The search for articles was carried out in public databases published in English and Russian before December 31, 2022. The following terms or their combinations were used as search queries: in Russian – «гипотермия», «анестезия», «непреднамеренная интраоперационная гипотермия», «периоперационная гипотермия», «озноб», «согревание», «активное согревание», «абдоминальная хирургия», «онкология», «онкологическая хирургия», «кардиальные осложнения», «абдоминальная онкологическая патология», «послеоперационное осложнение», «ишемическая болезнь сердца», «нарушения ритма сердца», «ишемическая болезнь сердца»; in English – "hypothermia", "anesthesia", "intraoperative perioperative hypothermia", "unintentional intraoperative hypothermia", "shivering", "warming", "active warming", "abdominal surgery", "oncology", "oncological surgery", "abdominal oncological pathology", "postoperative complication", "cardiac events", "ischemic heart disease" , "cardiac arrest", "cardiac arrhythmia", and "cardiac complications". To ensure the quality of the search, the selection of relevant studies was done manually, with a selection of articles published predominantly in the past five years.

Incidence, factors and mechanisms for the development of unintentional hypothermia during surgery and anesthesia. According to Sabbag et al., the proportion of patients with temperatures <35°C was 19.1%, those with temperatures <36°C – 64% [27]. In 2017, in a multicenter retrospective study, the all-Russian public organization "The Federation of Anesthesiologists and Resuscitators" analyzed 5,733 case histories of patients in intensive care units (ICU) at various clinical centers of the Russian Federation (Moscow, Novodvinsk, Arkhangelsk, Krasnodar, Yakutsk, and Chita). The study showed that more than 70% of the patients were moved to the recovery room in a state of hypothermia, with the average body temperature of the patients upon admission to the ICU at 33.6°C (between 32.9 and 34.3°C) [13].

Unlike therapeutic hypothermia, which is used in a number of neurosurgical or cardiovascular procedures, UIH occurs spontaneously and is due to multiple factors. First of all, it is a microclimate of the operating room. A decreased temperature of the operating room in winter can be caused by structural defects in walls and windows, as well as insufficient heating; whereas in summer it is due to climate equipment used for creating comfortable working conditions for the surgical team [28]. Therefore, with no passive heating by additional surgical clothing starting from the preoperative room and active heating of the patient during the surgery, their body temperature decreases inevitably.

Undoubtedly, one of the main factors in the development of UIH is the duration and type of surgery. During long abdominal surgeries, UIH is much more frequent, as shown in a study by Sabbaq et al. [27]. During laparotomic surgical interventions, the area of heat dissipation due to the peritoneum increases, and moisture evaporates, which explains the incidence of UIH during open surgery. Surgical peritoneal lavage with saline or room-temperature chlorhexidine bigluconate solution also leads to a rapid decrease in core temperature [8]. However, with longer duration, UIH can also be observed during minimally invasive operations. UIH occurs in 29% of the patients undergoing abdominal closed surgery, according to a study by Chen et al. [32]. In a study by Li, Liang and Feng, hypothermia was detected in 72.7% of adult patients during video-assisted thoracoscopic surgeries [28]. During endoscopic operations, dry and cold carbon dioxide is injected into the abdominal cavity. Carboxyperitoneum may be a risk factor for UIH, since insufflation of dry and cold carbon dioxide reduces the patient's core temperature, as shown by a prospective observational study by Groene et al. [22]. Again, the time factor is of decisive importance. Operations lasting more than 2 hours are characterized by a higher incidence of UIH, which can be considered as a specific risk factor for the development of UIH.

The initial state of the patient, the severity of the underlying pathology for which the surgery is performed, as well as the severity of concomitant pathology and factors such as old age, excessive weight, and others are also of significant importance for the development of UIH [27].

Concomitant chronic pathologies, like diabetes mellitus, hypothyroidism, cardiovascular diseases, worsen the body's thermoregulatory capabilities. Asthenic patients with severe malnutrition are initially most prone to hypothermia, due to metabolic disorders in the body. In this category of patients, preoperative hypothermia guarantees a continued decrease in temperature during surgery and can lead to serious consequences.

In elderly patients, hypothermia is more pronounced, since anesthetics demonstrate more pronounced vasodilatory effect on people over 60 years of age [18]. Today, such preoperative characteristics as age, height, weight, high scores by the ASA (American Society of Anesthesiologist) scale, heart rate and systolic blood pressure are considered to be prognostic signs of UIH [17].

Of direct importance in the initiation of hypothermia is anesthetic support, which contributes to violated thermoregulation. In a healthy awake person, the body temperature is maintained by behavioral and vegetative regulation when the threshold temperature is reached. During anesthesia, there are no behavioral reactions and only the vegetative mechanism of body defense and external control of thermoregulation are implemented [5]. Normally, the threshold temperatures for vasoconstriction and shivering are 36.5-36°C, respectively, which decrease by 2-3°C during general anesthesia. In addition, autonomic reactions worsen, since most anesthetics increase thermal response and reduce cold threshold reactions; the "inter-threshold range" can increase 10-fold (from 0.3°C to 2-4°C), which delays the start of the thermoregulatory defense mechanism.

The main mechanism of hypothermia during general anesthesia is vasodilation (with simultaneous suppression of vasoconstriction), which occurs in response to numerous drugs that are part of the premedication (opioid analgesics, benzodiazepines), induction (propofol, sodium thiopental), or inhalation anesthetics to maintain anesthesia (sevoflurane, isoflurane, desflurane) [5]. Vasodilation shifts the centralized blood flow to the periphery, which disrupts the leading mechanism for maintaining temperature homeostasis. In the periphery, warmed central blood consumes the accumulated heat through irradiation, which leads to a gradual decrease in core temperature of the body [5]. In addition, the infusion of insufficiently heated solutions reduces the temperature of circulating blood [28].

There are three phases in the development of hypothermia during anesthesia: the initial rapid decrease, the slow linear decrease, and the plateau phase [5]. The first phase is observed in the 1st hour of anesthesia and is characterized by a decrease in CT by 0.5-1.5°C and a simultaneous increase in peripheral

temperature from 33 to 35°C due to vasodilation and redistribution of heat from the center to the periphery. The second phase of hypothermia, in the next 2-4 hours of the surgery, is due to the excess of heat loss over metabolic heat production. After 3-4 hours, the plateau phase follows, when body temperature stabilizes, since peripheral vasoconstriction initiated by a decreased body temperature reduces both metabolic heat production and heat transfer from the core to the periphery [26].

Violation of thermoregulation is also observed during neuraxial anesthesia, when the patient's behavioral response and the autonomic defense mechanism are also excluded. Like with general anesthesia, spinal anesthesia is characterized by decreased threshold values of thermoregulation and thermal redistribution from the core to peripheral tissues. With the use of this method of anesthesia, another mechanism of hypothermia is the blockade of the sensory impulse about a reduced temperature from blockade zones to thermoregulatory centers. A significant predictor of core hypothermia during spinal anesthesia is the level of blockade. The correlation between high blockade and low core temperature during spinal anesthesia is consistent with the known physiological effects of spinal anesthesia: the larger the area of blockade, the greater the expected impaired thermoregulation [26].

Given these data, it can be assumed that the combination of general anesthesia with regional anesthesia will increase the risk of intraoperative decrease in body temperature.

An analysis of the literature on UIH shows that the problem of hypothermia is also relevant for cancer patients. Thus, a study by the Morozumi's group revealed that intraoperative hypothermia occurs quite often and, moreover, can be an important predictor of recurrence and survival in stage II muscle invasive bladder cancer [23]. In this study, 68 (55%) of 124 patients who experienced hypothermia during radical cystectomy, with no difference in the number of postoperative complications, had a higher recurrence rate within 12 months (p=0.013).

Analyzing the results of two-year survival after radical cystectomy in 852 patients, the study by Timothy et al. led to opposite conclusions [16]. In this study, despite active rewarming with the Bair Hugger patient warming system, UIH was registered during surgery in 274 (32%) patients, among whom 37 (4.3%) patients had profound hypothermia (t<35.0°C). At the same time, there was no statistically significant association of hypothermia with two-year survival, excluding UIH as a predictor of cancer outcomes among the patients undergoing radical cystectomy.

An analysis of 1,547 colorectal procedures revealed that the incidence of intraoperative hypothermia was 67.0% and was higher for laparoscopy than for laparotomy (71.23% vs. 63.16%; chi-square $P = 0.001$). In addition, there were significant differences in the severity of hypothermia [25].

The relationship between body weight and the incidence of UIH is evidenced by a study by Motamed et al., who showed that the average incidence of hypothermia was 21% in the patients operated on for breast neoplasms. At the same time, the body mass index (BMI) was significantly lower in the hypothermia group – 23.5 ± 4.1 compared to 26.4 ± 6.1 kg/m^2 in normothermic patients (p<0.05) [21].

In 2018, a group of researchers led by Tai conducted a similar study on mice, the results of which indicated that there is a significant risk of metastases in case of sepsis induced by hypothermia and massive blood loss [33].

Thus, cancer patients may represent a specific group in terms of the risk for developing perioperative hypothermia.

Complications of unintentional intraoperative hypothermia. Hypothermia during surgery is not a physiological condition and can be accompanied by adverse effects both during surgery and lead to undesirable consequences in the postoperative period. It is now recognized that perioperative hypothermia can have a negative impact on many vital systems of the human body [5]. Hypothermia has been proven to be involved as a factor that reduces the activity of the blood coagulation system, increases the likelihood of cardiac arrhythmias, myocardial ischemia, increased blood loss, increased duration of postoperative wound healing, the occurrence of septic complications, which together increases the total number of complications, the consumption of medications, the duration of hospitalization, and postoperative mortality [16].

With UIH, there are also violations of the blood coagulation system. The studies by Tsarev revealed a dependence of the risk for developing coagulopathy (decrease in the values of the international normalized ratio) on hypothermia in patients with polytrauma [2]. A decreased activity of the coagulation system factors results in bleeding of wounds, which in the future leads to repeated interventions and the need for blood transfusions.

A meta-analysis of 384 studies conducted by a group of scientists from the PRC showed that perioperative hypothermia can significantly increase the risk for surgical infection [15]. Poveda et al. did a detailed review with a meta-analysis of 956 publications of 9 studies that were devoted to the study of the relationship between intraoperative warming of patients and infectious complications and are available in the PubMed, CINAHL, LiLACS, CENTRAL and EMBASE databases. They came to the conclusion that additional randomized clinical trials are needed. trials to evaluate the effectiveness of UIH prevention as a factor in preventing infection in the surgical site [24].

Hypothermia leads to prolonged effects of anesthetics and muscle relaxants, later awakening time and delayed extubation of patients.

Hypothermia changes the level of potassium in the blood serum. An analysis of 50 clinical and experimental studies evaluating the effect of hypothermia on potassium levels, performed by Buse et al. identified the main pathophysiological mechanisms that explain fluctuations in blood potassium levels with temperature [30]. At the beginning of hypothermia, hypokalemia is observed, associated with its intracellular shift due to increased functioning of the Na, K-ATPase, beta-adrenergic stimulation, pH shift, and membrane stabilization. Then, with aggravation of hypothermia due to insufficient activity of enzymes, an increased level of potassium occurs.

The most formidable complications of hypothermia occur in pronounced, severe forms of hypothermia, when core temperature drops below 35.2°C. A decreased body temperature leads to spasm of the coronary vessels, an increased oxygen consumption by the heart muscle, which can lead to myocardial ischemia. Cardiac arrhythmias are potentially life-threatening for the patient and may result in cardiac arrest [10]. Hypothermia during surgery leads to a slower impulse conduction, which can lead to varying degrees of atrioventricular block. Postoperative shivering and tachycardia also negatively affect the cardiovascular system, increasing myocardial oxygen demand and exacerbating existing cardiac pathology, especially in debilitated patients [12].

Currently, cardiology guidelines consider adequate correction of perioperative hypothermia and prevention of postoperative shivering to be important components of anesthesia management as a method of preventing myocardial injury [4].

There is no doubt that the number of negative consequences of UIH will depend not only on the surgical access, but also on the number and depth of comorbidities. Thus, high-risk patients should include oncological patients, who are characterized by weight loss, residual intoxication due to chemotherapy prior to surgery, and often concomitant chronic pathologies, cardiovascular diseases in particular [10], with 78.2% of oncological patients having concomitant coronary artery disease [12]. Hypothermia is also often accompanied by cardiac arrhythmias and arterial hypertension, and triples the incidence of myocardial ischemia [11].

It should be noted that in most cancer patients, surgical treatment is preceded by radiation or chemotherapy. At the same time, the basic chemotherapy drugs used in the treatment of neoplasms have a wide range of toxic effects, including cardiotoxicity, causing damage to cardiomyocytes, endocardium and heart valves, development of myocardial dysfunction and/or heart failure. The incidence of complications, the onset of clinical manifestations, and the severity of manifestations of toxicity vary depending on the selected anticancer treatment, the dose of the drug, and the presence of concomitant cardiovascular diseases. The combination of chemotherapy or their combination with radiation therapy can aggravate the cardiotoxic effect [7].

As proven by the study on the impact of UIH on the cardiac complications incidence, unintentional hypothermia is an independent risk factor for early postoperative complications. Thus, a retrospective analysis of 121 cases of radical esophagectomy revealed that 51 (96.2%) out of 53 patients with early postoperative complications had UIH. Among the complications, 8 cases of cardiac arrhythmia were recorded, making 11.1% of all early postoperative complications.

The study of UIH as a predictor of early postoperative complications in patients with bladder cancer who underwent cystectomy showed its significance in the development of various complications [11]. The study involved 124 patients, of which 68 (54.8%) patients experienced hypothermia during surgery. Complications were observed in 22.1% of the patients who experienced hypothermia and in 14.3% of the patients without hypothermia. At the same time, cardiac complications were noted in 12.5% and 6% of cases, respectively, in groups with and without hypothermia.

Therefore, the presented data show a close relationship between UIH and postoperative complications, including cardiac complications, in cancer patients, which indicates the importance of preventing hypothermia at the stages of surgery and anesthesia.

Prevention of unintentional intraoperative hypothermia and methods of warming patients. Undoubtedly, the methods of active rewarming of patients in the perioperative period significantly affect the severity of UIH and its complications [4]. However, at present, despite the relevance of the problem with UIH, intraoperative thermometry has not yet become a routine practice in anesthesiology, like a control of hemodynamics or respiration. Studies around the world indicate low compliance with recommendations for temperature management in the perioperative period. For instance, according to the European group TEMMP (Thermoregulation in Europa Monitoring and Managing Patient Temperature), which studies the compliance with the temperature regime of patients undergoing surgical interventions, special warming methods in the perioperative period are applied to only 20% of patients [18].

Currently, UIH prevention uses methods aimed at various mechanisms of hypothermia in each phase of the perioperative period [34, 29]. In order to prevent the occurrence of UIH, a number of clinics have developed guidelines for the personnel of operating and anesthesia teams [20]. The FAST TRACK strategy for accelerated surgical treatment and rehabilitation, which is widely implemented in world and Russian medicine, includes mandatory temperature management and prevention of perioperative hypothermia [6]. The latest orders of the Ministry of Health of the Russian Federation on the procedure for providing medical care in the field of "anesthesiology and resuscitation" regulate the presence of insulating blankets in the operating room and in the recovery room of the intensive care unit [8, 9].

Today, it is recommended to start warming from the moment the patient enters the preoperative room [14]. For example, following the recommendations of the Association of Scientific Medical Societies in Germany on pre-warming of patients has led to a considerable and clinically significant reduction in the incidence of UIH. Currently, the incidence of hypothermia in German clinics is 15.8% during surgery and 5.1% after surgery [17].

To preserve the accumulated heat, the patient is covered with heat-insulating blankets before the surgery; special blankets with active warming mechanisms and blankets with chemical reagents have been developed, the operation of which does not require an additional power source or heating control unit [36]. Active heating systems are also applied, using warm air blown under clothing or a drape, which further reduces the severity of hypothermia during surgery [31].

After providing vascular access, at the stage of anesthesia induction, it is proposed to use heated solutions for intravenous infusions, since a certain amount of heat is spent on warming the infusion media entering the body. Therefore, rewarming with infusion solution can be used as an additional measure to rewarm the patient [3]. According to the data obtained by Stolyarov and his colleagues, correction of perioperative hypothermia by heating solutions in emergency surgical patients in 92.4% of cases prevents perioperative hypothermia and its clinical manifestations [4]. Beccera et al. believe that even short-term, for 5-15 minutes, preliminary active warming of the patient before laparoscopic urological surgery allows maintaining a significantly higher temperature during the intraoperative period compared to patients who are not pre-warmed [33]. Yoo et al. consider active forced heating with air at a temperature of 47°C at the heater output from the induction period as a simple and effective method for preventing UIH during surgeries lasting more than 120 minutes [19].

In open surgical interventions, the use of heated solutions for cavity irrigation is proposed, which reduces the combined losses caused by moisture evaporation and heat convection from the cavity. In closed surgical interventions, it is also necessary to use heated and humidified carbon dioxide during insufflation into the abdominal or pleural cavities. The heated gas prevents the development of intraoperative hypothermia, maintains the patient's basal body temperature, and may even increase it [35].

A noteworthy fact is the development of hypothermia during surgery even in patients who are subjected to active warming methods. In fact, hypothermia occurs not only during the surgery itself, but begins to develop even at the preparatory stage for the surgery: during the preparation of the patient, introduction to anesthesia, and can also continue in the first hours after the surgery in the recovery room or in the intensive care unit. Therefore, many researchers studying UIH emphasize the need to develop methods for predicting and identifying the patients at risk of intraoperative hypothermia; optimizing measures for thermoregulation; and the need for additional research on studying methods of active warming and

introducing innovations in the technology of active warming of patients [1].

Conclusion. Hypothermia in the perioperative period remains one of the relevant issues in modern anesthesiology [3]. An analysis of the studies presented in the literature on the incidence of UIH and its role as a factor contributing to the development of complications affecting the treatment outcomes for surgical patients shows that UIH is often studied in a generalized group of patients, without distinguishing individual nosological categories. To date, in oncological patients, the temperature profile when using various methods of intraoperative active warming and the effect of UIH on the development of cardiac complications have not been sufficiently studied, and data on core temperature depending on the methods of surgical access in the perioperative period have not been presented. There is inconsistency in the data, which can be explained by the heterogeneity of the sample of study groups in terms of underlying and concomitant diseases, types of surgeries, age, and other factors.

Considering the foregoing, monitoring of core temperature and assessing the effect of UIH on the development of cardiovascular complications in patients with oncological pathology is relevant, and prevention of cardiac complications based on maintaining normal core temperature in the perioperative period seems a promising and needed research.

Reference

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DOI 10.25789/YMJ.2023.82.22

УДК: 577.112.856: 611.018.74: 616.12

O.N. Poteryaeva, I.F. Usynin

ENDOTHELIAL PROTECTIVE FUNCTIONS OF HIGH DENSITY LIPOPROTEINS

The development of cardiovascular diseases inversely depends on the cholesterol level of high density lipoproteins (HDLs). On the other hand, it is known that pathogenesis of many cardiovascular diseases is based on endothelial dysfunction. Such facts indicate a special role of this class of lipoproteins in the functioning of endothelial cells. Upon binding to various receptors on endothelial cells, HDLs initiate the induction of endothelial nitric oxide synthase, enhance the production of NO, and stimulate the synthesis of prostacyclin, thus leading to vasorelaxation. By suppressing the synthesis of intercellular adhesion molecules, HDLs prevent the migration of leucocytes and monocytes/macrophages into the vascular wall, exerting anti-inflammatory action. HDLs inhibit the production of reactive oxygen species, prevent apoptosis, and stimulate the proliferation and migration of endothelial cells. Understanding the mechanisms of the protective action of HDLs on vascular endothelium is a necessary stage in the development of new therapeutic agents with the endothelial protective properties.

Keywords: endothelial cells, high density lipoproteins, apoptosis, angiogenesis, cardiovascular diseases.

Introduction. Vascular endothelium, which is located on the boundary between circulating blood and cells of organs and tissues, performs not only the barrier function. It is the key regulator of vascular homeostasis, which maintains a balance between vasodilation and vasoconstriction, inhibition or stimulation of the migration and proliferation of myocytes, fibrinolysis and thrombosis, and is involved in the regulation of intercellular adhesion and aggregation of thrombocytes. Endothelial dysfunction underlies the pathogenesis of many cardiovascular diseases [3].

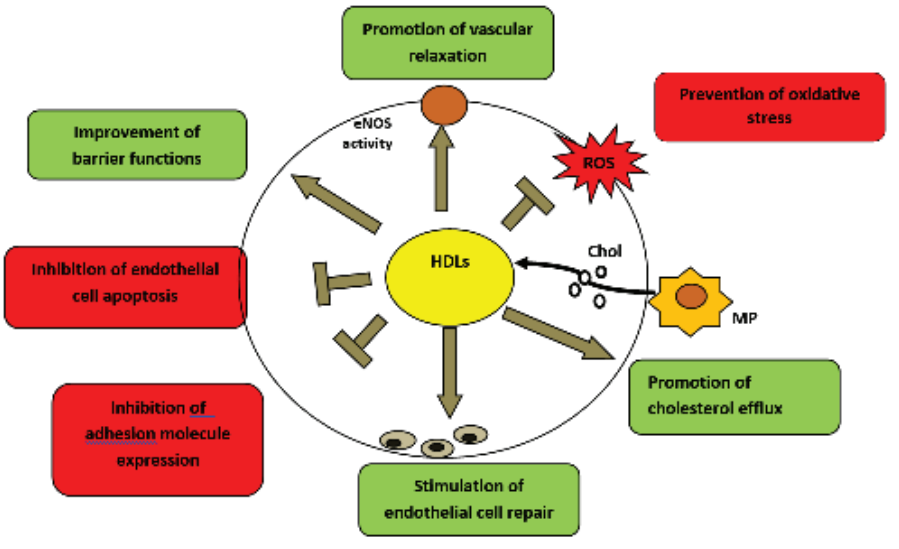
Reactive oxygen species (ROS), oxidized low density lipoproteins (LDLs) and very low density lipoproteins (VLDLs), and free radicals disturb the ability of endothelium to synthesize nitric oxide (NO). The action of inflammation mediators and proinflammatory cytokines leads to the synthesis of P- and E-selectins on the endotheliocyte membrane as well as monocytic chemotactic protein-1 (MCP-1), tumor necrosis factor a**The regulation of vascular tone.** It is known that binding, internalization and transport of HDLs through endothelial cells are carried out by the following proteins: scavenger receptor class B type I (SR-BI), ATP-binding cassette transporter G1 (ABCG1), endothelial lipase, and ecto-F1-ATPase. Each of them contributes to vascular homeostasis. In addition, endothelial cells express sphingosine-1-phosphate receptors (S1PR) for S1P – the bioactive lipid, 50-70% of which is transferred by HDLs (S1P-HDLs) [43]. The binding of HDL/apoA-I or S1P to receptors not only leads to transendothelial transport of lipids, but also triggers some intracellular signaling events that are accompanied by potential vasoprotective effects (Fig.1). HDLs initiate the induction of endothelial nitric oxide synthase (eNOS), which enhances the synthesis of NO and leads to vasorelaxation; they suppress the synthesis of endothelial adhesion molecules and prevent the migration of leucocytes and monocytes/macrophages into the vascular wall, thus exerting anti-inflammatory action. HDLs inhibit reactive oxygen species and apoptosis; they provide the proliferation and migration of endothelial cells, angiogenesis and re-endothelialization [35,49]. It was shown that HDLs in obese persons with diabetes and dyslipidemia lose their ability to endothelial protection [52].

Nitric oxide is the key signaling molecule for maintaining the normal functioning of vessels by regulation of the vascular tone. It prevents the endothelial inflammation and activation of thrombocytes and also diminishes the growth of myocytes. The activity of eNOS can be regulated by physiological concentrations of HDL/apoA-I through phosphorylation by Ser1179 enzyme [36]. The HDL-induced release of NO decreased under the action of N-nitro-L-arginine methyl ether (L-NAME) – a nonselective inhibitor of eNOS. In bovine aorta endothelial cells (BAEC), colocalization of apoA-I and eNOS was revealed using confocal microscopy with immunostaining of these proteins. The interaction between apoA-I and eNOS proceeds most likely in the perinuclear region rather than on the membrane [24]. Antibodies to apoA-I block the HDL-induced activation of eNOS in isolated plasmatic membranes of endothelial cells [35].

The ability to stimulate eNOS in endothelial cells can be mediated by different HDL binding sites. The molecular mechanism of activation can start from the interaction of HDLs with SR-BI and stimulation of phosphatidyl inositol-3-kinase (PI3K), which in its turn gives rise to the parallel activation of serine/threonine protein kinase B (Akt) and mitogen-activated protein kinase (MAPK)/Erk1/2 with subsequent activation of eNOS, which generates NO and vascular relaxation [36,37]. In aortic cell culture of transgenic mice expressing apoA-I$^{-/-}$, a decrease in the amount of phosphorylated proteins Akt and Erk1/2 was observed [25]. This signaling pathway was partially suppressed when SR-B1 was knocked down using small interfering RNA (siRNA), and induction of SR-BI by inhibitors of HMG-CoA reductase (statins) enhanced the activation of eNOS [32].

Another activation mechanism of eNOS starts from the interaction of S1P-HDL with S1PR. S1P exerted a strong vasodilatory action on the wild-type mice aorta. In HUVEC cells, the effects of S1P on phosphorylation of Akt and eNOS depended on its concentration and disappeared completely after the pretreatment with L-NAME. In endothelial cells of mice with a deficit of S1P, phosphorylation of Akt and an increase in [Ca^{2+}] in response to HDLs and S1P were considerably diminished. Earlier it was shown that activation of eNOS is achieved due to mobilization of [Ca^{2+}] from internal supplies and is a prerequisite for the Akt-dependent

POTERYAEVA Olga Nikolaevnам – MD, leading researcher at the Laboratory of mechanisms of intercellular interactions, Institute of Biochemistry, Federal Research Center of Fundamental and Translational Medicine, http://orcid.org/0000-0003-1068-2431; **USYNIN Ivan Fedorovich** – MD in Biology, Head of Laboratory of mechanisms of intercellular interactions, Institute of Biochemistry, Federal Research Center of Fundamental and Translational Medicine, https://orcid.org/0000-0003-1752-9034



Vasoprotective effect of HDLs on the functions of endothelial cells [adapted from 49]. MP – macrophage, ROS – reactive oxygen species, eNOS – endothelial nitric oxide synthase, Chol – cholesterol, and HDLs – high density lipoproteins.

activation of eNOS and NO-dependent vasorelaxation [26].

By now, the effect of HDLs on the NO production via ABCG1 receptors and their interaction with the structural component of caveolae, a cholesterol-binding protein – caveolin, has been studied. Caveolae and caveolins are the most important modulators of signaling transduction in the cell. Caveolins interact with the signaling molecules and modulate their activity; in most cases, they serve as inhibitors [2]. An increase in the cholesterol content in endothelial cells of the lungs of mice on a high-cholesterol diet enhances the interaction between caveolin and eNOS, thus suppressing the catalytic activity of the enzyme and diminishing the release of NO. HDLs abolished the inhibition of eNOS in endothelial cells, but had no effect in mice with a deficit of caveolin. Stimulation of the outflow of cholesterol and oxysterols, which are detected in a large amount in atherosclerotic plaques, with ABCG1 decreases the interaction of eNOS with caveolin and the subsequent phosphorylation of eNOS [14].

It was shown recently that ecto-F1-ATPase is the single receptor involved in the stimulation of NO production by endothelial cells and NO-dependent vasorelaxation, which are induced by the lipid-free apoA-I. The activation of ecto-F1-ATPase under the action of apoA-I in human endothelial cells and in mice aorta led to Ser1179 phosphorylation of eNOS protein through the signaling pathway of various kinases, including Akt, protein kinase A, AMPK, and calcium/calmodulin-dependent protein kinase type II (CaMKK2) [38].

An increase in the level of eNOS protein under the action of HDLs was demonstrated also in endothelial progenitor cells (EPC), which are a subpopulation of stem cells that can differentiate into mature endothelial cells and take part in re-endothelialization and neovascularization processes [12]. In addition, HDL/apoA-I can increase the amount of eNOS protein not by changing the gene transcription, but rather by increasing the half-life of the eNOS protein in endothelial cells of human vessels via the activation of PI3K, Akt and p42/44 MAPK [38].

Studies with experimental *in vitro* and *in vivo* models showed that HDLs stimulate endothelial cells to produce prostacyclin, thus increasing the inflow of arachidonic acid and the expression of cyclooxygenase-2 (COX-2), which produces prostacyclin (PGI2) – a metabolite of arachidonic acid, the vasoactive endothelial lipid mediator. Prostacyclin is a powerful factor that prevents the aggregation of thrombocytes and initiates vasodilation. Different HDL_2 and HDL_3 subtypes promoted the release of PGI2 in endothelial cells in a dose-dependent manner, which was blocked by a specific inhibitor of COX-2 – rofecoxib. The knockdown of SR-B1 receptors also significantly decreased the release of PGI2 [5]. In diabetes mellitus type 2, glycated HDLs lose their ability to enhance the expression of COX-2 and the release of PGI_2 in HUVEC endothelial cells. However, the addition of S1P to dysfunctional HDL restores this ability. The regulation of COX-2 expression included phosphorylation of the signaling pathway MAPK/ERK, which increased phosphorylation of the nuclear transcription factor CREB [51]. The potential contribution of HDLs to vascular homeostasis via increasing the synthesis of PGI2 can be enhanced by statins [33]. Along with the favorable effect on vasodilation, HDL inhibits the synthesis of thromboxane A2, which is a powerful vasoconstrictor of endothelial cells [40].

In persons with familial hypoalphalipoproteinemia and a low level of HDLs, a sharp decrease in both the basal and stimulated activity of NO was observed; this was accompanied by endothelial dysfunction, which was estimated using venous occlusion plethysmography. A single injection of the reconstructed HDLs (rHDLs) consisting of apoA-I and phosphatidylcholine resulted in complete restoration of vasomotor functions [41]. The *in vivo* effect of HDLs was proved also with the use of apoA-I mimetic peptides, which are the artificially synthesized peptides possessing the biological properties of native apoA-I. Oral administration of the peptide imitating the action of apoA-1 (D-4F) to mice with the knockout of LDL receptor (i.e. hypercholesterinemia) improves the endothelium-dependent vasodilation and decreases the thickness of the arterial wall [18].

The regulation of apoptosis. The oxidized LDLs cause a stable increase in the concentration of intracellular calcium, which leads to the death of endothelial cells. It was shown that the rHDLs, which consist of apoA-I, cholesterol and phospholipids, inhibited the apoptosis in endothelial cells. Therewith, the enrichment of rHDLs with plasmalogens or sphingomyelins enhanced their anti-apoptotic activity [46]. Besides, HDLs retained the anti-apoptotic activity also after the knockdown of eNOS with the use of its inhibitor – L-NAME, which testifies that anti-apoptotic activity of HDLs does not depend on the activation of eNOS [42].

HDLs prevent the apoptosis of HUVEC endothelial cells caused by various stimuli. The mechanism of the endothelial anti-apoptotic effect of HDLs depends on the stimulus of apoptosis. Suppression of the apoptosis induced by TNF-α is related to a decrease in the induction of caspase 3, which is a component of all primary apoptotic pathways. The inhibition of apoptosis in the absence of growth factors in a medium is associated with weakening of the mitochondrial pathways of apoptosis. Therewith, HDLs decrease spreading of mitochondrial potential, generation of ROS, release of cytochrome C into cytoplasm, and activation of caspases 3 and 9. By activating Akt, HDLs give rise to phosphorylation of

the Akt target – BAD (Bcl-2, the associated agonist of cell death), which facilitates the detachment of Bcl-2 from Bcl-xL and suppression of apoptosis [35,42]. It was revealed that HDLs isolated from the blood of patients with stable myocardial ischemia or acute coronary syndrome, which contain an increased amount of apolipoproteins C-I or C-III, transformed into strong inductors of apoptosis in vascular myocytes and endothelial cells due to the increased activity of Bcl-2 and expression of proapoptotic protein tBid [37].

The overwhelming majority of proofs of the anti-apoptotic action of HDLs on endothelium were obtained from observations of cell cultures. The effect of HDLs *in vivo* was established when studying the apoA-I mimetic (D-4F) as the anti-apoptotic agent in a rat model of diabetes; D-4F was shown to improve the vascular reactivity and decrease the fragmentation and desquamation of endotheliocytes [16].

The regulation of angiogenesis. Endothelial cells are able to proliferate, migrate and participate in angiogenesis; this ability underlies neovascularization and maintains integrity of the vascular wall. HDLs (in the concentration of 50, 100 and 500 μg/ml) in a dose-dependent manner for 72 h increased the proliferation of HUVEC cells by a factor of 2-5 compared to the control group. Therewith, already in 24 h HDLs reliably increased the migration of such cells and enhanced their ability to form vessel-like endothelial tubes [23]. Fluorescence microscopy using Alexa-568 dye demonstrated a significant increase in lamellipodia (a sign of cell migration) in endothelial BAEC cells under the action of HDLs, which was comparable with the action of vascular endothelial growth factor (VEGF), the main regulator of angiogenesis [7]. D-4F restored re-endothelialization, which was disturbed in the presence of oxidized HDLs, thus promoting the proliferation and migration of human aortic endothelial cells (HAEC) and the formation of lamellipodia. Proliferation of the cells was revealed by immunostaining on PCNA (the nuclear factor involved in replication and reparation of DNA in proliferating cells). The endothelial migration of cells and re-endothelialization were verified by wound repair and transwell analysis (a test for investigating the migration reaction of endothelial cells to angiogenic inductors or inhibitors) [7].

The mechanisms controlling the angiogenic reactions in response to HDLs are related to an increase in the number of receptors for VEGF (VEGFR) and its rapid phosphorylation, i.e. activation. VEGFR2 was shown to be the main receptor for VEGF that mediates angiogenesis in endothelial cells under the action of HDLs. The expression of VEGFR2 depended on the time and HDL dose. The blockade of VEGFR2 activation by SU1498 inhibitor significantly abolished the proangiogenic ability of HDLs. Moreover, S1P3 inhibitor (suramin) prevented the expression of VEGFR2 as well as the subsequent migration of endothelial cells and formation of new vessels, whereas S1P1 agonist (CYM-5442) and S1P2 (JTE-013) inhibitor did not exert any effect [23]. Under normal conditions, HDLs induce through various receptors (ABCG1, S1P and SR-BI) the activation of many signaling pathways that are necessary for physiological angiogenesis, particularly PI3K/AKT, Gi/Ras/ERK and eNOS. This enhances the migration and proliferation of endothelial cells, mobilization of EPC, re-endothelialization, and tubulogenesis [50].

HDLs play the key role in the regulation of angiogenesis caused by hypoxia. When the content of intracellular oxygen decreases, HDLs modulate post-translational modification of HIF-1a (the transcription factor induced by hypoxia 1-a), after which it is translocated into the nucleus. This stimulates the expression of proangiogenic mediators, such as VEGF, angiopoietin, fibroblast growth factor and others. HDLs, after binding to SR-BI receptor on the cell surface, mediate angiogenesis at hypoxia via the signaling pathway PI3K/Akt, modulation of HIF-1α/VEGF, and enhancement of the eNOS activity [50]. The introduction of rHDL/apoA-I increased the levels of VEGF mRNA, facilitated an increase in the density of sural capillaries in the ischemic hind limbs in mice with streptozotocin-induced diabetes mellitus. Local administration of HDLs restored the angiogenesis and formation of coronary collaterals and facilitated wound healing [28].

To confirm re-endothelialization *in vivo*, the area of vascular wall denudation after perivascular electrical injury is measured. This model made it possible to demonstrate that the vascular endothelialization disturbed by electrical injury can be restored by inserting the human apoA-I gene into somatic cells of transgenic mice expressing apoA-I$^{-/-}$ [27]. The effect on the restoration of endothelium *in vivo* was demonstrated in mice on a high-cholesterol diet with the carotids injured by electricity. Healing was slower in the experimental group compared to the control mice: in the 5th day, healing constituted only 27.8% against 48.2%, respectively. The introduction of D-4F enhanced the restoration of endothelium in mice up to 43.4%. In the process, a considerable inverse correlation between healing of endothelium and plasma markers of oxidative stress was observed [13].

In addition, HDLs can stimulate re-endothelialization and neovascularization at the injured sites through differentiation of EPC into mature endothelial cells and their adhesion to the vessel walls [12]. The apoA-I mimetic, D-4F, increased the amount and functional activity (proliferation, migration and formation of capillary tubes) of mice and human EPC [19]. HDLs promoted the angiogenesis disturbed by ischemia via stimulating the differentiation of EPC using the signaling pathway PI3K/Akt [22].

ApoA-I increased the expression of angiopoietin 4 (the protein growth factor that stimulates the formation of blood vessels from the earlier existing ones) in human aortic endothelial cells. This signaling pathway acted through PI3K/Akt/FOX01 (forkhead box protein O1 – a transcription factor) [30].

In mice with the brain injure similar to human amyotrophic lateral sclerosis, apoA-1 decreased the death of endothelial cells in the mice brain (mBEC) via the signaling pathway PI3K/Akt, which was verified by the inhibition with wortmannin (the PI3K inhibitor). Therewith, apoE did not exert such an effect on the cell culture. A considerable increase in the death of endothelial cells upon inhibition of the apoA-I action by monoclonal antibodies has been proved [19].

The effect on barrier functions. HDL-S1P ensure stability and permeability of vessels [22]. In genetically modified mice (apoM–/–), a strong decrease in the plasmatic level of S1P increased plasma exudation into the extravascular tissues [31]. Besides, in apoM–/– mice, two-photon microscopy, which is employed to obtain brain images *in vivo*, revealed an increase in hematoencephalic barrier permeability for small molecules (fluorescent albumin, 45 kDA) and a flow of large proteins mediated by transcytosis (sodium fluorescein, 365 Da and Alexa fluor488, 643 Da). For example, the transfer of fluorescent albumin in arterioles increased by a factor of 3-10. The S1PR1 agonist (SEW2871) rapidly normalized the disturbed permeability and maintained it in all brain microvessels [10]. Earlier it was shown that the absence of apoM in mice disturbed the endothelial barrier in the lungs and brown adipose tissue [47].

HDLs facilitate the integrity of the HUVEC endothelial barrier via the process including S1PR1 and activation of Akt [43]. Recently it was revealed that stimulation of the barrier integrity in human

endothelial cells of microvessels under the action of S1P signaling leads to phosphorylation of AMPK [45].

D. Svensson [11] analyzed the ability of the wild-type apoA-I to weaken the detrimental effect of cathelicidin peptide (LL-37) on the viability of HUVEC endothelial cells. LL-37 is synthesized by granulocytes, lymphocytes and monocytes; after binding to the cell membranes it can initiate the formation of pores in the membrane, thus reducing the cell viability. The apoA-I binding to LL-37 led to a structural rearrangement of the peptide, which decreased its antibacterial action and cytotoxicity. The siRNA knockdown of the apoA-I gene for decreasing the expression of protein in the HepG2 cells, which produce apoA-I, increases the LL-37-induced cytotoxicity.

The anti-inflammatory action. An important property of HDL is the direct action on endothelium, which is associated with its anti-inflammatory effect. In particular, HDLs weaken the expression of adhesion molecules VCAM-1, ICAM-1 and E-selectin in cultured endothelial cells [9,53]. This process is mediated by SR-BI and S1P receptors, PI3K and eNOS [35]. The S1P-HDL induced phosphorylation of AMPK in inhibiting the expression of adhesion molecules was confirmed *in vitro* in HUVEC cells and *in vivo* in mice aortic endothelial cells. The introduction of the AMPK activator (AICAR) in mice under natural conditions for three days stimulated the phosphorylation of AMPK followed by activation of eNOS and inhibiting the expression of VCAM-1, migration of monocytes and their adhesion to endothelial cells. Therewith, the activation of AMPK and eNOS was completely suppressed by siRNA to CaMKK2 lying above the AMPK level, or STO-609, the specific inhibitor of CaMKK2 [34].

The key role in the modulation of cell responses to inflammation belongs to the nuclear transcription factor NF-kB. In the inactive state, the NF-kB factor forms a heterodimeric complex localized in cytosol; the complex consists of two subunits, p50 and p65, which are associated with the inhibiting protein IkB. When the NF-kB factor is activated by high concentrations of glucose, reactive oxygen species, and inflammatory cytokines, the IkB protein is phosphorylated and becomes degraded. As a result, the release of p50/p65 heterodimer occurs, which is translocated into the nucleus and initiates the nuclear transcription of the genes involved in the development of inflammatory reactions of endothelium, particularly the cell adhesion molecules (ICAM-1 and VCAM-1), Willebrand factor, and E- and P-selectins; this leads to dysfunction of endothelial cells [20]. The anti-inflammatory action of HDLs is associated with inhibiting the production of various factors, including E-selectin, cell adhesion molecules and cytokines [6]. It was shown that apoA-I decreased the activation of NF-kB induced by palmitate in cultured endothelial cells [35]. Positive effects associated with the anti-inflammatory action of apoA-I in different cells were discussed in recent reviews [20,43].

In endothelial cells, delipidized apoA-I and rHDL suppressed the expression of adhesion molecules, thus enhancing the activity of heme oxygenase-1. Heme oxygenase-1 (HO-1) is an inducible enzyme that neutralizes ROS, which are formed by NADPH oxidase under the action of oxidized LDLs. In HUVEC cells, D-4F increased the expression of HO-1 in dependence on the dose and time of action. The action mechanism of D-4F is associated with the activation of HO-1 enzyme through the route Akt/AMPK/eNOS/HO-1 [48]. D-4F suppressed the accumulation of oxidized LDLs and migration of monocytes and inhibited the expression of adhesion molecules and MCP-1, which led to the restoration of migration and reparation of HAEC cells *in vitro* [13]. In Sprague Dawley rats, oral administration of D-4F *in vivo* decreased the iodixanol-induced inflammation by inhibiting NADPH oxidase, production of ROS and formation of peroxynitrite ($ONOO^-$) [17]. Thus, D-4F *in vitro* and *in vivo* prevents endothelial dysfunction, oxidative stress, and inflammation.

HDL decreases inflammation in endothelial cells by increasing the expression of annexin (lipocartin) in them, which is followed by inhibiting the activation of phospholipase A2. In TNF-α activated endothelial HUVEC cells, HDLs increased the level of annexin A1 via the SR-BI receptor by involving the ERK, p38MAPK, Akt and PKC signaling pathways. The HDL-induced annexin A1 inhibited the expression of cell adhesion molecules (VCAM-1, ICAM-1) and E-selectin as well as the secretion of MCP-1, IL-8, VCAM-1 and E-selectin, thus suppressing the adhesion of monocytes. Special inhibitors of the above listed signaling pathways decreased *in vitro* the inhibiting effect of HDLs on the adhesion of monocytes to the TNF-α activated endothelial cells. In the *in vivo* experiments, HDLs (10 mg/kg) induced the expression of annexin in thoracic aorta endothelial cells and prevented its decrease under the action of TNF-α [6].

Conclusion. The facts presented in the review testify to the essential role of HDLs in the functioning of endothelial cells. HDLs carry out their regulatory function via receptors on the membrane of endothelial cells (SR-BI, ABCG1 and S1PR) and also via the activation of endothelial lipase and ecto-F1-ATPase. Thus, binding of HDLs to receptors is necessary not only for transendothelial transport of lipids, but also for triggering the intracellular signaling events, particularly PI3K/Akt, AMPK and MAPK. As a result, HDLs initiate the induction of endothelial nitric oxide synthase, enhance the production of NO, and stimulate the synthesis of prostacyclin, thus leading to vasorelaxation. By suppressing the synthesis of intercellular adhesion molecules, HDLs prevent the migration of leucocytes and monocytes/macrophages into the vascular wall and exert the anti-inflammatory effect. HDLs inhibit the production of reactive oxygen species and prevent apoptosis; they also stimulate the proliferation and migration of endothelial cells. Understanding the mechanisms of the protective action of HDLs on vascular endothelium is a necessary stage in the development of advanced therapeutic agents with the endothelial protective properties.

Financial support. This work was supported by the Ministry of Science and Higher Education of the Russian Federation within the governmental order (project 1021050400914-1-1.6.4).

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N.A. Chulakova, A.F. Potapov, A.A. Ivanova

PROADRENOMEDULLIN AS A BIOMARKER OF COVID -19 POOR OUTCOME: META-ANALYSIS AND SYSTEMIC REVIEW

DOI 10.25789/YMJ.2023.82.23

УДК 616.9-03

The aim of this study was to analyse and summarise all researches about proadrenomedullin (pro-ADM) prognostic value as covid-19 severity and mortality early predictor. After a literature search and selection, we found 19 articles eligible to inclusion in a meta-analysis. We found pro-ADM had significantly high values in patients both admitted to the general department and ICU-patients with unfavourable outcomes. The Pro-ADM measurement in the admission or early stages of the hospitalisation can be used for the patient's risk stratification, to making a decision and a differential treatment approach.

Keywords: coronavirus disease COVID-19, biomarker, proadrenomedullin, severity score, mortality prognosis.

Introduction. The objective assessment of the disease severity and outcome prediction are essential components to make a decision in the patient's management and appropriate treatment definition. Stratification problems and patient's transferring based on the disease severity and the risk of unfavourable outcomes acquired crucial and priority when there are large number of cases and inevitable excessive burden on healthcare systems. These requirements are particularly relevant in diseases with a wide variability of clinical course and rapidly developing severe complications, an example of which was the new coronavirus infection COVID-19.

Currently, different prognostic scales (APACHE II, SOFA, SAPS II, CURB-65, NEWS) and laboratory biomarkers (leukocytes and platelets level, D-Dimer, C-reactive protein (CRP), Interleukin-6, Interleukin-10, Tumor Necrosis Factor-α, procalcitonin and etc.) are used in the Covid-19 severity assessment [8]. However, none of these scale and laboratory tests has any benefits in Covid-19 prognostic effectiveness with low sensitivity and specificity, it requires further searches of reliable predictive biomarkers of disease's severity.

Covid-19 pathways investigation found the key role of endothelial damage which correlate with infection's severity [16]. Therefore, findings of early indicator with high predictive value considering covid-19-associated endothelitis are reasonable. One of the newest biomarker is adrenomedullin (ADM) – hormone with cytokine-like effects, it consist of 52 amino acid peptide and released by endothelial and vascular smooth muscle cells and widely distribute in tissue and this production increased during infections [1]. ADM has vasodilative immunomodulate and anti-inflammatory effects, it's used as early marker in lower respiratory tract infections, community-acquired pneumonia and sepsis [2]. ADM has low metabolic stability and brief half-life, its splits in 1:1 ratio with precursor called mid-regional proadrenomedullin (pro-ADM) and it can proportionally represent the ADM level and it is used in tests. The biomarker showed direct correlation with increased procalcitonin level and prognostic scales (APACHE II, SOFA, SAPS II, CURB-65, NEWS). The Pro-ADM level in sepsis and septic shock were 1,8 (0,4-5,8) nmol/L and 4,5 (0,9-21,0) nmol/L respectively [2,4]. In several single studies pro-ADM level interpretation and combination with other biomarkers and scales demonstrated efficiency in making a decision about admission in ICU or save transferring out, antibiotics escalation or de-escalation and poor prognosis prediction [1].

In view of the above, our research summarised current studies to evaluate proADM as an early biomarker of severity and mortality predictions.

Purpose of the study was combined and analyse articles to assess pro-ADM prognostic ability as an early marker of severity and mortality in Covid-19 patients.

Materials and Methods. In PubMed, EMBASE (Experta Medica), Cochrane Central Register of Controlled Trials, Scholar Google and e-library we selected article were had been published in English and Russian till 25.11.2022 with pro-ADM levels, severity and outcomes information. The search strategy was used with key words and combinations: «new coronavirus infection», «COVID-19», «predict», «midregional

CHULAKOVA Nadezhda Alexandrovna – 3-year postgraduate student of the Department of Anesthesiology, Resuscitation and Intensive Care with the course of Emergency Medicine of the Faculty of Postgraduate Training of Doctors of the Medical Institute of M.K. Ammosov North-Eastern Federal University; e-mail: chulakovan@list.ru; **POTAPOV Alexander Filippovich** – MD, Associate Professor of Anesthesiology, Resuscitation and Intensive Care Department with the course of Emergency Medicine of the Faculty of Postgraduate Training of Doctors of the Medical Institute of M.K. Ammosov North-Eastern Federal University; e-mail: potapov-paf@mail.ru; **IVANOVA Albina Ammosovna** – MD, Associate Professor and Head of Anesthesiology, Resuscitation and Intensive Care Department with the course of Emergency Medicine of the Faculty of Postgraduate Training of Doctors of the Medical Institute of M.K. Ammosov North-Eastern Federal University; e-mail: iaa_60@mail.ru

proadrenomedullin», «proadrenomedullin» and Russian translations.

Eligibility criteria were clinical trials with covid-19 confirmed diagnosis, adults only (18 years old and more).

Exclusion criteria were case studies, clinical trials with less than 5 patients, preclinical studies, opinion articles, thesis, studies with pregnant or patients with decompensation chronic diseases.

For quality assurance the manual article selecting was used following PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses, www.prisma-statement.org). All included studies had patients' characteristics, laboratory data, course of disease and outcomes. For all patients the measurement of pro-ADM was performed in the plasma by TRACE technique (Time-Resolved Amplified Cryptate Emission, KRYPTOR «BRAHMS»).

All article were divided on two main groups: 1 group include patients admitted to hospital in the general (or infectious diseases) department and 2 group with severe or critically ill patients admitted to the ICU. In each group the proADM level in the survivors and the deceased was compared. In all studies, the main characteristics, laboratory data and assessment by scales (SOFA, NEWS, CURB-65) at admission were collected.

The meta-analysis was conducted according to the Cochrane Collaboration recommendations using the Review Manager (RevMan) program, version 5.4.1 (2020). The meta-analysis included eligibility studies: patients were already separated or they could be grouped into two - survivors and non-survivors; each group had information about total population number, pro-ADM mean level on the 1st day of hospitalization with standard deviations and 95% confidence interval (CI) (or these indicators were converted by us on the website math.hkbu.edu.hk, from available data – sample size, median, maximum, minimum and interquartile interval); statistical significance set up at $p<0.05$. The calculation of the weighted average (arithmetic) is also carried out.

Heterogeneity was evaluated by χ^2 (Chi square) with considered level of p-value 0,10 and I^2 index with values for heterogeneity level indication were 0-40%-low, 30-60% - moderate, 50-90% - significant, 75-100 – high [5]. To assess overall mean difference (MD) fixed effects model was chosen for moderate heterogeneity, and a random effects model was chosen for significant heterogeneity. To assess the publication bias in the meta-analysis with 5 or more studies, a funnel-plot was constructed. The assessment of methodological quality was carried out according to the Russian version of the Newcastle-Ottawa scale for all studies included in the meta-analysis [7].

Result and discussion. After systemic literature search using keywords, 68 publications were selected from a total of 572 references, after initial screening and removing de-duplication publications, 28 articles (5 Russian and 23 foreign studies) were selected. Further selection and check of these articles, 19 articles (3 Russian and 16 foreign studies) were included in the meta-analysis (Fig. 1).

Among selected articles (n=19), 3681 patients were analysed: 3096 patients admitted in the general department and 585 ICU patients.

Meta-analysis of studies with general department patients. 3096 patients admitted in the general department were enrolled, 580 patients deceased (mortality rate was 18,7%). After synthesis the weighted average pro-ADM level in survivors was 0,83 nmol/L, in non-survivors was – 1,57 nmol/L, the mean difference (MD) was statistically significant MD = -0,87 (95% CI: -1,08, -0,67), $p<0,00001$. Heterogeneity was significant ($I^2=86\%$, $\chi^2=76,4$, $p<0,00001$) (Fig. 2,4).

Meta-analysis of studies with ICU patients. Meta-analysis incorporated 585 ICU patients (169 deceased with mortality rate 28,9%). The weighted average pro-ADM level in survivors was 0,90 nmol/L, in non-survivors was – 2,11 nmol/L, the mean difference was statistically significant, MD = - 0,71 (95% CI: -0,80, -0,61), $p<0,0004$. In contrast with results of group with patients hospitalized in the general department, the heterogeneity of the data in ICU patients was moderate ($I^2=44\%$, $\chi^2=10,68$ $p=0,10$) (рис. 3, 4).

Therefore, result of the meta-analysis shows that the level of pro-ADM in non-survivors is significantly higher than survivors in both patients of the general department ($p<0.00001$) and ICU patients ($p<0.0004$). It should be noted that the values of pro-ADM in ICU patients with severe COVID-19 are initially higher even in survivors.

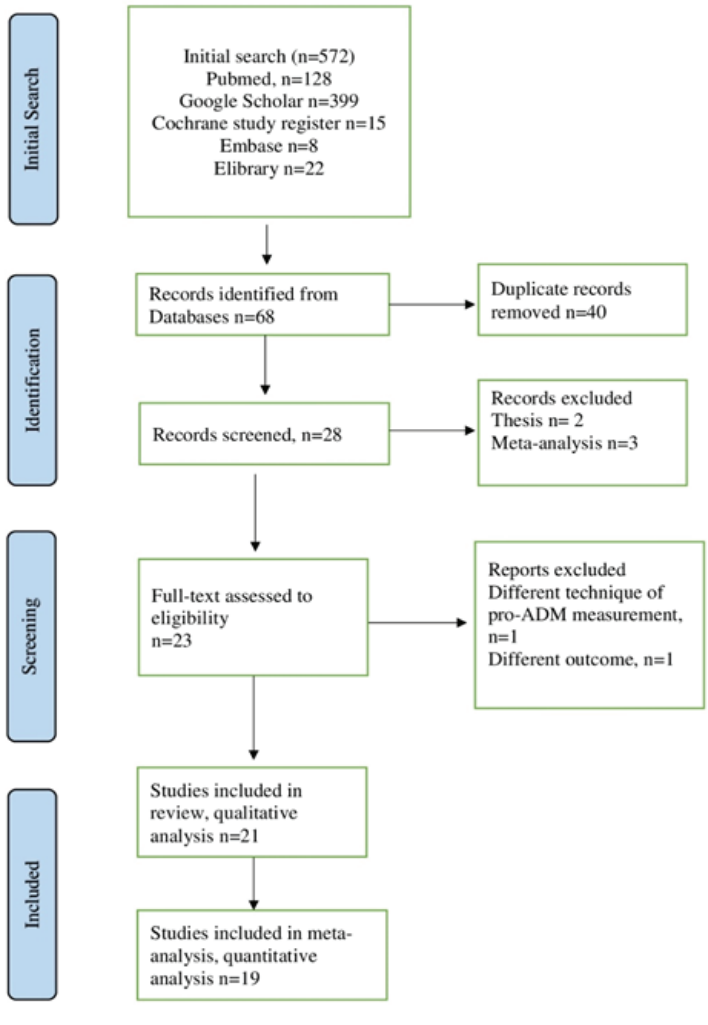

Fig. 1. PRISMA Flow diagram

The main characteristics of articles included in the meta-analysis

Author, Year Country	Type of study	Period	Number of patients survivors/deceased (mortality rate)	Department	AUC (95% ДИ)	Cut Off nmol/L	Findings
Gregoriano C. et al., Italy 2021 [29]	Prospective Observational	February-April 2020	89 72/17 (17.2)	General department	0.78	0.93	Pro-ADM mmeasured daily during hospitalization (till 8 day) Increased in deceased
Mangioni D. et al. Italy, 2022 [26]	Prospective Observational	February-October 2020	100 87/13 (13)	General department	0.87 (0.79–0.94)	1.04	With γ-interferon, it prognostic value was low. Pro-ADM prognostic value increased on day 7
Indirli R. et al., Italy, 2021 [25]	Retrospective Observational	March- June 2020	116 95/21 (18.1)	General department	0.79	1.00	With copeptin, it showed prognostic effectiveness
Méndez R. et al., Spain, 2021 [9]	Prospective	March- June 2020	210 183/27 (12.9)	Emergency department	-	1.16	With proendothelin, it showed correlation with severity and mortality
Lo Sasso B. et al., Italy, 2021 [11]	Retrospective Observational	September- October 2020	110 96/14 (12.7)	General department	0.95 (0.86–0.99)	1.73	The highest pro-ADM level among all studies, wide variability of pro-ADM level
de Guadiana-Romualdo L.G. et al., Spain, 2021 [10]	Prospective	August-October 2020	359 327/32 (8.9)	General department	0.832 (0.77-0.894)	0.8	Pro-ADM level had correlation with SOFA score
Minieri M.,et al., Italy, 2022 [28]	Retrospective Observational	April-December 2020	321 224/97 (30.2)	Emergency department	0.85	1.105	NIMV/IMV need assessed
Попов Д.А. и др., Россия, 2020 [6]	Prospective Observational	May-June 2020	97 83/14 (14.4)	General department	0.75 (0.59—0.91)	0.895	The highest prognostic significance among all indicators
de Guadiana-Romualdo L.G. et al., Spain, 2021 [24]	Prospective Observational	March - April 2020	99[17] 85/14 (14.1)	General department	0.905 (0.829-0.955)	1.01	Separation between severe and non-severe Predicting the progression of the disease
Moore N. et al., UK, 2022 [23]	Prospective Observational	April-June 2020	135 105/30 (22.2)	General department	0.844 (0.776- 0.912)	1.54	Only CRP and Pro-ADM had prognostic value among all indicators. ICU admission and IMV need assessed
Sozio E. et al., International 2022 [17]	Retrospective	March-April 2020	1278 986/292 (22.8)	Emergency department	0.786	0.911	Pro-ADM, CRP and LDH assessed for stratification into group - hospitalization need or not and poor outcome prediction

End of the table

Atallah N. J. et al., USA, 2022 [21]	Retrospective Observational	April-June 2020	182 173/9 (4.9)	General department	0.76 (0.59–1.17)	0.87	IMV need, ICU admission, disease progression and poor outcome assessed
Montrucchio G. et al., Italy, 2021[12]	Retrospective Observational	March-June 2020	57 26/31 (54.4)	Intensive care unit	0.95 (0.86–0.99)	1.8	Comparison with CRP, PCT, LDH Measurement on first 48 hour, 3, 7 and 14 days were done
Попов Д.А. и др., Россия, 2022 [19]	Prospective Observational	No information	135 115/20 (14.8)	Intensive care unit	0.78 (0.66–0.90)	0.895	The highest prognostic significance among all indicators
Малинина Д.А. и др., Россия, 2020 [3]	Retrospective Observational	May-August 2020	37 18/19 (51.3)	Intensive care unit	-	-	The highest prognostic significance among all indicators
Benedetti I. et al. et al., Italy, 2021 [15]	Observational	March-April 2020	21 10/11 (52.4)	Intensive care unit	0.91	1.07	Measurement on 1, 3, 5days were done. The highest prognostic significance among all indicators
Van Oers J.A.H. et al., Netherlands, 2021 [13]	Prospective Observational	March-May 2020	105 75/30 (28.6)	Intensive care unit	0.84 (0.76-0.92)	1.57	With C-terminal proendothelin-1
Oblitas C.M. et al., Spain, 2021 [22]	Prospective Observational	August-November 2020	95 83/12 (12.6)	Intensive care unit	0.73 (0.63–0.81)	1.0	1,0 With methemoglobin and carboxyhemoglobin it prognostic values were low
Montmollin E.et al., France, 2022 [20]	Prospective	April 2020-May 2021	135 89/46 (34.1)	Intensive care unit	0.744	1.0	Measurement on 1, 3, 7 days were done

It's essential to note significant heterogeneity in patients of the infectious department (I^2=86%) is due to the results presented by B. L. Sasso [11], in which the average values of pro-ADM in patients with an unfavorable outcome were noticeably higher and amounted 2.62 nmol/L. In reviewed studies mortality rate in the ICU was high, in three studies it exceeded 50% [3, 12, 15]. The age median among patients of the general department varied 53,3 to 67 years, and among ICU patients - 63,3 to 70,9 years, and male prevailed (in the general department were 51,9-65,2%, in the ICU were 67,4-87,7%).

The results of our systematic review and meta-analysis confirm the conclusions of other researches, which present a significant difference in the values of pro-ADM in surviving and deceased patients. Thus, in the meta-analysis of G. Montruccio et al., the mean difference of pro-ADM among survivors versus non-survivors ICU patients was - 0.96 nmol/L (95% CI:-1.26,-0.65, heterogeneity I^2=0% p<0.00001) [25]. Lippi et al. conducted the meta-analysis with mixed-unfavorable outcomes (ICU-admission, renal replacement therapy, invasive mechanical ventilation, acute respiratory distress syndrome, death) and the same result was obtained – the mean difference between unfavorable/favorable outcomes was 0.67 nmol/l (95% CI: 0.42-0.93, heterogeneity I^2=81% p<0.001) [18]. The meta-analysis by Lampsas et al. included studies about endothelial dysfunction biomarkers among ICU patients, also showed the efficiency of the pro-ADM (the mean difference between the deceased/survivors was 0.71 nmol/L, 95% CI: 0.22, 1.20 nmol/L, p=0.02) [29].

In addition, pro-ADM were measured several times and its increase was noted with the progression of the disease in some studies [12, 15, 27, 30]. In all studies high level of pro-ADM was correlated with other severity indicators (high level of CRP, LDH, D-dimer, lymphocytopenia) and high scores of APACHE II и SOFA scales.

In some studies, other markers investigated such as γ-interferon [27], copeptin [26], proendothelin [9], C-terminal proendothelin-1[13], methemoglobin and carboxyhemoglobin [22]. In reviewed studies pro-ADM mortality prognostic value with ROC-analysis were demonstrated the highest sensitivity and specificity among other predictors (AUC varied 0,73 to 0,95).

Some authors recommended the inclusion pro-ADM measurement in the required diagnostic complex when a

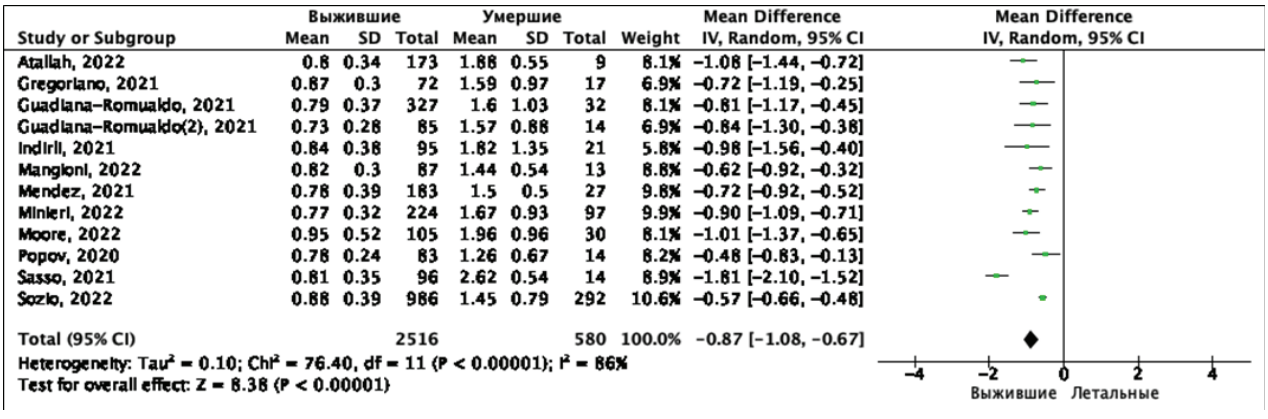

Fig. 2. Meta-analysis results and Forest Plot, general department patients.

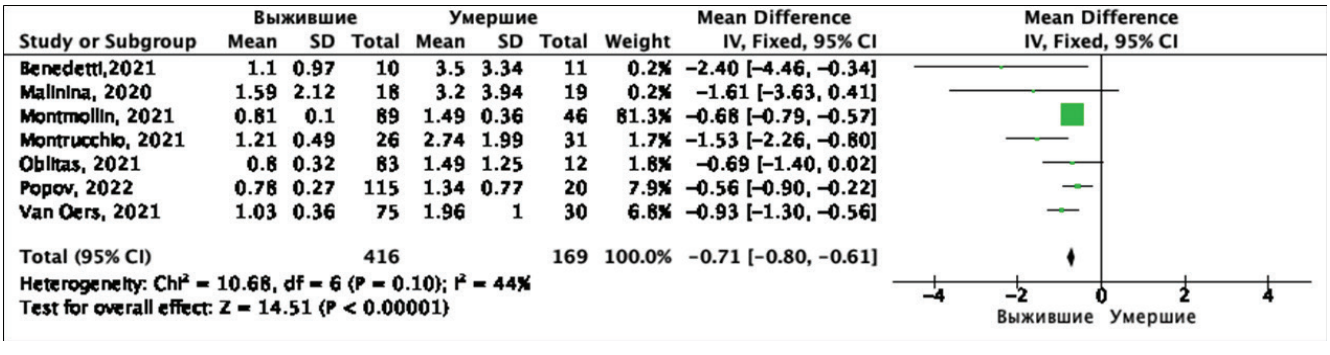

Fig. 3. Meta-analysis results and Forest Plot, ICU patients

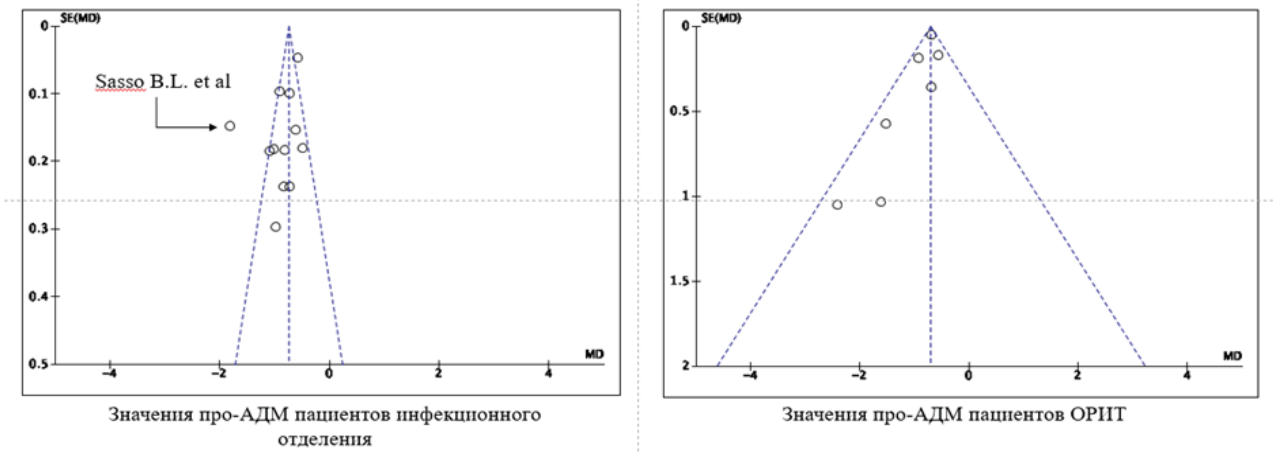

Fig. 4. Funnel Plot of the meta-analysis for assess publication bias

patient is admitted to the hospital. Thus, Sozio et al. found the high prognostic significance of pro-ADM in predicting outcome and making a decision about hospitalization in the infectious department, they proposed to count pro-ADM blood level [17]. Note that there was other opinions. For example, Zaninotto et al. demonstrated no noticeable increase in prognostic informativeness with pro-ADM addition: the prognostic significance of criteria such as age and the index of neutrophils to lymphocytes ratio amounted to AUC was 0.916 (95% CI 0.853-0.979), but pro-ADM AUC was 0.900 (95% CI 0.827-0.974) [14].

Currently, despite the first positive results about the high prognostic informativeness of pro-ADM as an early marker of an unfavorable outcome in COVID-19 compared with other laboratory indicators, the question of it use as a single or additional marker remains open.

Conclusion. Results of our meta-analysis indicate prognostic efficiency of pro-ADM as an early predictor of the unfavorable outcome of COVID-19. Determination the level of pro-ADM as a promising biomarker with high sensitivity and specificity in severity and mortality prediction seems to be optimal at the early stages preferably at the emergency department for patients' stratification according to their severity, making a decision of hospitalization and differentiated approach of medical care.

Of course, the attractiveness of patients's stratification by only one highly sensitive and specific biomarker is obvi-

ous, it will improve the quality and effectiveness of diagnosis and treatment, as well as ensure the economic feasibility of the entire treatment process. However, further studies are required to select such markers in diseases and particular in COVID-19. At the same time, it's necessary to conduct further trials, to compare pro-ADM with other biomarkers, to determine the level of its threshold values accounting comorbidity, pregnancy and other groups of patients.

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DOI 10.25789/YMJ.2023.82.24
УДК 575.176

N.I. Pavlova, V.A. Alekseev

GENETIC FACTORS IN OBESITY

The rapidly increasing prevalence of obesity in the population in recent decades is an important public health issue because it increases the risk of diabetes, heart disease, stroke and other serious diseases. Its causes include excessive consumption of high-calorie foods, as well as a sedentary lifestyle. It has been proven that, in addition to energy imbalance, 40–70% of the development of obesity is influenced by hereditary factors. As a rule, obesity results from the interaction of certain gene polymorphisms with the environment. Only a small number of cases of obesity are the result of the presence of mutations in certain genes (monogenic obesity), causing Mendelian syndromes with a very low frequency in the population. This review describes the genetic causes of obesity, including syndromal, monogenic and polygenic causes.

Keywords: Obesity, genes, polymorphisms.

Introduction. Obesity is a source of chronic energy imbalance in a person who constantly receives more carbohydrates from food than is necessary to fuel the metabolic and physical functions of the body. The rapidly growing prevalence of obesity is characterized by free access to a high-calorie product and a simultaneous decrease in physical activity. Also, as a result of the severe acute respiratory syndrome 2 (SARS-CoV-2) pandemic, which causes a new coronavirus disease COVID-19, quarantine restrictions were introduced around the world [2], which caused changes in lifestyle, diet, as well as the occurrence of hypodynamia, which in turn led to the occurrence of energy consumption and the incidence of obesity [27]. Various studies show that minor changes in body weight over relatively long periods of time increase body weight and eventually approach a significant increase in body weight [18; 40]. If current reserves are maintained, 1 billion adults (nearly 20% of the world's population) are expected to be obese by 2025. Of particular concern is the global rise in obesity among children and adolescents; more than 7% were obese in 2016 compared to less than 1% in 1975 [49].

In recent years, the prevalence of obesity in Asian countries has been steadily increasing due to unbalanced diet and lack of exercise. The Asian type of obesity is different and is characterized by high body fat and low skeletal muscle mass. At the same time, there is excessive accumulation of fat in the abdomen and liver (non-alcoholic fatty liver disease), which is associated with an increased cardiometabolic risk [6].

In the Russian Federation, the share of the population belonging to the Mongoloid race and of Mongolo-Caucasoid mixed origin is about 9%. If the majority of the population of the European part of Russia belongs to the Caucasoid race, then in the Asian part of the country the proportion of representatives of the Mongoloid race increases significantly. Representatives of various Asian ethnic groups live on the territory of the Russian Federation, such as the Yakuts and Buryats, representing the two largest Siberian ethnic groups.

In recent years, the Republic of Sakha (Yakutia) has seen an increase in the prevalence of obesity, which is associated with the loss of traditional methods of managing and the transition of the indigenous population from the usual protein-lipid type of nutrition to the carbohydrate-lipid type [13]. Sofronova S.I. and co-authors conducted a study to determine the prevalence of overweight and obesity among representatives of the indigenous population of Yakutia (Evenks, Dolgans, Evenks, Yukaghirs, Chukchis, Yakuts) living in the northern uluses. 37.8% of Yakuts are overweight, they found that obesity is more common in men. A strong relationship has been found between BMI and systolic blood pressure. Yakuts and Evenks had higher mean systolic blood pressure than other ethnic groups [11]. Marinova L.G. et al. studied children living in Yakutsk and found a high prevalence of first-degree obesity, mostly in boys. Abdominal obesity was observed in 86% of children. One case of metabolic syndrome has been reported. In the work done, there is no information about the ethnicity of the children participating in the study [5].

Obesity is associated with premature mortality and is a major public health threat, accounting for a significant portion of the burden of noncommunicable diseases worldwide. Diabetes mellitus, dyslipidemia, hypertension, non-alcoholic fatty liver disease, cardiovascular disease, cancer [10;12] and severe COVID-19 are more common as the main risk factor for a large number of serious complications in obese people, which lead to higher adult mortality rates.

It has been proven that, in addition to energy imbalance, 40–70% of the development of obesity is influenced by hereditary factors [9]. As a consequence, genetic approaches can be used to characterize the underlying physiological and molecular mechanisms that control body weight. Since 2007, genome-wide association studies (GWAS) have identified about 250 genes associated with obesity [47].

Monogenic and syndromic obesity. There are about 30 Mendelian disorders with obesity as a clinical feature, often in association with mental retardation, dysmorphic features, and organ-specific developmental anomalies (eg, pleiotropic syndromes) [41]. Monogenic obesity is inherited according to the Mendelian type and, as a rule, rare, early and severe. Mutations in the genes of leptin (*LEP*), leptin receptor (*LEPR*), type 4 melanocortin receptors (*MC4R*), proopiomelanocortin (POMC), prohormone convertase 1 (*PCSK1*), brain-derived neurotrophic factor (*BDNF*), and type tyrosine kinase receptor lead to the development of monogenic obesity. 2 (*NTRK2*), the *SIM1* gene, and the Ras suppressor kinase type 2 gene (*KSR2*). Mutations in these genes impair appetite control and lead to hyperphagia, which ultimately leads to obesity [7]. Defects in the *MC4R* gene are the most common known monogenic form of childhood obesity, accounting for about 6% of cases of monogenic obesity.

Syndromic obesity is when obesity occurs in the clinical context of a particular set of associated clinical phenotypes. This is obesity caused by chromosomal rearrangements such as Prader-Willi

PAVLOVA Nadezhda Ivanovna – PhD in Biology, Leading Researcher – Head of the Laboratory of Hereditary Pathology, Department of Molecular Genetics, Federal State Budgetary Scientific Institution Yakut Science Center for Complex Medical Problems; e-mail: solnishko_84@inbox.ru; **ALEKSEEV Vladislav Amirovich** – junior researcher of the Arctic Medical Center, Federal State Budgetary Scientific Institution Yakut Science Center for Complex Medical Problems; e-mail: vldslvalekseev@gmail.com

syndrome, WAGR syndrome, SIM1 syndrome and pleiotropic syndromes, including Bardet-Biedl syndrome, Fragile X syndrome, Cohen syndrome, etc. [47]. Children with syndromal obesity have extreme obesity, physical dysmorphology, and mental retardation, some of them with indeterminate neuroendocrine abnormalities. It is the latter anomaly that is thought to be responsible for adverse effects on the function of the hypothalamus, which serves as the brain center of appetite that regulates energy balance through food intake and energy expenditure, as a result, children with syndromal obesity are usually characterized by severe hyperphagia and a decrease in satiety, which contributes to weight gain [45] .

Polygenic obesity. The most common form of obesity is polygenic obesity. A number of obesity-prone gene loci have been identified in various populations using the GWAS approach. The GWAS approach helps to identify common SNPs that contribute to a relatively low risk (measured by odds ratio [OR]<1.5) of the growth of complex diseases and phenotypes, including phenotypes associated with obesity, such as diabetes and hypertension [42].

One of the first obesity genes detected by GWAS in European patients with type 2 diabetes is the *FTO* gene. The researchers identified important SNPs in the first intron of *FTO* that were associated with obesity. These initial screenings were carried out in 2007 among the European population [14; 23;48], and then confirmed in other populations, including Hispanics [46], East Asians [32], Africans [21] of the Middle East [20; 26]. Previously, we also confirmed the association of this gene with obesity in the Yakut population [1; 28]. The *FTO* gene is involved in the regulation of the diet, encodes a protein involved in energy metabolism and affecting metabolism in general [4]. According to the results of the studies, allele A of the *FTO* gene is associated with reduced lipolysis, lack of satiety after an adequate meal, and impaired appetite control. The phenotypic manifestation of the A allele of the *FTO* gene is overweight, obesity due to overeating.

The *PPAR* family genes are mediated through specific receptors called *PPARs*, which belong to the steroid hormone receptor superfamily. *PPARs* affect the expression of target genes involved in cell proliferation, cell differentiation, as well as in immune and inflammatory responses [38]. Three closely related subtypes (alpha, beta/delta and gamma) have been identified. PPAR proteins are able to bind to various ligands, including fatty acids, drugs (fibrates, thiazolidinediones) [43]. Members of the PPAR family have distinct tissue distribution patterns and tissue-specific functions. PPAR-α is predominantly present in the liver, where it plays an important role in the regulation of nutrient metabolism by stimulating the uptake and oxidation of fatty acids. PPAR-γ is mainly expressed in adipose tissue. It is induced during adipocyte differentiation and is a regulator of fat cell formation and lipid accumulation. PPAR-δ is abundantly expressed throughout the body and is thought to be involved in adipogenesis and energy metabolism [22].

PPARα is activated by various natural agonists, including unsaturated fatty acids and eicosanoids, while fibrate preparations act as synthetic agonists. In the liver, *PPARα* plays a key role in fatty acid catabolism by upregulating numerous genes involved in mitochondrial fatty acid oxidation, peroxisomal fatty acid oxidation, and many other aspects of cellular fatty acid metabolism [30]. Regulates the expression of genes encoding enzymes and transport proteins that control lipid homeostasis, which ultimately leads to stimulation of FA oxidation and improved lipoprotein metabolism [19]. This gene may be specifically involved in the lipolytic process and in weight loss induced by an exercise program [35].

The PPARG2 protein is abundant in fat cells and plays a key role in their formation. The main function of this protein is the activation of genes associated with fat accumulation, differentiation of adipose tissue cells and myoblasts. It stimulates the differentiation of adipose tissue resident preadipocytes into adipocytes and promotes the mobilization of circulating bone marrow progenitor cells into white adipose tissue and their subsequent differentiation into adipocytes [29]. It plays an important role in the formation of the sensitivity of various tissues to insulin. It is the *PPARG2* gene that determines lipid metabolism [36]. The *PPARG* gene counteracts obesity-induced inflammation by controlling the inflammatory response either by downregulating pro-inflammatory genes or by influencing lipid metabolism. The ability to reduce inflammatory cell infiltration further highlights the central role of *PPARG* in obesity-induced inflammation. During the inflammation process, *PPARG* can direct cells to adipocyte differentiation, which leads to the maintenance of inflammation genes in a suppressed state in adipose tissue in obesity [31].

The function of *PPARD* is to regulate genes associated with fat accumulation (triglyceride synthesis), differentiation of adipocytes (fat cells) and myoblasts, insulin sensitivity, osteoblast and osteoclast activity (growth regulation) [37]. Also, *PPARD* is directly related to the development of obesity, is involved in wound healing, cell growth, and lipoprotein metabolism. Participates in the control of peroxisome proliferation, which are responsible for fatty acid oxidation and energy metabolism, is expressed in many tissues and organs. By stimulating fatty acid oxidation, *PPAR* β/δ activation results in fat mass loss in various mouse models of obesity [33]. In addition to enhancing fatty acid oxidation, *PPAR* β/δ activation in muscle also increases the number of type I muscle fibers, leading to increased endurance [39].

The intestinal fatty acid binding protein FABP2 binds saturated and unsaturated long-chain fatty acids and is involved in the synthesis of triglyceride-rich lipoproteins [44]. The affinity of *FABP2* for long chain fatty acids is doubled when the Ala54Thr polymorphism is present in the *FABP2* gene. Ala54Thr polymorphism increases triglyceride (TG) secretion and free fatty acid transport in vitro [34]. In a study by Baier L.J. et al. (1996) found that this polymorphism affects lipid transport and secretion. They suggest that individuals expressing the Thr54 genotype process more long-chain fatty acids into chylomicron triglycerides than individuals with the Ala54 genotype. Either or both of these processes will result in a decrease in the rate of insulin-mediated glucose uptake and an increase in the rate of insulin release from pancreatic β-cells, consistent with the observed insulin resistance and hyperinsulinemia found in subjects with the Thr54 genotype [17].

The leptin receptor (*LEPR*) gene, which secretes a single transmembrane cytokine protein located on chromosome 1p31, plays a critical role in the regulation of body weight by stimulating energy expenditure and inhibiting food intake. Mutations in the *LEPR* gene are associated with monogenic forms of severe early obesity and hyperphagia. Numerous studies in various populations have confirmed the association of Q223R (rs1137101) polymorphism with obesity rates [15; 16; 50].

The Q223R polymorphism reduces leptin binding and thus impairs leptin signaling, thus playing an important role in the pathogenesis of obesity through its direct effects on lipid and glucose metabolism, adipose tissue metabolism, body fat control, and cardiovascular function.

Increased appetite is the driving force behind weight gain. A large amount of literature data indicates that changes in

the hormone ghrelin play an important role in fluctuations in appetite after eating [24; 25]. Ghrelin is the gut hormone with the strongest orexigenic signal that helps the body respond to changes in metabolic status by binding to growth hormone secretion-stimulating receptors (GHSR) [3]. It is synthesized predominantly in the stomach and is found in the bloodstream of healthy individuals. The minor allele 178C>A of the polymorphic *GHRL* gene is associated with obesity, the effect occurs due to an increase in the level of ghrelin, forming an early feeling of hunger [8].

Conclusion. Considering the genetic causes and understanding the growing evidence of epigenetic changes influencing the growing epidemic of obesity provide valuable tools in the treatment of obesity. The ability to identify individuals at high risk could facilitate targeted obesity prevention strategies with increased impact and cost-effectiveness.

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DOI 10.25789/YMJ.2023.82.25

УДК 571.27+612.821.2+612.821.5

A.A.Gulyaev, K.A. Drobyaskina, I.A. Sinyakin, T.A. Batalova

NEUROINFLAMMATION AND BRAIN FUNCTION: POSSIBLE IMPLICATIONS IN CHILDREN INFECTED WITH COVID-19

COVID-19, the disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), affects children differently than adults, with milder symptoms. However, several cases of neurological manifestations with neuroinflammatory syndromes, such as multisystem inflammatory syndrome (MIS-C), have been reported following infection. As with other viral infections such as rubella, influenza, and cytomegalovirus, SARS-CoV-2 causes a massive release of pro-inflammatory cytokines that affect microglial function, which can be critical for brain development. Along with viral induction of neuroinflammation, other non-infectious conditions may interact to cause additional inflammation, such as imbalances in fatty acid and polyunsaturated fatty acid diets and alcohol consumption during pregnancy. In addition, transient thyrotoxicosis caused by SARS-CoV-2 has been reported, with secondary autoimmune hypothyroidism that may go unnoticed during pregnancy. Together, these factors may represent an additional risk of infection by influencing neurodevelopmental mechanisms such as synaptic pruning and the formation of neuronal ensembles. In this review, we discuss these conditions to consider and the possible occurrence of neurodevelopmental disorders in children infected with COVID-19.

Keywords: neuroinflammation, children, COVID-19, synapse formation, brain development, nutrition.

GULYAEV Artem Alexandrovich – 6 year-course student of the Amur State Medical Academy, 610 group, Medical Faculty, Blagoveshchensk, Amur State Medical Academy of the Ministry of Health of Russia, e-mail: Superstar533129@gmail.com; **DROBYASKINA Ksenia Anatolyevna** – 3 year-course student of the Amur State Medical Academy, 321 group, Pediatric Faculty, Blagoveshchensk, Amur State Medical Academy, e-mail: drobyaskinak@gmail.com; **SINYAKIN Ivan Alekseevich** – 5 year-course student of the Amur State Medical Academy, 505 group, Medical Faculty, assistant-researcher of the Laboratory of mechanisms of etiopathogenesis and restorative processes of the respiratory system in non-specific lung diseases of the Far Eastern Scientific Center of Physiology and Pathology of Respiration, Blagoveshchensk, the Amur State Medical Academy, e-mail:sinyakin.ivan2016@yandex.ru; **BATALOVA Tatyana Anatolyevna** – Professor, Doctor of Biological Sciences, Head. Department of Physiology and Pathophysiology of the Amur State Medical Academy. Blagoveshchensk, Amur State Medical Academy, e-mail: batalova_ta@mail.ru.

Introduction. COVID-19 is a systemic disease caused by severe acute respiratory syndrome coronavirus 2, which belongs to the betacoronavirus genus [3]. The most common neurological symptoms in response to SARS-CoV-2 infection include: headache, anosmia, impaired consciousness, infectious encephalopathies, and neuroinflammatory syndromes such as acute demyelinating encephalomyelitis [1]. A biomarker study (NfL, intraaxonal marker of neuronal injury; glial fibrillar acidic protein; GFAp, marker of astrocytic activation/damage) also provided evidence of neuronal damage and glial cell activation in patients with COVID-19 [39], strongly suggesting that SARS-CoV-2 has neurotropic activity. In addition, SARS-CoV-2 has been shown to be able to infect human neu-

ral progenitor cells [57]. Like SARS-CoV, SARS-CoV-2 uses the angiotensin-converting enzyme receptor (ACE2) for cell invasion by binding to it via the spike (S) protein [10]. In the central nervous system (CNS), glial cells and neurons express this receptor [19]. It is not yet known which pathway SARS-CoV-2 uses to reach the nervous system, but there are 2 theories. According to the first one, the virus enters the CNS by the hematogenous route, in which it can penetrate into leukocytes and cells of the blood-brain barrier (BBB), or, in the second case, the virus can migrate to the CNS via axonal transport [67].

SARS-CoV-2 infection in children. Children are less likely to develop severe COVID-19, but the main question that causes much controversy is related to the long-term consequences of mild or subclinical infection remains unresolved. In the child's brain, complex neural networks are subject to intensive modernization, which modulates the activity of neurons and immunological complexes of the CNS, such as microglia, cytokines, chemokines, the complement system, and peripheral immune cells [18], which further leads to synaptic pruning (pruning) and the formation functional neuronal ensembles [63]. In pathological conditions, some maternal cytokines and leukocytes cross the placenta and may impair fetal development [28]. In addition, ACE2 expression is intense in the placenta [5], suggesting a possible route for fetal infection with SARS-CoV-2 via vertical transmission [59]. There are now several case reports suggestive of intrauterine infection [49,66], and placental viremia has been confirmed by r-PCR and the presence of inflammatory cells in the cerebrospinal fluid along with neurological manifestations consistent with those described in adult patients [66]. In addition, during maternal infection, fetal microglia can be directly activated by viruses or cytokines and microchimeric maternal cells [28].

Since the beginning of the COVID-19 pandemic, it has been observed that in children, "subclinical infection" is either asymptomatic or mild [6]. Children with subclinical symptoms are potential carriers of the virus, but with a lower rate of infectivity than adult patients with a pronounced clinical picture, as was characteristic of the influenza virus [62]. In addition, children and adolescents with asymptomatic COVID-19 may develop a condition called multisystem inflammatory syndrome (MIS-C), with clinical and laboratory features that are not similar to those seen in Kawasaki disease and toxic shock syndrome [38]. Among the main symptoms associated with general systemic inflammation in blood vessels throughout the body, Kawasaki syndrome can cause a severe acute complication of encephalopathy [31]. The generalized vascular disorder caused by Kawasaki syndrome, as well as the complications that affect the body of a child infected with COVID-19, can also potentially alter the function of the neurovascular block, which plays an important role in brain development, and thereby contribute to an increased risk of late disorders. development of the nervous system. As with COVID-19, severe forms of H1N1 influenza are also characterized by a cytokine storm and multiple organ failure as a result of increased vascular permeability. Wang S. et al. it was theorized that BBB damage is the result of systemic exposure to pro-inflammatory cytokines produced in the lungs [30].

An additional possible complication in the mother's body during COVID-19 infection is associated with the expression of the ACE2 receptor in the thyroid gland, which has one of the highest expression levels of this receptor [20]. It has been described that SARS-CoV-2, like many other viral infections, may be associated with the development of subacute thyroiditis (SAT), which, although a self-limited and generally undiagnosed condition, can subsequently lead to autoimmune hypothyroidism [61]. The development of hypothyroidism in pregnant women deserves special attention, since congenital fetal hypothyroidism is the main cause of non-genetic treatable mental retardation in children [4]. The thyroid hormones thyroxine (T4) and triiodothyronine (T3) are essential for normal brain development [54], and their deficiency is associated with a delay in the development of sensory, motor and cognitive skills [33], reflecting the involvement of the latter in several processes such as neurogenesis, cell differentiation, migration, synaptogenesis and myelination, as well as the mechanisms of synaptic plasticity [13]. In addition, thyroid hormones may influence the development and function of microglia, as it has been demonstrated that hypothyroidism can change microglia morphology to a pro-inflammatory phenotype [52] and microglial function in general [16]. Thus, hypothyroidism secondary to viral invasion and the development of subacute thyroiditis may be a very strong endogenous correlate involved in fetal brain dysfunction.

Neuroinflammation and microglial dysfunction affect brain development and plasticity. Localization of the ACE2 receptor in microglia [62] increases the possibility of its direct activation by SARS-CoV-2, which may increase the risk of late neurodegenerative diseases, as shown for other viral infections [18]. Viruses such as Zika virus (ZIKV), cytomegalovirus, and rubella are able to cross the placental barrier and/or BBB and reach the CNS [37]. In ZIKV infection, along with damage to progenitor cells, an increase in neuroinflammation is observed, which disrupts the physiological role of microglia during brain development [69]. The same is true for other RNA viruses, such as cytomegalovirus [2]. It is possible that these data suggest that the inflammation caused by a viral infection will be more detrimental to the development of the nervous system than the direct cytopathic effect of the virus on neurons.

At the end of the gestational and early early postnatal periods, the homeostatic function of microglia plays an active physiological role in synaptic pruning and neural network formation [35], being highly reactive to its microenvironment. Abnormal microglial responses during synaptic remodeling during critical periods of development can lead to the emergence of inadequate neural networks that increase the risk of developing neurological and psychiatric disorders [46]. Thus, prenatal or perinatal infections can lead to impaired physiological functions of microglia, which is an important risk factor for the late onset of diseases such as schizophrenia, autism spectrum disorder (ASD), and attention deficit/hyperactivity disorder (ADHD) [18].

Viral infections affecting the brain induce the phagocytic activity of microglia, which is involved in the elimination of pathogens and cellular debris [14]. Microglia can also promote neurogenesis and induce neurotoxicity through the release of oxidants, which in turn can activate inflammation [40]. A triggering receptor expressed on myeloid cells 2 appears to be required for microglia-mediated synaptic pruning during brain development [63]. In a mouse model of coronavirus infection, it was shown that the microglia-associated triggering receptor expressed on myeloid cells 2 and DAP12 (12 kDa DNA activating protein) were among the most highly expressed genes [8]. Taken together, these studies suggest that microglial function is modulated by viral infections during development and may be associated with long-term complications in children infected with COVID-19.

The formation of microglia can also be influenced by T-lymphocytes involved in

its various functions at the early stages of development [36]. Indeed, the population of T cells that act as “catchers” in the CNS, localized both in the brain parenchyma and in the choroid plexus and meninges, are associated with the maintenance of functional neuroplasticity in a healthy brain. These T cells can also stimulate peripheral immune cells through a complex signaling pathway with the choroid plexus, releasing IFN-Y [27], and promoting plasticity through the release of IL-4 [51]. However, the “cytokine storm” mechanism in the pathogenesis of SARS-CoV-2 infection can disrupt the normal cytokine-mediated cross-pooling in the choroid plexus, when IFN-Y, together with IL-6, is one of the main active molecules of the pro-inflammatory profile. Also, a group of scientists found high levels of IL-6 and INF-Y in the CNS of K18-hACE2 transgenic mice infected with SARS-CoV [58].

Dietary modulation of neuroinflammation. Localization of the ACE2 receptor in microglia [62] increases the possibility of its direct activation by SARS-CoV-2, which may increase the risk of late neurodegenerative diseases, as shown for other viral infections [18]. Viruses such as Zika virus (ZIKV), cytomegalovirus, and rubella are able to cross the placental barrier and/or BBB and reach the CNS [37]. In ZIKV infection, along with damage to progenitor cells, an increase in neuroinflammation is observed, which disrupts the physiological role of microglia during brain development [69]. The same is true for other RNA viruses, such as cytomegalovirus [2]. It is possible that these data suggest that the inflammation caused by a viral infection will be more detrimental to the development of the nervous system than the direct cytopathic effect of the virus on neurons.

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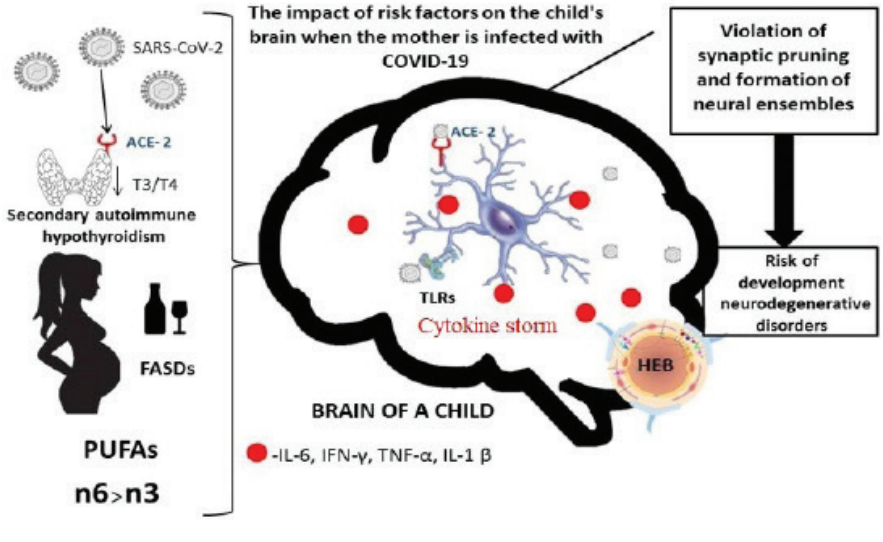
Relationship between maternal alcohol use during pregnancy and neuroinflammation in COVID-19. Fetal alcohol spectrum disorders include several pathologies and side effects associated with alcohol use by pregnant women [11]. Some of the neurocognitive impairments seen in alcohol spectrum disorders include: memory or visuospatial decline, low behavioral self-control, rapid mood changes, impulsive behavior, loss of adaptive functions such as speech and communication, poor social interaction, and movement disorders [68]. Alcohol can interfere with the development of the fetal nervous system through changes in a number of events such as neurogenesis, gliogenesis, myelination, and impaired development of functional neural networks [34]. Thus, the teratogenic effects of ethanol during pregnancy are considered as a risk factor for the development of brain anomalies [25], and there is a strong correlation between alcohol use during pregnancy and ADHD and ASD [45, 44].

Ethanol-induced brain malformations are often associated with microglial activation via toll-like type 4 receptor (TLR4) [65] and release of pro-inflammatory cytokines and chemokines [29]. TLR4 activation can induce inflammation through a MyD88-dependent signaling pathway



Infectious and noninfectious factors alter the microglial function and contribute to developmental brain disorders. SARS-CoV-2 - severe acute respiratory syndrome coronavirus 2; FASDs- fetal alcohol spectrum disorder; HEB - hematoencephalic barrier; PUFAs - polyunsaturated fatty acids; ACE2 - angiotensin-converting enzyme 2; IL-6 - interleukin 6; IFNY - interferon gamma; TNFa - tumor necrosis factor alpha; IL1ß - interleukin 1 beta.

that interacts with nuclear factor kappa-bi (NF-kB) [17]. In addition, maternal alcohol consumption during pregnancy contributes to the development of newborn infections [32], reducing the immune response to the fight against viral and bacterial infections [7] with impaired adaptive immunity and altered B-cell responses, leading to an increase in the severity of viral infections [23]. It has recently been reported that SARS-CoV-2 also interacts with TLR receptors that induce pro-inflammatory cytokines [55]. Thus, SARS in COVID-19 and alcohol use during pregnancy may interact in converging inflammatory pathways. A generalized scheme of the impact of risk factors on the brain of a child when a mother becomes infected with COVID-19 is shown in Figure.

Clinical presentation in children with MIS-C temporally associated with SARS-CoV-2. In a retrospective study, which took place in the UK at the Great Ormond Street Hospital [48], scientists selected 58 case histories of children (mean age, 9 years [interquartile interval {IQR}, 5.7-14]; 33 girls [57%]) that met MIS-C criteria. PCR tests for SARS-CoV-2 were positive in 15 of 58 patients (26%), and IgG test results were positive in 40 of 46 (87%). A total of 45 of 58 patients (78%) had evidence of current or previous SARS-CoV-2 infection. All children had fever and nonspecific symptoms, including vomiting (26/58 [45%]), abdominal pain (31/58 [53%]), and diarrhea (30/58 [52%]). Rash was present in 30 of 58 (52%) cases and conjunctival injection was present in 26 of 58 (45%) cases. Laboratory evaluation indicated a marked inflammatory response, such as C-reactive protein (229 mg/L [IQR, 156-338] estimated in 58 of 58) and ferritin (610 μg/L [IQR, 359-1280] estimated in 53 out of 58). Of the 58 children, 29 developed shock (with biochemical signs of myocardial dysfunction) and required inotropic support and hospitalization in the intensive care unit. Of those admitted to the ICU, 23 of 29 [79%] received mechanical ventilation. Eight patients (14%) developed dilatation or aneurysm of the coronary arteries. Comparison of PIMS-TS with Kawasaki syndrome and toxic shock syndrome showed differences in clinical and laboratory characteristics, including older age (mean age, 9 years [IQR, 5.7–14] vs. 2.7 years [IQR, 1.4– 4.7] and 3.8 years [IQR, 0.2-18] respectively) and greater elevations in inflammatory markers such as C-reactive protein (median, 229 mg/L [IQR 156-338] vs 67 mg/L [IQR, 40-150 mg/L] and 193 mg/L [IQR, 83-237], respectively).

Conclusion. Since the onset of the COVID-19 outbreak, children have remained less susceptible to infection in most cases with subclinical manifestations and a mild course. Despite reports of MIS-C syndrome, parents and pediatricians are not fully aware of the possible long-term effects of inflammation on brain development and possible interactions between viral infections and non-infectious conditions such as nutritional imbalances of FAs and PUFAs and alcohol consumption during pregnancy. Transient thyroiditis caused by SARS-CoV-2 has also been reported, which can lead to autoimmune hypothyroidism. In the present review, we hypothesize that these conditions may interact to cause an increase in neuroinflammation, which may alter the physiological role of microglia by influencing the mechanisms of synaptic pruning and neural circuit formation that occur from 2 years of age through adolescence. Thus, it should be noted that autoimmune hypothyroidism, malnutrition, and maternal alcohol consumption during pregnancy may be considered risk factors in children infected with COVID-19, who may be more susceptible to neurodevelopmental disorders such as schizophrenia, autism, ADHD and cognitive impairment. Therefore, attention should be paid to possible interactions between risk factors that can lead to long-term brain developmental abnormalities and occur in the next few years. Therefore, careful monitoring of children exposed to SARS-CoV-2 or born to infected mothers is strongly recommended, and future studies that could identify additional risk factors are highly recommended.

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DOI 10.25789/YMJ.2023.82.26

УДК 577.2.612.43

A.A. Grigoryeva, E.D. Okhlopkova

THE ROLE OF MELATONIN IN DISORDERS OF THE PSYCHO-EMOTIONAL SPHERE

An analysis of the works of domestic and foreign literature devoted to the study of the effect of melatonin on the psycho-emotional state of the organism was carried out. According to most researchers, the trend towards an increase in the prevalence of depressive disorders continues. Currently, there is a search for new approaches in the treatment of depression. The relationship between melatonin and the occurrence of depressive disorders requires further study.

Keywords: melatonin, depression, circadian rhythm, neuroinflammation, nervous system, chronotype.

Introduction. According to the World Health Organization (WHO), the June 2022 World Mental Health Report noted that 1 billion people in the world suffer from mental disorders, including 15% of working age. During the coronavirus pandemic, the prevalence of depression increased by 25%. Depression remains a major problem in the modern world. Despite research on depressive disorders and their treatment with antidepressants, about 80% of inpatients with depression and 70% of outpatients complain of sleep disturbances. Currently, there are several available hypotheses for the occurrence of depressive disorders. Among them are neurotransmitter dysfunction hypotheses and chronobiological concepts, i.e. altered circadian rhythms mediated by melatonin. Melatonin is a universal biological regulator of vital rhythms for all living organisms, as evidenced by its secretion in all animals, starting with unicellular [1,2].

The history of the discovery of melatonin (MT) is associated with the name of Aaron Lerner, a professor of dermatology at Yale University, who studied the nature of vitiligo. Having reviewed the publication of C. McCord and F. Allen (1917), who found that the use of an extract of the pineal glands of cows led to a lightening of the cover of tadpoles by compressing the dark epidermal melanophores. Professor A. Lerner came to the conclusion that a substance responsible for pigmentation and destruction of pigments is formed in the pineal gland, and thought that this substance would help in the treatment of skin diseases. In the early 1950s a group of scientists led by Lerner succeeded in isolating an extract from cow pineal glands that brightens the skin of frogs. The experiment was delayed, so it was decided to complete work on it, but shortly before the end of the term, scientists managed to isolate and determine the structure of the main substance - it turned out to be N-acetyl-5-methoxytryptamine, which was named melatonin. The resulting discovery was described by Lerner in an article published in 1958 in the Journal of the American Chemical Society [17].

Melatonin performs important antioxidant and chronobiotic functions for the body, but also affects carbohydrate metabolism, secretion of insulin, leptin, adiponectin, adipocyte proliferation, and eating behavior. The mechanism of action of melatonin lies in its amphiphilicity, which allows it to penetrate through cell and nuclear membranes and directly interact with intracellular organelles. The antioxidant function of MT can be distinguished, and it consists in the inhibition of the formation of hydroxyl radicals, the protection of lipids, proteins and DNA, and cellular apoptosis. Melatonin also has the ability to limit oxidative stress and regulate energy metabolism. Including body weight, insulin sensitivity and glucose tolerance. The effects of MT are realized at the stages of energy consumption (nutrition), redistribution of energy reserves and energy consumption. Synchronization of human eating behavior with metabolic processes also occurs with the participation of melatonin.

It has been found that melatonin is synthesized in the human body in the cells of the bone marrow, intestines, on the skin and in the retina of the eye. According to the first assumptions, melatonin was considered a hormone involved in the regulation of circadian rhythm mechanisms in living beings, but later it was found that, in addition to this hormonal function, MT is involved in the regulation of seasonal and lunar cycles in animals and humans. The level of melatonin in human blood fluctuates during the day: during daylight hours

Federal State Budgetary Scientific Institution Yakut Science Centre of Complex Medical Problems, Yakutsk: **GRIGORYEVA Anastasia Anatolyevna** – junior researcher Arctic Medical Center, nastiagrigoryeva@gmail.com; **OKHLOPKOVA Elena Dmitrievna** – Ph.D., senior researcher, elena_ohlopkova@mail.ru.

it does not exceed 10 pg/ml, at night its concentration rises, reaching a maximum at 2-4 am to 200 pg/ml or more. It is known that in breast milk the level of tryptophan, the precursor of MT, also has a circadian rhythm, which determines the rhythm of sleep and wakefulness of the newborn. Recently, studies have shown that enterochromaffin cells of the intestinal mucosa are the source of melatonin. Moreover, most of them are in the duodenum and rectum. The transport function of melatonin in the blood is carried by the protein albumin. At night, the concentration of melatonin rises in the fluid of the spinal cord, ovarian follicles, seminal fluid and fluid of the anterior chamber of the eye. After being released from the bond with albumin, melatonin binds to specific receptors on target cells and penetrates into the cell nucleus. The main metabolite of melatonin in urine is 6-hydroxymelatonin sulfate, the level of excretion of which in the urine corresponds to the concentration of melatonin in serum [45].

Depressive disorders currently occupy the fourth place among morbidity in the world population, and according to WHO data, by 2023 they will be in second place, second only to pathologies of the cardiovascular system. According to available data, up to 10% of the world's population suffer from depression, and up to 45% of people have experienced a depressive episode at least once in their lives [1].

In recent decades, a number of studies have been carried out in which it has been shown that melatonin secretion is impaired in depressive disorders. It has been found that people with depression have a decrease in plasma melatonin at night. Scientists believe that low levels of melatonin in people with depression are a sign of a decrease in the concentration of norepinephrine and serotonin in the brain. This fact shows that a low value of melatonin can serve. as a marker of the balance of neurotransmitters in the brain. As a result of a detailed study of melatonin secretion in disorders associated with the nervous system, the concept of "low melatonin syndrome" has been described. According to this concept, low secretion of melatonin may be a biological marker of predisposition to depressive disorders [5].

In recent decades, a number of studies have been carried out in which it has been shown that melatonin secretion is impaired in depressive disorders. It has been found that people with depression have a decrease in plasma melatonin at night. Scientists believe that low levels of melatonin in people with depression are a sign of a decrease in the concentration of norepinephrine and serotonin in the brain. This fact shows that a low level of melatonin can serve as a marker of the balance of neurotransmitters in the brain. As a result of a detailed study of melatonin secretion in disorders associated with the nervous system, the concept of "low melatonin syndrome" has been described. According to this concept, low secretion of melatonin may be a biological marker of predisposition to depressive disorders [5].

The works of foreign researchers have shown that in people with manifestations of depression, melatonin secretion depends on the time of day, according to some results, the level of melatonin increases at night [4], according to others, an increase was noted regardless of the time of day, both in the daytime and at night [2]. A study by Szymanska A. [12] showed that the level of daytime melatonin depends on the severity of depressive manifestations (according to the Hamilton Depression Scale) and is higher in people with severe depression, however, the level of night melatonin was increased in all groups with both severe and severe depression. and with mild depression.

A number of studies have shown that people with a late chronotype are more at risk of developing disturbances in the psycho-emotional state, sleep patterns, and seasonal mood swings [34]. Rosenthal N. B co-authors revealed the relationship of seasonal depressive disorders with the violation of biological rhythms [43]. The increase in the risk of depressive disorders in the winter is due to low light during the daytime, which leads to sleep disturbance and is associated with factors such as daytime fatigue and hypersomnia. The etiology of any depression has the same characteristics such as: impaired cognitive functions (ability to concentrate, memory), mood, relationship between sleep, physiological cycles.

In addition, in depressive disorders, a decrease in the range of fluctuations of some rhythms was revealed. It was found that some patients have a shift in the phase of the circadian rhythm to an earlier time (early awakening and activation of the secretory rhythms of melatonin, rapid falling asleep, cortisol and norepinephrine in the early morning hours), while the rest of the individuals have a phase delay (late falling asleep and awakening). This leads to a decrease in the release of melatonin, cortisol, serotonin and thyroid-stimulating hormone, as well as fluctuations in the number of heartbeats and body temperature [3].

Thus, the early signs of depression should not be ignored, it is necessary to consult a specialist and undergo treatment.

The effect of melatonin on the human body in depressive disorders. Melatonin acts on various proteins, cellular and molecular pathways involved in depression [22, 6]. One of the most important mechanisms of action of melatonin in depression is to reduce oxidative stress. Oxidative stress is known to play a role in the pathophysiology of depression [39, 46]. Oxidative stress caused by overproduction of reactive oxygen species (ROS) and/or a defective antioxidant triggers a series of oxidative damage [47]. Oxidative stress easily induces neuronal apoptosis, neurological deficits, and neurotoxicity, thereby accelerating the onset and development of neuropsychiatric disorders, such as depression, Alzheimer's and Parkinson's diseases [44]. It has been proven that the antioxidant function of melatonin is associated with its ability to prevent excessive formation of ROS and with an increase in the level of antioxidants [35]. Melatonin has the function of removing free radicals and an indirect antioxidant effect, and also increases the activity of antioxidant enzymes [23, 41]. Melatonin also regulates the levels of glutathione peroxidase (GP), catalase (CAT), and superoxide dismutase (SOD), antioxidant enzymes that prevent ROS-induced damage [24, 48]. Melatonin upregulates SOD and Cat levels in rats with copper-induced oxidative stress, along with alleviation of depressive-like behavior [25]. In addition, melatonin suppresses serum ROS levels and alters redox signaling molecules in the brain in lipopolysaccharide-treated mice [26]. Recent studies have shown that melatonin greatly increases the activity of SOD, GP and Cat, and reduces the level of malondialdehyde (MDA) [36]. Melatonin improves Cat activity and SOD enzymes and subsequently inhibits lipid peroxidation damage to hippocampal neurons [27]. Melatonin also neutralizes the effect of oxidative stress and restores the activity of SOD, GP, and Cat [28]. Therefore, melatonin exhibits neuroprotective and antidepressant effects through stimulation of the antioxidant system and suppression of intracellular oxidative stress.

Melatonin can improve neuronal survival and neurogenesis [13]. Melatonin promotes neuronal differentiation [14] and exhibits high antioxidant and antiapoptotic properties [29]. Agomelatine increases cell proliferation and neurogenesis in the ventral dentate gyrus and improves

the survival of newly formed cells in both the dorsal and ventral dentate gyrus [7]. As is known, in some neuropsychiatric disorders, such as depression, the hippocampus is severely weakened during neurogenesis [30]. Depression is also associated with induced stress, where there is a decrease in dentate gyrus neurogenesis [8]. Thus, melatonin may exert antidepressant effects by facilitating neurogenesis and preventing apoptosis in the hippocampus.

Melatonin reduces neuroinflammation by reducing IL-1β and TNFα, which contributes to a positive effect on depression [48]. Neuroinflammation is involved in the pathophysiology of depression [37]. Dysfunction of the immune system, showing pro-inflammatory conditions, in patients with severe depressive disorders [21, 40].

It is important to note that studies of patients with depression have demonstrated changes in the structure and function of brain regions during pro-inflammatory processes [20]. Previous studies have shown increased levels of pro-inflammatory cytokines such as IL-1β, IL-6 and TNF-α in depression [10, 31]. With an increase in the concentration of IL-6, there is a decrease in the volume of the hippocampus in patients with depression. Plasma IL-1, IL-6, IL-2 receptors, IL-6 receptors, and acute phase protein concentrations are increased in patients with major depressive disorders [49].

Recent clinical and preclinical evidence suggests that neuroinflammation is a key factor interacting with three neurobiological correlates of depressive disorder: serotonin depletion in the brain, dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis, and disruption of the continuous production of sex hormone-forming neurons in dentate gyrus of the hippocampus [21].

Depression also increases levels of inflammatory markers, including TNF-α, IL-1β, IL-2, and IL-6 [19]. It has been shown that melatonin, through anti-inflammatory processes, contributes to the reduction of free radical damage [16]. Melatonin reduces the levels of TNF-α, IL-1β, and oxidative stress mediators in various parts of the rat brain after intracerebroventricular administration of lipopolysaccharides [9].

Melatonin also reduces the lipopolysaccharide-induced increase in TNF-α in maternal serum and fetal brain [15]. In addition, chronic administration of agomelatine suppresses pro-inflammatory cytokines such as IL-6 and IL-1β both in the periphery and in the brain of LPS-exposed rats [32]. Chronic treatment with agomelatine also attenuates depression and suppresses inflammatory signals in epileptic rats. [42]. In mice exposed to lipopolysaccharide, melatonin induces an antidepressant effect by reducing the levels of TNFα, IL-1β, and IL-6, as well as weakening autophagy [26]. Melatonin reduces oxidative stress, NF-κB activation, and depressive behavior after lipopolysaccharide administration to mice [11]. Previous studies have shown that lipopolysaccharide-induced depressive behavior is significantly associated with elevated TNF-α levels, while melatonin administration prevents this mechanism [33]. On the other hand, pro-inflammatory cytokines suppress the release of melatonin. The introduction of recombinant IL-1β reduces the level of melatonin in the blood serum of rats [38]. In addition, suppression of nocturnal melatonin in mothers with mastitis is significantly associated with an increase in TNF-α production [18]. Thus, the antidepressant effect of melatonin may be associated with the suppression of neuroinflammation.

Thus, the analysis of the literature indicates that melatonin has multifunctional biological properties, has a multifaceted beneficial effect on the human body, and its effectiveness and safety in the treatment of symptoms of depression and jet lag is beyond doubt. Currently, any quick search on the biomedical research search engine Pubmed reveals that there are 1948 articles on melatonin and depression. Such a number of articles for the development of the study of melatonin is not enough in our time, so this direction requires further study.

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POINT OF VIEW

DOI 10.25789/YMJ.2023.82.27

УДК 616.31-089.844:159.9

I.S. Pinelis, Y.I. Pinelis, I.D. Ushnitsky

CHARACTERISTICS OF PSYCHO-EMOTIONAL STATE OF PATIENTS REQUIRING PLASTIC SURGERY

The results of psychoemotional state assessment of patients who consulted a plastic surgeon con-cerning aesthetic operations in the maxillofacial region (MF) (ear, nose, blepharoplasty, facelift, etc.) are presented. The work evaluated their personality characteristics, which influence the deci-sion-making for aesthetic operation.

The **aim** of the research is to identify the characteristics of psychoemotional tension in patients who applied to a plastic surgeon for aesthetic operations.

Materials and methods. 145 patients who consulted a plastic surgeon for aesthetic surgery of the face and neck were surveyed. The research was done at Chita State Medical Academy Clinic of the Ministry of Health of the Russian Federation.

Results. The article presents the data concerning the peculiarities of such patients' appearance perception, estimation and pretensions level. The main directions of their psychological support before the aesthetic surgery with the purpose of increasing the efficiency of the performed cos-metic intervention are considered. Meanwhile, inflammatory and oncological diseases of the face cause the highest situational and personal anxiety, while the general level of anxiety is the lowest among the patients dissatisfied with their appearance. At the same time, a close connection of emotional, characterological and behavioral reactions with a person's appearance caused by con-genital and acquired facial defects and deformations was determined.

PINELIS Iosif Semenovich – MD, Professor of the Department of Surgical Dentistry, the Chita State Medical Academy; e-mail: pinelis1@mail. ru; **PINELIS Yury Iosifovich** – MD, Associate Professor, Head of the Department of Surgical Dentistry of the Chita State Medical Academy; e-mail: pinelisml@mail.ru; **USHNITSKY Innokentiy Dmitrievich** – PhD, MD, pro-fessor, head of the Department of Therapeutic, Surgical, Orthopedic Odontology and Odontolo-gy of Children's Age, M.K. Ammosov North-Eastern Federal University, Yakutsk, e-mail: in-cadim@mail.ru

Discussion. Dominance, self neglet, and friendliness predominate in personality traits, and the level of situational and personality anxiety is defined as average, which predetermines to refer such patients to the risk group. However, at the same time, psychopathological disorders (dys-morphomanic syndrome, neurosis-like disorders, and psychopathies) were found to be signifi-cantly rare in the examined individuals. In such situations, dysmorphophobia should be excluded, the likelihood of which increases in individuals who claim to have surgery in atypical areas, re-peated surgical interventions, etc.

Conclusion. This research determined that the risk factors for developing a mental disorder for aesthetic surgery patients are: more often female sex, lack of marital relationships, chronic psy-chotraumatic situation, long-term and ineffective surgical treatment, psychiatric disorders in the past, low level of meaningfulness in life, etc. It was established that most of the patients applied for elimination of cosmetic defects by clinical and aesthetical causes. In this regard, the participa-tion of a psychologist and psychiatrist is essential in deciding the appropriateness of cosmetic surgery as well as in conducting psychotherapeutic preparation of patients for surgery.

Keywords: aesthetic surgery, situational and personality anxiety, psychoemotional state, maxillo-facial area, psychological support.

Introduction. Dissatisfaction with one's appearance affects significantly the psychological and emotional state of a person, his character and social adaptation, psychological vulnerability, resentfulness, irritability, etc. Often patients try to eliminate this dissatisfaction and psychological problems with plastic surgery [1, 2, 10]. Meanwhile, it should be noted that their decision of cosmetic surgery is not always a conscious one.

Plastic surgeries usually result in a rapid and lasting improvement of a person's appearance, which contributes to self-esteem changes and social significance increase [11]. However, aesthetic surgeries are not indicated for people with mental illnesses (delusions, dysmorphobia, etc.), which requires a prior psychiatric examination [3, 2]. Sometimes, psychoemotional tension of various degree (PET) is detected in such patients at psychiatric pathology absence [6, 9]. It affects the patient's preparation for surgical intervention, the course of the postoperative period, and the assessment of the quality of surgical results [4, 5]. PET manifests as addictive behavior, characterized by a desire to change one's appearance by surgical procedures, distorted perception, and pathological dissatisfaction with one's face. It develops on the basis of insecurity, low self-esteem, distorted perceptions of one's own appearance and beauty ideals, and requires surgery with no obvious defects [7]. PET is assessed by a psychiatrist, psychologist, and plastic surgeon during an initial consultative interview with the patient. Treatment includes cognitive-behavioral psychotherapy, participation in support groups, and medication correction [8]. In view of the above, we chose the direction for our research.

The objective of the research is to reveal peculiarities of psycho-emotional tension in people who have undergone aesthetic surgeries to the plastic surgeon.

Materials and methods of the research. The study involved 145 patients who consulted a plastic surgeon about aesthetic operations on the face and neck. The study was conducted at the clinic of Chita State Medical Academy of the Ministry of Health of the Russian Federation. To achieve the goal of the study we determined the dominating motives, personal features, and anxiety level of the reconstructive and plastic surgery patients that will help to expand the knowledge on their psychosocial peculiarities, determine the risk groups for the development of mental disorders and specify the algorithms of PET correction. Patients who had consulted a plastic surgeon were examined and questioned. Cosmetic defects in 79 patients (54,5%; χ^2=11,10; p=0,02) were explained by the consequences of trauma, inflammatory and oncologic diseases; the rest patients had age-related changes of the face or were not satisfied with their own appearance. All examined patients were: women - 82.0% (χ^2 =6,10; p=0,03), men - 18%, the age varied from 17 to 61 years old and older (under 20 years old -13 (9%), 21-40 years old - 73 (59%), 41-60 years old - 39 (27%), over 61 years old - 20 (13.8%) (Table 1.).

There were anonymous questionnaires that contained questions about age, gender, education, profession, marital status, reasons for going to a plastic surgeon, etc. R.Cattell's multifactorial questionnaire, Leary's questionnaire, and Spielberger's test were used to determine personal situational anxiety.

The study was conducted with the ethical principles of scientific medical research involving human subjects, as defined by the Declaration of Helsinki of the World Medical Association (1964, ed. 2000), and the requirements of the regulatory documents of the Russian Federation on clinical trials. All patients gave voluntary informational consent to conduct the research.

The obtained data were processed

Table 1

Distribution of patients by sex and age

Sex		Age			
Males	Females	Under 20 years old	21-40 years old	41- 60 years old	From 61 years old and older
26 (17.93%) p=0.02	119 (82.06%) p=0.03	13 (9%) p=0.02	73 (59%) p=0.001	39 (27%) p=0.01	20 (13.8%) p=0.02

Table 2

Distribution of patients according to the degree of psycho-emotional instability depending on the cause of defects and deformations in the maxillofacial region (TM)

Etiological factor	Degree of psycho-emotional instability
Patients with the face trauma consequences	Significant prevalence of emotional instability (χ2 =49.09; p=0.0001)
Patients with cancerous defects of the craniofacial cavity	Significant prevalence of emotional intemperance with low ability to predict the consequences of their actions (82.3%; χ2 =21.10; p=0.002)
Individuals with age-related changes	Moderate psycho-emotional instability (χ2 =15.40; p=0.0001)

using Statistica 6.0 statistical analysis program (Stat Soft, USA). We used Pearson's χ2 test to estimate the significance of differences (p) to compare relative values. Starting with a p-value equal to or less than 0.05, the differences were assessed as significant.

Results and Discussion. According to the data obtained, aesthetic surgery patients often had PET, but they rarely consulted a psychiatrist or psychologist. Meanwhile, they were diagnosed with psychopathology, more often of neurotic level, consequences of severe stress and reactive states, depression, superficial sleep, dysmorphophobia, personality disorder, etc. Thus, the intellectual and emotional-volitional features, communicative properties and types of interpersonal interaction in the majority of observations were at the level of average values and were within the norm according to R. Cattell's multifactorial questionnaire during the study of personality characteristics. At the same time, the study of personal characteristics revealed a significant prevalence of emotional instability in patients with the trauma consequences (χ^2 =49,09; p=0,0001), and the signs of emotional intemperance with low ability to predict the consequences of their actions were markedly dominant in those with cancer consequences (82.3%; χ2 = 21.10; p=0.002) (Table 2). At the same time, people who wanted to look younger showed signs of increased trustworthiness, inner relaxation, ability to get along with people, and lowered self-esteem.

The data from the Leary's questionnaire indicated that unselfishness and friendliness were the leading features in most of the patients (84,9%; χ^2=22,7; p=0,001). At the same time, dominance in interpersonal relationships, assertiveness, and aggressiveness (65,7%, χ^2 =17,10; p=0,002) prevailed in patients with the consequences of facial injuries, and unselfishness and increased submissiveness (χ^2 =1,81; p=0,06) prevailed in those with the consequences of cancer.

Spielberger's questionnaire assessment revealed that the level of situational anxiety did not exceed average values in the overall group of patients, while the level of personal anxiety was slightly above average (χ^2 =14,41, p= 0,036). Patients with the facial trauma consequences had a lower level of situational anxiety than the level of personal anxiety. The level of situational anxiety exceeded the level of personal anxiety in patients with the consequences of oncological diseases. The level of personal anxiety significantly exceeded the level of situational anxiety in the group of patients dissatisfied with their appearance.

Almost half of the patents were single (72), 20 were divorced (13.8%), 46 (32%) were married or married, and 7 (4.8%) were widowed. Among those who responded 4.8% had incomplete secondary education, 54.4% had specialized secondary education, 13.8% had secondary education, and 27% had higher education.

Consequently, it can be noted that inflammatory and oncological diseases of the face cause the highest situational and personal anxiety, while patients dissatisfied with their appearance have the lowest general level of anxiety.

On the whole, the study of the patients' personality features convincingly shows a close connection of emotional, characteristic and behavioral reactions with human appearance due to congenital and acquired facial defects and deformities. Dominance, unselfishness and friendliness dominate in their personal features, and the level of situational and personal anxiety is defined as average. Such patients should be classified as a risk group for PET. At the same time, psychopathological disorders (dysmorphomanic syndrome, neurosis-like disorders, psychopathies) were rarely revealed in the examined patients. However, dysmorphophobia should be excluded in them, the probability of which increases in those who pretend to operate in atypical areas, repeated surgical interventions, etc. Risk factors for psychiatric disorders for aesthetic surgery patients are: more often female sex, absence of marital relations, chronic psychotraumatic situation, long-term and ineffective surgical treatment, psychiatric disorders in the past, low level of meaning in life, etc.

Conclusion. The majority of patients appealed for cosmetic defects correction due to evidently justified indications. Meanwhile, the definition of indications for plastic surgery is one of the most important stages in the plastic surgeon's activity. To some extent, it depends on the individual and psychological characteristics knowledge of those who consulted a surgeon. That's why the participation of a psychologist and psychiatrist is essential when deciding on the advisability of cosmetic surgery, as well as for psychotherapeutic preparation of patients for surgery.

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N.V. Zaitseva, A.A. Subbotina, O.V. Dolgikh

BONE METABOLISM MARKERS AND *ENOS* GENE POLYMORPHISM IN CHILD POPULATION OF EASTERN SIBERIA WITH DISEASES OF THE MUSCULOSKELETAL SYSTEM

DOI 10.25789/YMJ.2023.82.28

УДК 616.71

At present, it seems quite relevant to study genetic predictors of all classes of diseases in children including diseases of the musculoskeletal system.

The aim of this study was to examine markers of musculoskeletal diseases associated with specific indicators of bone metabolism and the G894T polymorphism of the *eNOS* gene in children from Eastern Siberia.

Materials and methods. We examined two groups of children aged 3-7 years from Eastern Siberia. The main group was made of 69 children with diseases of the musculoskeletal system. The comparison group included 47 children without such diseases. The study involved investigating biochemical (N-osteocalcin, ionized calcium, and alkaline phosphatase) and molecular genetic (the G894T polymorphism of the *eNOS* gene) indicators; they were identified by using relevant methods (PCR in real time and ELISA tests).

Results. We comparatively analyzed the result of bone remodeling in both groups and established that the children from the main group had up to 2.6 times higher levels of N-osteocalcin, ionized calcium, and alkaline phosphatase than their counterparts from the comparison group ($p<0.05$). These indicators have a pathogenetic association with bone metabolism disorders. Our analysis of the G894T polymorphism of the *eNOS* gene revealed that frequency of the allele T equaled 27.5% in the main group and was almost 2 times higher than in the comparison group ($p<0.05$).

Conclusion. We established significantly excessive levels of N-osteocalcin, ionized calcium, and alkaline phosphatase in children with diseases of the musculoskeletal system as well as more frequent G894T polymorphism of the *eNOS* gene (T-allele) associated with such diseases (OR=2.37; 95% CI 1.18-4.74; $p=0.01$). This indicates developing demineralization of bone tissue in this group and allows considering the analyzed markers to be predictors of musculoskeletal diseases in children from Eastern Siberia.

Keywords: diseases of the musculoskeletal system, the G894T polymorphism of the *eNOS* gene, N-osteocalcin, ionized calcium.

Introduction. The health of the child population is one of the most sensitive indicators of the state of society. At the moment, there is a high level of childhood morbidity in all groups of nosologies [1]. There are high growth rates of the pathology of the musculoskeletal system in the preschool children - 2.6 times [12].

Violation of the formation of bone mass in childhood creates the prerequisites for the development of osteoporosis in adulthood. Among a number of reasons for the formation of osteopenic conditions in children, genetic factors, disorders of mineral and vitamin metabolism, malnutrition, various pathological processes, etc., are of the greatest importance [6].

The use of some biochemical indicators allows assessing the state of bone tissue metabolism, identifying its violations, leading to the development of damage to the musculoskeletal system [9].

One of the promising and frequently studied markers of bone formation is the protein osteocalcin. It is the main protein of the bone matrix (molecular weight 5800 Da) and is synthesized mainly by osteoblasts. The content of this protein in blood serum reflects the state of bone metabolism in general [3, 10]. Ionized calcium is calcium that circulates freely in the blood and is not bound to proteins. The level of ionized calcium better reflects calcium metabolism than the level of total calcium [4]. Alkaline phosphatase is an enzyme involved in the metabolism of phosphoric acid. Its activity increases with the development of pathological processes in bone tissue [8]. To date, of particular relevance are studies to identify associations of genetic predisposition to the development of pathological processes in bone tissue and markers of its phenotypic implementation, that is, the identification of genetic and metabolomic predictors of pathological conditions.

The aim of this study was to examine markers of musculoskeletal diseases associated with specific indicators of bone metabolism and the G894T polymorphism of the *eNOS* gene in children from Eastern Siberia.

Materials and methods. 116 children living in the territory of one of the regions of Eastern Siberia were selected for the study. The observation group included 69 children aged 4-7 years (34 boys, 35 girls) with pathology of the musculoskeletal system (mainly dorsopathy and dorsalgia). The comparison group included 47 children aged 3-7 years (21 boys, 26 girls) without pathologies of the musculoskeletal system.

In the course of the study, a complex of biochemical and molecular genetic methods was used. To do this, the following biological media were taken from the examined children after signing the informed voluntary consent to medical intervention by the parents: blood to determine the levels of N-osteocalcin by ELISA using monoclonal antibodies to osteoprotein, ionized calcium by the ion-selective method and alkaline phosphatase by the kinetic colorimetric method; buccal epithelium to determine the genotype by polymorphism G894T (Glu298Asp) of the *eNOS* gene by polymerase chain reaction with further allelic discrimination of amplification curves.

Statistical processing of the obtained quantitative data was performed in the Statistica 6.0 program and presented

ZAITSEVA Nina Vladimirovna – academician of the Russian Academy of Sciences, MD, Professor, Scientific supervisor of FBSI Federal Scientific Center for Medical and Preventive Health Risk Management Technologies, e-mail: znv@fcrisk.ru; ORCID: 0000-0003-2356-1145; **SUBBOTINA Alena Alexandrovna** – junior research assistant of the Department of Immunobiological Diagnostics of the FBSI Federal Scientific Center for Medical and Preventive Health Risk Management Technologies, e-mail: alena-osa@bk.ru; ORCID: 0000-0002-3579-4125; **DOLGIKH Oleg Vladimirovich** – MD, Head of the Department of Immunobiological Diagnostics of FBSI Federal Scientific Center for Medical and Preventive Health Risk Management Technologies, e-mail: oleg@fcrisk.ru; ORCID: 0000- 0003-4860-3145.

as X±SE - the arithmetic mean (X) and the standard error of the mean (SE). Differences were considered significant if the corresponding p values were less than 0.05. Calculation of the frequency distribution of genotypes and alleles and observance of the Hardy-Weinberg equilibrium in the observation and comparison groups was carried out using the Gen-Expert online program. The association strength of the analyzed traits was determined using the odds ratio (OR). For OR, a confidence interval (CI) was calculated at the 95% significance level.

Results. The state of bone metabolism was assessed by the level of the main markers of bone tissue metabolism – N-osteocalcin, ionized calcium and alkaline phosphatase. When comparing these indicators, it was found that in the main group, the level of N-osteocalcin was statistically significantly higher than similar indicators in the comparison group by 2.6 times (p<0.05), while 50% of children in the observation group exceeded the reference level. The concentration of ionized calcium was increased in all children of the observation group relative to the reference level and 1.2 times relative to the comparison group. At the same time, the values of alkaline phosphatase were in the range of the physiological norm in 90% of children, but 1.2 times higher than in the comparison group (table 1).

Genetic analysis for the establishment of the genotype according to the G894T polymorphism of the *eNOS* gene revealed that the distribution of genotype frequencies in groups corresponds to the Hardy-Weinberg equilibrium, so the data can be analyzed using the general and multiplicative models of inheritance (for the observation group, p = 1.00; for the comparison group, p =0.16).

The study of the occurrence of genotypes established the following frequencies in the group of children with diseases of the musculoskeletal system: G/G - 52.2%; G/T - 40.6%; T/T - 7.2%. At the same time, the presence of the G/T and T/T genotypes increased the association with diseases of the musculoskeletal system by 3.9 and 1.15 times relative to the comparison group (p<0.05) (Table 2).

The analysis of alleles according to the multiplicative inheritance model showed that the occurrence of the T allele in the observation group is 27.5%, which is almost 2 times higher than in the comparison group (p<0.05) (table 2).

Discussion. Currently, dorsalgia and dorsopathy of childhood and adolescence are becoming more common [11]. In our study, dorsopathy occurred in 93% of children 4-7 years old with pathology of the musculoskeletal system.

Both high and low levels of osteocalcin in the blood can indicate bone loss. Animal studies suggest that high levels of osteocalcin are due to its reabsorption from bone tissue, which releases it, increasing blood levels [16]. Also, an increase in the level of osteocalcin in the blood is associated with fractures [13], osteoporosis [17], and bone softening [14].

One of the pathogenetically important criteria for calcium and phosphorus metabolism is the level of ionized calcium and the content of alkaline phosphatase. Previous studies have shown that in children with diseases of the musculoskeletal system, excess concentrations of ionized calcium in the blood serum reached 1.17±0.01 mmol/dm³ [5] and alkaline phosphatase in patients of the orthopedic-traumatology department - 486.25 U/l with a reference level of 129-417 U/l [2].

Nitric oxide, the formation of which is responsible for NO-synthase, is involved in cellular processes responsible for the renewal of bone tissue. It promotes the proliferation and differentiation of osteoblasts, and also plays a key role in osteoclast activity, as a decrease in NO levels has been shown to enhance osteoclastogenesis and associated bone resorption [15].

When studying the distribution of G894T polymorphic genotypes and alleles of the *eNOS* gene, significant differences were obtained among children with undifferentiated connective tissue dysplasia and healthy children in St. Petersburg. So, in all groups of children with undifferentiated connective tissue dysplasia, characterized by defects in fibrous structures and the main substance involved in the construction of cartilage and bone tissue, the T/T genotype and the T allele were more common [7].

Conclusion. Thus, it has been established that in children with pathology of the musculoskeletal system, significantly increased blood levels of N-osteocalcin, ionized calcium and alkaline phosphatase should be attributed to the features of bone tissue metabolism, which indicates a lower mineralization of the bones of the skeleton in this category of patients according to compared with the group of children without pathology (p<0.05). One of the etiological factors in the development of this condition is the factor of genetic determination of the G894T polymorphism of the candidate endothelial NO synthase (*eNOS*) gene. According to the results of this study, the occurrence of the T allele of the *eNOS* gene in the observation group was significantly higher than in the comparison group (OR=2.37; 95% CI 1.18-4.74; p=0.01).

Table 1

Comparative analysis of indicators of bone metabolism in children with pathology of the musculoskeletal system and healthy children

Indicator	Reference level	Observation group (n=69)	Comparison group (n=47)
N-osteocalcin. ng/cm³	27.92-67.95	72.93±11.30*/**	27.70±2.79
Ionized calcium. mmol/dm³	1.03-1.10	1.26±0.01*/**	1.06±0.02
Alkaline phosphatase. U/dm³	71.00-645.00	501.21±32.24**	405.94±26.88

* – differences are significant relative to the reference level (p <0.05);
** – differences are significant relative to the comparison group (p <0.05).

Table 2

Frequencies of genotypes and alleles of the *eNOS* gene in subjects with pathologies of the musculoskeletal system and healthy children

Gene	Genotypes/ alleles	Observation group (n=69)	Comparison group (n=47)	OR (95% CI)	p
eNOS (rs1799983)	G/G	0.522	0.787	0.29 (0.13-0.69)	0.01
	G/T	0.406	0.149	3.90 (1.53-9.95)	
	T/T	0.072	0.064	1.15 (0.26-5.04)	
	G	0.725	0.862	0.42 (0.21-0.85)	0.01
	T	0.275	0.138	2.37 (1.18-4.74)	

Conflict of interest. The authors of the article report that there is no conflict of interest.

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CLINICAL CASE

A.A. Budaev, V.B. Bondarev, N.V. Borovkova, A.M. Fine, M.S. Makarov, A.Yu. Vaza, O.L. Evdokimova, M.V. Neznanova, M.V. Storozheva, A.S. Mironov, A.Yu. Nikolaev, Yu.V. Andreev, I.N. Ponomarev

DOI 10.25789/YMJ.2023.82.29

УДК 616.6-089.843-032

RECONSTRUCTION OF THE ANTERIOR CRUCIATE LIGAMENT WITH CRYOPRESERVED ALLOGENEIC TIBIALIS ANTERIOR TENDON GRAFT

Aim of study. To evaluate the effectiveness of allogeneic tendon, sterilized with supercritical carbon dioxide, in restoration of the anterior cruciate ligament (ACL) during clinical example.

Material and methods. The patient was admitted at the N.V. Sklifosovsky Research Institute for Emergency Medicine with the diagnosis: an old rupture of the anterior cruciate ligament of the right knee joint, rupture of the internal meniscus, chondromalacia of the inner condyle of the right femur 2-3 art., according to the ICRS classification. From the anamnesis, it became known about the previous 2 operations for ruptures of the ligaments of the right knee joint and the use of autologous tendons as a plastic material.

A conversation was held with the patient about all possible methods of ACL repair and a choice was made in favor of using a cryopreserved allogeneic tibialis anterior tendon graft as a graft due to a shortage of autologous tissues. Allogeneic tendons were taken from tissue donors, treated with 10% dimethyl sulfoxide, sterilized with supercritical carbon dioxide and frozen at -80°C. After confirming the absence of toxicity, as well as the results of bacteriological examination, the allogeneic tendon graft was used for ACL repair.

Results. The early postoperative period was uneventful. On the 12th day postoperative wounds healed by primary intention. An MRI examination revealed a graft of the anterior cruciate ligament in the formed bone canals; the graft was not thickened and had a homogeneous structure.

After 28 days, the patient gradually began active and passive development of movements in the knee joint. After 6 weeks the range of motion and strength in the right knee joint was comparable to the left knee joint. After 4 months, according to magnetic resonance imaging, ligamentation of the allogenic graft and "synovial sleeve" formation were observed.

Conclusion. According to the clinical course and time of rehabilitation, the technique of using allogeneic tendons during ACL plastic surgery was comparable to the use of an autologous graft. The clinical experience of using allogeneic tendons at the early postoperative stage could be assessed as successful. Further observation is necessary to fully assess the reconstruction and integration of the tendon.

Keywords: anterior cruciate ligament, arthroscopic repair, graft, tendons, allogeneic, sterilization.

Introduction. Rupture of anterior cruciate ligament (ACL) is a frequent injury among knee joint injuries, which, in the absence of surgical treatment, leads to chronic instability and degenerative changes in the joint [5]. Arthroscopic reconstruction is considered to be the optimal method of surgical treatment of such injuries, which includes replacing the damaged ligament with autologous tissues, allogeneic grafts or synthetic implants. The choice of plastic material depends on the clinical situation, the preferences of the surgeon, and the capabilities of the medical institution [3]. Autologous tissues are the most commonly used material in arthroscopic operations for knee joint ligament ruptures. Nevertheless, this method of plastic surgery does not avoid negative effects, mainly associated with the presence of donor site [1]. The next promising method based on using conserved allogeneic tendons. Conservation process could use freezing, freeze-drying and chemical solutions treatment [4]. Conservation may dramatically change the structure of the tendon tissue, which leads to unsatisfactory strength characteristics of such transplants. Earlier in our publications it was described that penetrating cryoprotectors and their combinations allow preserving the overall architecture of tendons, the structure of collagen fibers and cellular composition, and also do not change the physic-mechanical characteristics of the graft [2]. At the same time, the choice of sterilization method remains an unsolved problem, since known methods (gas sterilization, gamma irradiation, etc.) negatively affect the viability of cells in the graft and indirectly damage of collagen fibers [6]. In our opinion, it is optimal to use supercritical carbon dioxide for sterilization, which allows preserving the structure of biological objects. Preclinical tests have shown sterility, absence of toxicity in cell culture, as well as preservation of physical and mechanical properties and structural integrity of allogeneic tissue that has undergone such treatment. This allowed, on the basis of a positive decision of the local ethics committee, within the framework of scientific work, to proceed to limited clinical trials on the use such transplants in situations, where it is impossible to use autologous material.

The aim of the study is to demonstrate in clinical example the effectiveness of using cryopreserved allogeneic tendon sterilized with supercritical carbon dioxide in the restoration of the anterior cruciate ligament.

BUDAEV Anton Arkadyevich – research associate, https://orcid.org/0000-0002-5864-5683; BudaevAA@sklif.mos.ru; **BONDAREV Vasily Bridzhevich** – research associate, https://orcid.org/0000-0002-1183-3644, **BOROVKOVA Natalia Valeryevna** – MD, Head of Scientific Department, https://orcid.org/0000-0002-8897-7523, **FINE Alexey Maksimovich** – MD, Head of the Scientific Department; Professor of A.I. Evdokimov Moscow State Medical University, Ministry of Health of the Russian Federation, https://orcid.org/0000-0001-8616-920X; **MAKAROV Maxim Sergeevich** – PhD in Biology, senior researcher, https://orcid.org/0000-0002-2184-2982; **VAZA Alexander Yulievich** – PhD, visiting research fellow, https://orcid.org/0000-0003-4581-449X; **EVDOKIMOVA Olga Liveryevna** – head. Radiosurgery Center, https://orcid.org/0000-0001-8099-9544; **NEZNANOVA Maria Viktorovna** – radiologist, https://orcid.org/0000-0002-0635-6783; **STOROZHEVA Maya Viktorovna** – research associate, https://orcid.org/0000-0003-1927-2404; **MIRONOV Alexander Sergeevich** – PhD, Head of Department, https://orcid.org/0000-0001-9592-7682; **ANDREEV Yuliy Vadimovich** – PhD, Senior researcher, https://orcid.org/0000-0001-8151-9

Clinical example. The patient K.V.M., female, born in 2000, was admitted to the Department of emergency traumatology of the musculoskeletal system of N.V. Sklifosovsky Research Institute for Emergency Medicine on 25.09.2022 with the diagnosis: an old rupture of the anterior cruciate ligament of the right knee joint, rupture of the internal meniscus, chondromalacia of the inner condyle of the right femur 2-3 art. according to the ICRS classification (ICD code-10 T93.3).

From the anamnesis: the patient is professionally engaged in sports, in 2018 during the competition she suffered an injury to the right knee joint – a rupture of the posterior cruciate ligament (PCL) and the external collateral ligament (ECL), for which she was operated a few months later. Arthroscopic restoration of the PCL was performed with autograft from the tendons of the popliteal muscles and the external collateral ligament with an autograft from the tendon of the long fibular muscle. A year after the operation and rehabilitation measures, the patient returned to active sports. In 2021 during training, she suffered a repeated injury to her right knee joint. On MRI from 12.04.2022, the following were diagnosed: rupture of the anterior cruciate ligament, linear rupture of the internal meniscus. The grafts in PCL and ECL were not damaged.

Since this injury no longer required emergency surgery, on 09.20.2022 K.V.M. applied for a planned consultation at of N.V. Sklifosovsky Research Institute for Emergency Medicine, where all possible options for surgical treatment, as well as options for plastic materials for ligament restoration, were explained to her. As a result of previously performed operations on the right knee joint to restoring PCL and ECL with tendons of the right lower limb, the patient had a deficiency of autologous plastic material that can be used for plastic surgery of the ACL on the right lower limb. After discussion and the voluntary informed consent of the patient, it was decided to perform plastic surgery with an allogeneic tendon, which was cryopreserved and sterilized with supercritical carbon dioxide. The use of a preserved allogeneic tendon graft (plastic material) for the treatment of patients with ligamentous apparatus defects has been approved by the Committee on Biomedical Ethics of the N.V.Sklifosovsky Research Institute of SP DZM (extract from Protocol No. 6-21 of 06.15.2021).

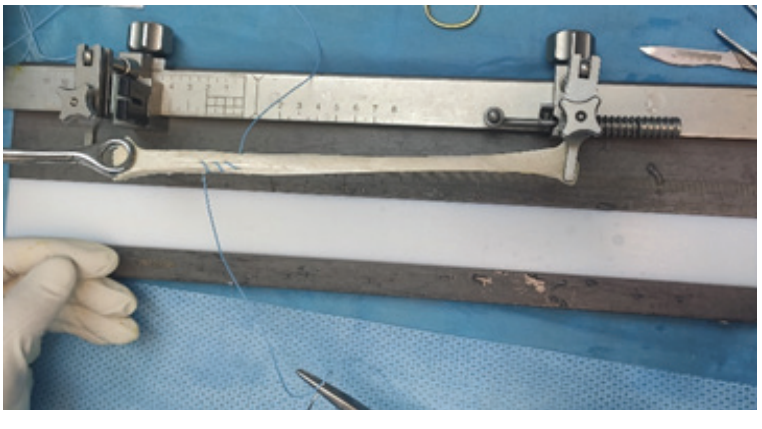
Allogeneic cryopreserved tibial tendon grafts were harvested as part of research work on the basis of the department of tissue preservation and transplant production. Tendon grafts in sterile conditions were explanted and mechanically processed according to the standard operating procedure. Allografts were treated with 10% solution of cryoprotector dimethyl sulfoxide, after that tendons were packed and sterilized with supercritical carbon dioxide. The next step was to freeze the bags with tissues at a temperature of -80 ^{0}C. Satellite samples of allografts, passed all stages of processing together with the transplant, were examined in the culture of multipotent human mesenchymal stromal cells. The prepared allograft was not toxic to the cells and also did not damage their structure. During bacteriological examination, the sterility of the tendon allograft was confirmed. After receiving the results for the absence of hemotransmissive infections in the tissue donor and thanatological examination, the individual transplant was prepared for clinical use (Fig.1).

On 09.26.2022 the patient underwent arthroscopic restoration of the ACL with an allogeneic tendon graft of the anterior tibial muscle sterilized with supercritical carbon dioxide with controlled decompression (Fig. 2,3). Also, during the operation resection of the inner meniscus of the right knee joint was performed. Due to the possible occurrence of conflict between the drilled channel in the tibia and the already formed channel for the PCL, it was decided to slightly change its location.

The early postoperative period proceeded without peculiarities. On the 1st day after operation we performed X-ray control of the position of the titanium but-

Fig. 1. Packed and sterile allogenic tendon graft

a



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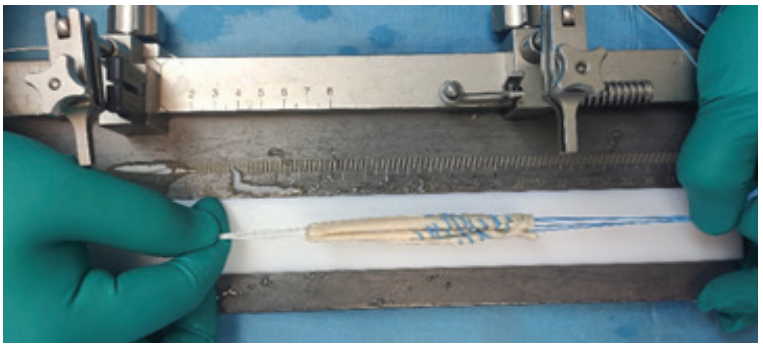

Fig. 2. Allograft preparing before arthroscopic restoration. a – graft stitching, b- graft before insertion into bone channels

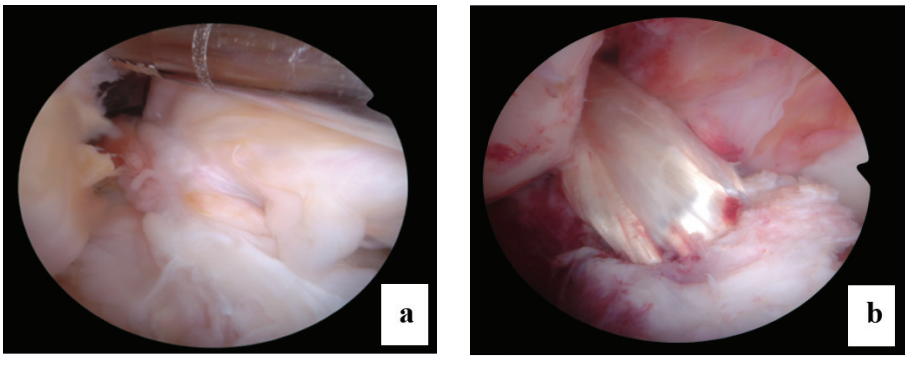

Fig. 3. Arthroscopic picture before (a) and after (b) allograft restoration

tons, fixing graft (Fig. 4). On the X-ray control the position of the fixators and bone channels was satisfactory.

The lower limb was fixed with an adjustable articulated orthosis in the position of full extension of the knee joint for 28 days. To prevent thromboembolic complications from the first day after surgery the patient wore compression knitwear. After receiving the results of ultrasound Dopplerography of veins of the lower extremities from 09.27.2022, on which no echo signs of thrombosis of the veins of the lower extremities were detected, walking with crutches without support on the operated limb was allowed. Also,

Postoperative wounds were treated with 10.25% Povidone-Iodine solution (Fig. 5), and for more effective, rapid reduction of edema, lymphatic drainage applications with kinesiotapes of the knee joint area (Fig. 6) and a standard protocol of therapeutic physical culture were used.

On 12th day the postoperative wounds healed by primary tension, the stitches were removed. After the sutures were removed, myostimulation in TENS (Transcutaneous electrical nerve stimulation) and EMS (Electrical muscle stimulation) modes was used to prepare the thigh and lower leg muscles for movements in the knee joint.

On the 14th day after the operation, the patient underwent magnetic resonance imaging on the GE Signa Hdx 3.0T device, in 3 projections, in T1, T2, PD FS modes. In the formed bone channels allograft is determined, located parallel to the Blumensaat line. The graft is not thickened, homogeneous structure. In PDF mode, trabecular edema of the knee joint bones is detected at the level of metal fixers, the MR signal from paraarticular soft tissues is increased (Fig. 7).

On the 28th day, the patient independently bent the right knee joint by 45° without pain, after which a step-by-step development of movements was

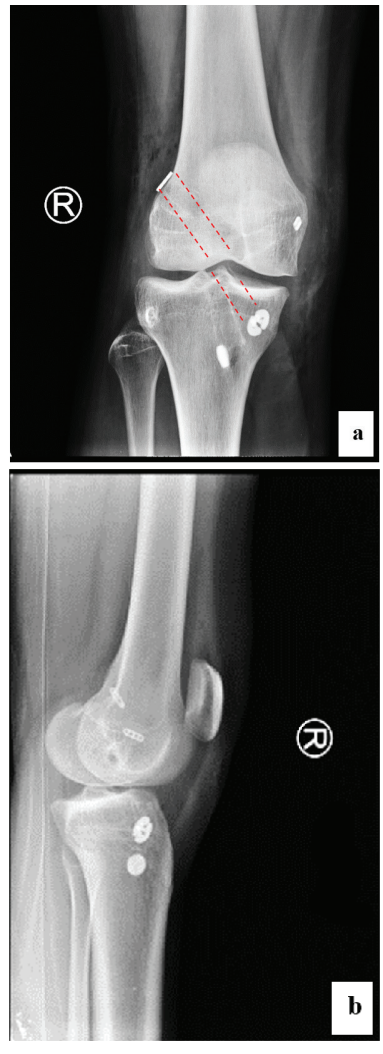

Fig. 4. X-ray examination of the right knee joint in straight (a) and lateral (b) projections, dotted lines show the formed bone channels

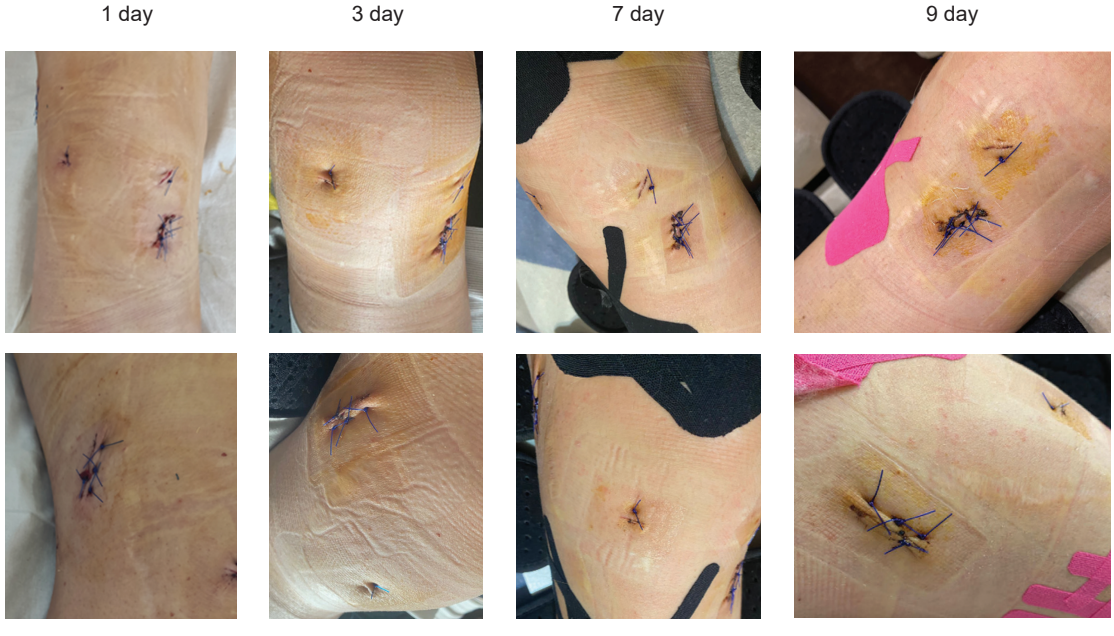

Fig. 5. Dynamic of postoperative wounds reparation Динамика заживления послеоперационных ран. Upper line – top view, lower line– lateral view

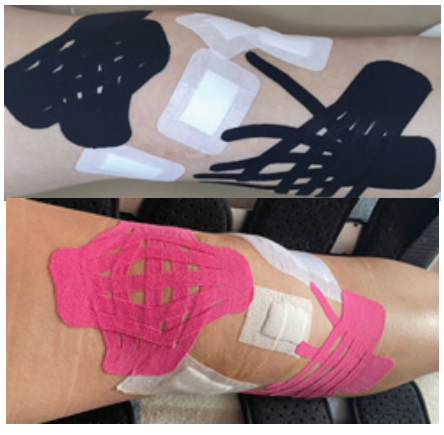

Fig. 6. Lymphatic drainage applications with kinesiotapes during injure recover

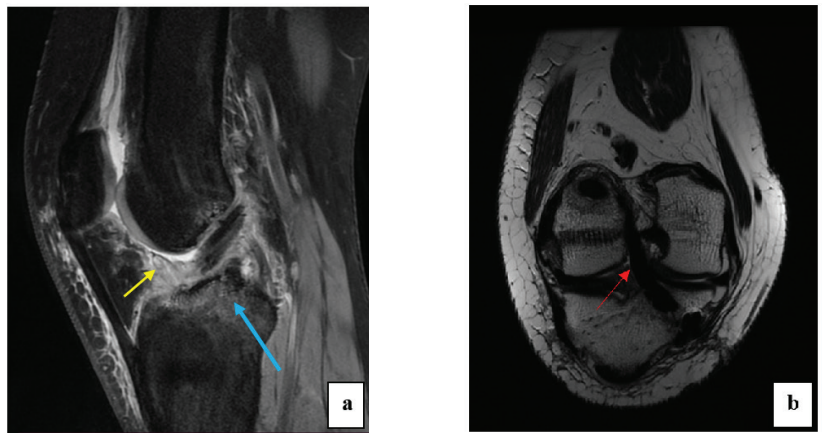

Fig. 7. Magnetic resonance imaging of the right knee joint on the 14th day after surgery: a – increased signal from the pararticular soft tissues (yellow arrow) and bone structures (blue arrow) on PD FS in sagittal projection

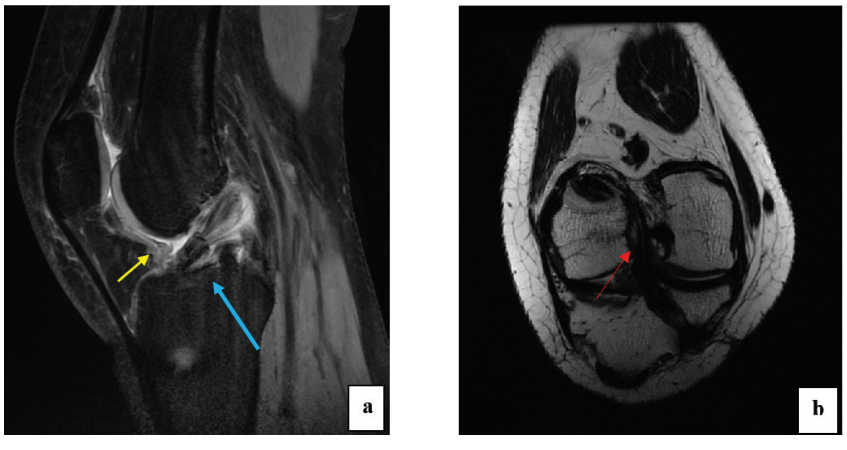

Fig. 8. Magnetic resonance imaging of the right knee joint 4 months after surgery: a – minimal edema of the pararticular soft tissues (yellow arrow) and bone structures (blue arrow) remains on PD FS in sagittal projection; b – an increase in the MR signal from the graft on T2 VI in the oblique coronary projection (red arrow)

started before the onset of pain with a flexion step from 10 to 20 in orthosis. According to the chosen rehabilitation protocol, from that moment on, first metered, and then full support on the lower limb when walking on crutches was offered.

At the end of 5 week the flexion amplitude in the knee joint was 90°, the patient also began to walk without crutches with full support on the right lower limb.

By 7 week the range of motion and strength in the right knee joint was comparable to the left knee joint. At this stage the patient did not complain and returned to her usual lifestyle.

After 4 months, magnetic resonance imaging of the right knee joint was repeated in order to assess tissue rearrangement (ligamentation) and integration of the allograft into the bone channels. The graft is thickened, there is an increase in the MR signal in the T2 VI mode throughout. The fibers are traced with the presence of fluid accumulations in the graft structure. A decrease in paraarticular edema and trabecular edema of bone structures at the level of metal fixators was revealed (Fig. 8). According to the MR study, we assume that the ligamentation of the allogeneic PCC and the formation of the "synovial sleeve" has begun.

Conclusion. This clinical case demonstrates the first experience of using frozen allogeneic graft, treated with cryoprotector and sterilized with supercritical carbon dioxide with controlled gas decompression, in patient with a rupture of the ACL. According to the clinical course and time of rehabilitation, the proposed method of using allogeneic tendons at this stage of observation is comparable to use of autologous graft. But it should be noted that the monitoring of such patients should continue until the complete reconstruction of the tendon and its integration into the bone channels.

The clinical case is demonstrated with the consent of the patient.

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S.A. Evseeva, M.E. Nikiforova, O.N. Ivanova, N.A. Danilov, A.F. Zhelobtsova, I.S. Ivanova, T.E. Burtseva

A CLINICAL CASE OF HAMARTOMA OF THE GRAY TUBERCLE OF THE HYPOTHALAMUS IN A 7-YEAR-OLD CHILD

DOI 10.25789/YMJ.2023.82.30

УДК 616-006.03

The article is devoted to an unusual clinical case which is hamartoma of the gray tubercle of the hypothalamus in a 7-year-old child with clinical signs of premature pubertal development. Congenital hamartomas of the hypothalamus is extremely rare in practice: about 1 case per 200 thousand live-born children. This is a congenital non-tumor heterotopia, which is located on the wall or bottom of the III ventricle of the brain, manifested by seizures of epilepsy, impaired intelligence and behavior, signs of precocious puberty.

Keywords: symptoms, hamartoma, defect, hypothalamus, heterotopias, puberty, child, neurology, tumor, pediatrics.

Introduction. A hamartoma of the hypothalamic grey tubercle is a congenital non-tumorous heterotopia that is located on the wall or floor of the third ventricle of the brain, and presents with epilepsy attacks, intellectual and behavioral disorders, and signs of precocious puberty [1]. According to rare studies in HH, epilepsy is the most common (87%), and intellectual and behavioral disorders and precocious puberty are less common (67.2%). Moreover, in HH children, signs of premature pubertal development have an earlier debut and a more malignant course [3]. According to magnetic resonance imaging in children with premature pubertal development, HH is detected in 30% of cases [2]. Therapy for preterm puberty in children with HH is aimed at suppressing hormonal activity with luleburin analogues.

Clinical example: From the life history: baby girl born in 2014 from the 1st pregnancy, which proceeded without features. Delivery at 40 weeks, operative. Birth weight was 4420 g, body length was 57 cm. Apgar score of 8/9. She screamed immediately, it was a loud scream. Natural feeding up to 12 months of age. Introducing complementary feeding from the age of 6 months. Psychomotor development to 1 year of age. Her head is holding since 2 months, he was turning since the age of 4 month, sitting since the age of 6 months, walking since the age of 12 months, her first teeth appeared in the age of 6 months, her first words were in the age of 1.5 years.

Prophylactic vaccinations by age.

Transferred diseases: SARS, angina, chicken pox.

No injuries or surgeries.

Allergic history: not aggravated.

Hereditary history: Grandmother had diabetes of the 2nd type on her mother's side.

Past medical history: since August 2016 (at the age of 2 years) parents noticed bloody vaginal discharge. The child was urgently hospitalized to the Department of Endocrinology and Gastroenterology of the Pediatric Center of Hospital No.1-NCM, where a complete clinical, laboratory and instrumental examination was performed.

The patient's condition was assessed as satisfactory. The patient's well-being was not impaired. The skin was clean and pale. The patient had no fever. The pharynx was calm. Peripheral lymph nodes were not palpated. Nasal breathing was free, no discharge. Breathing in the lungs was weakened in the lower parts: puerile, no rales. Respiratory rate (HR) was up to 22 per minute. Heart tones were clear, rhythmic, no murmurs were heard. Heart rate was 120 beats per minute. The abdomen was soft and painless. The liver and spleen were not enlarged. Sexual development: increase in mammary glands within the last 2 months. On examination, the mammary glands were enlarged in size (corresponding to grade 2 according to Tanner); sparse, weakly pigmented pubic hair. Within 6 months, the child has an acceleration of growth - more than 10 cm in 6 months, height exceeding the 97th percentile for that age and sex. Stool and diuresis were not disturbed.

Paraclinically: general blood test, biochemical blood test, general urinalysis, hormonal profile were without pathology.

Ultrasonic examination of the pelvic organs: pear-shaped uterus, increased in size (length 18 mm, cervix 8 mm, M-echo 2.2 mm), right ovary 13x7 mm, left ovary 12x6.5 mm, echostructure fine-cellular, normal echogenicity. Conclusion: enlarged uterus and ovaries.

X-ray of the left hand and wrist: bone age was ahead of the passport age and corresponded to 2 years 6 months.

MRI of the brain with contrast: volumetric mass of homogeneous structure with clear contours of 1-1.5 mm, which does not accumulate contrast substance, is visualized. In T1 mode the focus is hypointense, and in T2 mode it is variable hyperintense.

Based on the complaints, medical history, clinical signs of premature pubertal development, and data of laboratory and instrumental methods of investigation, the clinical diagnosis was made: Gamartoma of the gray tubercle of the brain. Gonadotropin dependent precocious puberty. Treatment was recommended: diferelin (synthetic decapeptide, analogue of natural gonadotropin-releasing hormone) 3.75 mg v/m once every 28 days.

In the dynamics in 2018, the child was routinely admitted to the Department of

EVSEEVA Sardana Anatolievna – PhD, senior researcher, Yakut Science Center of Complex Medical Problems, sarda79@mail.ru; **NIKIFOROVA Margarita Egorovna** – head of the Department of Endocrinology and Gastroenterology of Pediatric Center of the Republican Hospital No.1-NCM; **IVANOVA Olga Nikolaevna** – MD, Professor, Department of Pediatrics and Pediatric Surgery, Medical Institute, North-Eastern Federal University; **DANILOV Nikolay Andreyevich** – endocrinologist, Department of Endocrinology and Gastroenterology, Pediatric Center of the Republican Hospital No.1-NCM, postgraduate student of the Medical Institute of the North-Eastern Federal University; **ZHELOBTSOVA Ayana Fedotovna** – endocrinologist, Endocrinology and Gastroenterology Department, Pediatric Center of the Republican Hospital No.1-NCM; **IVANOVA Irina Semyonovna** – the 3rd year student of the Faculty of Medical Care, Medical Institute of the North-Eastern Federal University; **BURTSEVA Tatiana Egorovna** – MD, Professor, Department of Pediatrics and Pediatric Surgery, Medical Institute of the North-Eastern Federal University; head of laboratory, Yakut Science Center of Complex Medical Problems

Endocrinology and Gastroenterology at the age of 4 years.

Paraclinically: level of sex hormones: lutein-stimulating hormone (LH) - 0.86 U/L (RI: 0.7-2.9 U/L), follicle-stimulating hormone basal - 2.13 U/L (RI: 0.11-1.6 U/L), 17-OH-progesterone - 5.11 nmol/L (RI: 0.22-5.15 nmol/L). Conclusion: elevated levels of follicle-stimulating hormone.

Ultrasonography of the pelvic organs: pear-shaped uterus, increased in size (length 25 mm, cervix 10 mm, thickness 16 mm, width 10 mm), right ovary 25x12 x15 mm, left ovary 24 x13 x14 mm, fine-cellular echogenic structure, normal echogenicity. Conclusion: enlarged uterus and ovaries.

Hand X-ray: Bone age corresponds to 8 years old.

In December, 2020, the girl was admitted to the Endocrinology and Gastroenterology Department of Pediatric Center of the Republican Hospital No.1-NCM at the age of 6 years.

Sex hormone levels: lutein-stimulating hormone (LH) - 0.73 U/L (RI: 0.7-2.9 U/L), basal follicle-stimulating hormone - 1.62 U/L (RI: 0.11-1.6 U/L), 17-ON-progesterone - 5.2 nmol/L (RI: 0.22-5.15 nmol/L). Conclusion: elevated levels of 17-OH progesterone.

Brain MRI: volumetric neoplasm of homogeneous structure with clear contours of 1-1.4 mm, which does not accumulate contrast substance, has no signs of invasive growth is visualized. Tumor size did not increase in dynamics, and due to stabilization of clinical symptoms, it was recommended to continue the administration of Dyferelin in a dose of 3.75 mg per month once every 26 days.

The last hospitalization was in April 2022. The child was 8 years old. No active complaints on admission. Body temperature was 36.4; height was 138 cm; weight was 47 kg. The condition was of moderate severity, caused by the underlying disease. Feeling of well-being was not impaired. The consciousness was clear. The child was active. The appetite was heightened, and the sleep was calm. The physique is hypersthenic. Subcutaneous fat was significantly expressed, visceral type. BMI=24.6 kg/m2, SDS height= +3.15 standard deviations. Skin was clean, pale pink and moist. Conjunctiva of the eyes was pale pink. Peripheral lymph nodes were not palpated. There were no peripheral edemas. The musculoskeletal system was not changed. Growth was high. The pharynx was pink. The palatine tonsils were not enlarged, calm. Nasal breathing was free. Percussion, clear pulmonary sound, auscultation, vesicular breathing, no rales, BPM 18 per minute. Percussionally, the boundaries of relative bluntness were unchanged. The heart tones were clear, rhythmic, heart rate was 90 per minute, BP was 110/59 mm Hg. The tongue was clean and moist. The abdomen was not enlarged, soft and painless on palpation. The liver was not palpated, and the spleen was not enlarged. Stools were daily, regular. Free urination. Urine was light and transparent. Endocrine system: the female genital organs were formed correctly. Thyroid gland was not enlarged on palpation, clinically euthyroid. Mammary glands enlarged to Tanner 2. Nervous system: focal symptomatology was negative. Meningeal signs were negative.

Paraclinically: general blood test normal.

Biochemical blood count: alanine aminotransferase - 10.30 units/L (RI: 0.00-39.00 units/L), aspartate aminotransferase - 24.90 units/L (RI: 0.00-47.00 units/L), albumin - 42.10 g/L (RI: 38.00-54. 00 g/l), total bilirubin - 5.60 μmol/l, (RI: 3.40-17.10 μmol/l,), phosphorus - 2.04 mg (RI: 1.45-1.75 mg), total cholesterol - 5.57 mmol/l (RI: 1.20-5.20 mmol/l), total protein - 75.4 g/l (RI: 60.00-80.00 g/l). Conclusion: increased blood cholesterol levels in the biochemical blood test.

Hormone profile: Level of sex hormones on June 8, 2020 Lutein-stimulating hormone (LH) 0.53 U/L (RI: 0.7-2.9 U/L), follicle stimulating hormone basal 1.62 U/L (RI: 0.11-1.6 U/L), 17-ON-progesterone 5.4 nmol/L (RI: 0.22-5.15 nmol/L). Conclusion: Increased levels of 17-OH-progesterone, decreased levels of lutein-stimulating hormone.

Oral glucose tolerance test: fasting glucose 4.59 mmol/l, glucose after one hour - 8.95 mmol/l, glucose after 2 hours - 8.28 mmol/l. Conclusion: Impaired glucose tolerance was observed.

EEG: no changes or pathology detected.

ECG: atrial rhythm HR 84 beats per min, normal position of EOS. Elevated voltages of left ventricular complexes.

Ultrasonography: The uterus was in the normal position, with dimensions of 42 mm length + cervix, 17 mm thickness, 20 mm width. The uterine contour was smooth, the myometrial echostructure was homogeneous, the uterine cavity was not enlarged, and the ovaries were typically located. the right ovary was 21.2x10.5 õ12.3 mm, the left ovary was 17.7x21.3 õ12.3 mm, the echostructure was preserved, echogenicity was normal. Conclusion: No pathology revealed.

Thyroid gland ultrasound: no echopathology detected.

OPD ultrasound investigation: gallbladder deformity.

EchoCG: regurgitation on IC of the 1st degree, on AC of the 0-1 degree.

Skull X-ray: X-ray signs of intracranial hypertension.

Hand X-ray dated April 16, 2022: Bone age corresponds to 11 years.

Treatment: TABLE #15, Dyferelin 3.75 mg x 1 p p/cm - 16.01.2020, Iodomarin 150mcg x 1 p/day for prophylactic purposes.

Clinical diagnosis: Main: Gamartoma of the grey tubercle of the brain. Gonadotropin dependent precocious puberty.

Concomitant: Obesity of the 1-2 degree.

Given recommendations: local pediatrician observation, glucofage (metformin, Siofor) 250 mg 1 time a day in the evening constantly for 6-9 months, therapy with LH-RH analogues (3.75 Mg) diferelin (triptorelin) in/m 1 time in 26 days, uninterruptedly.

Conclusion: A child diagnosed with a gonadotropin-dependent premature puberty hamartoma was observed from 2016 to 2022. Since 2016, no growth of the hamartoma was detected on examination. Therefore, therapeutic treatment and follow-up was recommended. Clinically, the child's hypothalamic gray tubercle hamartoma manifested as symptoms of premature puberty without signs of delayed psychomotor development and epilepsy. Early diagnosis and timely treatment improve the prognosis and quality of life of the patient. Gamartomas are benign tumors with no risk of malignization, and the prognosis is favorable with early diagnosis and timely treatment.

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E.E. Voropaeva, A.A. Alieva, L.S. Ishchenko, Y.V. Khaydukova, E.A. Kazachkova, E.L. Kazachkov, A.F. Vasilenko

NEW CORONAVIRUS INFECTION COVID-19 IN A PREGNANT WOMAN COMPLICATED WITH ACUTE TRANSVERSE MYELITIS

DOI 10.25789/YMJ.2023.82.31

УДК 618.3-06:616.98:578.834.1-06:616.832-002.1

The incidence of various neurological complications among hospitalized patients with novel coronavirus infection (NCI) COVID-19 ranges from 13% to 40% according to the literature. To date, the variants and nature of clinical manifestations, the impact of neurological complications of NCI COVID-19 on pregnancy are poorly understood due to the insufficient number of stud-ies on this issue. The article presents a clinical case of the development of acute transverse myeli-tis in a pregnant woman against the background of NCI.

Keywords: COVID-19, new coronavirus infection, pregnancy, neurological complications, acute transverse myelitis.

Introduction. After two years of new coronavirus infection (NCI) COVID-19 pandemic it became evident that it is a multisystem disorder. SARS-CoV-2 binds to the cell receptor of angiotensin transforming enzyme-2 (ACE2), activating its S-protein necessary for the SARS-CoV-2 intrusion into a cell. ACE2 is located in cytoplasmic membrane of numerous cell types of a human, including II-type alveolar cells in the lungs and entherocytes of the small intestine, endothelial cells of the arteries and veins, cells of the smooth musculature of the heart arteries, adrenal glands, bladder, brain and others [1], which predetermines the polyorganic nature of the disorder.

According to the data of various scientists, neurological complications of the NCI, are observed in 13-40% of hospital patients [2, 3]. A number of researchers revealed a correlation between the NCI and frequency of occurrence of this or that neurological pathology due to hypoxia associated with respiratory deficiency [5, 6]. However, at the current stage numerous pathogenic factors of the SARS-CoV-2 damage to the central (CNS) and peripheral (PNS) nervous systems are being discussed. Both the virus invasion, and its mediating effect are being studied taking into account the presence of ACE2 in neurons and glial cells of the brain and spinal cord [7, 8]. Apparently, SARS-CoV-2 has a very high neuro-invasive potential relative to the previous coronaviruses.

Acute transverse myelitis (ATM) is one of the neurological complications of the NCI. It is an acute focal inflammatory autoimmune disease of the spinal cord that leads to motor, sensory and autonomic dysfunction [12, 13]. Frequency of ATM occurrence is 1-8 cases per million people per year [14]. There exist two types of the latent period before the onset of clinical manifestations. The first type is a short latent period (from 2 hours to 5 days). It is caused by direct neurotropic effect of the virus. The second type is a long latent period (from 10 days to 6 weeks). It is indicative of the post-infectious neurological complication [2]. Currently 43 cases of ATM, that have developed against the background of NCI, have been described [15, 16]. Notably, only single cases of the first type ATM development together with the signs of NCI such as fever, cough and general weakness, were depicted [17, 18, 20]. We have not come across the information of the NCI complicated by ATM in pregnant women in the available published papers.

We present our own clinical case report of the favorable outcome of the NCI COVID-19 complicated by community-acquired bilateral polysegmental pneumonia and ATM with short latent period.

A pregnant woman M., 24 years old, resident of Magnitogorsk. It was the patient's first wanted pregnancy. The patient was under regular medical check-up from the 7-8th week. The pregnancy course was complicated by threatened miscarriage at the 11th, 19th and 22nd gestation week (the patient received treatment in the day-stay hospital). At the 24th gestation week an increase in the level of transaminase (ALT up to 45 IU/L) and mild hypochromic anemia were registered. The patient started to receive iron therapy. At the 25th week she fell down on the buttocks (the patient "slipped"), did not seek treatment, did not suffer from pain in the back, locomotor functions were not impaired. Specifics of the somatic anamnesis: infrequent cold-related diseases; in 2015 she had combined trauma due to road accident (by patient's own account – she had pelvic fracture, complete recovery). She did not have NCI COVID-19 prior to maternity hospital admission, and had negative epidemiological anamnesis for NCI.

The patient had an acute onset of a disease on 23 February 2022 when there appeared pain in the back, ostalgia, the temperature increased to 38.6°C. In 40-60 minutes after the disease onset the patient noted sharp pains in the lumbar region with irradiation to the thoracic spine; then tensive lower abdominal pain, weakness in the lower extremities that increased within the next 20-30 minutes. The patient could not stand up unassisted, felt sensory loss and numbness in her lower extremities. She was transported to the maternity hospital of Magnitogorsk city by an ambulance team. No pregnancy pathology was detected. In accordance with the intra-hospital patients' routing she was hospitalized to the

South Ural State Medical University of the Ministry of Health of Russia, Chelyabinsk: **VOROPAEVA Ekaterina Evgenievna** – MD, Associate Professor, Professor; Deputy Chief Physician of the Regional Clinical Hospital No. 2, ORCID: 0000-0002-9055-102X; **ALIEVA Alina Avzalovna** – postgraduate student; obstetrician-gynecologist of Regional Clinical Hospital No. 2, ORCID: 0000-0001-6270-8707; **ISHCHENKO Lyudmila Stanislavovna** – PhD, Associate Professor, associate professor; obstetrician-gynecologist of Regional Clinical Hospital No. 2, Chelyabinsk lyudalyn@mail.ru , ORCID: 0000-0002-9405-0134; **KHAYDUKOVA Yulia Vladimirovna** – postgraduate student; head of department, Regional Clinical Hospital No. 2, Chelyabinsk; **KAZACHKOVA Ella Alekseevna** – MD, Prof., ORCID: 0000-0002-1672-7058; **KAZACHKOV Evgeny Leonidovich** – MD, Professor, Head of the Department, ORCID: 0000-0002-4512-3421; **VASILENKO Andrey Fedorovich** – MD, Prof., ORCID: 0000-0001-5799-7233.

department of infectious diseases where she got a positive SARS-CoV-2 ICT (immunochromatographic test for quantitative detection of a virus antigen).

Multispiral computed tomography (MSCT) of the chest organs has been performed: CT-signs of the interstitial changes of both lungs, medium probability of COVID-19, degree of severity CT-1 (5%). MSCT of the lower thoracic and lumber regions of the spine has been performed: no signs of haematomyelia have been registered; manifestations of the advanced degenerative disc disease, spondylarthrosis, L_4-S_1 disc protrusion, degenerative joint disease of sacroiliac joints were registered. Objective status: the status of the pregnant woman is that of medium severity due to lung damage, intoxication syndrome, plegia. Respiratory rate is 18 breaths per minute, SpO_2 98% without respiratory support.

Neurological status: the face is symmetrical, midline tongue protrusion, no bulbar disorders. The upper extremities have full range of motion. There is no motion in the lower extremities. Foot clonus. Decrease in sensation due to impaired conduction function: on the right side D_{10}, on the left side D_{11}-D_{12}; pain on palpation at D10, bitter pain on palpation at L_3-L_4. Pelvic organ dysfunction is present: no urge to defecate and urinate.

Laboratory evaluation revealed: leukocytosis ($12.3x10^9$/L), mild anemia (Hb 94 g/L); C reactive protein (32.7mg/L), procalcitonin <0.1 ng/mL, positive SARS-CoV-2 (PCR) dated 23 February 2022.

Diagnosis at admission: pregnancy 27 weeks. NCI COVID 19, virus identified, moderate severity. Community-acquired bilateral viral pneumonia, CT-1, respiratory deficiency 0. Unspecified acute transverse myelitis at the thoracic spine. Lower paraplegia with decrease in sensation due to impaired conduction function. A differential diagnosis between spinal stroke and myelitis has been performed.

Magnetic resonance imaging (MRI) of the cervical, thoracic and lumber section of the spinal cord reveals the following: MRI signs of initial dystrophic changes of the cervical part of the spinal cord, focal changes of the Th_{11} vertebral body (probably a small hemangioma), MRI signs of synovitis of sacro-iliac joint, initial MRI signs of dystrophic changes of the lumber section of the spinal cord. Lumbar puncture has been performed, 1.5 ml of colorless clear fluid has been taken, protein 0.099 g/L, cytosis 1.2 x 10^6/L, glucose 4.9 mmol/L. SARS-CoV-2 (PCR) from cerebrospinal fluid was negative. The treatment was started. The patient received: antiviral therapy (interferon alfa-2b, 10000 IU/mL, endonasal, 3 drops per each nasal passage 5 times/day (t/d), antibacterial therapy (intravenous ceftriaxone 2 ml x 1 (t/d), subcutaneous unfractionated heparin 5000 unit/day, metabolic therapy (cytoflavin, intravenous bolus injections), nootropic agents (citicoline, 1000 mg x 1(t/d), intravenous fluid drip). Under advice of the regional multidisciplinary case management team, the pregnant woman was routed to maternity hospital of the Chelyabinsk Regional Clinical Hospital № 2 (RCH №2). On 3 April, 2020 the hospital was repurposed to an infectious diseases hospital to provide medical assistance to pregnant, parturient women, and puerperants with NCI as well as their newborns in the territory of the Chelyabinsk city and Chelyabinsk Region. In accordance with the recommendations of the case management team the patient was also given the following drugs: methylprednisolone 1000 mg intravenous fluid drip, 1 t/d for 5 days; omeprazole 20 mg capsules x 2 t/d per os; subcutaneous nadroparin 0.4 ml x 2 t/d; IV infusion of monoclonal antibodies etesevimab 1400 mg + bamlanivimab 700 mg, antiviral therapy remdesivir 200 mg, IV fluid drip.

25 February 2022, on the 3rd day from the disease onset the patient was transported to the maternity hospital of the RCH №2 by helicopter, was presented to the emergency department with complaints of acute highly intensive pain in the low back, without irradiation that increased with movement, lower limb weakness up to the impossibility to produce motion in them. By the woman's own account against the background of the received therapy she noted some improvement – she noted single spontaneous movement in left limb. The patient's status on hospital admission is that of moderate severity.

The patient was awake, Glasgow coma scale (GCS) score of 15, oriented to place, time and space. NEWS score of 0. Body mass index – 29.7. Symmetric face, midline tongue protrusion, no bulbar disorders. The upper extremities have full range of motion, decrease in sensation of the due to impaired conduction function on the right side D_{10}, on the left side - D_{11-12}. Pain on palpation at D_{10}, acute painfulness at L_3-L_4. No motion in the lower extremities. Unlabored nasal breathing. SpO_2 - 98% without respiratory support. Blood pressure of 110/70 (110) mm Hg, heart rate of 98 beats per minute. The abdomen is enlarged due to pregnant uterus in accordance with gestational age, non-tender in all the sections. The uterus is normally toned. Active fetal movements. Fetal heart rate is clear and even, of 135-144 beats per minute.

Neurological status of the pregnant woman: lower paraparesis with decrease in muscular strength in the left limb up to 1 score, plegia in the right limb. The muscle strength score in hands is 5. The tendon reflexes in the legs are brisk to clonus, symmetrical, no pathological reflexes. Coordinating tests: the patient performs finger - nasal test accurately, without intention, D=S, impaired conduction function from the level T12 on the left, L1 on the right. Autonomic functions are not violated. Meningeal syndrome is negative. Pronounced pelvic disorders with no urge to urinate or defecate. Laboratory evaluation revealed the following: mild anemia (Hb 104 g/L, hematocrit 29%), stab shift up to 24%, C reactive protein – 18 mg/L, activated partial thromboplastin time 40.1%, Lactate 3.2 mmol/L, Aspartate aminotransferase - 45 (unit/L), Alanine aminotransferase 34 (unit/L). The levels of leukocytes, thrombocytes, glucose, bilirubin, creatinine, ferritin, D-dimer, lactate dehydrogenase, procalcitonin, hemostatic profile were within the normal range.

Ultrasound screening of the fetus, Doppler examination of the uteroplacental and fetal blood flow did not reveal any pathology. On 25 February 2022 a regional multidisciplinary case management team (obstetrician-gynecologist, neurologist, anesthesiologist-intensivist, infectious disease specialist, neurological surgeon) meeting was held. It was recommended to continue the NCI treatment with the change in antibacterial drug (meropenem 1 g x 3 t/d, IV), methylprednisolone pulse therapy, metabolic therapy (cytoflavin 10 mg/day), nootropic agents (citicoline, 1000 mg x 1(t/d) IV), thromboembolic complication prophylaxis (nadroparin 0.6 s/c, daily), anti-anemic therapy (Iron(II) sulfate / ferrous sulfate 100 mg + ascorbic acid 60 mg tablet x 2 t/d per os).

Positive dynamics was observed in the course of the treatment: motions appeared in the right foot toes, the strength in the proximal part of the left limb increased (the patient started to bend the leg in the knee joint, to hold it in this position on the bed). Partial regress of the sensory impairment was also registered. The border of the hypostesia lowered from both sides: 1segment on the right side, 2 segments on the left side. Position sense was still impaired. However, this impairment could be evaluated as the one of moderate degree of manifestation (it was difficult for the patient to name the toes of her left foot and movement

direction in them, she could tell the toes of the right foot, sometimes mixed up the movement direction). The patient already could sit in the bed unassisted for a long time with her legs down, started to feel the urge for urination.

On 27 February 2022 paraesthesiae appeared in the lower limbs, sensory impairment decreased, she could raise her pelvis a little bit above the bed, leaning on her legs. Weakness in the lower limbs, tingling in the heels persisted. General status is of moderate severity due to focal neurological pathology. GCS score of 15. NEWS score of 0. SpO_2/FiO_2 – 466. A positive dynamics was observed in the neurological status: gradual recovery of the sensation and increase in the motion volume in the lower extremities. The patient was verticalized with the assistance of the healthcare workers. The defication and urination were under full control. Upon the end of methylprednisolone pulse therapy, the patient was given prednisolone per os at an initial daily dose (based on her body mass) 70 mg in the morning with a 5 mg decrease every three days until complete cessation.

On 3 March 2022 taking into account the positive dynamics in the patient's status, absence of the damage to the bronchopulmonary system, normal body temperature along the whole course of treatment, absence of changes in the laboratory parameters, negative SARS-CoV-2 (PCR) from nasopharyngeal swab dated 1 March 2022, she was dismissed from the maternity hospital of the RCH №2 with subsequent admission to the inpatient neurological department of the Clinics of the South Ural State Medical University of the Chelyabinsk city. The patient was transported by medical ambulance. By 11 March 2022 a regress in the clinical symptoms of the ATM and patient's complaints was observed. She was dismissed from the hospital in a satisfactory condition and recommended to continue taking prednisolone 10 mg/day per os every other day. Further on, she was under ambulatory observation of neurologist, obstetrician-gynecologist, including the use of telemedicine consultations. Stable positive dynamics was noted in terms of ATM. The pregnancy proceeded without obstetric complications.

On 18 May 2022 the patient was examined by a neurologist. Diagnosis: residual symptoms of the previous acute transverse myelitis at the level of thoracic spine, cone of the spinal cord (as of 22 February 2022). Syndrome of central lower spastic paraparesis (decrease in the algesthesia along the anteroexternal surface of the femur and shin till the lower third of the left leg shin, femur and knee to the shin – on the right leg, pelvic organ function was not disturbed. Delivery without active pushing phase is recommended. On 23 May 2022 the patient performed delivery at the gestation age 38-39 weeks on a scheduled basis via cesarean section without complications. A girl was born weighing 3,000 g, 50 cm in height, Apgar score 8/9. They were discharged from the hospital on the 4th day after the delivery in a satisfactory condition. Morphological characteristics of placenta: involutional-distrophic and compensatory reactions in placenta corresponded to the gestation age.

Discussion. In view of the NCI pandemic, researchers pay special attention to the infectious myelitis, enteroviruses, herpes-zoster virus, herpes simplex virus of type 1, and zika virus being considered its etiological agents in the pre-covid era [17]. Specialists assume a pathogenetic link between the NCI complicated by ATM and cytokine storm mechanism, explaining the clinical picture of the disease by inflammatory reaction with the release of various inflammation mediators. Moreover, if activated immune cells enter the CNS, then immune mediated inflammation of the brain or spinal cord develops [17, 20, 21]. Direct entry of the virus into the spinal cord is possible especially if the permeability of the blood-brain barrier is increased. We assume that in this particular clinical case, NCI-associated ATM was caused by systemic inflammatory response despite negative SARS-CoV-2 PCR in the CSF. This is evidenced by an obvious curative effect of methylprednisolone pulse therapy together with complex NCI COVID-19 therapy. We have studied the majority of the described cases of NCI-associated ATM in the non-pregnant state. What calls attention to itself is the fact that the disease manifestations develop after a patient's discharge from the hospital or days/weeks after the viral infection symptoms relief [2, 14, 20, 21, 27]. In this particular case symptoms of ATM (clinical picture of sensory and motor disorders together with pelvic organ dysfunction) developed together with the acute onset of NCI COVID-19. Such disease development variant is rather rare. Much more often, lower paraparesis and urine retention gradually increase within several days [27, 29].

According to the published research data, the diagnosis of ATM is based on clinical presentations, spinal cord MRI report and CSF analysis. A number of researchers described patients with NCI and clear clinical manifestations of ATM whose spinal cord MRI did not reveal any pathological foci just as in the given clinical case report [17, 29]. In the CSF analysis in patients with NCI and ATM different mild shifts in protein content and cytosis were observed. In all the available case descriptions the CSF glucose level was within the normal range [17, 18, 23, 30]. In the reported clinical case the patient's CSF demonstrated normal cytosis and protein content against the elevated glucose level. Different outcomes of NCI-associated myelitis in non-pregnant state were reported: from complete recovery of the lost functions to persistent neurological deficit and even deaths [20, 31].

Conclusion. In the presented case report what attracts the attention is the peracute onset of ATM against the background of NCI COVID-19 in a pregnant woman with the developed clinical manifestations of sensory and motor impairment combined with pelvic organs dysfunction, quick persistent positive dynamics, symptoms regression and recovery of the impaired functions of the spinal cord against the background of the timely adequate comprehensive therapy of NCI and ATM which was approved by specialists of the multidisciplinary telemedicine case management team.

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DOI 10.25789/YMJ.2023.82.32

УДК 617-089

E.N. Kirilova, T.I.Nikolaeva, S.A. Myreeva, T.A. Sosina

ORGAN-SPARING TREATMENT FOR ENDOMETRIAL CANCER (CLINICAL CASE)

Endometrial cancer ranks among the leading malignant diseases of the female reproduc-tive system and remains a pressing issue in oncogynecology. Although endometrial cancer is pre-dominantly a disease of the menopausal period, 10-14% of cases are diagnosed in patients of re-productive age. Most of these women do not yet have children, making fertility-preserving treatment a priority. As such, if a patient wishes to maintain her reproductive function, standalone hormone therapy may be conducted. Hormonal treatment induces atrophy of the tu-mor-altered endometrium, after which estrogen-progestin medications restore its function. The article presents a clinical case of successful realization of generative function following a favora-ble outcome of endometrial cancer treatment. A 41-year-old patient with low ovarian reserve and unrealized reproductive potential was offered fertility-preserving treatment, involving hyster-oscopic resection followed by hormone therapy. In deciding on fertility-preserving treatment, the presence of favorable prognostic factors was considered: positive receptor status of the tumor and tumor differentiation degree. After treatment, follow-up examinations revealed no recur-rence or disease progression, and the patient was advised to plan for pregnancy using assisted reproductive technologies. However, this was not carried out because of the patient's spontane-ous pregnancy. During gestation, the patient underwent regular oncogynecological examinations according to the established schedule, and no recurrence or disease progression was detected. At 35 weeks of gestation, a surgical delivery was performed, resulting in the birth of a girl weighing 2820 g, with a height of 48 cm and an Apgar score of 8/8 points. This clinical case demonstrated that fertility-preserving treatment for early-stage endometrial cancer not only cured the malignant process but also subsequently fulfilled the generative function, without reducing the effective-ness of the treatment.

Keywords: endometrial cancer, organ-preserving treatment, hormone therapy, clinical case.

Introduction. In contemporary society, a significant proportion of women of childbearing age are susceptible to gynecological cancer, many of whom wish to preserve their fertility for the future [25]. Uterine body cancer (UBC) is one of the leading malignancies affecting the female reproductive system. According to statistics, in 2019, 62,000 new cases of endometrial cancer (EC) were diagnosed worldwide, and alarmingly, both the incidence and mortality rates are projected to increase by 1-2% annually [2, 16, 17, 23]. For comparison, American healthcare statistics indicate the annual identification of approximately 40,000 new cases, and an increase in the frequency of UBC by about 50% over the past 20 years [9]. The lowest mortality rates due to EC are recorded in Central and South Asia, while the highest rates are observed in African countries [15]. UBC survivors may experience treatment-related issues, including infertility, early menopause, sexual dysfunction, and lymphatic edema of the lower extremities [26]. In Russia, EC remains the most common oncogynecological disease, ranking first in the structure of gynecological cancer. In 2021, there were 22,951 patients registered [3]. Among them, 84.4% were diagnosed at stages I-II with a 5-year survival rate of up to 73.1%. Women aged 45 to 74 are more frequently affected, with an average age of diagnosis at 62 years [18]. However, in rare cases, EC can be diagnosed in patients of reproductive age. In such situations, the oncogynecologist faces not only the challenge of treating the young patient but also the possibility of preserving fertility [13, 21, 22]. In 5.2% of cases, the diagnosis was made in patients of reproductive age (18 to 45 years), amounting to an absolute number of 1,317 individuals. Over the past 10 years (2006-2016), there has been not only a steady increase in the incidence of EC,

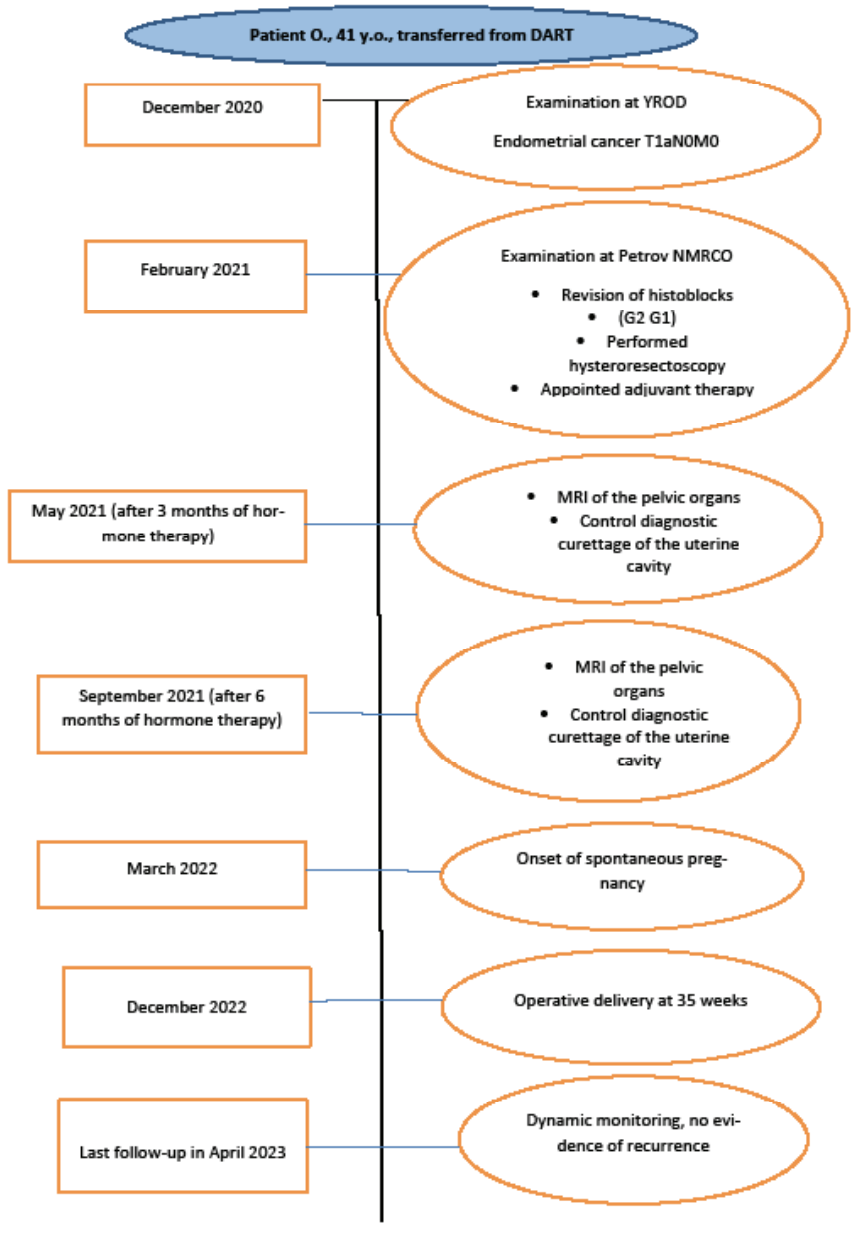
KIRILOVA Eleonora Nikolaevna – oncologist - gynecologist of the Outpatient Department at Yakutian Republican Oncological Dispensary, Yakutsk, e-mail: elya_kirillova1@icloud.com; **NIKOLAEVA Tatyana Ivanovna** – PhD, Associate Professor of the Department of Oncology at the Medical Institute of the M.K. Ammosov North-Eastern Federal University, Chief Physician at YROD, Yakutsk, e-mail: NTI_Nika@mail.ru; **MYREEVA Svetlana Anatolyevna** – PhD, Head of the Department of Oncogynecology at Yakutian Republican Oncological Dispen-sary, Yakutsk, e-mail : myreeva2011@yandex.ru; **SOSINA Tuyara Anatolyevna** – oncologist-gynecologist of the Polyclinic Department at Yakut Republican Oncological Dispensary, Ya-kutsk, e-mail: integraka22@gmail.com

with a growth rate of 38.45%, but also a "rejuvenation" of the pathology [5, 11].

Due to various socio-economic reasons, a significant proportion of women postpone the birth of even their first child to an age older than 35 years [5, 11, 19]. As a result, over the past few decades, preserving fertility in young patients with malignant tumors of the reproductive system has remained one of the priority directions in oncology. Currently, American (NCCN), European (ESMO), and Russian clinical guidelines have been developed for the treatment of early-stage endometrial cancer with fertility preservation in young patients who have not yet realized their reproductive function. However, there are no unified standards in the schemes and duration of hormone therapy [14, 20]. In particular, the 2022 recommendations of the Russian Society of Clinical Oncologists and the 2020 Association of Oncologists of Russia indicate that fertility-preserving treatment for uterine cancer should be performed in institutions with such experience [1, 7]. Patients should be informed about the risks associated with conservative treatment for fertility preservation, specifically the high frequency of relapses, as well as the need for future uterine removal. In Russia, since the mid-1970s, the P. Herzen Moscow Research Oncology Institute has been actively involved in fertility-preserving treatment for precancerous conditions and early-stage endometrial cancer, and research on this treatment method and the search for possible ways to improve oncological and reproductive outcomes continue in the clinic to this day [5].

According to the classic model proposed by Y.V. Bokhman in 1983, there are two types of endometrial cancer (EC) [24, 27]. Patients with Type I pathogenetic variant of endometrial cancer are characterized by obesity, high estrogen levels in pre- and postmenopause, basal and reactive hyperinsulinemia, and a tendency to develop highly differentiated endometrial adenocarcinoma with high levels of estradiol and progesterone receptors in tumor tissue. Patients with Type II pathogenetic variant of endometrial cancer usually occur in elderly women, have a high degree of malignancy with an unfavorable prognosis. Considering the leading role of excessive estrogen influence on endometrial tissue and progesterone deficiency in the development of EC, hormonal therapy for early-stage EC involves the administration of high doses of gestagens, which leads to atrophic changes in the endometrium and disease remission, against which it is possible to perform therapy aimed at restoring reproductive function [6, 8]. It should be noted that fertility-preserving treatment is only possible with certain characteristics of the malignant neoplasm: the initial stage of tumor development (stage IA without myometrial invasion), a high degree of tumor cell differentiation, the absence of signs of metastasis and tumor invasion into the lymphovascular space [10, 12].

Clinical case. Patient O., 41 years old, sought consultation at the Yakutsk Republican Oncological Dispensary (YROD) in December 2020, following a referral from the Assisted Reproductive Technology (ART) department, where she was undergoing examination for primary infertility. From the medical history: in March 2020, endometrial hyperplasia was diagnosed as part of the examination for entering the ART program with a diagnosis of female infertility, tubal factor, chronic bilateral salpingo-oophoritis in remission, chronic endometritis, pituitary microadenoma, hyperprolactinemia, advanced reproductive age, and low ovarian reserve. Diagnostic curettage was performed at the ART department, and moderately differentiated endometrial adenocarcinoma was revealed by histological examination. Gynecological history: menarche at 12 years old, immediately established to last for 4 days with a 26-day cycle, regular, no pregnancies, gynecological diseases: chronic salpingo-oophoritis, registered for pri-



Schematic Representation of the Clinical Case of Patient O., 41 Years Old (DART — Department of Assisted Reproductive Technologies)

mary infertility. Primary examination and bimanual examination: external genitalia developed correctly, female-type hair distribution. Narrow, nulliparous vagina, vaults free, elastic. Cervix of normal size, conical in shape, mucous membrane is not visually changed, external pharynx is of a punctate form, without pathological changes. Uterus is anteverted, of normal size, pear-shaped, of usual consistency, painless upon palpation, adnexa not enlarged, parametrium soft. Pelvic ultrasound revealed increased endometrial thickness, depleted follicular apparatus in both ovaries. Pelvic and extraperitoneal space magnetic resonance imaging: uterus not enlarged, measuring 4.5 cm in length, 6.0 cm in thickness, 5.2 cm in width, with clear, even contours, homogeneous myometrium; uterine cavity not dilated, endometrium of heterogeneous structure, without reliable signs of myometrial invasion; cervix and ovaries not enlarged; pelvic and extraperitoneal lymph nodes not enlarged. Multislice computed tomography of thoracic and abdominal organs: no focal pathology in the structure of parenchymal abdominal and extraperitoneal organs detected; diffuse changes in the pancreatic parenchyma; perigastric lymphadenopathy; hemangioma of L5 vertebral body; lungs without fresh or infiltrative changes. Other examinations: superficial gastritis detected by fibrogastroscopy; no visible pathology detected by rectosigmoidoscopy. Infectious and allergic diseases: not detected. Family history: at 16 years old, patient's sister was diagnosed with acute myeloblastic leukemia.

At the multidisciplinary council, endometrial cancer staging was performed using the TNM system (UICC, 8th revision, 2016) and FIGO classification (2009): cT1aN0M0 Stage IA. Considering the patient's wishes and unrealized reproductive potential, a consultation was held with the N.N. Petrov National Medical Research Center of Oncology (NMRCO) of the Ministry of Health of the Russian Federation, where an organ-sparing treatment option was proposed, consisting of hysteroscopic resection of the tumor followed by hormone therapy. The patient was informed about the risks associated with organ-sparing treatment and referred for the surgical stage to the N.N. Petrov National Medical Research Center of Oncology of the Ministry of Health of the Russian Federation. A review of the histological blocks was conducted at the federal institution, concluding: low-grade endometrioid adenocarcinoma against the background of an endometrial polyp. The patient underwent hysteroscopy with targeted endometrial resection. Postoperative histological examination: reactive changes in stratified squamous non-keratinizing epithelium without underlying stroma, cervical epithelium with focal mature squamous cell metaplasia. Against the background of endometrium with pronounced secretory changes, foci of hyperplasia with architectural atypia without cytological atypia were observed. Subsequently, the patient was prescribed hormone therapy for 6 months: Depo-Provera (medroxyprogesterone acetate) 500 mg following the treatment plan and tamoxifen 20 mg.

After 3 and 6 months of hormone therapy, as part of the control examination, hysteroscopy with separate diagnostic curettage of the cervical canal and uterine cavity was performed. Histological conclusion: fragments of endometrium without signs of malignant growth. According to magnetic resonance imaging of the small pelvis and the retroperitoneal space, there is no reliable evidence of progression of the primary disease. A fluid formation is observed in the projection of the right ovary, with more data indicating a cyst.

The patient was referred for an in-person consultation at the N.N. Petrov National Medical Research Center of Oncology, considering the tubal factor of primary infertility, low level of anti-Mullerian hormone, low number of antral follicles, high interest in pregnancy, and low probability of spontaneous conception. Assisted reproductive technology was recommended for pregnancy planning, which had not been used due to spontaneous pregnancy. During gestation, the patient underwent control examinations with an oncogynecologist according to established terms, with no evidence of recurrence or disease progression. A telemedicine consultation was conducted with the N.N. Petrov National Medical Research Center of Oncology before delivery. Conclusion: Delivery can be performed through natural birth canal if there are no obstetric indications for cesarean section.

Following existing clinical guidelines of the Ministry of Health of the Russian Federation, the patient should be informed about the risks associated with conservative treatment for fertility preservation, specifically the high frequency of recurrence when preserving the uterus, the risk of metastasis, and the need for prophylactic hysterectomy after reproductive realization or reaching an age where pregnancy planning is no longer relevant. The decision on the extent of surgical treatment should be made after delivery and examination within 3-4 months. In the absence of a tumor process in the uterus or preservation of the minimal degree of tumor process spread, it is possible to discuss the issue of hysterectomy with salpingectomy and ovarian preservation based on the histological review of surgical material in the patient with low-grade endometrioid adenocarcinoma, which is associated with low risk of metastasis to regional lymph nodes. In the absence of data on regional lymph node involvement and preservation of the disease stage, lymphadenectomy may not be performed.

In case the patient refuses prophylactic hysterectomy, after delivery, it is recommended to perform a histological examination of the endometrium and, in the absence of pathological changes, insert an intrauterine hormonal system with mandatory subsequent histological and ultrasound monitoring of the endometrium every 6 months.

At 35 weeks of gestation, an operative delivery was performed, resulting in the birth of a girl weighing 2820g, with a height of 48cm, and an Apgar score of 8/8 points. During the operation, a diagnostic curettage of the uterine cavity was performed, followed by a histological examination. The results showed no evidence of malignant growth in the material, with the presence of blood, fibrin, and small fragments of decidual tissue.

In the postpartum period, the patient declined prophylactic hysterectomy, and the monitoring continues: Figure 1.

Conclusion. The presented clinical case demonstrates the high efficacy of hormone therapy application. Organ-preserving treatment allows for the subsequent realization of reproductive function. A thorough consideration of indications and contraindications for the upcoming treatment and proper patient selection are essential. In most cases, after organ-preserving treatment, the risk of tumor recurrence is low, and women regain the chance to conceive and give birth to a healthy child. The decision to perform organ-preserving treatment should be made by a multidisciplinary team involving an oncologic gynecologist, radiation oncologist, obstetrician-gynecologist, reproductive endocrinologist, and perinatologist.

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M.S. Savvina, V.M. Argunova, T.E. Burtseva, V.B. Egorova, V.P. Shadrin, A.A. Yakovlev

A CASE OF MULTISYSTEM INFLAMMATORY SYNDROME

DOI 10.25789/YMJ.2023.82.33

УДК 616-053.2 (571.56)

The article presents a clinical case of children multisystem inflammatory syndrome (MSI-Ch) associated with a new coronavirus infection (COVID-19) in a 10-year-old child complicated by pancarditis: endomyopericarditis, unspecified bronchopneumonia, systemic inflammatory response syndrome of infectious origin with an organic disorder: with a lesion heart, kidney, liver, multiple organ failure, secondary antiphospholipid syndrome. The correspondence of the clinical picture, laboratory and instrumental data to the diagnosis of MSI-Ch, and differential diagnosis are discussed.

Keywords: multisystem inflammatory syndrome in children (MSI-Ch), Kawasaki disease, Covid-19, children.

Introduction: Multisystem inflammatory syndrome in children (MSI-Ch) is a severe condition that occurs after suffering Covid-19, in which internal organs become inflamed - lungs, heart, eyes, kidneys, brain, gastrointestinal tract, skin. This disease has created a diagnostic problem as it shares similarities with Kawasaki disease and Kawasaki syndrome. With MSI-Ch, there is a persistent increase in temperature (100%), a rash on the skin, conjunctivitis, swollen lymph nodes, and edema on the hands and feet. Most also have gastrointestinal symptoms - abdominal pain, vomiting, loose stools. On the part of the respiratory system, signs of damage are observed in less than half of the patients [9,12]. A quarter of children with MSI-Ch have heart disease. There are two types of pathological process in the cardiovascular system. In the first case, this is the expansion of the coronary arteries, as a rule, without myocarditis. It is detected incidentally on ultrasound of the heart and rarely manifests as an acute coronary syndrome. In the second case, there is a clinic of acute myocarditis, which develops over several days and manifests itself as cardiogenic shock [16].

The main laboratory sign of MSI-Ch is hyperproduction of inflammatory markers - C-reactive protein, ferritin, procalcitonin, fibrinogen, D-dimer, triglycerides [14, 1, 11, 15, 8]. Thrombocytopenia can also be observed, which is not typical for Kawasaki disease and lymphopenia [1, 16]. Almost all children with MSI-Ch will have elevated D-dimer levels, but despite this, thrombosis is rare [17]. Elevated troponin levels indicate cardiac involvement [9,11]. Thus, elevations in inflammatory and cardiac biomarkers not only suggest a diagnosis of MSI-Ch but may reflect disease severity [6,10].

According to recent reports, the Council of State and Territorial Epidemiologists (CSTE) and the US CDC Centers for Disease Control and Prevention have updated the criteria for the diagnosis of MSI-Ch to distinguish this disease from other similar conditions. Thus, MSI-Ch occurs in patients younger than 21 years old, with a coronavirus infection within 60 days before or during hospitalization, a fever of 38C and above for any duration. The only laboratory sign is now considered to be an increase in CRP levels of 3.0 mg/dl and above. The diagnosis of MSI-Ch requires at least two signs of involvement of organs and systems: damage to the heart, skin and mucous membranes, gastrointestinal tract, changes in hematological parameters and shock [5].

Of the instrumental methods of investigation, echocardiography can be useful for prompt recognition of patients with cardiac dysfunction to adjust therapy or to determine alternative diagnoses [6].

Treatment of MSI-Ch is carried out primarily with immunomodulators, since the disease is caused by dysregulation of the immune system [13]. This is treatment with high doses of intravenous immunoglobulins (IVIG) alone or in combination with a corticosteroid. At the same time, antiplatelet agents and anticoagulants are prescribed [15,8]. In some cases, the appointment of inhibitors of interleukin-6 and antitumor necrosis factor is required [7].

According to many researchers, 50-60% of patients have changes in the cardiovascular system. Most children with MSI-Ch have coronary artery disorders, which is significantly higher than in Kawasaki disease, in which the degree of involvement of the coronary vessels is 10-15% [6,9,10]. Most patients, even with severe cardiovascular disease, recovered without consequences [15,6,10]. Since the development of coronary aneurysms occurs in the recovery stage, it is necessary to conduct control echocardiographic studies even in patients without pathology of the cardiovascular system in the acute stage of the disease [15,8].

The pathophysiology of multisystem inflammatory syndrome remains largely unclear. Apparently, it is based on a virus-induced hyperimmune reaction [2,3,4,9]. The most important role in the pathogenesis is played by the activation of T-lymphocytes, hyperproduction of pro-inflammatory cytokines. (TNF-α, interleukins 1, 2, 6, 8, 10, granulocyte-macrophage colony-stimulating factor), deposition of immune complexes in the vascular wall. The mechanism of immune complex deposition in the vascular wall determines the development of a multisystem inflammatory response and explains most of the clinical and laboratory signs of the syndrome, such as fever, hyperferritinemia, coagulopathy, and an increase in inflammation markers [15,16].

The purpose of the study: to describe the clinical and laboratory picture

SAVVINA Maya Semenovna – PhD, senior researcher, Laboratory for Monitoring Children's Health, Yakut Science Centre of Complex Medical Problems; maya_savvina@mail.ru; **ARGUNOVA Vera Maichna** – doctor of the Cardio-Rheumatological Department of the Pediatric Center of the State Autonomous Institution of the Republic of Sakha (Yakutia) RB No. 1-National Centre of Medicine; **BURTSEVA Tatyana Egorovna** – MD, Professor of the Department of Pediatrics and Pediatric Surgery, NEFU Medical Institute, bourtsevat@yandex.ru; **EGOROVA Vera Borisovna** – PhD, Associate Professor of the Department of Pediatrics and Pediatric Surgery, M.K. Ammosov NEFU Medical Institute, veraborisovna@yandex.ru; **SHADRIN Viktor Pavlovich** – PhD, Associate Professor of the Department of Anesthesiology, Resuscitation and Intensive Care with the course of emergency medical care, M.K. Ammosov NEFU Medical Institute; **YAKOVLEV Aleksandr Aleksandrovich** – pediatrician, resident for 2 years, Department of Hospital Pediatrics, St. Petersburg State Pediatric Medical University, alexander.med18@gmail.com.

of multisystem inflammatory syndrome in children.

Material and research methods. A retrospective analysis of the medical history of a patient who was hospitalized in the cardio-rheumatology department of the Pediatric Center of the SAI RS (Yakutia) " Republican Hospital No. 1 - National Center of Medicine" was carried out, where an in-depth examination and treatment was carried out according to all standards and clinical recommendations.

Clinical case. Patient S., 10 years old, gender - male, was admitted to the cardio-rheumatology department of the Pediatric Center of the SAI RS (Yakutia) " Republican Hospital No. 1 - National Center of Medicine" for examination and treatment. Complaints at admission: an increase in body temperature up to 38.5 C, pain in the abdomen, in the knee joints, neck, rash all over the body, swelling of the feet and hands.

Anamnesis vitae: A child from 1 pregnancy, which proceeded with the threat of termination, physiological birth, on time, birth weight - 2800g. Psycho-motor and physical development by age, was on mixed feeding.

Table 1

Biochemical parameters of blood serum

Indicators	4.12.2020	15.12.2020	11.01.2021	Reference values
ALT (u/l)	19.30	17.40	34.70	0-39
AST (u/l)	49.40	17.60	13.90	0-47
Urea (mmol/l)	6.2	7.9	6.6	1.8-6.4
Bilirubin total (μmol/l)	5.2		6.6	3.4-17.1
Creatinine (μmol/l)	55.15	45.12	47.81	27-62
Albumin (g/l)	19.6	35.8	40.60	38-54
Total protein (g/l)	81.4	93.90	72.20	60-80
Glucose (mmol/l)	6.14	4.81	4.37	3.3-5.6
GGT (gamma-glutamyl transferase) (u/l)	25.5	27.2	30.50	0-17
Phosphorus (mmol/l)	1.11	1.35	1.59	1.45-1.78
Lactate dehydrogenase (u/l)	536.7	191.6	288.0	0-295
CPK (u/l)	229.5			0-154
Calcium total (mmol/l)	1.95		2.47	2.2-2.7
Sodium (mmol/l)	130.0		132.7	138-145
Potassium (mmol/l)	4.47		5.47	3.4-4.7

Table 2

Complex ultrasound of the heart (M-and B-mode, TsDK, dopplerography)

Дата	Conclusion
03.12.2020	Decreased systolic function of the myocardium of the left ventricle. Ejection fraction - 47%. A slight expansion of all cavities of the heart, the aorta in the area of the sinuses, the trunk of the pulmonary artery. Sealing of the leaflets of the mitral valve. Insufficiency of the mitral valve of the 1st degree, the valve of the pulmonary artery of the 1st degree. Tricuspid valve insufficiency associated with mild pulmonary hypertension (estimated pressure 36.13 mmHg). Separation of the sheets of the pericardium along the anterior wall of the right ventricle, the posterior wall of the left ventricle.
04.12.2020	Open oval window 0.35 cm. Insufficiency of the tricuspid valve of the 3rd degree, the mitral valve of the 2nd degree. Additional trabecula in the cavity of the left ventricle. Slight expansion of the cavities of the right atrium and left ventricle. Ejection fraction - 45%.
06.12.2020	Signs of pulmonary hypertension of the 1st degree. Expansion of the left and right coronary arteries. Separation of the sheets of the pericardium.
14.12.2020	Mitral valve insufficiency of the 1st degree, tricuspid valve of the 1st degree. The cavities of the heart are not dilated. Ejection fraction - 67%.
30.12.2020	Splitting of the pericardium

Past diseases: acute respiratory infections rarely, up to 7 years of age, purulent tonsillitis, chickenpox, in February 2020 - protracted bronchitis.

Vaccinations received according to the calendar. There were no injuries or operations. There is no allergy. Heredity, according to the mother, is not burdened.

Anamnesis morbi: fell ill acutely on November 27, 2020 - fever up to 40C, weakness, body aches. He was treated on an outpatient basis, took paracetamol, arpeflu, there was no improvement. He was hospitalized in the central ulus hospital on November 30, 2020, where he stayed until December 1, 2020 diagnosed with bronchopneumonia. He was transferred to the Children's Infectious Diseases Clinical Hospital (ChIDH) with a risk of covid pneumonia with lung damage up to 8%. On ELISA - elevated Ig G, positive Ig M from 12/01/2020. From 01.12.20 - 03.12.20 was in hospital at the ChIDH. Due to the severity of the condition and the lack of proper treatment in the ChIDH, he was transferred to the pediatric center of the Pediatric Center of the SAI RS (Yakutia) "Republican Hospital No. 1 - National Center of Medicine". Upon admission, the condition was assessed as very serious, in the mind, the state of health is reduced. Body temperature - 38.8 C, respiratory rate - 32 per minute, heart rate - 140 beats per minute. Blood pressure - 98/43 mm Hg. Contact, adequate. General hyperesthesia. Lips are bright and dry. The tongue is lined, the tip is "raspberry". Hyperemia of the conjunctiva of both eyes. Nasal breathing is difficult, there is no discharge. Throat is hyperemic. Enlarged cervical lymph node, 3*3cm, painful. Integuments - abundant maculopapular rash on the

back, back of the thighs, over the elbow and knee joints, hemorrhagic rash on the feet, over the knee joints, not abundant. Feet and hands are moderately edematous, hyperemic. Soreness of large joints - hip, knee, elbow, ankle. Pain in the femoral muscles at rest and on palpation. In the lungs, breathing is carried out in all fields, hard, no wheezing, dry cough, infrequently. The boundaries of the heart are not expanded, the tones are the gallop rhythm, loud, sonorous. The abdomen is soft, swollen, painful on palpation along the intestines, peristalsis is alive. The liver is enlarged by 2.5-3 cm, dense. Stool once, liquid. There are no meningeal symptoms. The peripheral pulse is symmetrical.

The patient was in the Department of Anesthesiology and Intensive Care from 04.12.20 till 08.12.2020, then transferred to the cardio-rheumatology department for further treatment.

Research results. In the general blood test at admission: an increase in ESR to 55 mm per hour, neutrophilia (leukocyte formula shift to the left), lymphopenia. Biochemical analysis of blood - cytolysis, increased levels of LDH, CPK, uric acid (Table 1). Immunological blood test dated 07.12.20. - an increase in CRP to 72.60 mg / l, ASLO - 424.60 IU / ml. Ferritin from 04.12.20. - 689.71 μg/l (normal - 7-140), troponin - 0.04 ng/l (normal up to 0.03), D-dimer - 1.58 μg/ml (normal - 0-0.5).

On X-ray computed tomography of the chest organs dated 04.12.2020, there were no reliable data for viral pneumonia. Effusion in both pleural cavities with the formation of subsegmental, compression atelectasis. Stagnation in the pulmonary circulation.

Ultrasound examination of the abdominal organs from 12/04/2020: Hepatosplenomegaly. A small accumulation of free fluid in the pelvis. Dopplerographic increase in the linear velocity of blood flow in the superior mesenteric artery.

On the basis of clinical, anamnestic, laboratory and instrumental studies, the main diagnosis was made: Multisystem inflammatory syndrome, COVID-associated. Pancarditis: endomyopericarditis. Complication: Bronchopneumonia, unspecified; Syndrome of a systemic inflammatory response of an infectious origin with an organic disorder: with damage to the heart, kidneys, liver, multiple organ failure. Secondary antiphospholipid syndrome. Differentiation from Kawasaki disease is indicated by the age of the patient, the severity of the course of the disease, heart damage, involvement of the skin and mucous membranes and the gastrointestinal tract, and a high level of CRP.

Treatment: intravenous immunoglobulin "Privigen", glucocorticoids ("Metipred", "Dexamethasone"), digoxin, aspirin, heparin, veroshpiron, spironaloctone. Physiotherapeutic treatment was also carried out - magnetotherapy on the chest.

Discharged on the 40th day with improvement. When discharged, there are no complaints, the condition is satisfactory and stable. It is recommended to observe a pediatrician and an infectious disease specialist at the place of residence, a scheduled examination by a cardiologist, rheumatologist and hematologist in a month with a repeat of the immunogram and coagulogram. Continue exercise therapy. Exemption from physical education for 1 year.

Conclusion. This clinical case is a complication of coronavirus infection, when the clinic of multisystem inflammatory syndrome developed.

The severity of the course of the disease is due to the defeat of the cardiovascular system - the expansion of the coronary vessels, the splitting of the sheets of the pericardium. The difficulty in treatment is associated with the lack of effective etiotropic therapy.

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