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ORIGINAL RESEARCH

N.A. Barashkov, A.M. Cherdonova, A.V. Solovyov,
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ANALYSIS OF THE FREQUENCY OF HETEROZYGOUS CARRIAGE OF SIX MUTATIONS OF AUTOSOMAL RECESSIVE DISEASES AMONG RUSSIAN OLD-SETTLERS OF YAKUTIA

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Among the populations of Eastern Siberia, the Russian old-settlers of the village of Russkoe Ustye (Allaikhovsky district of Yakutia) occupy a special place: their origin is relations with people from the old possessions of Novgorod the Great (presumably around 1570). The investigation of the hypotheses of the origin of the people of Russkoe Ustye, are of considerable scientific interest for the study of the colonization process of Northern Eurasia. In this study analysis of the carrier frequency of the six mutations responsible for autosomal-recessive diseases in 30 unrelated individuals from the village of Russkoye Ustye was performed. In the population of the Russkoe Ustye we did not find any heterozygous mutations, which are the cause of phenylketonuria (0/30), Wilson disease (0/30), congenital cataract (0/30), progressive deafness (0/30), and methemoglobinemia (0/30). In this Siberian population with a carrier frequency of 6.7%, the c.35delG mutation of the GJB2 gene (2/30) responsible for autosomal recessive deafness 1 A was detected. The absence of local East-Siberian variants of founder mutations associated with congenital cataract, progressive deafness, and methemoglobinemia, which are prevalent among Turkic-speaking Yakuts, indicates that this Turkic component in population of Russkoe Ustye is absent or represented to a small extent. Increasingly, than the Turkic component in the in population of Russkoe Ustye is represented a common West-Eurasian component, as indicated by the presence of the c.35delG mutation of the GJB2 gene, which is common in Europe.

Keywords: Russkoe Ustye, carrier frequency, Wilson disease, phenylketonuria, congenital deafness, congenital cataract, juvenile deafness, methemoglobinemia.

Introduction. Among the populations of Eastern Siberia, the Russian old-settlers of the village of Russkoe Ustye (Allaikhovsky district of Yakutia) occupy a special place: their origin is relations with people from the old possessions of Novgorod the Great (presumably around 1570), who fled from the persecution of the guardsmen of Ivan the Terrible. However, in chronicles the first mention of the village dates back to 1638, it was in this year that a Cossack detachment led by the Tobolsk Cossack Ivan Rebrov opened the sea route to Indigirka [6]. To date, there are several versions of the origin of the residents of Russkoe Ustye their ancestors could be either Novgorodians who fled from the oprichnina of Ivan the Terrible, or descendants of Cossacks and industrial people (in particular, from the village of Pokhodsk, it was from this village that Cossack explorers began their campaigns to meet with non-peaceful Chukchi), or settlers from the city of Zashiversk, liquidated by a royal decree in 1805 [1, 3]. Hypotheses of the origin of the people of Russkoe Ustye, are of considerable scientific interest for ethnography, history, anthropology and the study of the processes of settlement of Northern Eurasia.

In addition to the generally accepted archaeological and ethnographic approaches, of molecular genetics methods have become widely used in recent years to resolve the issues of the origin of individual ethnic groups and restore the evolutionary history of various regions. The study of the gene pool of populations can include both classical markers based on typing of paternal (Y-chromosome) and maternal lines (mtDNA) and analysis of autosomal markers. From the autosomal markers, one of the most interesting markers is mutations of autosomal recessive diseases, which are characterized by the founder effect. An analysis of the carrier frequency of founder mutation to some extent allows us to characterize the relationship with certain migration processes of the past, and can be used in the study of the population genetic cluster of autosomal markers. On the other hand, the analysis of the carrier frequency of hereditary diseases is important for assessing the burden of hereditary diseases, and can be applied in clinical genetics and the epidemiology of hereditary diseases.

In this regard, the aim of this work was to analysis of the carrier frequency of six major founder mutations responsible for the occurrence of autosomal recessive diseases in the population of Russkoye Ustye.

Material and methods. Genomic DNA of 30 unrelated individuals from the village of Russkoye Ustye (Allaikhovsky District of Yakutia) was isolated using phenol-chloroform extraction with informed written consent of all participants of this study. Amplification of the desired fragments was carried out by polymerase chain reaction (PCR) on a programmable thermal cycler BioRad T100 Thermal Cycler (Bio-Rad Laboratories, Inc., USA) using the original sequences of oligonucleotide primers. Methods for detecting major mutations that are the main cause of six autosomal recessive diseases are presented in Table 1.

Brief description of investigated autosomal recessive diseases.

Phenylketonuria (OMIM 261600) is an autosomal recessive disease associated with impaired activity of the enzyme phenylalanine-4-hydroxylase [12], which normally catalyzes the conversion of phenylalanine to tyrosine. If left untreated (excludes phenylalanine from the diet), it leads to the accumulation of phe-

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Table 1

Methods for detecting major mutations, which are the main cause of six autosomal recessive diseases

Disease (OMIM)	Gene	Mutation	Primers sequencing	Size of PCR-product (bp)	Restriction ferment	Size after restriction
Congenital cataract (610019)	*FYCO1*	c.1621C>T p.(Gln541*)	F: TTGGCCTGCCGGAGCTCTT R: AGTGACCTGGAGGAGCAGAAGAAGCAGC**C**CATT	266	*Pct* I GAATGCN↑ / CTTAC↓GN	wt/wt 239/27 wt/mut 266/239/27 Mut/mut 266
Methemoglobinemia (613213)	*CYB5R3*	c.806C>T p.(Pro269Leu)	F: CCTCAGTTGCCACATCTGTAA R: TCATGGGTGGGGGGCCACACATCAGCACC**C**G	448	*Sfr*303 I CCGC↑GG / GG↓CGCC Режет при норме	wt/wt 30/418 wt/mut 448/418/30 mut/mut 448
Progressive deafness (607293)	*CLIC5*	c.1121G>A (p.Trp374*)	F: CGTCATCTCAGCCGGGATATCATAGTTGCG R: TGCTGGTATCATGGGAACTCCA	293	*Bsc4* I CCNNNNN↑NNGG GG↓NNNNNCC	wt/wt 258 wt/mut 293/258 mut/mut 293
Phenylketonuria (261600)	*PAH*	c.1222C>T p.(Arg408Trp)	F: TCCAAATGGTGCCCTTCAC R: GGACACAAGGGTAATTCTGACCT	351	*Mnl* I CCTC(N)7↑ / GGAG(N)6↓ Режет при норме	wt/wt 59/73/217 wt/mut 59/73/217/297 Mut/mut 297/59
Autosomal-recessive deafness 1 A (220290)	*GJB2*	c.35delG p.(Gly12fs)	F: ACTCAGGTGAACAAGCTACT R: TCTTTCCAATGCTGGTGGAGTGTTTGTTC**C**CA	337	*Bsc4* I CCNNNNN↑NNGG / GGN-N↓NNNNNCC	wt/wt 303/34 wt/mut 334/303/34 Mut/mut 334
Wilson disease (27900)	*ATP7B*	c.3207C>A p.(His1069Gln)	F: ACCCTGAGATTGAACGACAGA R: CTTTACAGTATTTGGTGACTGCCACGCCCAC**C**G	337	*Dra* III CACNNN↑GTG/ GTG↓N-NNCAC Режет при норме	wt/wt 306/31 wt/mut 337/306/31 Mut/mut 337

Note: A mismatch substitution in the primer sequence is indicated by an underscore; wt/wt – norma, wt/mut – heterozygote, mut/mut – homozygote.

nylalanine and its toxic products, which affect, among other things, the central nervous system (phenylpyruvic oligophrenia). A decrease in the enzymatic activity of phenylalanine-4-hydroxylase is associated with homozygous and compound-heterozygous mutations in the *PAH* gene mapped at the locus 12q23.2 **(NC_000012.12)** causing this disease, which has spread widely in Europe as a result of the founder effect. Kalaydjieva et al (1991) found this mutation at high frequency in Bulgaria, Lithuania and Germany, where it was found in a common haplotype (haplotype-2). The c.1222C>T p.(Arg408Trp) mutation frequency exhibited a downward gradient from East to West, suggesting that its distribution is associated with the Balto-Slavic population of Eastern Europe [17]. The main reasons for this east-west gradient may reflect the migrations of early Slavic or Germanic peoples in the middle of the first millennium AD [9]. Another haplotype with this mutation was found in Northern Europe (with accumulation in Ireland) [10]. However, subsequent analysis of VNTR polymorphisms suggest of common founder haplotype in chromosomes with c.1222C>T p.(Arg408Trp), and its divergence is probably associated with early recombination and subsequent migrations in Europe [10].

Wilson disease (OMIM 277900) is an autosomal recessive disease characterized by accumulation of intracellular copper in the liver with subsequent hepatic and neurological disorders caused by homozygous or compound heterozygous mutations in the *ATP7B* gene mapped at the 13q14.3 locus (NC_000013.11). The most common cause of Wilson's disease in Europe was the c.3207C>A p.(His1069Gln) variant of the *ATP7B* gene. Presumably, this pathogenic variant arose as a result of a very ancient single mutational event on the territory of modern Europe [2,16]. The subsequent distribution of the variant c.3207C>A p.(His1069Gln) in the vector from northeast to southwest across Europe from the center of settlement in the territory between the Vistula and Elbe rivers in the 5th-6th centuries associated with West Slavic tribes [13].

Autosomal recessive deafness type 1A (OMIM 220290) is the most common form of hearing loss in most populations of the world. This form of the disease is caused by biallelic mutations in the homozygous or compound-heterozygous state in the *GJB2* gene **(NC_000013.11)** encoding the gap junction protein connexin 26 (Cx26). The c.35delG mutation accounts for up to 70% of all pathological *GJB2* alleles in Caucasians from

northern and southern Europe and North America, while the carrier frequency ranges from 1.3% to 2.8% [11]. It has been shown that the high frequency of c.35delG in the *GJB2* gene in populations of European origin is the result of the founder effect [7].

Methemoglobinemia (OMIM 250800) is a rare autosomal recessive disease caused by a deficiency in the methemoglobin repair system, which is caused by homozygous or compound heterozygous mutations in the *CYB5R3* gene mapped on chromosome 22q13.2 (**NC_000022.11).** Clinically, methemoglobinemia is manifested by headaches, dizziness, and shortness of breath, tachycardia, fatigue, and drowsiness, possibly a lag in physical and mental development as a result of constant cerebral hypoxia. In Yakutia, a missense substitution c.806C>T p.(Pro269Leu) of the *CYB5R3* gene, unique for this region, was identified [14]. Based on the analysis of haplotypes, the assumption of the existence of a founder effect for this disease was confirmed; the estimated age of distribution of the detected mutation was 285+135 years, which explains the high prevalence of this disease (1:1250) in this region of Siberia [14].

Congenital cataract (OMIM 607182) is the leading cause of vision loss in children worldwide, as it leads to clouding of the lens, which can therefore lead to various visual impairments, including complete loss of vision [15]. In Yakutia, a nonsense mutation c.1621C>T p.(Gln541*) specific to Eastern Siberia was found in exon 8 of the *FYCO1* gene (3p21.31, **NC_000003)** (involving in controlling of autophagy process), which leads to a premature stop codon and in the homozygous state is the main cause of congenital cataract (86% all cases of congenital cataract) [8]. Reconstruction of mutant STR-haplotypes with c.1621C>T indicates that the expansion of c.1621C>T (p.Gln541*) carriers in Eastern Siberia occurred as a result of the founder effect about 260±65 years ago [8].

Progressive deafness (OMIM 607293) is the second most common cause of hearing loss in Yakutia, after autosomal recessive deafness type 1 A, and is caused by the specific homozygous mutation c.1121G>A (p.Trp374*) of the *CLIC5* gene (6p21.1**, NC_000006.12)**. This transition leads to the formation of a premature stop codon at the 374th amino acid position (p.Trp374*), which terminates the synthesis of the polypeptide chain of the CLIC5 protein. Most patients with this form of the disease noted a late onset of hearing loss that occurred in the postlingual period (after acquiring speech skills) [4]. Haplotype analysis indicates that the c.1121G>A (p.Trp374*) mutation most likely spread as a result of the founder effect, mainly in the Arctic regions, among the Paleo-Asiatic, Tungusic and Turkic peoples of Yakutia, approximately in the middle of the 18th century [5].

Results and discussion. Among 30 unrelated individuals from the Russkoy Ustye, the analyses of carrier frequency of six major mutations causing of Mendelian diseases with autosomal-recessive pattern of inheritance was performed (Table 2). Three diseases (congenital cataract, progressive deafness, and enzipopenic methemoglobinemia) are associated with local variants of mutations that are common only in Eastern Siberia, mainly among Turkic-speaking peoples, and to a lesser extent among Tungus-speaking peoples, which is due to the founder effect [5,8,14]. The other three diseases (phenylketonuria, Wilson disease, and autosomal recessive deafness type 1A) are associated with common West-Eurasian variants that spread as a result of the founder effect, mainly during in the Neolithic period [2, 7, 9, 10, 13, 17].

As a result, of the six tested diseases, heterozygous c.35delG mutation in the *GJB2* gene was detected in two individuals (Table 2). The carrier frequency of the c.35delG mutation of the *GJB2* gene in the population of Russkoy Ustye was 6.7% (2/30). The c.35delG mutation leads to stop codon in the coding exon 2 of the *GJB2* gene, which leads to termination of the translation of the polypeptide chain of the connexin 26 protein. The expansion of this mutation was causing by common founder effect, and its approximate age was estimated at 10,000 years [7]. The initial center of distribution of this mutation probably is the territory of the Middle East, from where, during the Neolithic period, it was brought to Europe through

Table 2

The carrier frequency of major mutations of six autosomal recessive diseases in residents of the village of Russkoye Ustye

№	Disease (OMIM)	Gene	Mutation	Common origin / «age» of mutation	Carrier frequency
Eastern-Siberian variants of mutations					
1	Congenital cataract (610019)	*FYCO1*	c.1621C>T p.(Gln541*)	Founder effect in Yakut population / 250 years [8]	0/30
2	Progressive deafness (607293)	*CLIC5*	c.1121G>A p.(Trp374*)	Founder effect in Yakut, Even and Evenk populations / 350 years [5]	0/30
3	Methemoglobinemia (613213)	*CYB5R3*	c.806C>T p.(Pro269Leu)	Эффект основателя в популяции якутов / 285 лет [14]	0/30
West-Eurasian variants of mutations					
4	Phenylketonuria (261600)	*PAH*	c.1222C>T p.(Arg408Trp)	West-Eurasian common founder effect [17]/ the first millennium AD [9]	0/30
5	Wilson disease (277900)	*ATPB7*	c.3207C>A p.(His1069Gln)	West-Eurasian common founder effect [13] / 12 000 - 13 000 years [16] and 1600 years [13]	0/30
6	Autosomal-recessive deafness 1 A (220290)	*GJB2*	c.35delG p.(Gly12fs)	West-Eurasian common founder effect / 10 000 years [7]	2/30 (6.7%)

the Mediterranean Sea, and then, together with subsequent waves of migrations, spread to the territory of North and South America and Australia [7]. This study shows that this mutation is also found on the Arctic coast of Eastern Siberia among Russian old-settlers.

Conclusions

1) In the population of Russkoe Ustye we did not find major mutations that cause phenylketonuria (0/30), Wilson disease (0/30), congenital cataract (0/30), progressive deafness (0/30) and methemoglobinemia (0/30). With a carrier frequency of 6.7% the c.35delG mutation of the *GJB2* gene (2/30), causing of congenital autosomal recessive deafness type 1 A was found.

2) The absence of local East-Siberian variants of founder mutations associated with autosomal recessive diseases, which are prevalent among Turkic-speaking Yakuts, indicates that this Turkic component in population of Russkoe Ustye is absent or represented to a small extent. Increasingly, than the Turkic component in the in population of Russkoe Ustye is represented a common West-Eurasian component, as indicated by the presence of the c.35delG mutation of the *GJB2* gene, which is common in Europe.

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T.V. Borisova, V.G. Pshennikova, F.M. Teryutin, A.V. Solovyov, G.P. Romanov, S.A. Fedorova, N.A. Barashkov

ESTIMATION OF THE MUTATION AGE C.1621C>T P.(GLN541*) IN THE *FYCO1* GENE RESPONSIBLE FOR THE DEVELOPMENT OF AUTOSOMAL RECESSIVE CONGENITAL CATARACT IN THE YAKUT POPULATION

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The main cause of congenital or juvenile cataract with autosomal recessive inheritance in Yakutia is the c.1621C>T p.(Gln541*) nonsense mutation in the exon 8 of the FYCO1 gene. Previous studies have shown that the c.1621C>T p.(Gln541*) mutation has spread to the territory of Yakutia as a result of the founder effect. The initial assessment of the average "age" of the mutation using the data of linkage disequilibrium for three STR markers: D3S3685, D3S3582 and D3S3561 showed a result of ~10.4 ± 2.6 generations (260 ± 65.0 years). In the present study, we used a different approach to determine the "age" of the c.1621C>T p.(Gln541*) mutation using the DMLE+ 2.3 software based on the analysis of 25 SNP markers. The calculated DMLE+ 2.3 "age" of the mutation, taking into account the 95% confidence interval, varies from 25 to 67 generations (from 625 to 1675 years). Comprehensive data show that the c.1621C>T p.(Gln541*) mutation could have occurred between the 4th and 18th centuries with the most likely time of expansion from 11th century.

Keywords: congenital autosomal recessive cataract, CTRCT18, c.1621C>T (p.Gln541*), FYCO1, Yakuts, founder effect, Eastern Siberia.

Introduction. Congenital cataract is one of the main causes of childhood blindness [20]. It is known that from 8.3 to 25% of congenital or juvenile cataracts are inherited [11; 7; 16] by autosomal dominant type [20], less often by autosomal recessive and X-linked type [20; 13]. In the Yakut population, congenital or juvenile cataract with autosomal recessive inheritance is one of the most common orphan diseases, occurring at a frequency of 1 in 8257 people [22]. In this regard, we have previously conducted studies to find the main genetic cause of autosomal recessive cataract in Yakutia. Whole exome analysis revealed a new c.1621C>T p.(Gln541*) nonsense mutation exon 8 of the *FYCO1* gene (NM_024513.3) [9]. This substitution leads to the formation of a premature stop codon p.(Gln541*) in the functionally significant Coiled-coil domain and truncates the polypeptide chain of the FYCO1 protein [9]. Among the studied patients with congenital cataract in Yakutia, in 86% of patients (25 out of 29), the c.1621C>T p.(Gln541*) mutation was found in the homozygous state. Taking into account the significant contribution (86%) of the c.1621C>T p.(Gln541*) mutation to the etiology of the disease in the territory of Yakutia, haplotypes of mutant chromosomes were analyzed using 6 STR markers. The results of the study showed the unity of origin of all the studied mutant chromosomes, which indicates the spread of the c.1621C>T p.(Gln541*) mutation in the territory of Yakutia as a result of the founder effect. Phylogenetic analysis revealed the highest diversity of haplotypes in the central subpopulation of the Yakuts, which indicates the beginning of the spread of mutant chromosomes in the territory of Yakutia from the Lena-Amga interfluve [9]. The carriage frequency for this mutation in the population of Yakuts was 7.9%, in Evens - 2%, Evenks - 1.7%, in the populations of Russians, Yukagir, Dolgan and Chukchi this variant was absent.

Previously, the estimation of the "age" of the mutation was carried out using the method described in Risch et al. [12]. To determine the "age" of the mutation, the data of linkage disequilibrium for three STR markers were used: D3S3685, D3S3582, D3S3561 (~6.3 Mb). This method gives an estimate of the "age" separately for each marker under study and is based on the "genetic clock" approach [14]. The average "age" of the c.1621C>T p.(Gln541*) mutation in the Yakut population was ~10.4 ± 2.6 generations (260 ± 65.0 years), which indicated the start of the expansion of mutant chromosomes in the 18th century [9] and corresponded to the time when the first Russian explorers appeared in Yakutia. However, the results of screening indicated the absence of this mutation in the Russian population. In order to clarify the data obtained earlier, in this work, we applied a different approach to estimating the "age" of the c.1621C>T p.(Gln541*) mutation using the DMLE+ 2.3 software [19], which makes it possible to carry out calculations based on the linkage disequilibrium of several genetic markers.

Materials and methods. *Patients.* This study consisted of DNA samples from 24 Yakut patients with congenital cataract who had the c.1621C>T p.(Gln541*) mutation in the *FYCO1* gene in the homozygous state. DNA samples from 22 healthy Yakut patients constituted a control sample.

Estimation of the "age" of the c.1621C>T p.(Gln541) mutation in the FYCO1 gene using DMLE+ 2.3*

The DMLE+ 2.3 program allows to make a conclusion about the "age" of a mutation (in generations) based on the observed linkage disequilibrium of several genetic markers, using the Markov chain Monte Carlo algorithm [19]. To cor-

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rectly estimate the “age” of a mutation using the DMLE+ 2.3 software, it is necessary to calculate the proportion of mutant chromosomes in the sample (proportion of population sampled) and the population growth rate (d). The proportion of mutant chromosomes in the sample was calculated based on the frequency of heterozygous carriage of the c.1621C>T p.(Gln541*) mutation. Since the exact number of Yakuts is known only since the time of the First General Population Census of 1897 in the Russian Empire [4], there was not enough data to accurately calculate the population growth rate. In this regard, we used the population growth rate parameter d=0.085 used by Rannala and Reeve [18] to estimate the age of the mutation *SLC26A2* gene in the Finnish population, which, presumably, like the Yakut population, originated from a small ancestral population [14].

Results and Discussion. As a result of comparing the allele frequencies of two samples of individuals using the χ2 test, 25 SNP markers (69,851 kb) were identified for further analysis. We calculated the expected number of homozygotes in the Yakut population (0.3%) from the known frequency of heterozygous carriers of the c.1621C>T p.(Gln541*) mutation, which is 7.9% [9], and determined the proportion of mutant chromosomes in the sample to be 0.05. The average “age” of the c.1621C>T p.(Gln541*) mutation in the *FYCO1* gene with a proportion of mutant chromosomes in the sample of 0.05 was estimated at 38 generations (950 years), taking into account 95% CI, the “age” is within 25–67 generations (from 625 to 1675 years) (Fig. 1, A).

To assess the accuracy of the calculation of the “age” of the mutation and identify possible errors, we used two more values for the proportion of mutant chromosomes in the sample, 0.04 and 0.06 (Fig. 1, B and C). If the estimated proportion of mutant chromosomes is lower than the calculated one and equals 0.04, the average “age” of the mutation is 39 generations (975 years), taking into account 95% CI, is 28–72 generations (from 700 to 1800 years) (Fig. 1, B). If the proportion of mutant chromosomes is higher than calculated and equals 0.06, the “age” of the mutation is 37 generations (925 years), and at 95% CI it is 23–66 generations (from 575 to 1650 years) (Fig. 1, C). Thus, the change in the proportion of mutant chromosomes in the sample had little effect on the final estimate.

The average “age” of the c.1621C>T p.(Gln541*) mutation obtained in this study for 25 SNP markers (~950 years) significantly exceeds the result of the previous study (260 ± 65.0 years), where the calculation for 3 separate STR markers was used (Table 1). Both of these approaches are used in genetic studies and neither of them can be considered more reliable. However, a study by Clendenning et al [17] showed that the model for estimating linkage disequilibrium used by the DMLE+ 2.3 software [19] could be more effective to determine the origin of relatively "young" mutations in a population with a rapid population growth, in comparison with a method that gives a separate age estimate for each marker [12]. Perhaps the DMLE+ 2.3 approach is more appropriate for the Yakut population.

An earlier estimate of the “age” of the c.1621C>T p.(Gln541*) mutation at 260 years indicated that this mutation had spread during the period of exploration of Eastern Siberia by Russian explorers. However, given the absence of this mutation in the Russian population and the highest carrier rate among the Yakuts (7.9%) [9], it would be more likely to assume that this mutation is related to the ancestors of the Yakuts and to accept an earlier estimate of ~260 years as the lower threshold of “age ». Thus, the estimate of the maximum value of 1675 years obtained in this study can be considered an upper limit for the "age" of the mutation. If we accept both results of the “age” estimate, then the time of the appearance of the mutation is within the range from the 4th to the 18th centuries AD.

According to archaeologist A.I. Gogolev, the physical and linguistic features of the Turkic-speaking ancestors of the Yakuts took shape in the Baikal region from the 6th to the 10th centuries, then in the 13th century Yakuts moved to the valley of the middle reaches of the Lena river [3]. According to A.N. Alekseev, the southern ancestors of the Sakha penetrated the territory of Yakutia much earlier – back in the first half of the 1st millennium AD and by the 10th – 11th century, the ancestors of the Yakuts mastered significant territories of Prilenye [1, 2]. According to genetic data, the time of divergence of N3-lineages of the Y-chromosome of the Yakuts, based on microsatellite diversity, indicates a primary increase in the size of the ancestral population in ~4th century, followed by a secondary expansion starting from the 11th century. [8].

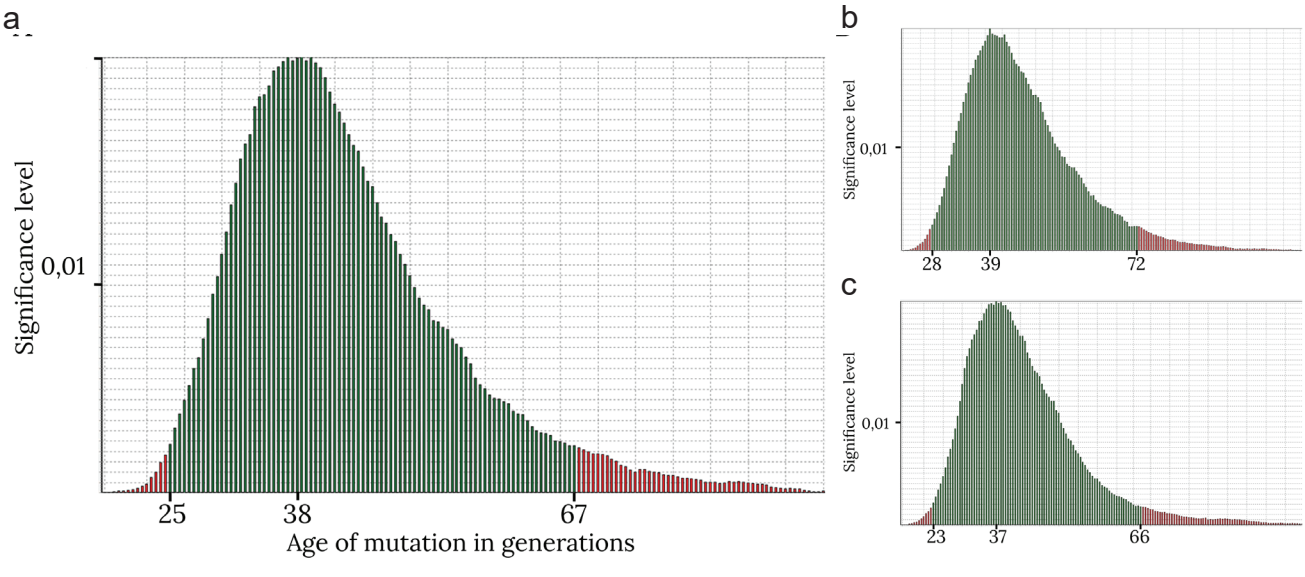

Fig. 1. Plots of estimation of the “age” of the c.1621C>T p.(Gln541*) mutation in the *FYCO1* gene using the DMLE+ 2.3 program [Reeve, 2002]. A - the "age" of the mutation with the value of the proportion of mutant chromosomes in the sample equal to 0.05; B - at 0.04; C - at 0.06.

Comparison of the results of age estimation of the c.1621C>T p.(Gln541*) mutation in the *FYCO1* gene

Method	Markers	Age of mutation (in generations)	Average age (in generations)	Reference
Risch et al., 1995 [12]	D3S3685	185 (~ 7,4)	260 ± 65,0 (10,4±2,6)	[9]
	D3S3582	317,5 (12,7)		
	D3S3561	277,5 (11,1)		
DMLE+ 2.3 [19]	25 SNP markers (69.851 kb)	625 – 1675 (25-67)	950 (38)	[Present study]

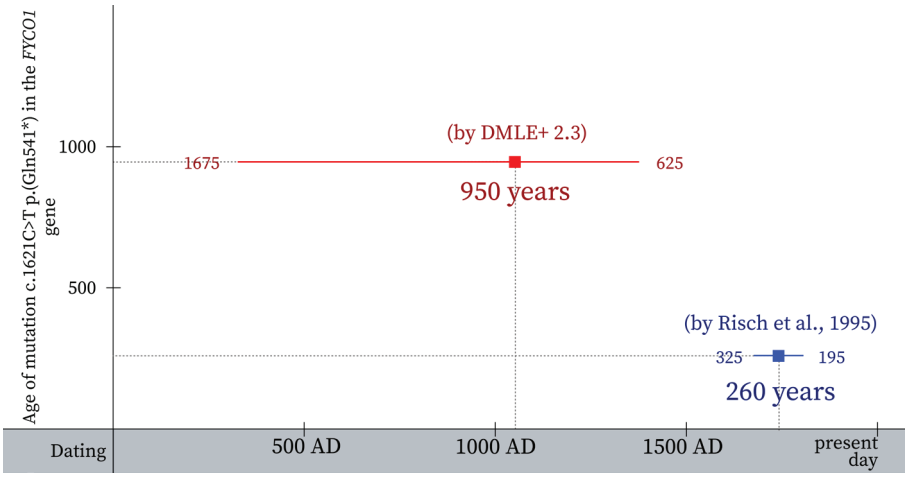

Fig. 2. The "age" of the c.1621C>T p.(Gln541*) mutation in the *FYCO1* gene, estimated using two methods.

Thus, the total period of occurrence of the c.1621C>T p.(Gln541*) mutation is from the 4th to the 18th centuries covers the time of the formation of the Sakha people [1]). At the same time, the average value of the age of the mutation ~950 years coincides with the appearance in the 10-11th centuries on the territory of Yakutia (in Namtsy and Ust-Aldan) sites of the Kulun-Atakh archaeological culture, directly associated with the ancestors of the Yakuts [2].

Conclusions. Thus, the time period from the 4th to the 18th centuries fully corresponds to the time of the formation of the Yakut people according to A.N. Alekseev, starting with the penetration of certain groups of southern pastoral tribes into the territory of Yakutia in the 3rd – 4th centuries. [6]. At the same time, the average mutation age of ~950 years coincides with the appearance in the 10 – 11th centuries. on the territory of Yakutia (in Namtsy and Ust-Aldan) sites of the Kulun-Atakh archaeological culture, directly associated with the ancestors of the Yakuts [2].

The authors declare no conflict of interest.

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ANALYSIS OF INTERFERON GENE POLYMORPHISM IN PATIENTS WITH HDV INFECTION IN THE REPUBLIC OF SAKHA (YAKUTIA)

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УДК 616.36-002.2

The aim of the research: to analyze the frequency of occurrence of polymorphisms rs8105790 of the IFNL3 gene, rs368234815 of the IFNL4 gene, rs1831583 of the IFNA1 gene in healthy people and patients with chronic viral hepatitis D among the ethnic group of Yakuts living on the territory of the Republic of Sakha (Yakutia).

Materials and methods of the study: to study gene polymorphisms in 157 individuals with chronic HDV infection and 160 apparently healthy individuals was used polymerase chain reaction (PCR). Analysis of the results included compliance with the Hardy-Weinberg law, Pearson's chi-squared test ($\chi 2$), odds ratio and its confidence interval.

Results: the people of young working age suffer more from HDV infection, wherein the development of cirrhosis from the moment of infection with the D virus is formed on average over 6.5 years. The high replicative activity of the HDV virus in 74.1% of cases is accompanied by suppression of HBV, but with an increase in the severity of fibrosis and the formation of cirrhosis and liver cancer, there is observed simultaneous replication of hepatitis B and D viruses. According to the data obtained, the risk of developing severe fibrosis in HDV is 1.7 times higher in carriers of the ΔG-allele of the rs368234815 polymorphism of the IFNL4 gene (OR=1.784; 95% CI 0.642–4.959) and 1.8 times higher in the carriers of the C-allele of the rs1831583 polymorphism of the IFNA1 gene (OR= 1.818; 95% CI 0.340–9.713).

Conclusion: the obtained results demonstrate that the C-allele rs1831583 of the IFNA1 gene and the ΔG-allele rs368234815 of the IFNL4 gene predispose to the formation of severe fibrosis in HDV infection, Yakutia.

Keywords: chronic hepatitis, liver cirrhosis, gene polymorphism, HDV, IFNL3, IFNL4, IFNA1.

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Introduction. Chronic viral hepatitis D (CHD) is caused by the hepatitis D virus and is characterized by a predominantly progressive course with the rapid development of liver cirrhosis (LC) than other hepatitis [1,5,4,10].

The Republic of Sakha (Yakutia) is one of the regions with a high prevalence of parenteral viral hepatitis [3,8]. The ongoing annual monitoring of the incidence of chronic viral hepatitis in the Republic of Sakha (Yakutia) shows an excess of the average for the Russian Federation.

Of the 14,975 patients registered in the electronic registry, chronic hepatitis D accounts for 7.8% (1176) of the total number of all chronic viral hepatitis, while HDV infection occurs in 40.8% of people with liver cirrhosis and in 38.5% with hepatocellular carcinoma (HCC). Chronic HDV infection is more often detected in the working-age population with a predominance of indigenous people. The high index of cirrhosis of HDV infection with the development of severe complications leading to early disability and death requires an in-depth study of the causes of liver fibrosis caused by the HD virus.

The mechanisms of genetic predisposition to chronic HDV infection have not yet been elucidated. The role of nucleotide polymorphic variants of interferon I (IFNA1) and type III (IFNL3, IFNL4) genes [2] in the pathogenesis of viral hepatitis is actively studied and is due to binding to cell receptors, as well as participation in the process of viral reproduction inside the cell [14]. Many studies prove the genetic determinism of the development of the chronic course of hepatitis in HBV and HCV infections [11, 16]. Despite the relevance of genetic predictors in the study of the development of chronic HDV infection, so far, no molecular genetic studies have been conducted in the Asian ethnic group in Russia.

Purpose: to analyze the frequency of occurrence of polymorphisms rs8105790 of the IFNL3 gene, rs368234815 of the IFNL4 gene, rs1831583 of the IFNA1 gene in healthy and sick individuals with chronic viral hepatitis D among the ethnic group of Yakuts living in the Republic of Sakha (Yakutia).

Materials and methods: the study was approved by the local ethical committee of the North-Eastern Federal University named after M.K. Ammosov", complies with the ethical principles of the Declaration of Helsinki of the World Medical Association (2013). The selection of the biomaterial was carried out on the basis of the infectious diseases department of the State Budgetary Institution of the Republic of Sakha (Yakutia) "Ya-

kut Republican Clinical Hospital" (chief physician, candidate of medical sciences Vasiliev N.N.), the infectious diseases department of the NEFU Clinic (director, candidate of medical sciences Ammosov V.G.) in the period from 2020 to 2022. Molecular genetic studies were carried out in the research laboratory "Molecular Medicine and Human Genetics" of the NEFU Clinic. Whole peripheral blood samples were used as a biomaterial for studying the polymorphism of the IFNL3 (rs8105790), IFNL4 (rs368234815), IFNA1 (rs1831583) genes. The clinical group included 157 patients with established HDV infection, 71 men and 86 women, aged 22 to 77 years, mean age 48±10.5 years, of the Yakut ethnic group permanently residing in the Republic of Sakha (Yakutia). At the same time, persons of the age category up to 44 years old accounted for 47%, up to 59 years old - 41%, 60 years and older - 12%. For comparison with the frequency of occurrence of the selected polymorphisms, 160 practically healthy individuals, 75 men and 85 women, aged 20 to 75 years old, average age 49±12.7 years old, were selected.

The demographic characteristics of patients (gender, age, place of residence), the form of hepatitis D disease (hepatitis, cirrhosis, cancer) were studied. The ethnic group was determined on the basis of questionnaire data. General clinical, serological and molecular biological methods for the study of hepatitis B and D viruses were carried out. Diagnosis and assessment of the stage of liver cirrhosis was carried out on a point scale in accordance with the Child-Pugh classification and the MELD survival scale. The diagnosis was established on the basis of clinical and laboratory data and the results of ultrasound, CT and MRI of the abdominal organs and fibroelastometry of the liver. Diagnosis and stage of HCC was carried out using the TNMB system in accordance with the Barcelona classification.

Isolation of DNA from peripheral blood was carried out using a commercial kit of OOO Evrogen (Moscow, Russia). For the molecular genetic study, the TagMan SNP Genotyping Assays Applied Biosystems, Thermo Scientific (USA) genotyping kits were used. Amplification was carried out on a CFX96 Touch Real-Time PCR Detection System (Bio-Rad, USA) in real-time mode.

Data processing was carried out using statistical criteria using the IBM SPSS Statistics 26.0 software package. To compare the means, we used T-test of independent samples (CI=95%; p> 0.05). The distribution of genotypes for the studied polymorphisms was checked for compliance with the Hardy-Weinberg equilibrium using Fisher's exact test. To compare allele frequencies between both sick and healthy groups, there was used Pearson's $\chi 2$ test with Yates' correction for continuity. Results were considered significant at $p<0.05$.

Results and discuss: distribution according to nosological forms of the study group revealed that in 36.9% (58) patients chronic hepatitis D was without severe fibrosis, in 63.1% (99) patients hepatitis D was in the stage of cirrhosis, including those with hepatocellular carcinoma 15 people.

When studying the epidemiological anamnesis of the clinical group, it was found that 46.5% of patients had a fact of surgical intervention, 44.6% of patients mentioned endoscopic and other invasive medical interventions. 33.1% of the surveyed do not exclude infection with HDV infection through blood transfusion, cases of intra-family contacts were observed in 32.1% of cases. A history of donation was noted in 2.3%, a possible sexual transmission in 5.3% of patients. Past acute viral hepatitis in history was registered in 39.5%. The duration of the disease was 19.2±11.7 years, the time from the moment of infection with the hepatitis D virus to the formation of liver cirrhosis is 6.5 years on average.

Clinical symptoms in most patients with HDV infection were manifested by asthenic syndrome in 100% of cases, skin manifestations in the form of telangiectasia and palmar erythema in 72.6%, and heaviness in the right hypochondrium in 68.8% of cases. Bleeding gums, nose, including episodes of bleeding from varicose veins of the esophagus were observed in 54.1% of patients, jaundice in 46.5%, edematous syndrome, including ascites, was observed in 40.8% of patients, splenomegaly was observed in 44 % and 41.4% of patients complained of pain in the joints. Among the most common comorbidities in patients were diseases of the gastrointestinal tract in 45.2%, diseases of the cardiovascular system in 21%, endocrine system in 11.5% and genitourinary system in 18.5% of cases.

In a laboratory study of peripheral blood of patients with CHD (n=157), pronounced cell cytolysis was observed in patients at the stage of liver cirrhosis. The level of serum aminotransferases revealed a 2.5-fold excess of ALT ($p<0.000$) in the initial manifestations of cirrhosis, also a 3-fold increase in AST activity ($p<0.000$) and a 4-fold increase in the level of bilirubin fractions ($p<0.000$) were detected in decompensated forms liver cirrhosis. A significant increase in the level of alpha-fetoprotein ($p<0.000$) in this group became a prognostically unfavorable factor in the course of severe liver fibrosis.

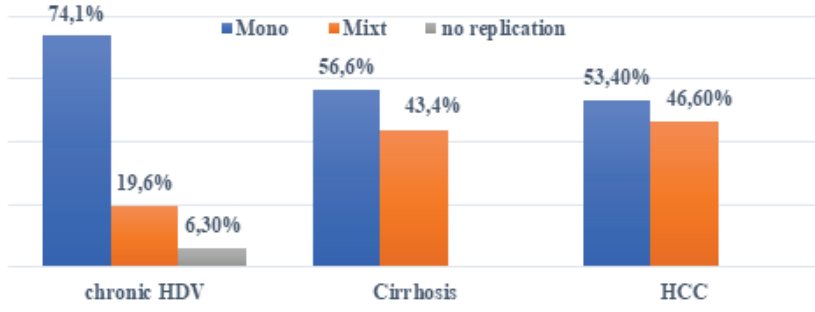
Previous studies on the incidence of chronic viral hepatitis B, C and D in the region has revealed a direct relationship between the incidence rate and the incidence of HCC in this category of patients [9]. The most aggressive course is detected in patients with chronic HDV infection, which is characterized by mixed replication of HBV and HDV viruses [6]. In the study of replicative activity by PCR (n=157), mono-replication of HDV was detected in 101 people, mixed replication of HDV in 51 people, 5 of the studied patients had no replication. In the CGD group without liver cirrhosis, mono-replication prevailed 74.1%, mixed replication was 19.6%, and 6.3% of patients were without replication. In the CGD group with liver cirrhosis, mixed replication was observed in 43.4% of cases, and mono-replication in 56.6%. In patients with HCC, the activity of the virus showed mixed replication in 46.6%, and mono-replication in 53.4% of cases (Fig. 1).

The genotype frequency of the examined patients corresponded to the Hardy-Weinberg equilibrium, which made it possible to carry out the distribution of alleles and genotypes of the polymorphic variant of the IFNL4 (rs368234815), IFNL3 (rs8105790) and IFNA1 (rs1831583) genes. The studied samples showed differences between the patients of the main group (n=157) and the control group (n=160) (Table 1). The sample of healthy patients was characterized by the largest number of carriers of homozygous AA - genotype rs1831583 of the IFNA1 gene, TT / TT - genotype rs368234815 of the IFNL4 gene and TT genotype rs8105790 of the IFNL3 gene without the presence of mutant allele, carriers of the homozygous AA genotype - genotype of the rs1831583 gene of the IFNA1 gene, TT/TT - genotype of the rs368234815 gene of the IFNL4 gene, TT genotype rs8105790 of the IFNL3 gene, but in the observed distribution, the proportion of heterozygous genotypes (carriers of the mutant allele) was higher than in the healthy group. Similar work was carried out by Karataylı S.C. and co-authors on the study of the rs8105790 polymorphism of the IFNL3 gene in patients with chronic HBV infection. Patients with the CC/TC genotype for rs8105790 ($p<0.0001$) were found to be more likely to be inactive HBsAg car-

riers [12].

In the studied groups, no significant differences were found in the frequency of genotype distribution (p>0.05), which indicates a fairly frequent occurrence of these markers among a healthy, uninfected population. The distribution of alleles showed significant differences for the C-allele of the rs1831583 locus of the IFNA1 gene (p=0.035) and the ΔG-allele of the rs368234815 locus of the IFNL4 gene (p=0.009). No significant differences were found in the rs8105790 polymorphism of the IFNL3 gene, which is probably due to the protective function of this gene, and it does not play a role in the development of chronic hepatitis D. According to the data, the probability of developing severe fibrosis in HDV is 1.7 times higher in carriers of the mutant allele of the polymorphism ΔG-allele of the rs368234815 polymorphism of the IFNL4 gene and 1.8 times higher in carriers of the C-allele of the rs1831583 polymorphism of the IFNA1 gene (Table 2). The research of the polymorphism of the genes we study is being carried out by many scientists [7,10,13]. A group of Chinese scientists examined 3128 people for the possibility of HBV infection with a number of polymorphisms of interferon genes in 14 loci, where the formation of HCC was associated with polymorphisms of the IFNA1 genes. In the studied groups of patients with liver fibrosis, the development of HCC was indicated in polymorphic variants of the IFNA1-rs1831583 and IFNA2-rs649053 genes [15]. There are indications that polymorphic variants of the IFNL3, IFNA1, and IFNL4 genes are associated with the development of liver cirrhosis in patients with chronic hepatitis B; polymorphic variants rs1831583 of the IFNA1 gene and rs649053 of the IFNA2 gene indicated the development of liver cancer [13]. There are studies that note the relationship between the development of liver cirrhosis and HCC in patients with HCV with the rs368234815 polymorphism of the IFNL4 gene [11], at the same time, unlike the Caucasians there is data that indicates the frequency of spontaneous clearance of HCV in carriers of the TT genotype of the rs368234815 polymorphism of the IFNL4 gene in representatives of the mongoloid race [7].

Conclusion: The Republic of Sakha (Yakutia) is one of the "endemic" regions of the Russian Federation in terms of the prevalence of viral hepatitis D. A feature of the course of HDV infection is the susceptibility to this pathology of people of indigenous nationality and of working age. The course of chronic hepatitis D



Share of HDV replication activity in patients with and without cirrhosis (n=157)

Table 1

The frequency of occurrence of polymorphisms of the studied genes in patients with CHD and healthy people of indigenous nationality

Genotypes. alleles	Chronic HDV n=157	healthy group n=160	$\chi 2$	*P*
Полиморфизм *rs1831583* гена *IFNA1*				
AA – genotype	95.5 (150/157)	96.8 (155/160)	p=0.565*	-
AC – genotype	3.8 (6/157)	3.1 (5/160)		-
CC – allele	0.6 (1/157)	0		-
A – allele (%)	97.0 (306/316)	98.0 (315/322)	-	p=0.035**
C – allele (%)	3.0 (8/267)	2.0 (5/250)	-	
Полиморфизм *rs8105790* гена *IFNL3*				
TT – genotype	85.3 (134/157)	79.4 (127/160)	p=0.078**	-
TC - genotype	14.6 (23/157)	20.6 (33/160)		-
CC – genotype	0	0		-
T – allele (%)	93.0 (291/313)	90.0 (287/319)	-	P=0.053**
C – allele (%)	7.0 (23/329)	10.0 (33/330)	-	
Полиморфизм *rs368234815* гена *IFNL4*				
TT/TT – genotype	87.2 (137/157)	87.5 (140/160)	p=0.598*	-
TT/ΔG – genotype	12.1 (19/157)	12.5 (20/160)		-
ΔG/ΔG – genotype	0.64 (1/157)	0		-
TT (%)	93.0 (293/316)	94.0 (300/320)	-	p= 0.009**
ΔG (%)	7.0 (21/300)	6.0 (20/334)	-	

* Analysis of arbitrary cross tables using the chi-square test;
**Analysis of four-field contingency tables. Pearson contingency coefficient (C)

Table 2

The incidence of liver cirrhosis in persons with HDV infection depending on the carriage of the mutant allele of the studied genes

Polymorphisms genes	Groups Research	Chances in groups	95%CI	OR
rs1831583 гена *IFNA1*	Carrier M	0.065	95%	1.818
	Not carrier M	0.036		
	Not carrier M	0.137		
rs368234815 гена *IFNL4*	Carrier M	0.206	95%	1.784
	Not carrier M	0.115		

Note: Carrier M - carrier of the mutant allele (XY, YY); Non-carrier M - homozygous for the normal allele (XX); OR- odds ratio (odds ratio), 95% CI - 95% confidence interval OR

is characterized by an aggressive nature and rapid development of complications. Decompensated forms of the disease are more often recorded in persons with HDV infection in the stage of cirrhosis and hepatocellular liver cancer. A prognostically unfavorable marker for the formation of liver fibrosis is the mixed replication of HBV and HDV viruses.

The obtained results demonstrate that the risk of developing severe liver fibrosis in HDV is 1.8 times higher in carriers of the C-allele rs1831583 of the IFNA1 gene and 1.7 times higher in carriers of the ΔG-allele rs368234815 of the IFNL4 gene. These groups of genes can become candidate genes for the formation of cirrhosis and liver cancer, which requires further study of other variants of the loci of the studied genes.

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ASSOCIATION OF POLYMORPHIC VARIANT RS1800849 OF THE UCP3 GENE WITH DEFICIT AND EXCESS OF BODY WEIGHT IN THE YAKUT POPULATION

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Genetic predisposition to obesity may play an important role in its etiology along with environmental influences. One of such potential therapeutic targets is uncoupling protein 3 (UCP3), is encoded by the *UCP3* gene. The main functions of UCP3 are considered to be proton transport in the presence of fatty acids, and a protective function, UCP3 can inhibit the actions of reactive oxygen species in mitochondria. The purpose of this study is to search for the association of polymorphism rs1800849 of the *UCP3* gene with body mass index (BMI) in the Yakut population. No significant differences in the distribution of alleles were found (χ^2 = 0.72; p = 0.397), so the frequency of allele (T) was 53%, and allele (C) was 47%. In females from the control group, it was founding that the heterozygous genotype was more common (p = 0.04) than in people with underweight. In males with overweight, the allele (C) is more common (p = 0.001), compared with the control group. The distribution of alleles of the rs1800849 polymorphism of the *UCP3* gene showed that in individuals with underweight (both females and males), the frequency of the allele (T) was significantly higher than the frequency of the allele (C) (p = 0.001). Allele (C) was more common in overweight males (p = 0.001), no such differences were found in females (p > 0.05). The article discusses the probable relationship of the distribution of rs1800849 alleles of the *UCP3* gene with the rate of basal metabolism affecting body weight, which is probably due to adaptation to a cold climate.

Keywords: UCP3, underweight, overweight, obesity, Yakut population.

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Introduction. The increase in the prevalence of overweight and obesity worldwide has aroused interest in the genetics of obesity. Genetic predisposition to obesity may play an important role in its etiology along with environmental influences. Thus, in a study by Nan et al., [14], it was shown that heredity explains most of the variations in body mass index (BMI, 61-80%) as the main indicator of obesity. Therefore, effective strategies for the prevention and treatment of obesity are currently being sought. One of such potential therapeutic targets is uncoupling protein 3 (UCP3), which is encoded by the *UCP3* gene (*SLC25A9*), and which belongs to the family of mitochondrial anion carriers. The *UCP3* gene is expressed mainly in skeletal muscles and in brown adipocytes [5, 24]. The main functions of UCP3 are considered to be proton transport in the presence of fatty acids [6, 15], and a protective function, UCP3 can inhibit the actions of reactive oxygen species in mitochondria [4]. UCP3 expression may increase when exposed to low temperatures and during prolonged physical exertion [22]. In addition, it is believed that UCP3 can participate in "uncoupling to survive" [9], in which energy from the oxidation of fatty acids is released as heat. Therefore, UCP3 is considered a good target for research aimed at manipulating energy consumption to combat obesity and type 2 diabetes (T2D).

Currently, three substitutions have been identified in the coding region of the *UCP3* gene - p.(Val102Ile), p.(Arg143Ter), p.(Arg70Trp), and one mutation of the splicing site – IVS6, leading to severe obesity and the development of T2D. Three mutations p.(Val102Ile), p.(Arg143Ter) and IVS6 were discovered by a group of scientists led by G. Argyropoulos in 1998 [11]. Mutation p.(Val102Ile) (rs2229707) is a missense substitution in exon 3, which was found in four siblings with obesity and T2D [11]. Mutations of p.(Arg143Ter) in exon 4 and mutation of the IVS6 splicing site (exon 6) were found in a compound heterozygous state in a 16-year-old adolescent suffering from pathological obesity [11]. Another missense mutation p.(Arg70Trp) (rs17848368) was found in China in a 15-year-old teenager with severe obesity and T2D [12].

In this regard, the purpose of this study is to search for the association of polymorphism rs1800849 of the *UCP3* gene with BMI in the Yakut population.

Materials and methods. The research sample comprised 279 people: 185 females and 94 males (with a mean age of 19.8±2.03 years). They presented no health issues at the time of the study and had completed a questionnaire in which they specified their sex, ethnicity,

and age. All participants gave written informed consent for participation in the study. This 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 (body weight in kilograms, height in centimeters) were measured for all participants by standardized methods. BMI was calculated by dividing body mass by the square of the body height. The sample was divided into three groups by BMI [16]: underweight (≤18.49 kg/m^2), normal weight (18.5–24.99 kg/m^2), and overweight/obese (≥25 kg/m^2).

Genomic DNA was isolated by phenol-chloroform extraction [18]. The amplification of the rs1800849 polymorphism was carried out by polymerase chain reaction (PCR) on a programmable BioRad T100 Thermal Cycler (Bio-Rad Laboratories, Inc., USA) using original sequences of oligonucleotide primers (F:5'-CCTTGTCACCAAGGAAGCGTCCACAGCTT-3' and R:5'- CTTCTGGCTTGGCACTGGTCTTATACACCC-3'). The primers were selected using the FastPCR program (http://primerdigital.com /). For amplification, a reaction mixture with a volume of 14 µl was used, including the following components: 1 µl of genomic DNA, 0.8 µl of Taq buffer with Mg^{2+} (700 mM Tris-HCl, pH 8.6, 166 mM $(NH_4)_2SO_4$, 25 mM $MgCl_2$), 0.5 µl of dNTP mixture (dATP, dGTP, dCTP, dTTP of 0.250 microns each), 0.24 µl of locus-specific oligonucleotide primers rs1800849F and rs1800849R, 0.2 µl (0.04 units) of *Taq* DNA polymerase (Silex, Russia), 5.51 µl of deionized H_2O and 5.51 µl of betaine (Sigma-Aldrich, USA). The detection was carried out in the standard amplification mode and the annealing temperature and amplification mode were selected individually. Genotyping of SNP markers was carried out using PCR-PDRF analysis, with enzymatic processing of PCR products by *SmaI* restriction endonuclease (SibEnzyme, Novosibirsk, Russia): CC genotype 185, 30 bp, CT genotype 215, 185 and 30 bp, TT genotype 215 bp. The separation of hydrolysis products was carried out in a 3% agarose gel (Figure 1). A single solution of TAE (40 mM tris (pH=7) was used as an electrode buffer.6), 20 mM glacial acetic acid, 1 mM EDTA). For gel electrophoresis, the BioRad Mini-Sub Cell GT System (Bio-Rad Laboratories, Inc., USA) was used as a standard horizontal camera at a

Table 1

Distribution of frequencies of genotypes and alleles of polymorphism rs1800849 of *UCP3* gene by gender

Genotype / Allele	Females (n=185)	Males (n=94)	χ^2	p
TT	57 (30%)	28 (29%)	0.031	0.861
CT	84 (45%)	43 (46%)	0.003	0.942
CC	44 (25%)	23 (25%)	0.016	0.9
T	0.54	0.53	0.02	0.888
C	0.46	0.47		

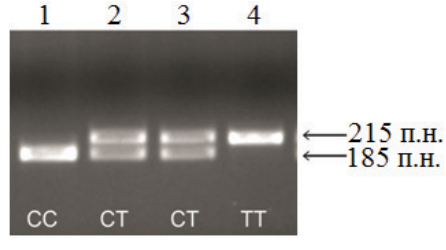

Fig.1. Electrophoregram of the results of PCR-PDF analysis of the rs1800849 polymorphism of the UCP3 gene.
Notes: Column 1 –CC genotype (185; 30 bp); columns 2, 3 –CT genotypes (215; 185; 30 bp); column 4 –TT genotype (215 bp).

Table 2

Comparative analysis of the frequency of occurrence of genotypes and alleles of the rs1800849 polymorphism of the *UCP3* gene between BMI groups

Genotype / Allele	Underweight	Control	χ^2	*p*	Overweight /obesity	Control	χ^2	*p*
ALL								
TT	16 (44%)	62 (29%)	3.437	0.06	7 (24%)	62 (29%)	0.294	0.588
CT	14 (39%)	100 (47%)	0.764	0.383	13 (45%)	100 (47%)	0.037	0.848
CC	6 (17%)	52 (24%)	1.008	0.316	9 (31%)	52 (24%)	0.616	0.433
T	0.64	0.52	2.956	0.09	0.47	0.52	0.5	0.48
C	0.36	0.48			0.53	0.48		
FEMALES								
TT	11 (44%)	42 (29%)	2.177	0.141	4 (25%)	42 (29%)	0.122	0.727
CT	**9 (36%)**	**67 (47%)**	**4.169**	**0.04**	8 (50%)	67 (47%)	0.007	0.792
CC	5 (20%)	35 (24%)	0.219	0.641	4 (25%)	35 (24%)	0.004	0.952
T	0.62	0.52	2.04	0.154	0.5	0.52	0.08	0.778
C	0.38	0.48			0.5	0.48		
MALES								
TT	5 (45%)	20 (29%)	1.27	0.26	3 (24%)	20 (29%)	0.165	0.685
CT	5 (45%)	33 (47%)	0.011	0.917	5 (38%)	33 (47%)	0.333	0.564
CC	1 (10%)	17 (24%)	1.27	0.26	5 (38%)	17 (24%)	0.09	0.764
T	0.68	0.76	1.587	0.208	**0.42**	**0.76**	**23.895**	**0.001**
C	0.32	0.24			**0.58**	**0.24**		

Notes: statistically significant differences are highlighted in bold (p<0.05)

voltage of 120 V. *pUC19/Msp I* (Silex, Russia) was used as a molecular weight marker. Electrophoregrams were visualized using Bio-Rad Universal Hood II Gel Documentation System (Bio-Rad Laboratories, Inc., USA).

Statistical analysis. The data obtained were analyzed using the Statistica 13.5 statistical program (TIBCO Software Inc., USA). The frequency of alleles and genotypes of the rs1800849 polymorphism of the *UCP3* gene was checked for compliance with the Hardy-Weinberg equilibrium. The frequency of alleles and genotypes between males and females, between the control group and with the groups of underweight and overweigh/obesity, as well as between the groups by BMI, was compared using the criterion χ^2.The values of p≤0.05 were considered statistically significant.

Results and discustion. Analysis of the frequency distribution of genotypes of polymorphism rs1800849 of the *UCP3* gene revealed that the heterozygous genotype CT = 45% (χ^2 = 30.581; p < 0.001) prevails in the Yakut population, and homozygous genotypes occur with the same frequency: TT = 30% and CC = 25% (χ^2 = 2.93; p = 0.09).No significant differences in the distribution of alleles were found (χ^2 = 0.72; p = 0.397), so the frequency of allele (T) was 53%, and allele (C) was 47%. There was also no difference in the frequency distribution of genotypes and alleles by gender (p > 0.05) (Table 1). The distribution of rs1800849 genotypes and alleles in the studied sample was in the Hardy-Weinberg equilibrium (χ^2 = 2.05; p = 0.152).

Comparative analysis revealed a tendency of a higher frequency of the TT genotype (p = 0.06) and allele (T) (p = 0.09) in individuals with underweight when compared with the control group (Table 2). In females from the control group, it was founding that the heterozygous genotype was more common (p = 0.04) than in people with underweight. In men with overweight, the allele (C) is more common (p = 0.001), compared with the control group.

When analyzing the distribution of allele frequencies in three groups (underweight, normal weight – control, overweight), it was founding that in Yakuts with underweight, the frequency of the T allele is significantly higher (p = 0.001) than the frequency of the allele (C), no such differences were found in the other two groups (Table 3). When dividing the sample by gender, it was found that in females and males with underweight, the frequency of distribution of the (T) allele was also significantly higher (p = 0.001) than the (C) allele. In males with overweight, on the contrary, the (C) allele is more common (p = 0.02).

Table 3

Comparative analysis of the frequency of occurrence of rs1800849 polymorphism alleles of the *UCP3* gene by BMI groups

BMI	Allele T	Allele C	χ^2	p
ALL				
Underweight	**0.64**	**0.36**	**15.68**	**0.001**
Control (Normal weight)	0.52	0.48	0.32	0.572
Overweight /obesity	0.47	0.53	0.242	0.623
FEMALES				
Underweight	**0.62**	**0.38**	**11.52**	**0.001**
Control (Normal weight)	0.52	0.48	0.32	0.572
Overweight /obesity	0.5	0.5	0	0.1
MALES				
Underweight	**0.68**	**0.32**	**25.92**	**0.001**
Control (Normal weight)	0.52	0.48	0.32	0.572
Overweight /obesity	**0.42**	**0.58**	**5.12**	**0.02**

The results of other studies on the effect of rs1800849 on BMI are too contradictory; therefore, its exact role in the etiology of obesity and concomitant diseases has not been established. For example, the allele (T) rs1800849 was associated with a higher BMI in the French and Spanish populations [1, 7], but negatively correlated with BMI in the UK population [23]. In other studies, rs1800849 was not significantly associated with obesity and BMI [3, 17, 21]. The discrepancies between the studies may be due to the fact that this polymorphism is located in the promoter region at a distance of 6 bp from the TATA box, and it affects only the transcription of the *UCP3* gene [10], but not the protein structure. It is believed that the allele (T) is associated with increased expression of UCP3 mRNA in skeletal muscles [2] and this overexpression positively correlates with resting metabolic rate [20]. It is interesting to note that the basal metabolic rate (BMR) is higher in people living in extremely cold conditions [8, 13, 19], which is one of the signs of human adaptation to a cold climate. Thus, in several studies it was found that the Yakuts BMR is 20% higher than the predicted values and in winter the metabolism can accelerate by 6% compared to the summer period [8, 19]. Similar results were found for the population of Evenks from Central Siberia, their BMR was higher compared to the population of Russians [13]. It can be assumed that carriers of the rs1800849 polymorphism allele (T) of the *UCP3* gene living in cold climatic conditions have a higher expression of UCP3 in skeletal muscles and this overexpression leads to a high metabolic rate at rest, the consequence of these processes is low weight. The presence of allele (C), on the contrary, probably leads to a reduced expression of UCP3 and to slower metabolism; therefore, carriers of this allele have an increased risk of developing obesity. In this regard, the relationship between BMR and polymorphism rs1800849 of the *UCP3* gene in the Yakut population requires further research.

Conclusion. In the Yakut population, both females and males had a strict association of the rs1800849 polymorphism allele (T) of the *UCP3* gene with underweight (χ^2 = 15.68, p < 0.001).On the contrary, the allele (C) was associated with overweight and obesity in males (χ^2 = 5.12, p < 0.02).Probably, the revealed dependence is due to the fact that carriers of the allele (T) have a higher BMR at rest, unlike carriers of the allele (C), whose BMR is lower. Accordingly, carriers of the allele (T) probably have a tendency to underweight, and carriers of the allele (C) probably have the opposite tendency associated with the risk of overweight.1.

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A NOVEL MUTATION IN THE *COL4A5* GENE IN A YAKUT FAMILY WITH ALPORT SYNDROME

Alport syndrome is a hereditary progressive kidney disease associated with sensorineural hearing loss and vision abnormalities, which is caused by mutations in the *COL4A3*, *COL4A4*, and *COL4A5* genes encoding the α3, α4, and α5 type IV collagen chains. This paper presents a case with a novel hemizygous mutation in the *COL4A5* gene in a Yakut family with Alport syndrome. The study involved 228 *GJB2*-negative patients with varying degrees of hearing loss and deafness living in the Republic of Sakha (Yakutia). Brothers were selected from this sample with a history of similar hearing and kidney impairments. For one of the sibs, a complete exome sequencing was performed, which resulted in the discovery of a new hemizygous mutation c.2375delA p.(Asp792fs) in exon 29 of the *COL4A5* gene on the long arm of the X chromosome (Xq22). This mutation was also detected in sibling using PCR-RFLP analysis.

Keywords: Alport syndrome, novel mutation, Yakutia, *COL4A5* gene

Introduction. Alport syndrome (SA) is a hereditary progressive kidney disease associated with sensorineural hearing loss and visual abnormalities [4]. The prevalence of AS is estimated at 1 in 50,000 newborns [16]. In Russia, the frequency of AS, according to epidemiological data, is 17:100,000 of the population [1]. AS is caused by mutations in the *COL4A3*, *COL4A4*, and *COL4A5* genes located on the long arm of the X chromosome (Xq22), which encode the α3, α4, and α5 chains of type IV collagen [10]. Mutations in these genes cause structural anomalies and dysfunctions of the basement membranes of the glomeruli of the kidneys, cochlea, and also cause some visual anomalies with damage to the cornea, lens, and retina [11].

There are three types of AS inheritance: autosomal recessive, autosomal dominant, and X-linked. The most common of these is the X-linked type of inheritance (OMIM #301050), which occurs in 80-85% of AS cases [9, 11]. This type of AS is caused by mutations in the *COL4A5* gene and, in some cases, by mutations in the *COL4A6* gene, which is located adjacent to the 5' end of the *COL4A5* gene [3]. ~14% of patients with AS have an autosomal recessive type of inheritance (OMIM #203780) caused by mutations in the *COL4A3* and *COL4A4* genes in homozygous or compound heterozygous states [9]. And approximately 1% of patients with AS have an autosomal dominant type of inheritance (OMIM #104200) due to mutations in the *COL4A3* and *COL4A4* genes [9].

Typical diagnostic features of AS are persistent hematuria, bilateral sensorineural deafness, family history of kidney disease, and visual anomalies [2]. Among patients with Alport syndrome, most often end-stage renal disease (ESRD) develops in men. Thus, ESRD can develop before the age of 40 in 90% of men and 12% of women with AS [16]. For 50% of men, dialysis or kidney transplantation is required before the age of 30 [16]. Deafness and visual anomalies are observed in 80–90% and 40% of men with X-linked Alport syndrome (XLAS), respectively [12]. However, it is rare to find cases of AS with all of the listed features, due to the age-related manifestation of some of them. For example, sensorineural hearing loss occurs at a later age, and approximately 90% of men and 10-15% of women lose their hearing by the age of 40 [19, 20].

In this article, we describe a case of a new hemizygous mutation in the *COL4A5* gene in a Yakut family with Alport syndrome.

Material and methods. *Patients.* The study involved 228 *GJB2*-negative patients with varying degrees of hearing loss and deafness living in the Republic of Sakha (Yakutia). Among them, 55.7% were women with an average age of 27 years, and 44.3% were men with an average age of 25 years. More than half of the patients (58.4%) were Yakuts, 19.5% were Russians, 9.3% were other nationalities, and 12.8% were metis. From this sample were selected brothers with a history of similar problems with hearing and kidneys (Fig.1, c, II:1, II:2). It was noted that the brothers were prescribed hemodialysis due to end-stage renal failure. During the collection of anamnesis, it was found that the mother (Fig.1, c, I:2) also had similar hearing and kidney disorders. Thus, in the total of the obtained anamnestic data, the diagnosis of Alport syndrome was suggested.

Molecular genetic analysis. For proband II:1 (Fig.1, c), the complete exome sequencing was performed. The analysis was carried out by the method of paired-end reading (2x100 bp) with an average coverage of at least 70-100x. For sample preparation, we used the technique of selective capture of DNA regions belonging to the coding regions of human genes. The sequencing data were processed using an automated algorithm, including alignment of reads to the reference sequence of the human genome (hg19), post-processing of the alignment, identification of variants, and filtering of variants by quality. The search for the c.2375delA p.(Asp792fs) mutation frequency in exon 29 of the *COL4A5* gene (chrX:107850101GA>G, NM_033380.2)

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was carried out using PCR-RFLP analysis. For amplification of fragments in exon 29 of the *COL4A5* gene (281 bp), mismatch primers (F) 5'-CCCCCATGGAAGGAAAAGTA-3' and (R) 5'-AATTCCAGACCTCAGGTGATCC-3' were used. For restriction, the *Hinf I* endonuclease with the G↑ANTC restriction site was used.

3D modeling of the α5 chain structure of type IV collagen. 3D visualization of the experimental spatial structure of the human protein Collagen alpha-5(IV) chain (UniProtKB - P29400 (CO4A5_HUMAN)) was carried out using the AlphaFold program (https://alphafold.ebi.ac.uk/entry/P29400). A PDB file of a protein with c.2375delA p.(Asp792fs) mutation was obtained using Colab notebook. Visualization of normal and truncated α5-chain collagen IV was carried out using the PyMol program (PyMOL Molecular Graphics System).

Ethical control. The study was approved by the local committee on biomedical ethics of the Federal State Budgetary Scientific Institution "Yakutsk Scientific Center for Complex Medical Problems", Yakutsk, Russia (Protocol No. 16, April 16, 2015).

Results and discussion. Whole exome sequencing revealed a novel hemizygous mutation in exon 29 of the *COL4A5* gene (transcript NM_033380.2) c.2375delA p.(Asp792fs). This deletion causes a frameshift, which results in the replacement of aspartic acid with valine at amino acid position 792 (Fig.1, b), which leads to the appearance of a premature stop codon in exon 30 at position 818 of the amino acid sequence.

The identified mutation was also found in the hemizygous state in proband II:2 (Fig.1, c). Affected family members had a history of similar symptoms (hearing problems and kidney disease). These data indicate that the identified new mutation in the *COL4A5* gene may be the cause of Alport's syndrome. In a sample of 226 *GJB2*-negative patients with hearing impairment, this mutation was not found.

The *COL4A5* gene consists of 51 exons and encodes the α5-chain of type IV collagen, consisting of 1685 amino acids. 3D modeling of the structure of the α5-chain of type IV collagen showed that as a result of the c.2375delA p.(Asp792fs) mutation, part of the collagen and the entire NC1 domain (Fig. 2b). It is known that the assembly of heterotrimers is initiated due to interactions of NC1 domains [5, 7, 15]. Deletion of this region of the gene can lead to disruption of the assembly of the necessary heterotrimer (α3α4α5) for

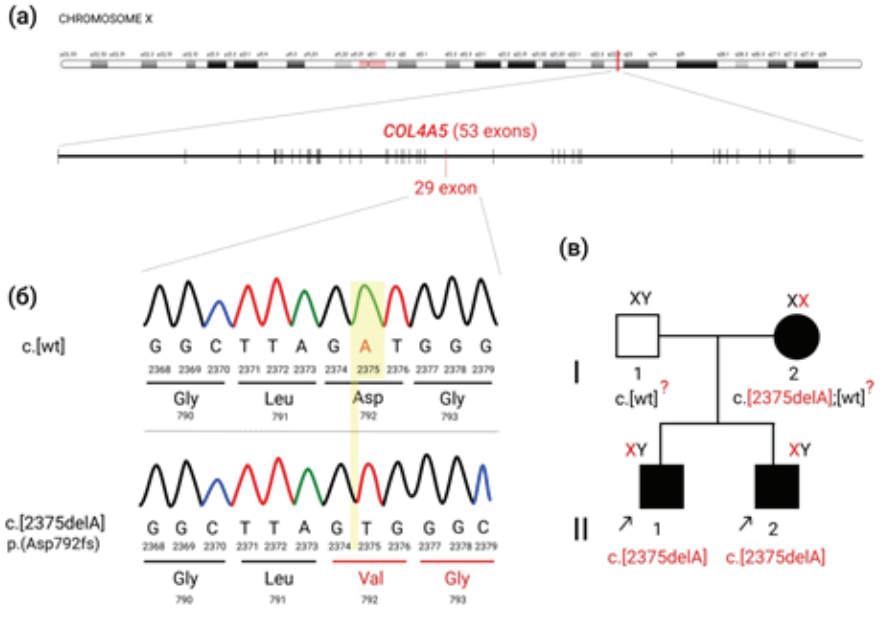

Fig. 1. Mutation with.2375delA p.(Asp792fs) of COL4A5 gene: a) — the localization of COL4A5 gene in the long arm of the X chromosome (q22.3); б) is the sequenogram of c.2375delA mutation with a frameshift, when the deletion of adenine (A) asparaginic acid (Asp - GAT) is replaced by valine (Val - GTG); в) - a pedigree with c.2375delA mutation : squares are men, circles are women; black are patients with mutation c.2375delA; arrows indicate probes; ? - no exact genotype known

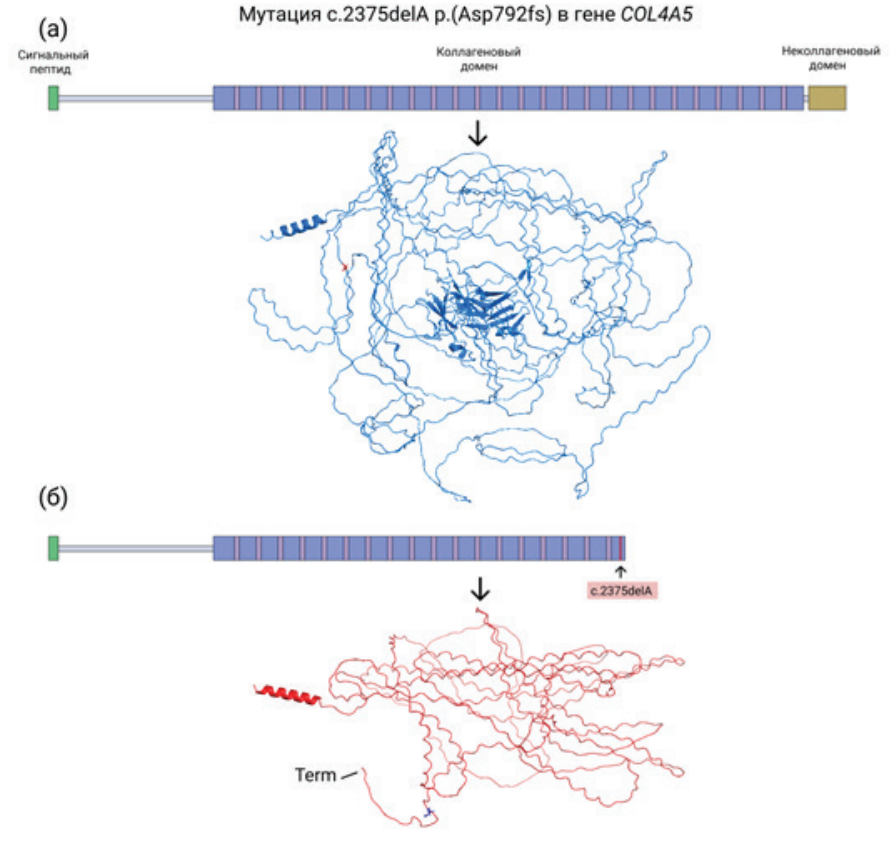

Fig. 2. Normal COL4A5 gene with c.2375delA p.(Asp792fs) mutation and the α5-chain of type IV collagen formed as a result of translation: a - schematic drawing of the COL4A5 gene and the resulting normal α5-chain of type IV collagen; b) shortened (part of the collagen domain and the entire NC domain is missing) α5-chain of type IV collagen as a result of the c.2375delA p.(Asp792fs) mutation in the COL4A5 gene

the proper functioning of the basement membrane in the tissues of the glomeruli of the kidneys, cochlea, and eyes.

It is known that only 10-15% of children with XLAS have *de novo* mutations [12]. In the case of our patients, this mutation was most likely passed from mother to both sons, which excludes the occurrence of this mutation *de novo* in the proband and sibling, but does not exclude the possibility of this variant *de novo* in their mother.

We have not been able to analyze the DNA of the parents of the proband, but it is known that their mother also suffered from kidney disease. Women heterozygous for mutations in the *COL4A5* gene can also have Alport syndrome: some of them may have all the overt signs of AS, as in men (hearing problems, kidney failure, visual abnormalities), others may be only minimally affected, and most can remain healthy throughout life [6, 17]. Thus, in one study, the characteristics of women and girls with proven mutations in the *COL4A5* gene were compared with the characteristics of hemizygous boys and men from 195 families [19]. It was shown that 95% of heterozygous girls and women had hematuria. Proteinuria, hearing loss, and ocular abnormalities developed in 75%, 28%, and 15% of heterozygotes, respectively. The probability of developing end-stage renal disease before the age of 40 was 12%, and deafness 10%. The risk of progression of end-stage renal disease in women increases after 40 years [19].

The reason for such a wide range of pathological phenotypes in women with heterozygous mutations in the *COL4A5* gene is presumably X inactivation, which is used by mammalian cells to equalize the dose of genes between female XX and male XY [8, 13]. At a very early stage of development in females, either the maternal or paternal X chromosome is randomly blocked by a complex cellular mechanism [13, 18]. This choice of inactivation is passed on to all offspring cells, resulting in a woman's body being a mosaic of cells with either an active maternal or paternal X chromosome [8, 13]. It is assumed that in heterozygous women with severe phenotypes, a healthy chromosome may be inactivated, which may explain the preferential expression of the mutant allele [14]. Thus, early, the amount of *COL4A5* mRNA was detected in the kidneys and leukocytes of a woman with two missense mutations in this gene, who suffered from kidney disease. At the same time, a correlation was found between the severe phenotype of Alport's syndrome (the patient underwent kidney transplantation) and the absence of detectable amounts of *COL4A5* mRNA with a normal sequence in the kidneys and leukocytes [14].

Conclusion. In conclusion, it should be noted that the identification of new mutations in AS and associated phenotypes is very important for disease prognosis, clarification of their clinical significance, early DNA diagnostics, and medical genetic counseling for families with AS in Yakutia. The results obtained complement the information available in the literature on the molecular genetic mechanisms of the occurrence of AS.

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FEATURES OF THE ENDOMETRIAL MICROBIOME OF INFERTILE WOMEN AND ITS EFFECT ON THE VIABILITY OF THE "IMPLANTATION WINDOW" PHASE

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Changes in the endometrial microbiome (dysbiotic type) were more often combined with dyschronism phenomena (inconsistency in the rate of the gland maturation with the secretory phase of the menstrual cycle) in women than with secretory changes. The data obtained allow us to state the dysbiotic type of microbiota appears to be a marker of violation of the morphological characteristics of the endometrium during the "implantation window" phase, mainly against the background of a chronic inflammatory process. The dysbiotic profile of the endometrium in infertile women is the cause of a violation of molecular mechanisms necessary for adequate blastocyst implantation. The studying endometrial microbiota increases the likelihood of predicting reproductive outcomes before embryo transfer and choosing an effective treatment strategy when changing quantitative and qualitative species characteristics.

Keywords: endometrium, endometrial microbiota, "implantation window" phase, lactobacillar (eubiotic) and dysbiotic microbiota types.

Introduction. The existing facts about the complexity and multifactorial conditionality of the implantation process have been supplemented by a new microbiological measurement of the human reproductive potential [5].

The idea of the complexity of microbial communities of various niches of the human body became possible with the introduction of the method of sequencing the ribosomal ribonucleic acid (rRNA) 16S gene, which is present in almost all bacteria [21].

The refutation of the dogma about the sterility of the uterus, separated from the lower infected part of the reproductive tract by the cervix, became possible with the isolation of bacterial deoxyribonucleic acid (DNA) taxa in 95% of endometrial samples after hysterectomy in non-pregnant women without signs of inflammation or vaginosis in conditions minimizing the risk of contamination [10].

Putting sequencing methods into practice made it possible to identify the relationship of microbiota disorders with various manifestations of reproductive dysfunction (from the formation of gametes in the gonads to implantation failures and/or pregnancy complications) and gynecological diseases [19,24].

However, a consensus on the microbial composition of the uterine nucleus of infertile and fertile women has not yet been formed, despite evidence of differences in the microbiota of the lower and upper parts of the reproductive system [1,28,34].

A vivid example of the abnormal endometrial microbiota with qualitative and quantitative transformations is chronic endometritis (CE) in 45% of infertile women with implantation failures and recurrent pregnancy losses [8,9,12,25,30].

Molecular detection of bacterial pathogens in endometrial samples showed an agreement between results using either polymerase chain reaction or sequencing in 77.0% [31].

The mechanism of the effect of the endometrial microbiota on the possibility of recurrent reproductive losses in CE remains unclear. The 16S ribosomal RNA sequencing method showed a large number of Phyllobacterium and Sphingomonas in the endometrium and a positive correlation of their content with immune B cells, a negative correlation with macrophages [22].

The features of the microbiome of women with endometrial polyp (EP) are believed to be associated with the possibility of their development on the CE background due to the continuous production of pro-inflammatory biological factors [3,6].

Data on the influence of the microbial community of the uterus on the effectiveness of the realization of the reproductive potential are contradictory due to small samples of patients, differences in assessment methods (a variety of Lactobacillus types, their relationship with opportunistic microorganisms, the presence of obligate pathogens).

Publications on the relationship between the predominance of Lactobacillus spp. in the endometrium (≥ 90% or ≥ 80%) by sequencing 16S ribosomal RNA with a significant increase in the frequency of implantation, clinical pregnancy, its prolongation and live birth [23] confirm the prospects of predicting the outcomes of infertility treatment/IVF programs based on the analysis of the uterine microbiome.

Controversial issues of the expediency and effectiveness of antibacterial therapy in infertile women are associated with the lack of clear ideas about the endometrial microbiota in each case. A recent meta-analysis of five studies (796 women) showed no differences in the reproductive performance of women with CE who received antibiotic therapy compared with the control group without treatment [13].

On the contrary, an increase in the frequency of implantation and pregnancy in IVF protocols of women with unexplained infertility, the restoration of fertility in case of recurrent implantation failures is associated with the CE antibiotic therapy [8,13,14].

The objective of the research: to study the microbiota and morphological characteristics of the endometrium in the phase of the "implantation window" of women with infertility of various genesis.

Materials and methods of the research: A prospective examination of 127 women of reproductive age who applied for infertility, including after ineffective attempts of in vitro fertilization (IVF), was performed.

The selection and examination of mar-

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ried couples were carried out on the basis of the Department of Assisted Reproductive Technologies of the Federal State Budgetary Institution "National Research Center of Endocrinology" of the Ministry of Health of the Russian Federation in Moscow, as well as the center for the emergency medical care of the Republican Hospital No. 2 in Yakutsk.

Criteria for inclusion in the study: age from 25 to 40; infertile women with verified CE, including in combination with endometrial polyp (EP); with tubal and idiopathic infertility; absence of male infertility factor; absence of infertility or fertility disorders of any other genesis; voluntary informed consent to 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, antibiotic therapy at least a month before inclusion in the study.

The first group consisted of women with unexplained infertility (idiopathic) (n=32), the second – with tubal infertility (n=38), the third – with infertility on the CE background in combination with EP (n=21), the fourth – with infertility on the CE background in general (n=36).

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 scope of the examination included assessment of complaints, anamnesis, general and gynecological examination, laboratory (clinical blood test, general urinalysis, biochemical method, hemostasiogram) and instrumental (hysteroscopy) studies.

Hysteroscopy was performed on days 7-9 of m.c. (when diagnosing the signs of PE and PE by ultrasound of the pelvic organs) with subsequent collection of material for morphological examination. The morphological signs of CE were presented by inflammatory infiltrates from lymphoid elements around glands and blood vessels, rarely diffusely; focal infiltrates in the form of "lymphoid follicles" in the basal and in all parts of the functional layer, consisting of leukocytes and histiocytes; the presence of plasma cells; focal stroma fibrosis; sclerotic changes in the walls of the spiral arteries of the endometrium.

CE was also verified immunohistochemically (markers CD 138+). In other cases, endometrial aspiration pipel-biopsy was performed during the "implantation window" phase (on days 20-24 of the menstrual cycle (ppm)).

Pathomorphological and immunohistochemical studies were performed according to the standard method.

Microbiological examination of the endometrium by real-time polymerase chain reaction (PCR) (Femoflor 16 tests, from Scientific and Production Association "50 DNA Technology" LLC (Russia)) was conducted to assess the presence and 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 from BIO-RAD (USA).

The protocol for patients' monitoring and the examination program were approved by the local ethics committee, 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 involving humans".

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

Qualitative variables were analyzed by constructing conjugacy tables using Pearson's chi-squared ($\chi 2$) test, with a small number of observations (less than 5) – Fisher's exact test.

Statistically significant differences were considered at $p < 0.05$.

Results and research methods. According to the histological study of endometrial biopsies in the "implantation window" phase, their compliance with the secretion phase was statistically sig-

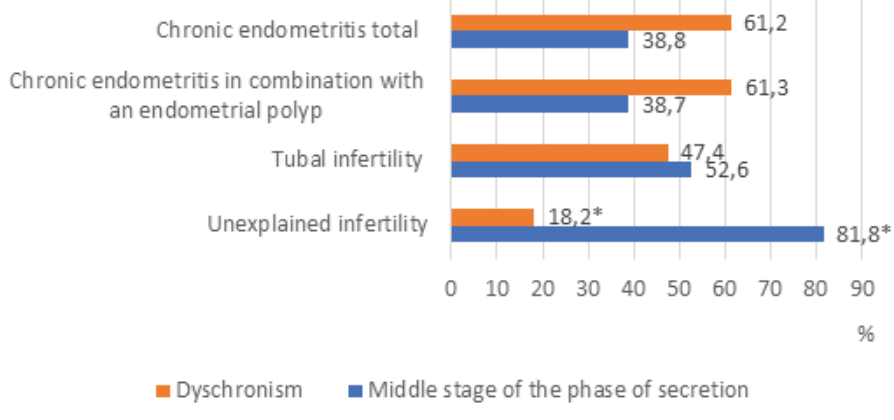

Fig. 1. Conclusions of morphological studies of endometrial biopsies of infertile women in the "implantation window" phase. *Differences in indicators are statistically significant from all groups ($p<0.05$)

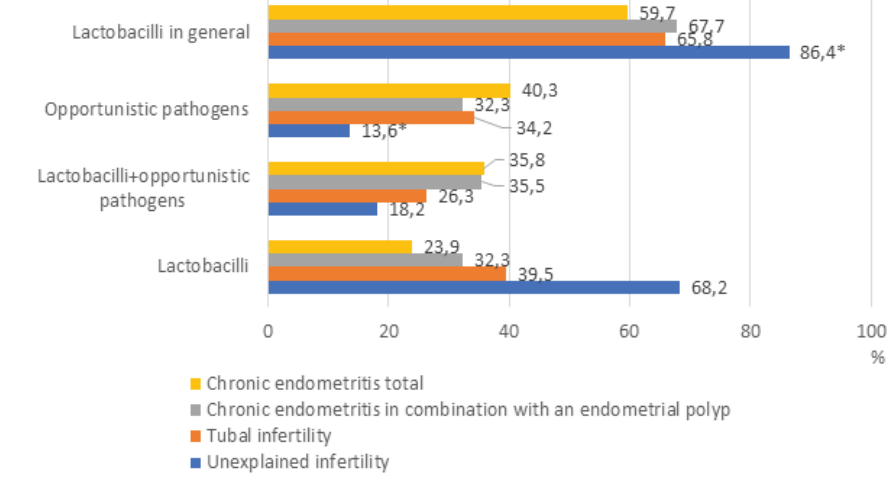

Fig. 2. Features of the endometrial microbiota of infertile women in the "implantation window" phase. *Differences in indicators are statistically significant between groups of women with unexplained infertility and CE ($p<0.05$)

nificantly more common in women with unexplained infertility – one and a half times than with TI (p=0.03, χ2=5.1), twice – with CE (p=0.00, χ2 =12.3), including in combination with EP (p= 0.002, χ2 =9.7) (Figure 1).

Figure 1 – Conclusions of morphological studies of endometrial biopsies of infertile women in the "implantation window" phase

Morphological signs of inconsistency between the architectonics of the endometrium and the "implantation window" phase, dyschronism in the maturation of the glandular epithelium and stroma, and uneven distribution of glands with accumulations in the perivascular zones in women with unexplained infertility were statistically significantly less common than in other groups: 2.6 times than with TI (p=0.03; χ2=5.1), 3.4 times – with CE (p=0.00; χ2=12.3) and its combination with EP (p=0.002; χ2=9.7).

In endometrial biopsies of almost half of women with TI, a picture of the middle stage of the phase of secretion was revealed, in the rest – dyschronism.

The features of the uterine microbiome of infertile women (Figure 2) were determined by the dominant type of bacteria detected: lactobacillar (more than 90%), mixed (less than 90% in combination with opportunistic microorganisms) and dysbiotic (absence of lactobacilli, predominance of opportunistic microorganisms).

The predominance of the lactobacillar type of microbiota in the endometrium of women with unexplained infertility was more common than in the other groups, but without statistically significant differences.

The frequency of a mixed microbiota profile (lactobacilli less than 90% of the total bacterial mass in combination with opportunistic microorganisms) in the endometrium of infertile women of different groups did not significantly differ. Bacterial communities with a high proportion of potential pathogens (isolated or mixed) in the endometrium of women with unexplained infertility were detected three times less often than with CE (p=0.03; χ2=5.3), twice as often as TI, however, no statistically significant intergroup differences were found.

The lactobacillar profile in the endometrium of women with unexplained infertility was detected more often than in the other groups, one and a half times than with CE (p=0.03; χ2=5.3).

Data on the occurrence of a variant of a reduced level of lactobacilli in combination with opportunistic microorganisms in groups of infertile women are presented in Figure 3

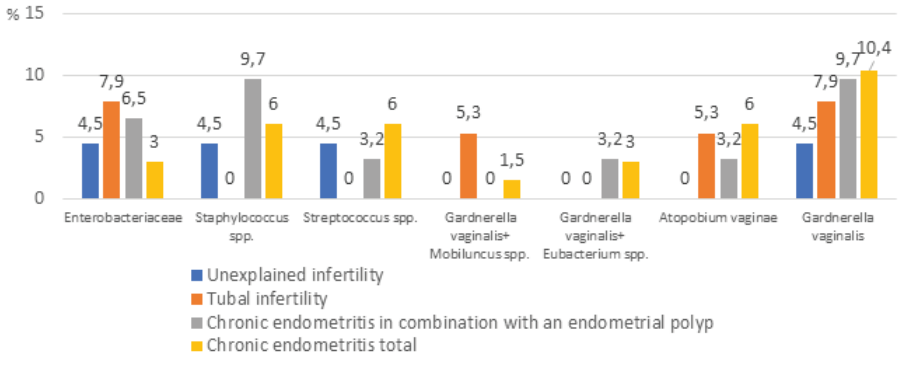

Fig. 3. Characteristics of the non-lactobacillar biotope of the endometrium of infertile women. There were no statistically significant differences between the groups

The spectrum of microorganisms with a mixed type of microbiota was represented mainly by monocultures with the highest proportion of Gardnerella vaginalis, Enterobacteriaceae and Staphylococcus spp. compared with other infections. Gardnerella vaginalis mixed with Mobiluncus spp. and Eubacterium spp. were found in separate groups of women with infertility (in a small number).

The characteristics of the endometrial biotope of infertile women with the dominant opportunistic microorganisms (dysbiotic) are shown in Figure 4.

The basis of the dysbiotic microbiome of the uterus of infertile women consisted mainly of monocultures (Gardnerella vaginalis, Mycoplasma hominis, Ureaplasma spp., Streptococcus spp. and Enterobacteriaceae), mixed facultative and obligate anaerobic bacteria were found in insignificant quantities. Obligate pathogens were not detected in the uterine cavity of infertile women.

The results of the analysis of the endometrial biotope of infertile women with different morphological characteristics of the endometrium in the "implantation window" phase are presented in Table 1.

The eubiotic profile of the endometrial microbiota was more common in women with its structure corresponding to the middle stage of the phase of secretion with the highest index in unexplained infertility: twice (p=0.03; χ2=5.8) than with TPI, three times – than with CE (p=0.00; χ2=14.0) and its combination with EP (p=0.04; χ2=9.1). The endometrial microbiome with a high proportion of lactobacilli in the presence of morphological signs of dyschronism was determined only in 6.3% of women.

In women with a mixed endometrial biotope (lactobacilli content less than 90% with the variability of opportunistic microorganisms), samples with morphological signs of the middle stage of the phase of secretion were detected in

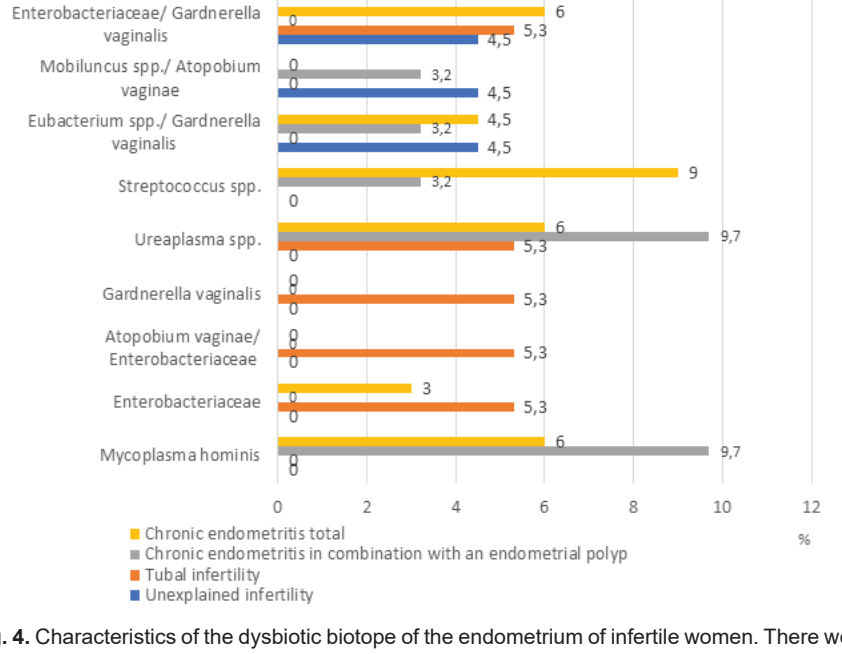

Fig. 4. Characteristics of the dysbiotic biotope of the endometrium of infertile women. There were no statistically significant differences between the groups

Morphological features of the endometrium in the "implantation window" phase in various variants of the uterine microbiome of infertile women

Groups		N	Lactobacilli		Lactobacilli+ opportunistic pathogens		Opportunistic pathogens	
			middle stage of the phase of secretion	dyschronism	middle stage of the phase of secretion	dyschronism	middle stage of the phase of secretion	dyschronism
Unexplained infertility	abs.	22	14*	1	3	1	1	2
	%		63.6	4.5	13.6	4.5	4.5	9.1
Tubal infertility	abs.	38	12	3	5	5	3	10
	%		31.6	7.9	13.2	13.2	7.9	26.3
Chronic endometritis in combination with an endometrial polyp	abs.	31	7	3	4	7	1	9
	%		22.6	9.7	12.9	22.6	3.2	29.0
Chronic endometritis total	abs.	67	14	2	9	15	3	24
	%		20.9	3.0	13.4	22.4	4.5	35.8

Note – * differences in indicators are statistically significant from other groups (p<0.05)

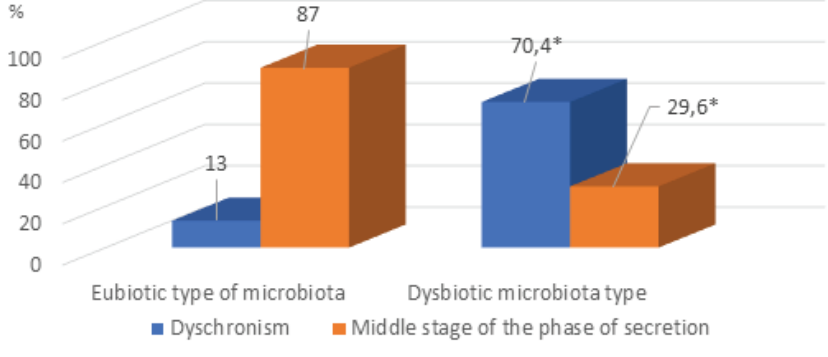

Fig. 5. Characteristics of the endometrial microbiome with various morphological characteristics in the "implantation window" phase. * Differences in indicators between groups are statistically significant (p<0.05)

13.3%, dyschronism – twice as high with CE as with TPI and unexplained infertility (22.5% vs. 8.8% on average). In the presence of potential bacterial pathogens in the endometrium, a discrepancy between its architectonics and the secretory phase of the menstrual cycle was more often noted. The morphological consistency of the endometrium during the "implantation window" phase was determined only in 5.0% of infertile women with a dysbiotic type of microbiota.

Data on the nature of the endometrial microbiota (eubiotic and dysbiotic types) depending on the morphological characteristics in the "implantation window" phase are presented in Figure 5.

The eubiotic type of microbiota in a sample of infertile women with a picture of the middle stage of the phase of secretion in endometrial samples was significantly more often than with incomplete secretory transformation of the stroma (p = 0.00; $\chi 2$=38.6). In case of endometrial glands and stroma dyschronism in the "implantation window" phase, most women had a dysbiotic microbiota type, almost five times more likely than with secretory changes (p = 0.00; $\chi 2$=38.6).

Our results suggest that the dominant Lactobacillus spp. biotope, modulating the function of endometrial cells and the local immunity system, is involved in the regulation of the fertile potential. It is probably the lactobacillar microbial profile that defines the endometrium as an immunologically favorable niche for blastocyst implantation [2,4]. The dominance of Lactobacillus (relative abundance> 90% relative to MBP) in the endometrial microbiota of infertile women is associated not only with the success of implantation, but also with the live birth rate in IVF protocols [15,18]. We believe that microbial homeostasis in the endometrium determines resistance to colonization by opportunistic flora, the ability to express genes that affect receptivity in the "implantation window" phase [27]. The data obtained allow us to state the concept of a complete remodelling necessary for a susceptible endometrium, mainly in the presence of a lactobacillar type of microbiota. The causes of infertility in these women may be associated with an imbalance of pro- and anti-inflammatory reactions in the endometrium.

Microbial diversity in the endometrium of infertile women with CE with a high proportion of Gardnerella vaginalis and Atopobium vaginae should be considered as a violation of protective physiological barriers and/or functional inferiority of cellular immunity components. Data on a lower frequency of Gardnerella vaginalis in women without CE allowed the authors to suggest their adverse effect on the possibility of pregnancy in natural cycles and IVF protocols [26].

The dysbiotic profile of the endometrium in 40.3% of infertile women with CE, a third – with TPI and 13.6% – with unexplained infertility seems, similar to the opinion of other authors [7,16,20], to be the cause of unfavorable molecular mechanisms for implantation. The presence of type IV CST (community state types) microbiota in the endometrium, especially Gardnerella and Streptococcus genra, is associated with a significant decrease in the frequency of implanta-

tion, pregnancy and miscarriage [18]. Changes in the endometrial microbiome of women with EP on the CE background (reduction of the lactobacillar profile by less than 90% in 22.6%; dysbiotic type – 29.0%) were associated with the phenomena of dyschronism (inconsistency of changes in the architectonics of the mucosa with the secretory phase of the menstrual cycle). Such correlations confirm the concept of a decrease in women's reproductive potential with a change in the molecular functions of endometrial microbes involved in the regulation of cellular metabolism, the immune system, and signaling cascades [11,29,32,33].

The pathological effects of bacteria on the endometrium at the dysbiotic profile are associated with a change in the functional activity of the local immune system and the development of an unfavorable environment for blastocyst implantation [3]. Obviously, the decision in favor of antibiotic therapy should be made after the confirmation of the inflammatory process modulated by the presence of non-lactobacillar microbiota in the endometrium.

Conclusion. Thus, the dysbiotic microbiota type appears to be a marker of a violation of the morphological characteristics of the endometrium during the "implantation window" phase, mainly on the background of a chronic inflammatory process of the endometrium. The effect of the non-lactobacillar biotope on the transformation of the endometrium in the "implantation window" phase and the possibility of inflammation depend on the balance of local immunoregulatory resources, which is the subject of further research.

The analysis of the composition of the endometrial microbiota is informative for predicting reproductive outcomes before embryo transfer, improving diagnosis and choosing a treatment strategy. The detection of lactobacillar type microbiota in the endometrium in infertile women with CE proves to be a reasoned rejection of antibiotic therapy.

Conflict of interests. The authors state no possible conflicts of interests.

Contribution of the authors

Polina M.L. conceived and developed the study. Polina M.L., Vitiazeva I.I., Douglas N.I., Zakharova P.N. participated in the collection, analysis and interpretation of data.

Polina M.L., Vitiazeva I.I., Zakharova P.N. wrote a draft report. All authors reviewed the report and approved of the final version before submission. All authors have read and agreed with the published version of the manuscript.

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INTENSITY OF LOCAL PROCESSES FREELY- RADICAL OXIDATION IN RED LICHEN PLANUS OF THE ORAL MUCOSA

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УДК 616.31-002.153

Lichen planus (CPL) occupies a special place among diseases of the oral mucosa (COPR) associated with chronic keratosis, which occurs in 50-75% of cases in the structure of mucosal pathologies. At the same time, despite extensive study, the problems of its treatment and prevention remain unresolved and the pathogenetic mechanisms have not been fully elucidated. In this regard, the study of the intensity of local processes of free radical oxidation at CPL COPR is an urgent task.

The purpose of the study: to evaluate the intensity of free radical oxidation processes in patients with CPL COPD in the oral fluid.

Materials and methods of research. 66 patients with a diagnosis of CPL COPR and 33 practically healthy individuals were examined. To characterize the intensity of SRO in the main and control groups, oral fluid was taken into test tubes after preliminary rinsing of the oral cavity with 10 ml of 0.9% sodium chloride solution. The intensity of free radical oxidation processes in the oral fluid was characterized by chemiluminescence (CML) on the LS 50B "PERKIN ELMER" spectrometer. Statistical processing of the results was carried out using methods of variational statistics.

Results and discussion. Studies of chemiluminescence of mixed saliva in patients with CPL SOPR showed an increase in the generation of oxygen radicals and lipid peroxides: the Ssp value, confirming the intensity of production of toxic radicals, exceeded the identical indicator of the control group by 1.4 times ($p<0.05$). Against the background of intensive generation of radical products (H) of mixed saliva in the main group, the indicator is 2.42 times ($p<0.05$) higher compared to the same indicator in practically healthy individuals, there was a decrease in the level of antioxidant antiradical protection (Sind-2) by 1.66 times ($p<0.05$), which indicates insufficient activity of the antioxidant defense system (AOS) of a patient with CPL SOPR. Chemiluminescence studies have shown that patients with CPL COPD revealed an imbalance between the processes of SRO and antioxidant protection, this is due to an increase in oxidant load and a decrease in antioxidant resources in mixed saliva in the main group compared with identical data in practically healthy individuals. The results obtained indicate the generation of free radical reactions in the group with CPL of the COPR.

Conclusion. An imbalance in the ratio of the processes of "formation of active metabolites – destruction of active metabolites" indicates hyperproduction of toxic SRO products in mixed saliva in patients with CPL COPR and disruption of work in the AOS system. The study of the activity of the SRO processes and the AOS system can be a condition for increasing the effectiveness of the therapy, by making adjustments to the standard treatment regimens of this category of patients, taking into account the functional state of the body's antioxidant barrier.

Keywords: chemiluminescence, lichen planus, oral fluid, free radical oxidation, antioxidant defense system.

Introduction. Lichen planus occupies a special place among diseases of the oral mucosa associated with chronic keratosis, which occurs in 50-75% of cases in the structure of mucosal pathologies [3].

The cause of the disease has not been studied enough. In many works, the opinion is given that numerous risk factors can be the impetus for the development of lichen planus: toxic-allergic, hereditary, viral and neurogenic origin. Based on clinical observations, researchers associate the occurrence of CPL with the influence of exogenous and endogenous factors on the tissues and organs of the oral cavity, taking certain medications, and general somatic pathology [1].

The clinical picture of CPL is characterized by the appearance of nodular elements on the SOPR with a combination of exudative-hyperemic, erosive-ulcerative, bullous and hyperkeratotic lesions [1]. One of the main mechanisms of destruction of cell membranes is oxidative stress (OS), which leads to hyperproduction of free radicals and has high activity. The intensification of free radical processes and the development of OS is one of the links in the inflammatory processes of immunogenesis in lichen planus.

Violation of the balanced state between the processes of free radical oxidation (SRO) and the activity of antioxidant protection (AOS) can lead to a violation of the integrity of the oral mucosa [5]. The biochemical characteristics of the intensity of SRO in blood serum and mixed saliva in patients with CPL COPR on the background of primary hypothyroidism were studied, indicating an increase in the activity of free radical processes with a decrease in antioxidant protection. The timely correction of the revealed pathobiochemical disorders in endocrine pathology is justified [4].

Variants of changes in the intensity of SRO in mixed saliva are a reflection of the general processes occurring in the oral cavity and related to the biochemical processes of the body as a whole [2].

The purpose of the study – to evaluate the intensity of free radical oxidation processes in patients with CPL COPR in the oral fluid.

Materials and methods of research. A survey of 66 patients diagnosed with CPL COPR (the main group), aged 35 to 65 years, including 18 men (27.2%) and 48 women (72.8%). The average age was 54.3±1.3 years. The control group consisted of 33 practically healthy individuals, aged 35 to 65 years, without lesions of the oral mucosa, aged 52.3±1.7 years, 25 (75.7%) women and 8 (24.3%) men.

To characterize the intensity of SRO in the main and control groups, oral fluid was taken into test tubes after preliminary rinsing of the oral cavity with 10 ml of 0.9% sodium chloride solution. Saliva was pre-frozen for 3-5 days, followed by defrosting and centrifugation with a rotation speed of 1500 rpm. The material for the work was a filler fluid.

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The intensity of free radical oxidation processes in the oral fluid was characterized by chemiluminescence on the LS 50B "PERKIN ELMER" spectrometer [2]. Spontaneous and induced Fe2+ CML were studied.

CML indicators were recorded: spontaneous glow reflecting the intensity of free radical formation (Ssp), after initiation (addition of an activator – iron), the value of a "fast" flash (h) of induced CML was determined, indicating the content of lipid hydroperoxides and the luminescence light sum (Sind-1) reflecting the rate of accumulation of lipid peroxide radicals.

The kinetics of CML initiated by H2O2 in the presence of luminol was analyzed by light sum in 1 min. luminol - dependent CML, indicating the intensity of free radical formation and the state of free radical peroxidation without external influence (Slum); by the maximum of H2O2 – induced luminescence (H) depending on the rate of oxidation and formation of reactive oxygen species in the medium and reflects the intensity of SRO; by the light sum for 2 min. H2O2 - induced CML (Sind-2), indicates the activity of AOS. The indicators were determined in relative units of luminescence intensity.

Statistical processing of the results was carried out using the methods of variational statistics with the calculation of the arithmetic mean (M), the standard deviation of the studied features (o) and the error of the arithmetic mean (m), 95% significance level was considered reliable. The parametric criterion of the reliability of Student-Fisher differences (t) was used to compare the averages with the normal distribution of the variant.

Results and discussion. Studies of chemiluminescence of mixed saliva in patients with CPL SOPR showed an increase in the generation of oxygen radicals and lipid peroxides: the Ssp value, confirming the intensity of production of toxic radicals, exceeded the identical indicator of the control group by 1.4 times (1,272±0.052 vs. 0.908±0.108 rel.units; P < 0.05). Studies of chemiluminescence of mixed saliva in patients with CPL SOPR showed an increase in the generation of oxygen radicals and lipid peroxides: the Ssp value, confirming the intensity of production of toxic radicals, exceeded the identical indicator of the control group by 1.4 times (1,272±0.052 vs. 0.908±0.108 rel.units; P < 0.05).

The toxic effect of free radicals is manifested in the intensification of lipid peroxidation processes in the main group, the activation of the production of lipid hydroperoxides (h) was found to be 1.69 times higher (2.036 ±0.088 vs. 1.203 ± 0.132 rel.units; P< 0.05) than the level of practically healthy individuals.

The leading mechanism for the development of hyperactivation of free radical oxidation is an increase in the rate of formation of lipid peroxide radicals (Sind-1), recorded in patients with CPL COPR, a significantly high level was noted 1.96 times higher than normal (4,117± 0.212 vs. 2,096 ± 0.254 rel.units; P < 0.05).

An increase in the concentration of hydroxyl radicals in mixed saliva was found in patients with CPL COPR, in contrast to the control group, which is reflected by an increase in the value (Slum) by 1.26 times (1.266 ± 0.051 vs. 1.002 ± 0.099 rel.units; P < 0.05).

Against the background of intensive generation of radical products (H) of mixed saliva in the main group, the indicator was 2.42 times higher (3.4±0.186 vs. 1.482±0.090 rel.units; P< 0.05) compared to the same indicator in practically healthy individuals, there was a decrease in the level of antioxidant antiradical protection (Sind-2) by 1.66 three times (3,555±0.120 vs. 2,137±0.066 rel.units; P < 0.05), which indicates insufficient activity of the AOS system of a patient with CPL SOPR.

Chemiluminescence studies have shown that patients with CPL COPR revealed an imbalance between the processes of SRO and antioxidant protection, this is due to an increase in oxidant load and a decrease in antioxidant resources in mixed saliva in the main group compared with identical data in practically healthy individuals.

The obtained results indicate an increase in the generation of free radical reactions against the background of weakening of antiradical protection in the group with CPL COPR, which indicates the development of local oxidative stress, which is one of the pathogenetic links of inflammatory processes occurring in the oral mucosa.

Conclusion. The revealed imbalance in the ratio of the processes of "formation of active metabolites – destruction of active metabolites" indicates hyperproduction of toxic SRO products in mixed saliva in patients with CPL COPR and disruption of work in the AOS system, requiring a differentiated approach to the appointment of corrective antioxidant therapy.

The study of the activity of the SRO processes and the AOS system can be a condition for improving the effectiveness of the therapy, by making adjustments to the standard treatment regimens in this category of patients, taking into account the functional state of the body's antioxidant barrier.

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DIAGNOSTIC AND TREATMENT METHODS

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THE CONDITION OF THE CORNEAL NERVE FIBERS AFTER EXTRACAPSULAR CATARACT EXTRACTION

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The term "phacosurgery" combines various surgical technologies for the complete or partial removal of the lens. The last decades are characterized by an almost complete transition from extracapsular cataract extraction to the so-called. microinvasive phacosurgery, in particular, standard ultrasound and hybrid (femtolaser) phacoemulsification. Nevertheless, in a number of cases (for example, when the so-called brown cataract is combined with severe dystrophic changes in the cornea), extracapsular extraction, which involves the removal of the “whole” nucleus through a wide surgical incision in the limbal zone of the cornea, is still the method of choice for phacosurgery. The condition of the cornea after phacosurgery remains one of the criteria for the success of the intervention. In this case, as a rule, attention is focused on the "loss" of cells of the posterior epithelium and violations of the optical regularity of the cornea. Changes in corneal nerve fibers (CNF) have been studied to a lesser extent.

The aim of the study was to study the structural changes in CNF after extracapsular cataract extraction based on laser confocal microscopy.

The studies were conducted on the clinical material of 20 operations of extracapsular cataract extraction, which were performed on 20 patients aged 52 to 74 years. The condition of CNF was assessed before and after 7-10 days, 2-2.5 and 6 months after surgery, using the length and density of the main branches of CNF (in mm/mm2 and units/mm2, respectively) in the central zone of the cornea.

Regardless of the timing of follow-up, a decrease in the length and density of CNF was revealed with a moderate tendency to recovery by the 6th month after the intervention. Despite this, a statistically significant decrease in the average length of the main CNFs persisted during all periods of observation, and in density - for 2-2.5 months.

Factors that induce changes in CNF after various phacosurgery techniques include their intersection during the surgical incision and the energy impact on the cornea. When using microinvasive techniques, taking into account the minimization of the length of the incision, ultrasound and/or laser radiation may be the dominant factor, and when using the extracapsular technique, the directly extended corneal incision, accompanied by the intersection of the CNF. In clinical practice, potential disturbances in the CNF structure after phacosurgery should be taken into account in situations accompanied by pronounced initial changes in CNF (condition after keratoplasty and excimer laser keratorefractive surgery, various types of systemic polyneuropathy).

Keywords: corneal nerve fibers, extracapsular cataract extraction, laser confocal microscopy of the cornea.

Introduction. The term "phacosurgery" combines various surgical technologies for the complete or partial removal of the lens. The last decades are characterized by an almost complete transition from extracapsular cataract extraction to the so-called. microinvasive phacosurgery, in particular standard ultrasound and hybrid (femtolaser) phacoemulsification [6, 4]. Possible limitations of the use of microinvasive phacosurgery techniques are associated with such technical elements of the intervention as energy fragmentation and emulsification of the nucleus, which are potential sources of complications caused by damage to the ligamentous-capsular apparatus of the lens and posterior corneal epithelium. The probability of these violations increases in a number of complicated situations, for example, with a combination of the so-called. brown cataract with severe dystrophic changes in the cornea. In these cases, the method of choice for phacosurgery is still extracapsular cataract extraction, which involves the removal of the “whole” nucleus through a wide surgical incision in the limbal zone of the cornea.

The condition of the cornea after phacosurgery remains one of the criteria for the success of the intervention. In this case, as a rule, attention is focused on the "loss" of cells of the posterior epithelium and violations of the optical regularity of the cornea. To a lesser extent, changes in the nerve corneal nerve fibers (CNF), an important structural component that provides the function of its sensitivity, have been studied. To date, a detailed study of the state of CNF is one of the directions for assessing the structural and functional state of the cornea. Structural analysis is based on various technologies of confocal microscopy, a method that allows, at a level close to morphological, to analyze the state of various anatomical layers and components of the cornea. According to the literature data based on confocal microscopy, the diameter [11, 10], the density and length of the fibers and their branches [17], the coefficients of anisometropia and directional symmetry [7] are used for the structural characteristics of CNF.

In a number of previous studies, changes in CNF have been studied in such systemic diseases as diabetes mellitus, amyloidosis, Parkinson's disease, past coronavirus infection, and other pathologies accompanied by the development of polyneuropathy [2,3,8,13,14]. The need to study CNF after various methods of phacosurgery is dictated by a number of circumstances: the presence of corneal incisions of various localization and length, the energy effect on the posterior layers of the cornea when performing microinvasive technologies, and, finally, the possibility of preoperative changes in the cornea of various origins, as well as a combination of pathological changes in the lens with various endocrinological and neurological diseases affecting the initial state of CNF. Taking into account the anatomical features of the CNF (“penetration” into the cornea in the limbus zone), the main cause of their

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damage during extracapsular cataract extraction can be the so-called. the corneal incision is rather long.

In previous studies, a decrease in the sensitivity of the cornea in the incision zone, a decrease in the density and thickening of the CNF after microinvasive ultrasonic phacoemulsification was noted [9,12,16]. When assessing the state of CNF after standard ultrasound and hybrid phacoemulsification, changes were revealed that, according to the authors, are comparable to age-related changes - a decrease in the anisotropy coefficient of directivity and an increase in the symmetry coefficient of directivity of CNF [5].

The aim of this study was to study the structural changes in CNF after extracapsular cataract extraction based on confocal microscopy.

Materials and methods. The studies were conducted on the clinical material of 20 operations of extracapsular cataract extraction, which were performed on 20 patients aged 52 to 74 years. Criteria for exclusion from the observation group: pathological changes in the cornea of any origin and systemic polyneuropathy of various etiologies. All patients gave informed consent to participate in the study.

In the process of extracapsular extraction at a distance of 0.5 mm from the limbus, a corneal incision was performed from 10:00 to 02:00 hours of the dial scale, tangential capsulotomy in the upper third of the anterior capsule, nuclear expression, lens sac lavage, intercapsular implantation of an intraocular lens, and removal of the central part of the anterior capsule. The incision was sealed with a continuous X-shaped nylon suture with a dipping knot.

The condition of CNF was assessed before and after 7–10 days, 2–2.5 and 6 months after the operation. When choosing the maximum period of postoperative follow-up (6 months), we took into account the known and noted in previous studies time period of reinnervation processes in case of CNF damage [15].

The previously developed algorithm for assessing the state of CNF is based on the analysis of images obtained using a laser confocal microscope (HRT III device with a corneal attachment Rostock Cornea, Germany) using the original Liner 1.2S software [1, 7]. As the main criteria for assessing the structure of the CNF, we used the length and density of the main branches of the CNF (in mm/mm2 and U/mm2, respectively) in the central zone of the cornea.

Statistical analysis and assessment of the reliability of the results obtained were carried out using Microsoft Excel 2010 and Statistica 8.0 software.

Average length and density of the main CNF (M ± d)

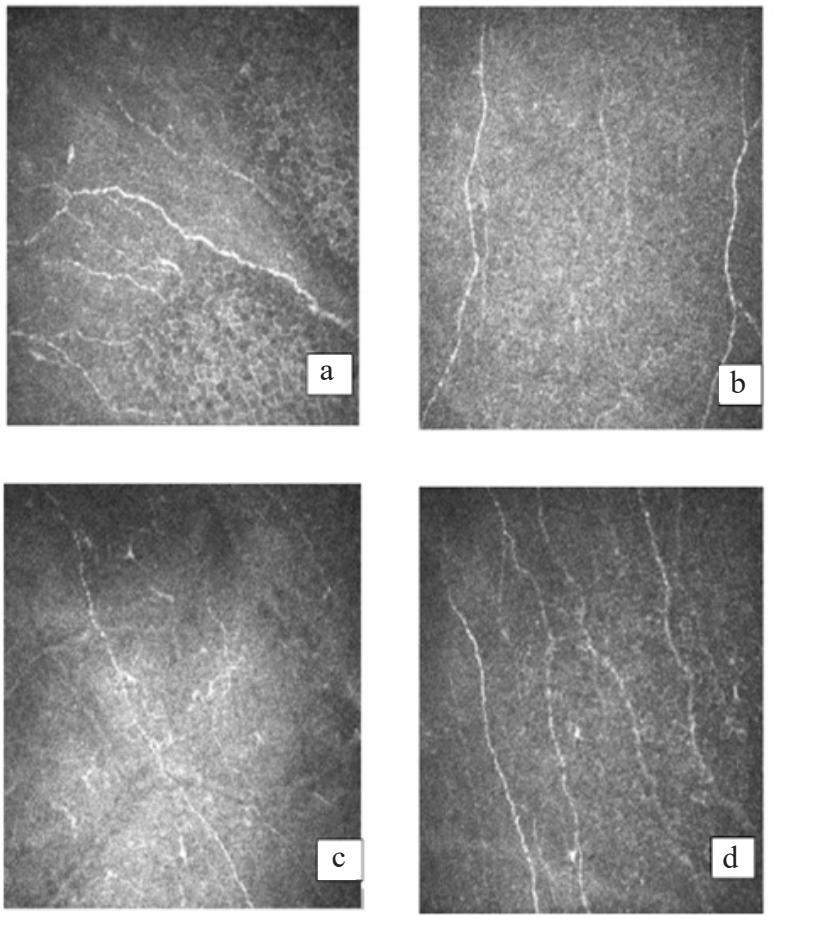
Index	Examination terms			
	1	2	3	4
Main CNF length (mm/mm$^{2)}$	34.4 ± 5.1	19.1 ± 3.9*	21.3 ± 4.3*	26.8 ± 3.4*
Density of main CNF (units/mm^2)	5.2 ±1.7	2.2 ±0.9*	3.4 ±1.3*	3.9 ±1.6

* differences compared to baseline are statistically significant ($p \leq 0.05$)

Results and discussion. Differences in the initial parameters were within the limits of age fluctuations, the average values of the length and density of the main branches of the CNF were 34.4 ± 5.1 mm/mm2 and 5.2 ± 1.7 units/mm2, respectively. The table shows the average values of the indicated indicators of the state of CNF at different periods of observation. Designations 1, 2, 3 and 4 in the table correspond to the terms of the examination (before and after 7–10 days, 2–2.5 and 6 months after the operation, respectively).

Regardless of the timing of follow-up, a decrease in the length and density of the main CNFs was revealed with a moderate tendency to recovery by the 6th month after the intervention. Despite this, a statistically significant decrease in the average length of the main CNFs persisted throughout the entire observation period, and the density of the main CNFs persisted for 2–2.5 months. As a clinical example, Figure 1 shows the results of confocal microscopy before and after extracapsular cataract extraction.

In a previous study, changes in the CNF structure after standard ultrasonic and hybrid phacoemulsification were



The results of laser confocal microscopy and the length (mm/mm2) and density (un/mm2) indices of the main CNFs, respectively, before (a) and at different times (b, c, d) after extracapsular cataract extraction: a) before surgery - 54.91 / 7, b) after 10 days - 18.69 / 3, c) in. after 2 months - 41.04 / 5, d) after 6 months - 54.10 / 6

analyzed using the directional anisotropy and directional symmetry coefficients characterizing the state of the CNF based on the analysis of their tortuosity and directionality [5]. Regardless of the operation technique, there was a tendency to a decrease in the directional anisotropy coefficient and an increase in the directional symmetry coefficient, which indicates an increase in the crimp of the fibers and a violation of their orientation. After hybrid phacoemulsification, the decrease in the directional anisotropy coefficient 2-2.5 months after the intervention turned out to be statistically less significant compared to the standard ultrasound technique, which may be due to a lower energy “load” on the cornea in the process of fragmentation and emulsification of the lens nucleus.

The factors that induce changes in CNF after various phacosurgery techniques include the intersection of fibers during the surgical incision and the energy impact on the cornea. When using microinvasive techniques, taking into account the minimization of the length of the incision, ultrasound and/or laser radiation may be the dominant factor, and when using the extracapsular technique, the directly extended corneal incision, accompanied by the intersection of the CNF.

Conclusion. With the obvious conditionality of comparing the results of assessing the state of CNF after extracapsular cataract extraction and microinvasive phacosurgery, it should be noted that in the first case there are more pronounced changes that persist even in the long term after surgery. In clinical practice, potential disturbances in the CNF structure after phacosurgery should be taken into account in situations accompanied by pronounced initial changes in CNF (condition after keratoplasty and excimer laser keratorefractive surgery, various types of systemic polyneuropathy).

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FAMILIAL HYPERCHOLESTEROLEMIA WITH MOLECULAR GENETIC CONFIRMATION IN THE REPUBLIC OF SAKHA (YAKUTIA)

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The article presents the first clinical cases with the results of molecular genetic confirmation of the mutation of the low-density lipoprotein receptor gene in patients of the Lipid Cabinet of the GAU RS(Ya) "RCBNo. 3" with a diagnosis of "probable" familial hypercholesterolemia.

Keywords: familial hypercholesterolemia, low-density lipoproteins (LDL), lipidogram, genetic mutation, statins, cascade screening.

Introduction: Familial hypercholesterolemia (CGHS) is characterized by an increase in the level of low–density lipoprotein cholesterol (LDL cholesterol) and it is the most common genetic cause of the early development of myocardial infarction and angina pectoris. This disease is caused by pathogenic mutations, more often in the LDL receptor gene (LDL), less often in the APOB and PCSK9 proteins. GHS significantly increases the risk of developing cardiovascular diseases of atherosclerotic genesis, the clinical manifestation of which occurs at a young age and leads to a 20-fold increase in the probability of cardiovascular mortality [1,6]. About 200 thousand patients die from potentially preventable heart attacks every year in the world [7,8], and timely initiated lipid-lowering therapy significantly prolongs the life of such patients [1,8,9].

According to the results of recent studies, the incidence of heterozygous familial hypercholesterolemia in European countries was on average 1 case per 500 people [8]. According to some literature data, this indicator can reach 1 case per 200-250 people, that is, from 14 to 34 million cases in the world [8,9].

Presumably, in Russia, the number of people with a heterozygous form of Familial hypercholesterolemia is more than 1 million. Nowadays, many of them have not been diagnosed [2,3]. This is due to the fact that in Russia the diagnosis of "Familial hypercholesterolemia" is very rare, there is no unified system of accounting for these patients, and therefore the true prevalence of the disease is still unknown [1,5,6]. According to the ESSE-RF study [4,6], which was conducted in 13 regions of the Russian Federation, the prevalence of "definite" and "probable" Familial hypercholesterolemia was studied only in 3 regions: in the Kemerovo and Tyumen regions it is 1 in 108 people, and in the Primorsky Territory the prevalence of Familial hypercholesterolemia was 1 in 172 people. In the remaining 10 regions, 16360 people were included in the study, of which 10% of the surveyed require further verification of Familial hypercholesterolemia [2]. According to the results of the 3-year work of the Russian RENAISSANCE Registry, 1,691 patients from 17 regions of Russia with diagnoses of "definite" and "probable" Familial hypercholesterolemia were included in the registry [2,3,5].

All over the world, the issue of early diagnosis of Familial hypercholesterolemia is more relevant than ever. The National Health System of Great Britain in its long-term plan (National Health System long-term plan) in 2019 stated that by 2023, 25% of the projected number of identified patients with Familial hypercholesterolemia will be treated [7,10]. This will be ensured by genetic testing of index patients diagnosed with Familial hypercholesterolemia and cascade screening of relatives [7].

Cascade screening has clinical and economic value because it provides identification of people suffering from familial hypercholesterolemia at a young age - before the onset of a cardiovascular catastrophe of atherosclerotic genesis. Early diagnosis provides the patient with the choice of an appropriate lifestyle and the possibility of prescribing lipid-lowering therapy, which gives a chance to reduce the risk of premature coronary heart disease, stroke and possible disability in the future.

Based on the above, the identification of patients with CFS in clinical practice is an important task of primary and secondary prevention of cardiovascular events [4].

Materials and methods: In the lipid cabinet of the Center for Predictive Medicine and Bioinformatics of the State Autonomous Institution of the Republic of Sakha (Yakutia) "Republican Clinical Hospital No. 3" for 2021 and 2022, we examined 535 patients, 355 of them women and 180 men. The average age of women was 63 years, for men – 67 years. 42 patients out of 535 examined persons were diagnosed with early myocardial infarction (men under 50 and women under 55). The clinical examination included: assessment of lipidogram, blood glucose, thyroid hormones, ECG; if available: Holter ECG monitoring, echocardiography, ultrasound examination of brachiocephalic arteries and vessels of the lower extremities, coronary angiography. The majority of the examined were of Yakut nationality and belonged to the

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polyclinic department of the GAU RS(Ya) "RCB No. 3". 9 patients were selected, according to Dutch diagnostic criteria with a diagnosis of "probable" and "definite" hereditary familial hypercholesterolemia, who underwent direct automatic sequencing of the promoter and exons of the LDLR gene on the methodological and instrument base of the sector for the study of monogenic forms of common human Diseases of the Institute of Cytology and Genetics SB RAS (Novosibirsk). According to the results of a molecular genetic study, pathogenic mutations in the LDLR gene in a heterozygous form were detected in 2 patients.

Results: Two clinical cases of Familial hypercholesterolemia are presented.

Patient 1. Woman C, 68 years old, in July 2021, applied for an appointment in the Lipid Cabinet for high blood cholesterol. It is known from the anamnesis that CHD has been suffering for a long time. She suffered a myocardial infarction in 2002. In 2003, the left ventricle was reconstructed with plastic surgery according to Dore, mitral valve plastic surgery according to Alfieri, mammary coronary bypass surgery of the anterior interventricular branch, coronary artery bypass grafting of the diagonal branch of the right coronary artery. In 2011, stenting of the proximal segment of the envelope artery. The last coronary angiography was performed in 2017, according to the results of which: Coronary sclerosis. Stenosis of the trunk of the left coronary artery up to 25%. Stenosis of the envelope artery in the middle segment is up to 30%, stenosis of the mouth of the branch of the obtuse edge is up to 40%. The left-handed type. The shunts are functioning.

In 2018, life-threatening cardiac arrhythmias were diagnosed, and therefore the patient was implanted with a biventricular EX with defibrillator function in the endocardial version of Medtronic Protecta CT-D PSF 625220S in DDD mode. The defibrillator was triggered in 2019. In 2020, an electrode displacement was diagnosed and its replacement was carried out. The first control after correction of the left ventricular electrode for resynchronizing stimulation of the heart was carried out in 2021.

Increase of total serum cholesterol to 14 mmol/L for a long time. Previously, familial hypercholesterolemia was not diagnosed. According to the Dutch criteria, 8 points were obtained – a "certain" SGHS.

Family history: the patient's mother had a myocardial infarction at 62, she died at 64, probably from a myocardial infarction. The patient's father died of an acute heart attack at the age of 60. Probanda's grandmother died in 84, there is no information about her grandfather. The patient's sister died at the age of 50, possibly from a vascular catastrophe. The patient's niece has elevated blood cholesterol up to 10 mmol/l. The patient (according to the words) has 3 children: the daughter (39 years old) has a moderate increase in cholesterol to 5 mmol / l, the sons 5.3 (44 years old) and 4.8 mmol / l (30 years old) – all are somatically healthy.

For genetic verification of the diagnosis, targeted direct sequencing of coding and non-coding regions of LDLR genes by Sanger was performed. Bioinformatic analysis of the obtained data revealed the replacement of rs773328511 (NM_000527.5 LDLR c.810C>A p.Cys-270Ter) in a heterozygous form in the LDLR gene.

Current status of the patient: Height: 170 cm, weight: 62 kg, BMI 21.5 kg/m2. At the moment, the patient is receiving treatment for coronary heart disease according to clinical recommendations, including valsartan + sacubitril 50 mg, against which stable hemodynamics is noted. According to the planned daily monitoring of the electrocardiogram in May 2022, the patient had short runs of ventricular tachycardia from 3 complexes. Brain natriuretic peptide 258 pg/ml. Exercise tolerance is moderately reduced, no further progressive decrease has been observed over the past 2 years. The level of total cholesterol is 10.3 mmol / l, LDL is 7.69 mmol / l while taking rosuvastatin 20 mg, the dose could not be increased due to signs of myopathy. When conducting a pharmacogenetic study of the SLCO1B1*5 gene, a combination of C.521CC alleles was found in the patient, which indicates a very high risk of liver damage and striated musculature. About 5 years ago, in combination with rosuvastatin 20 mg, ezetimibe was prescribed at a dose of 10 mg, the effectiveness of which could not be established retrospectively. The patient was unable to take this drug permanently due to the high cost. The patient-initiated combination therapy with rosuvastatin 20 mg per day and evolocumab 140 mg once every 2 weeks. After the first injection of evolocumab, the patient has complaints of myalgia, arthralgia, which limit daily activity. During examination: creatinine phosphokinase, ALT, AST, creatinine, and other biochemical parameters within normal values. Lipidogram indicators: OHS 8.33 mmol/l, LDL 6.22 mmol/L, TG 1.47 mmol/l, HDL 1.53 mmol/L. The patient resumed taking evolocumab 140 mg once every 2 weeks, the dose of rosuvastatin was reduced to 10 mg. Planned face-to-face consultation of the Research Institute of the PC named after Ak. Meshalkin E.N., further conservative management is recommended.

Clinical diagnosis: Heterozygous familial hypercholesterolemia, defined (the Dutch criteria were 16 points, according to S.Broome there is a definite diagnosis of heterozygous hypercholesterolemia). Coronary heart disease. Angina of tension. 2 Functional class. Post-coronary cardiosclerosis from 2002. Complete blockade of the left leg of the Gis bundle. Frequent ventricular extrasystole with unstable short paroxysm of ventricular tachycardia. Transient WPW syndrome.

Operations:

1. Reconstruction of the left ventricle with Douro plastic surgery, Alfieri mitral valve plastic surgery, mammary coronary bypass surgery of the anterior interventricular branch, coronary artery bypass grafting of the diagonal branch of the right coronary artery in 2003.

2. Selective coronary angiography from 2017: Coronary sclerosis. Stenosis of the trunk of the left coronary artery up to 25%. Stenosis of the envelope artery in the middle segment is up to 30%, stenosis of the mouth of the branch of the obtuse edge is up to 40%. The left-handed type. The shunts are functioning.

3. Implantation of an electronic pacemaker with defibrillator function in the endocardial version of Medtronic Protecta CT-D PSF 625220S in DDD mode in 2018. Defibrillator activation in 2019.

Background: Hypertension 3st. Controlled arterial hypertension. Risk of cardiovascular complications 4.

Complication: Chronic heart failure with an intermediate ejection fraction of 42% (S), stage 2a. Functional Class II (NYHA). Secondary dilation of the heart cavities.

Concomitant diseases: Dyscirculatory encephalopathy of the 2nd degree on the background of atherosclerosis and Hypertension. Genetically determined high risk of statin-induced myopathy.

The patient was taken on dispensary registration, included in the Republican Register of familial hypercholesterolemia. In terms of cascade screening (with the consent of the patient), evaluation of the effectiveness and safety of combined cholesterol-lowering therapy.

Patient 2. Male F., 29 years old. Married, no children. I applied to the lipid cabinet in December 2021 for a high level of total cholesterol up to 11 mmol/l. Obese 2 art . During physical examination of the corneal lipoid arch, no tendon xanthomas were detected. According to

the results of a biochemical blood test, the patient had high lipidogram values: total cholesterol: 9.72 mmol/l; TG 1.83 mmol/l; LDL:7.31 mmol/l. When collecting a hereditary history, it was found that the patient's father had an early non-fatal myocardial infarction in 42, but his further fate is unknown. There were no cases of early vascular catastrophes on the part of maternal heredity. The patient does not have siblings. According to the Dutch criteria, the patient has 6 points - "probable" familial hypercholesterolemia. In the future, the patient underwent ultrasound examination of the heart, brachiocephalic arteries, daily monitoring of the electrocardiogram, where deviations were not detected. Thyroid pathology and diabetes mellitus are excluded.

Current status of the patient: Height 185 cm, weight 123 kg. BMI 36 kg/m2. At the moment, he is taking treatment: atorvastatin 60 mg, adherence to therapy is low, does not follow a diet, the level of physical activity is low. According to the latest test results against the background of irregular intake of atorvastatin 60 mg: total cholesterol 9.28 mmol / l, LDL 6.76 mmol / l, HDL 1.5 mmol / l, TG2.92 mmol / l, ALT and AST indicators are normal. When conducting a pharmacogenetic study of the SLCO1B1*5 gene, a combination of alleles of C.521CC was obtained in the patient, which indicates a very high risk of liver damage and striated musculature. The dose of atorvastatin has been reduced to 40 mg and regular intake is strongly recommended. After evaluating the effectiveness and safety of statin therapy, the tactics of further drug treatment will be determined.

For genetic verification of the diagnosis, targeted direct sequencing of coding and non-coding regions of LDLR genes by Sanger was performed. Bioinformatic analysis of the obtained data revealed the replacement of rs121908038 (NM_000527.5 LDLR c.1202 T>A p.Leu401His) in a heterozygous form in the LDLR gene.

Clinical diagnosis: Heterozygous familial hypercholesterolemia, defined (the Dutch criteria were 14 points, according to S.Broome – a definite diagnosis of heterogeneous familial hypercholesterolemia). Obesity of the 2nd degree (BMI 36 kg/m2).

The patient was taken on dispensary registration, included in the Republican Register of familial hypercholesterolemia. The purpose of treatment is the primary prevention of atherosclerotic cardiovascular complications. Cascade screening is not possible. In terms of: genetic counseling on family planning, selection of effective lipid-lowering therapy.

Conclusion. Despite the absolute need for early detection of familial hypercholesterolemia for the primary prevention of atherosclerotic cardiovascular diseases, I would like to note that in our practice we are faced with the same problem that was identified in the RENAISSANCE study: despite the presence of familial hypercholesterolemia and pronounced hypercholesterolemia, patients do not want to take lipid-lowering therapy, and there is also a low the index patient's interest in conducting cascade screening [5]. This problem is being solved, but it requires an individual approach and long-term confidential contact with the patient.

Previously, no targeted identification of patients with familial hypercholesterolemia was carried out in the Republic of Sakha (Yakutia), the ethnic features of this disease remain unexplored, which requires further research.

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ANALYSIS OF HEARING THRESHOLDS IN PATIENTS WITH HEARING IMPAIRMENT ASSOCIATED WITH THE *GJB2* (Cx26) GENE MUTATIONS IN BURYATIA

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In this work, the analysis of the hearing condition in 26 patients with hearing impairment, in whom biallelic mutations of the GJB2 gene (Sh26) were detected in the Republic of Buryatia, was carried out. Genotype-phenotypic comparisons showed that for all 7 GJB2 genotypes: c.[35delG];[35delG]; c.[-23+1G>A];[35delG]; c.[-23+1G>A];[-23+1G>A]; c.[-23+1G>A];[516G>C]; c.[-23+1G>A];[327_328delGGinsA]; c.[35delC];[299_300delAT]; c.[235delC];[235delC] congenital (detected before one year – 76.0%), symmetrical (84.6%), sensorineural (100.0%) hearing loss is detected, variable in severity both between different GJB2 genotypes and within the same GJB2 genotype (grade III in 9.6%, grade IV in 17.3%, deafness in 73.1%). Based on the median hearing thresholds PTA0.5,1.0,2.0,4.0 kHz, three phenotypes were identified - "mild phenotype", "medium phenotype" and "severe phenotype". We conducted a genotype-phenotypic comparison, as a result of which GJB2-genotypes c.[-23+1G>A];[-23+1G>A] and c.[-23+1G>A];[516G>C] were assigned to genotypes with “average” (median 66.875 dB and 64.375 dB, respectively), and the remaining GJB2-genotypes: c.[35delG];[35delG]; c.[-23+1G>A];[35delG]; c.[-23+1G>A];[327_328delGGinsA]; c.[35delC];[299_300delAT]; c.[235delC];[235delC] - to genotypes with a "severe" form of the phenotypic effect. No genotypes with a "mild" phenotypic effect have been identified. At the same time, the hearing thresholds for GJB2 genotypes with the "average" form of the phenotypic effect (c.[-23+1G>A(;)-23+1G>A] and c.[-23+1G>A(;)516G>C]) were significantly (p=0.02268) better than in the reference group with GJB2 genotype c.[35delG];[35delG].

Keywords: deafness, GJB2 gene, genotype-phenotypic analysis, degree of hearing loss, Republic of Buryatia.

Introduction. It is known that up to 50% of cases of congenital deafness have a hereditary etiology [10]. At the same time, the genetic causes are extremely diverse, but the proportion of mutations in the *GJB2* gene (13q11-q12), encoding the protein of intercellular gap junctions - connexin 26 (26 kDa) [8], is significant and averages about 17.3% [5]. With the understanding of the etiology and pathogenesis of autosomal recessive deafness type 1A, studies aimed at genotype-phenotypic comparisons of hearing impairment in patients depending on the nature of mutational damage in the *GJB2* gene have become relevant. At the same time, of all known mutations of the *GJB2* gene, genotype-phenotypic studies mainly concerned the most common c.35delG mutation in Europe in the homozygous and compound-heterozygous state with other rarer pathogenic variants of the *GJB2* gene, as well as c.235delC and p.Val37Ile mutations common in Asia [3, 6, 15]. These studies have identified *GJB2* genotypes with a relatively "mild" phenotype. Thus, hearing loss in patients with genotypes: p.[Val37Ile];[Val37Ile] (median PTA 27dB), p.[Met34Thr];[Met34Thr] (median PTA 30dB) and p[Met34Thr];[Val37Ile] (median PTA 23dB) was significantly less than in patients with more “severe” genotypes – c.[35delG];[35delG] (median PTA 102dB), c.[35delG];[del(GJB6-D13S1830)] (median PTA 108dB) (p<.0001) and c.[235delC];[235delC] (median PTA 100.68dB) [6, 15]. In general, the authors concluded that the so-called truncating mutations (deletions, nonsense mutations) lead to greater hearing loss than non-truncating mutations (missense mutations) [9]. Genotype-phenotypic comparisons were also made for the homozygous *GJB2* genotype c.[-23+1G>A];[-23+1G>A], among deaf patients in Yakutia [1].

In 2021, studies were conducted aimed at diagnose of hereditary non-syndromic hearing loss in the Republic of Buryatia (Eastern Siberia). Using direct sequencing, in 165 individuals with hearing impairment the spectrum and frequency of mutations in the *GJB2* gene were determined. In the studied sample, 13 known allelic variants of the *GJB2* gene were found (c.-254C>T, c.-49G>A, c.-23+1G>A, c.35delG, c.79G>A, c.101T>C, c.109G>A, c.235delC, c.299_300delAT, c.327_328delinsA, c.341A>G, c.457G>A and c.516G>C). In general, the contribution of biallelic mutations of the *GJB2* gene to the etiology of hearing loss in the total sample of patients in Buryatia was 15.8% (26/165) [2]. Following the general trends in genotype-phenotypic comparisons, the purpose of this study was to analyze hearing thresholds in individuals with biallelic mutations of the *GJB2* gene in Buryatia.

Material and methods. *Study participants.* Informed voluntary examination by an audiologist-otorhinolaryngologist was performed by 165 deaf people. An examination of the ENT organs, audiometry, blood sampling from the cubital vein for molecular genetic analysis were carried out.

Audiometry. Pure tone audiometry (PTA) was performed using a AA222 tympanometer-audiometer (Interacoustics, Denmark) for air conduction at frequencies of 0.25, 0.5, 1.0, 2.0, 4.0, 8.0 kHz and for bone conduction at frequencies of 0.25, 0.5, 1.0, 4.0 kHz in steps 5.0 dB

DNA diagnostics. Samples (n=165) of genomic DNA were extracted from leukocytes. Amplification of the *GJB2* gene fragments, including exon 1 and exon 2 of the *GJB2* gene with flanking regions, was performed by PCR on MJ Mini thermal cycler (Bio-Rad) using the primer sequence described earlier [7, 11, 13]. The determination of the primary nucleotide sequence was carried out on an ABI Prism 3130XL Genetic Analyzer capillary sequencer (Applied Biosystems, USA) (Central Collective Use Center Genomics, Institute of Chemical Biology and Fundamental Medicine, Siberian Branch, Russian Academy of Sciences, Novosibirsk). The sequence variants were determined by comparison with the reference sequences of the *GJB2* M86849

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gene.2 and U43932.1 (GenBank) using the Chromas software.

Sample. Of the 165 examined deaf patients with hearing impairments, 26 were found to have biallelic mutations in the *GJB2* gene in the homozygous or compound heterozygous state. Among them, 7 different pathological *GJB2* genotypes were identified: c.[35delG];[35delG] (total 16 individuals, of which 9 men, 7 women aged 32 to 76 years, mean age 55± 12.25 years, all Russians), c.[-23+1G>A];[35delG] (total 5 individuals, of which 2 men, 3 women aged 19 to 59 years, average age 47± 17.33 years, all Russians), c.[-23+1G>A];[-23+1G>A] (1 Buryat, 58 years old), c.[-23+1G>A];[516G>C] (1 Buryat, 46 years old), c.[-23+1G>A];[327_328delGGinsA] (1 Buryat, 47 years old), c.[35delC];[299_300delAT] (1 Russian, 65 years old), c.[235delC];[235delC] (1 Buryat, 39 years old). Ethnicity was ascertained using a questionnaire (up to and including the third generation). To compare hearing thresholds, the reference group identified a sample of individuals with the *GJB2* genotype c.[35delG];[35delG] as the most numerous (n=16) and well studied.

Audiological analysis. Audiograms with breaks were normalized by adding maximum values(120.0 dB) in the places where the patient did not respond, according to the recommendations of the International Bureau of Audio Phonologists (www.biap.org.). The type of hearing loss was considered conductive - with an increase in air conduction thresholds on audiograms, sensorineural - with an increase in bone and air conduction thresholds on audiograms, mixed - with an increase in bone and air conduction thresholds with an interval exceeding a total of 20.0 dB in $PTA_{0.5,1.0,\ 2.0,4.0}$ kHz. Hearing loss was considered asymmetric when the difference between the hearing thresholds in the PTA of 0.5,1.0,2.0,4.0 kHz was more than 15.0 dB. The degree of hearing loss was assessed by the average hearing threshold in $PTA_{0.5.1.0.2.0.4.0}$ kHz according to the WHO classification: I degree - 26.0-40.0 dB, II degree - 41.0-55.0 dB, III degree - 56.0-70.0 dB, IV degree - 71.0-90.0 dB, deafness -> 91.0 dB. Phenotypes with I-II degree of hearing loss we attributed to "mild", with III-IV degree of hearing loss - to "medium", and deafness - to the "severe" form of the phenotype. For a detailed audiological analysis, the number of ears was used (26 patients = 52 ears), because the functional abilities of paired organs in humans can be different and the pathological effect of the genotype can be expressed in different ways.

Statistical analysis. StatPlus software (2021 AnalystSoft Inc.) was used for statistical processing. The median hearing thresholds of six *GJB2*-genotypes were compared in pairs with the reference group of the c.[35delG];[35delG] genotype using the Mann-Whitney U test. Differences were considered statistically significant at $p<0.05$.

Ethical control. The surveys included in the scope of the research work were conducted with the informed voluntary consent of the participants or their parents. This research work was approved by the local committee on biomedical ethics at the "YSC CMP" (Yakutsk, Protocol No. 16 dated April 16, 2009).

Results and discussion. In this study, out of 165 participants with hearing impairment, we selected the audiological data of 26 individuals with biallelic mutations of the *GJB2* gene (Cx26), identified in the homozygous or compound heterozygous state. Among the identified 26 patients, seven different *GJB2* genotypes were found: c.[35delG];[35delG], c.[-23+1G>A];[35delG], c.[235delC];[235delC], c.[- 23+1G>A];[-23+1G>A], c.[-23+1G>A];[516G>C], c.[-23+1G>A];[327_328delGGinsA], c.[35delC];[299_300delAT]. Assuming that severe hearing loss after birth would be recognized sooner than those with less severe hearing loss, we conducted a survey about the age at which hearing loss was diagnosed. This criterion can help reveal phenotype variability in different *GJB2* genotypes and even within the same *GJB2* genotype. As a result, it was found that the vast majority of *GJB2*-positive patients had predominantly early detection of hearing loss (up to one year

Table 1

Age of onset of hearing impairment in individuals with biallelic mutations of the *GJB2* gene

GJB2-genotype	Number of patients	Age of onset	Number of patients	Early diagnosis (before 12 mounts)	Late diagnosis. (before 12 mounts)
c.[35delG];[35delG]	16	In 1 years	11	11 68.7% (44.0-85.8%)	5 31.3% (14.2-56.0%)
		In 2 years	1		
		In 3 years	3		
		In 6 years	1		
c.[-23+1G>A];[35delG]	5	Before 12 mounts	5	**5** **100% (54.1-99.6%)**	**0** **0% (0.43-45.9%)**
c.[-23+1G>A];[-23+1G>A]	1	Before 12 mounts	1	1 100% (58.0-98.7%)	0 0% (0.13-84.2%)
c.[-23+1G>A];[516G>C]	1	Before 12 mounts	1	1 100% (58.0-98.7%)	0 0% (0.13-84.2%)
c.[-23+1G>A];[327_328delGGinsA]	1	Before 12 mounts	1	1 100% (58.0-98.7%)	0 0% (0.13-84.2%)
c.[35delC];[299_300delAT]	1	Before 12 mounts	1	1 100% (58.0-98.7%)	0 0% (0.13-84.2%)
c.[235delC];[235delC]	1	In 6 years	1	0 0% (0.13-84.2%)	1 100% (58.0-98.7%)
In total seven *GJB2*-genotypes	In total 26 patients (CI)			**20** **76% (57.7- 88.9%)**	**6** **23% (11.0-42.3%)**

Note: n - number of patients, CI - confidence intervals ($p<0.05$). Statistically significant differences are highlighted in bold.

Table 2

Severity of hearing loss in patients with different *GJB2*-genotypes

GJB2-genotype	Degree of hearing loss	Median hearing thresholds in $PTA_{0.5.1.0.2.0.4.0\ кГц}$	Mann-Whitney U test *p*	Phenotypes
c.[35delG];[35delG] (n=16. 32 уха)	IV (n=5)	115.0	Reference group	Sever
	Deafness (n=27)			
c.[-23+1G>A];[35delG] (n=5. 10 ушей)	IV (n=3)	106.25	0.23097	Sever
	Deafness (n=7)			
c.[-23+1G>A];[327_328delGGinsA] (n=1. 2 уха)	IV (n=1)	96.875	0.23320	Sever
	Deafness			
c.[35delC];[299_300delAT] (n=1. 2 уха)	III (n=1)	92.5	0.24774	Sever
	Deafness			
c.[235delC];[235delC] (n=1. 2 уха)	Deafness (n=2)	117.5	0.24372	Sever
c.[-23+1G>A];[-23+1G>A] (n=1. 2 уха)	**III (n=2)**	**66.875**	**0.02268**	**Median**
c.[-23+1G>A];[516G>C] (n=1. 2 уха)	**III (n=2)**	**64.375**	**0.02268**	**Median**

Примечание. Жирным шрифтом выделены статистически достоверные различия (p<0.05).

of age, inclusive) - 76.0% (CI 57.7%-88.9%), late detection (after one year) was reported in 23.0% (CI 11.0%-42.3%) (p<0.05) (Table 1). With genotypes c.[-23+1G>A];[35delG], c.[-23+1G>A];[-23+1G>A], c.[-23+1G>A];[516G>C], c.[-23+1G>A];[327_328delGGinsA], c.[35delC];[299_300delAT] (n=5) hearing loss was detected up to one year (Table 1). Late detection of hearing impairment (at six years) was registered in one case with genotype c.[235delC];[235delC]. The most numerous genotype c.[35delG];[35delG] (n=16) was found to be the most variable in terms of detection of hearing impairment. So, in 11 patients with this genotype, hearing loss was detected before one year, in 5 patients - at two and three years, and in one case at six years. It is interesting to note that in the c.[-23+1G>A];[35delG] genotype, hearing loss was recorded earlier (up to one year - 100.0%, CI 54.1-99.6%) significantly more often than late detection (0%, CI 0.43% - 45.9%) (Table 1). Based on the fact that all patients with biallelic *GJB2* mutations were born before the introduction of audiological screening of newborns and children of the first year of life, it can be assumed that most of the observed *GJB2*-genotypes lead to predominantly severe and, accordingly, early detected hearing loss.

Audiometric data in individuals with pathological GJB2-genotype. The results of tone threshold audiometry showed that all detected *GJB2*-genotypes (100%) were associated with sensorineural type of hearing loss (n=26 individuals, 52 ears). At the same time, the majority in the sample (n=22; 84.6%) had symmetrical hearing loss. The exception was four individuals with different *GJB2* genotypes (15.4%) who, in the absence of a visible otological problem, had asymmetric hearing thresholds. In these patients, the interaural hearing threshold difference ranged from 36.6 dB for the c.[35delG];[35delG] genotype, 20.0 dB for the c.[-23+1G>A];[35delG] genotype, 31.5 dB for the c.[-23+1G>A];[327_328delGGinsA] genotype; up to 45.0 dB for the c.[35delC];[299_300delAT] genotype. In general, in the sample of *GJB2*-positive patients, hearing loss ranged from grade III to deafness: grade III sensorineural hearing loss in 9.6% (n=5 ears), grade IV sensorineural hearing loss in 17.3% (n=9 ears), sensorineural deafness in 73.1% (n=38 ears).

For genotype-phenotypic comparison between identified *GJB2*-genotypes, the c.[35delG];[35delG] genotype was chosen as the reference genotype, as the most well-studied in the world and the most frequent in our sample. In the reference group of patients, the median hearing threshold in the PTA of $_{0.5,1.0,2.0,4.0}$ kHz was at the level of 115.0 dB. The comparison of the median hearing thresholds in $PTA_{0.5,1.0,2.0.4.0}$ kHz of the reference group with other identified *GJB2* genotypes demonstrated comparable hearing thresholds for *GJB2* genotypes: c.[-23+1G>A];[35delG] (median 106 .25 dB), c.[-23+1G>A];[327_328delGGinsA] (median 96.875 dB), c.[35delC];[299_300delAT] (median 92.5 dB) and c.[235delC];[235delC] (median 117.5 dB) (p>0.05). The median hearing thresholds of the reference and these four *GJB2* genotypes corresponded to the degree of hearing loss - deafness. In this regard, these genotypes c.[-23+1G>A];[35delG], c.[-23+1G>A];[327_328delGGinsA], c.[35delC];[299_300delAT], c.[235delC];[235delC], including the reference c.[35delG];[35delG], were classified as genotypes with a severe phenotypic effect (Table 2).

Significantly better hearing thresholds compared to the reference genotype were demonstrated by two *GJB2* genotypes: c.[-23+1G>A];[-23+1G>A] (median 66.875 dB) and c.[-23+1G>A];[516G>C] (median 64.375 dB) (p=0.02268) (Table 2). Median hearing thresholds for these two *GJB2* genotypes corresponded to grade III hearing loss (Table 2). As a result, we assigned these two *GJB2* genotypes c.[-23+1G>A];[-23+1G>A] and c.[-23+1G>A];[516G>C] to genotypes with " average" form of the phenotypic effect. In accordance with the data obtained, no genotypes with a "mild" phenotypic effect, corresponding to I-II degrees of hearing loss, were identified (Table 2).

Previously, it was shown that for patients with the c.[35delG];[35delG] genotype, the median in $PTA_{0.5,1.0,2.0}$kHz was 102 dB [9]. It is likely that the observed difference (~13 dB) in the depth of hearing loss between our study and that of Snoeckx et al. [9] is due to the difference in frequencies taken into account. In Snoeckx et al. study [9], the frequency range was limited to three measured frequencies ($PTA_{0.5,1.0,2.0}$kHz), while in our study, measurements were made at four frequencies ($PTA_{0.5,1.0,2.0,4.0}$kHz). In addition, in this multicenter study, it was shown that with the c.-23+1G>A mutation in the compound with the c.35delG mutation [9] a significantly milder phenotype is recorded. In the present work, it was shown that the c.-23+1G>A mutation in the homozygous state (median 66.875 dB) and in the compound-heterozygous state with the c.516G>C mutation (me-

dian 64.375 dB) also has a significantly (p=0.02268) less hearing loss (moderate phenotype) compared to the phenotypically more "severe" reference genotype c.[35delG];[35delG] (median 115.0 dB) (Table 2). However, it should be noted that earlier on a larger sample of patients with genotype c.[-23+1G>A];[-23+1G>A], with a median of 85.41 dB (which corresponds to IV degree of hearing loss), it was shown wide individual variability of hearing thresholds, ranging from mild hearing loss to deafness [1].

Conclusion. Thus, the analysis of the state of hearing in individuals with mutations in the *GJB2* (Cx26) gene in Buryatia revealed the following:

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ASSOCIATION OF POLYMORPHISMS OF THE GENES *HTR2A* AND *5-HTT* WITH SMOKING IN YAKUTS

Tobacco smoking is the most common form of addiction worldwide. The aim of our study was to determine whether the rs6311 polymorphisms of the *HTR2A* gene and the 5HTTLPR and rs25531 polymorphisms of the *5-HTT* gene are associated with smoking in the Yakut population. The study involved 223 people, including 115 smokers and 108 non-smokers. The results of the analysis of the relationship between the rs6311 polymorphism of the *HTR2A* gene and smoking showed that in the group of smokers, allele A was somewhat more common than in the group of non-smokers (OR - 1.138, 95% CI = [0.742-1.138]). Analysis of the distribution of alleles and genotypes of the 5-HTTLPR polymorphism of the *5-HTT* gene showed the predominance of the short S allele (74.8–89.4%) and the SS genotype (61.7–81.5%) in both samples. Smokers had a significantly ($p<0.05$) higher frequency of the risk allele L (OR -2.830, 95% CI= [1.674 -4.783]) compared to non-smokers. When analyzing the 5-HTTLPR and rs25531 polymorphisms grouped into groups, it was found that the frequency of the L' allele was four times higher in the sample of smokers ($p<0.001$) than in the sample of non-smokers (OR -4.844, 95% CI = [2.503-9.372]). An analysis of the distribution of combinations of genotypes of both studied genes showed the predominance of people with a combination of GG genotypes of the *HTR2A* gene and S'S' of the *5-HTT* gene, which showed a protective effect on smoking (OR -0.550, 95% CI = [0.319-0.948]). A significant association with smoking was shown by a combination of heterozygous genotypes AG of the *HTR2A* gene and L'S' of the 5-HTT gene (OR -13.637, 95% CI= [1.752-106.144]). This study established a significant association of *5-HTT* gene polymorphisms with smoking in the Yakut population. In connection with the equivalent serotonin expression of the S and LG alleles, 5HTTLPR and rs25531 polymorphisms, it is more informative to carry out their generalized analysis.

Keywords: nicotine addiction, smoking, 5-HTT, HTR2A, rs6311, 5HTTLPR, rs25531, Yakut population

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Introduction. Nicotine addiction is the most common and severe disease, included in the ICD-10 F17 group of diseases - "Mental and behavioral disorders associated with the use of psychoactive substances" [1]. Dependence arises on the basis of the entry of nicotine into the reaction with alpha-4-beta-2-acetylcholine receptors in the brain, which leads to the formation of a steady craving for tobacco, withdrawal syndrome that develops when smoking is stopped, and a number of side effects.

Nicotine dependence is closely related to psychological and social factors [2, 5, 6]. Individual differences in proneness to addictive behaviors, including nicotine addiction, are partly mediated by genetic factors. Current estimates of the heritability of all major addictive disorders range from 40% to 80% [3]. The share of genetic factors of nicotine addiction accounts for 50 to 75% [9, 12].

One of the effects of nicotine is to alter serotonin levels, hence genes encoding receptors or transporters involved in serotonergic pathways are potential candidates in the mechanism of nicotine addiction [18].

The *HTR2A* gene encodes the serotonin receptor, which is the key to the monoaminergic regulation of the body, which determines the biological functions and behavior of a person. The polymorphic variant rs6311 (-1438 A>G) is potentially associated with impaired efficiency of post-transcriptional processes and is considered a risk factor for neuropsychiatric and cognitive pathologies [20]. The presence of the A allele increases the transcriptional activity of the gene; several studies have shown that this allele is associated with smoking [13, 14, 15, 16].

The 5-HTTLPR polymorphic region (rs4795541) is a functional insertion/deletion polymorphism of 44 base pairs in the promoter region of the serotonin transporter gene, *5-HTT* [7]. This polymorphism was identified by two allelic variants, long (L with 16 repeats) and short (S with 14 repeats) forms, which affect the transcriptional efficiency of the *5-HTT* gene. The rs25531 polymorphism is within this polymorphism and results in two higher-expressing L-LA allele variants and lower-expressing LG variants—indicating that 5-HTTLPR may be functionally triallelic rather than biallelic [19]. Despite a large number of association studies of this polymorphism with nicotine dependence, data on which 5-HTTLPR allelic variant contributes to smoking are ambiguous [12].

The aim of our study was to determine whether the rs6311 polymorphisms of the *HTR2A* gene and the 5HTTLPR and rs25531 polymorphisms of the *5-HTT* gene are associated with smoking in the Yakut population.

Material and research methods. The experimental part of the study was carried out in the Laboratory of Hereditary Pathology of the Department of Molecular Genetics of the Yakut Scientific Center for Complex Medical Problems (YSC CMP). The material of the study was DNA samples from the collection of biomaterial (DNA) of the populations of the Republic of Sakha (Yakutia) YSC CMP, using the UNU "Genome of Yakutia" (reg. No. USU_507512). The study included participants who filled out a questionnaire approved by the Local Committee on Biomedical Ethics at the YSC CMP and voluntarily signed an informed consent to conduct a genetic study.

A total of 223 DNA samples of volunteers without chronic diseases (56 women and 167 men) of Yakut nationality were studied, the average age of which was 46 ± 0.08. For molecular genetic analysis, genomic DNA samples were isolated from whole blood using a Newteryx commercial DNA isolation kit (Yakutsk, Russia).

The study of polymorphisms was carried out by polymerase chain reaction (PCR) followed by restriction fragment length analysis (RFLP). The conditions for amplification and restriction are presented in table 1.

Statistical analysis of the results of the study was carried out using the program: "Office Microsoft Excel 2010", "Statistica 8.0". The frequencies of rs6311, 5-HTTLPR and rs25531 were determined by direct counting.

When analyzing the contingency of the frequency of an unfavorable allele with smoking, a four-field contingency table and a Yates-adjusted χ-square test were used. The following formula was used to calculate the odds ratio:

$$OR = \frac{A*D}{B*C}$$

where OR is the odds ratio; A, B, C, D are the number of observations in the cells of the contingency table. To assess the significance of the odds ratio, the boundaries of the 95% confidence interval (95% CI) were calculated. Results were considered significant at $p<0.05$.

Results and discussions:

An analysis of the distribution of allele and genotype frequencies of the polymorphic variant of the HTR2A gene (rs6311) in the group of smokers and non-smokers did not reveal significant differences; the G allele and the homozygous GG genotype prevailed in both groups (Table 2).

The results of the analysis of the relationship between the rs6311 polymorphism of the HTR2A gene and smoking showed that in the group of smokers allele A was somewhat more common than in the group of non-smokers (OR - 1.138; 95% CI = [0.742-1.138]), however, the significance of the differences was not significant (p=0.628) and the confidence interval showed a wide range.

Analysis of the distribution of alleles and genotypes of the 5-HTTLPR polymorphism of the 5-HTT gene showed the predominance of the short S allele

Table 1

Conditions for PCR-RFLP analysis

Polymorphism	Primer structure	Amplicon length	Annealing conditions	Restriction enzyme	Interpretation
rs6311	F:AACCAACTTATTTCCTACCAC R:AAGCTGCAAGGTAGCAACAGC	469 bp	57°C	*Msp* I	Genotype AA – 469 bp; Genotype GG – 243+226 bp; Genotype AG - 469, 243+226 bp
5-HTTLPR	F:GAGGGACTGAGCTGGACAAC-CCAC R:GGCGTTGCCGCTCTGAATGC	486 bp, 529 bp	62°C	-	Allele S - 486 bp, Allele L - 529 bp
rs25531				*Msp* I	Genotype LA -125+62+343 bp; Genotype LG - 125+62+ 174+167 bp Genotype SA - 125+62+ 299 bp

Примечание. п.н. – пар нуклеотидов.

Table 2

Calculation of the odds ratio of the rs6311 polymorphism of the HTR2A gene with smoking

Genotype and Allele	Smokers n (%)	non-smokers n (%)	X^2	OR (95 % CI)	P value
Genotype AA	4 (3.5)	4 (3.7)	0.650	1.138 (0.742-1.746)	0.723
Genotype AG	53 (46.1)	44 (40.7)			
Genotype GG	58 (50.4)	60 (55.6)			
Allele A	26.5 ± 0.029	24.1 ± 0.029	0.235		0.628
Allele G	73.5 ± 0.029	75.9 ± 0.029			

* n - is the number; Chi-square test with Yates correction; OR is the odds ratio; CI - confidence interval

Table 3

Calculation of the odds ratio of biallelic 5-HTTLPR polymorphism with smoking

Genotype and Allele	Smokers n (%)	non-smokers n (%)	X^2	OR (95 % CI)	P value
Genotype LL	14 (12.2)	3 (2.8)	12.323	2.830 (1.674-4.783)	0.002
Genotype SL	30 (26.1)	17 (15.7)			
Genotype SS	71 (61.7)	88 (81.5)			
Allele L	25.2 ± 2.863	10.6± 2.099	14.943		0.000
Allele S	74.8 ± 2.863	89.4± 2.099			

Table 4

Calculation of the odds ratio of grouped 5-HTTLPR and rs25531 polymorphisms with smoking

Genotype and Allele	Smokers n (%)	non-smokers n (%)	X^2	OR	P value
Genotype L'L'	11 (9.6)	1 (0.9)	20.204	4.844 (2.503-9.372)	0.000
Genotype L'S'	29 (25.2)	10 (9.3)			
Genotype S'S'	75 (65.2)	97 (89.8)			
Allele L'	22.2 ± 2.739	5.6 ± 1.559	24.009		0.000
Allele S'	77.8 ± 2.739	94.4 ± 1.559			

Table 5

Distribution of the combination of genotypes of the HTR2A gene and the 5-HTT gene with the calculation of the odds ratio to smoking

HTR2A	*5-HTT*	Smokers n (%)	non-smokers n (%)	X^2	OR	P value
AA	L'L'	2 (1.7)	0 (0.0)	0.444	-	0.506
AA	L'S'	1 (0.9)	0 (0.0)	0.001	-	0.975
AA	S'S'	1 (0.9)	4 (3.7)	0.953	0.228 (0.025-2.074)	0.33
AG	L'L'	3 (2.6)	0 (0.0)	1.228	-	0.268
AG	L'S'	13 (11.3)	1 (0.9)	8.508	**13.637 (1.752-106.144)**	0.004
AG	S'S'	37 (32.2)	43 (39.8)	1.101	0.717 (0.414-1.242)	0.295
GG	L'L'	6 (5.2)	1 (0.9)	2.11	5.890 (0.697-49.750)	0.147
GG	L'S'	15 (13.0)	9 (8.3)	0.843	1.650 (0.690-3.946)	0.359
GG	S'S'	37 (32.2)	50 (46.3)	4.094	**0.550 (0.319-0.948)**	0.044

(74.8–89.4%) and the SS genotype (61.7–81.5%) in both samples (Table 3).

Smokers had a significantly ($p<0.05$) higher frequency of the risk allele L (OR -2.830; 95% CI= [1.674 -4.783]) compared to non-smokers.

Due to the equivalent serotonin expression of the S and LG alleles, the 5HTTLPR and rs25531 polymorphisms, and a more accurate analysis of the association with smoking, the LGLG, LGS, and SS genotypes were combined into the S'S' genotype group. The LALA genotype into the L'L' genotype, and the LALG and LAS genotypes into the L'S' group (Table 4).

When analyzing the 5-HTTLPR and rs25531 polymorphisms grouped into groups, it was found that the L' allele frequency ($p<0.001$) was four times higher in the sample of smokers than in the sample of non-smokers (OR -4.844; 95% CI = [2.503-9.372]).

To study the relationship of both genes (HTR2A and 5-HTT) with smoking, in samples of smokers and non-smokers, various combinations of genotypes were analyzed (Table 5).

An analysis of the distribution of genotype combinations showed the predominance of people with a combination of GG genotypes of the HTR2A gene and S'S' 5-HTT gene, which showed a protective effect on smoking (OR - 0.550; 95% CI = [0.319-0.948]). A significant association with smoking was shown by a combination of heterozygous genotypes AG of the HTR2A gene and L'S' of the 5-HTT gene (OR -13.637; 95% CI= [1.752-106.144]).

Due to the different frequency of occurrence of the studied polymorphisms in different ethnic samples, we cannot statistically reliably state that the relationship between these polymorphisms and smoking that we found is suitable for all people. This pilot experiment allows us to draw only small conclusions on the study of the above genes in the Yakut population. Thus, our results are consistent with the studies of Lerman C et al. (2000) and Ishikawa H et al. (1999), who also found a protective effect of the S allele on smoking [16, 17]. In studies by Siemińska A. et al. (2008) among the Polish population, they did not find a link between 5-HTTLPR polymorphism and smoking [18]. Many researchers have studied this polymorphism with addictive behavior, and in the works of Stefan Bleich et al. (2007) they found an association of the L – allele with obsessive-compulsive craving for alcohol [19]. In a study by Gerra G et al (2005), the frequency of the short S genotype was higher in smokers than in non-smokers [20].

Conclusion. This study established a significant association of 5-HTT gene polymorphisms with smoking in the Yakut population. In connection with the equivalent serotonin expression of the S and LG alleles, 5HTTLPR and rs25531 polymorphisms, it is more informative to carry out their generalized analysis. For the rs6311 polymorphism of the HTR2A gene, no statistically significant association with smoking was found, probably due to the fact that the frequency of the A allele has a relatively rare occurrence, which must be taken into account and, possibly, later validation of the population is required. Despite the discovery of an association between 5-HTT gene polymorphisms and smoking, additional studies are required in similar populations but with larger sample sizes to further explore interactions with other candidate genes and addictive behaviors.

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ORGANIZATION OF HEALTHCARE, MEDICAL SCIENCE AND EDUCATION

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BIOETHICAL ISSUES IN THE ORGANIZATION OF SPECIALIZED MEDICAL CARE FOR PATIENTS WITH MOTOR NEURON DISEASE IN THE REPUBLIC OF SAKHA (YAKUTIA)

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Amyotrophic lateral sclerosis (ALS) is a severe neurodegenerative disease in which the death of central and peripheral motor neurons occurs, leading to an inevitable fatal outcome. Bioethical problems arise at any stage of the disease when a patient with ALS goes to a doctor. The help of psychologists, the activities of bioethical committees in medical organizations in informing patients and their relatives about the disease, the awareness of neurologists about social support measures from the state can help in organizing medical and social assistance measures to improve the quality of life of patients with ALS.

Aim: to solve the bioethical problems of patients with ALS to improve the organization of specialized medical care

Keywords: bioethics, motor neuron diseases, amyotrophic lateral sclerosis, artificial lung ventilation, palliative care.

Introduction. ALS is a serious disease of unknown etiology and pathogenesis, accompanied by the death of central and peripheral motor neurons, steady progression and inevitable death. The incidence of ALS in the world is 1.89 per 100 thousand population, and the prevalence is 5.2 cases per 100 thousand population [17]. 7% of ALS patients have been ill for more than 5 years, the average life expectancy is 2.5 years with bulbar and 3.5 years with spinal debut of ALS. In recent years, there has been an increase in the incidence of MND in the world [11].

The disease is manifested by the development of motor disorders, bulbar and pseudobulbar syndrome, as a result of which the patient becomes immobilized, cannot eat and talk. The main cause of death in patients with ALS is restrictive or restrictive-obstructive respiratory failure, which develops due to paralysis of the diaphragm muscles, respiratory muscles and aspiration of food and saliva in bulbar disorders[7].

Managing a patient with ALS is a very difficult task for a doctor because of the severity, rapid progression and incurable course of the disease. In a challenging complex of medical and social objectives, one of the main ones are the bioethical problems that accompany a patient with ALS until the end of his life. The complexity of bioethical problems arises at all stages of the provision of specialized medical care (SMC) by a neurologist. At the 1st stage of outpatient SMC, if ALS is suspected, the doctor is faced with a dilemma: whether to inform the patient and his relatives about his suspicions or await the final diagnosis after the examination. When providing SMC at stage 2 in an inpatient hospital, when differential diagnosis with ALS-like diseases has been carried out and the final diagnosis of ALS has been established, the attending physician also faces the difficult task of communicating the diagnosis to the patient and his relatives. At the same time, psychological work should be carried out separately with the patient and his relatives who will take care of him in the future. The decision on palliative therapy, social, financial issues, as well as the patient's preliminary orders should be made long before there is a need for enteral nutrition or auxiliary ventilation of the lungs. All this requires the use of a complex of social, medical, and legal measures, the main of which is a bioethical issue affecting the moral aspects both on the part of the doctor and on the part of the patient and his relatives. In the Russian Federation, there is no single standard for the management of patients with incurable and fatal diseases, including ALS.

Materials and methods of research. The study included patients with ALS (n=11), their relatives (n=11) and neurologists (n=30). 3 out of 11 patients needed periodic artificial lung ventilation. All study participants gave written informed consent to participate in the study.

Inclusion criteria:

1. patients with clinically reliable ALS using El Escorial criteria [5];
2. relatives of ALS patients who do not have a mental illness;
3. doctors with a specialization in neurology

Exclusion criteria:

1. patients with ALS-mimicking syndromes;
2. patients and their relatives who have not signed a written informed consent for the study;
3. patients with severe MND who cannot participate in the study on their own;
4. relatives of patients with mental illnesses.

Research methods

1. The clinical method included a study on El Escorial criteria to establish ALS and to assess somatic status
2. Using the Hospital Anxiety and Depression Scale (HADS) to study the psycho-emotional state of patients and their close relatives.
3. The method of medical interview was conducted with patients and close relatives caring for a patient with ALS to determine the degree of readiness to accept information about their disease.
4. The questionnaire method was conducted for neurologists.
5. Statistical analysis of the research results was carried out using Excel to determine the average values.

Results. The sample of patients included 11 patients with reliable ALS according to El Escorial criteria. By ethnicity, 8 (72.7%) people are representatives of the Yakut ethnic group, 3 (27.3%) pa-

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tients are of the Russian ethnic group. The average age was 53±11.8 years. The age varied from 30 to 72 years. In 5 patients, the diagnosis of "ALS" was established for the first time. The remaining 6 patients were diagnosed from 1.5 to 5 years ago.

A survey on the HADS scale showed that all patients experience clinically pronounced anxiety and depression. However, a survey on the HADS scale of relatives of patients did not reveal clinical signs of depression, while the level of anxiety also significantly exceeded normal indicators. Thus, in the group of patients with ALS, the anxiety level averaged 17.74± 0.48 points, depression - 13.60± 0.68. In relatives of patients with ALS, the anxiety level was 13.8 ± 0.32 points. The average level of depression in family members was 5.9±0.62 points.

Thus, our study shows that all patients and their family members experience pronounced psycho - emotional stress associated with the disease.

In addition, in none of the cases was the help of a clinical psychologist provided, because in outpatient clinics to which these patients were attached, the staffing of this specialist is not provided due to the absence of a compulsory medical insurance (CMI) tariff for this medical service. In inpatient hospitals, excluding hospitals of the regional vascular center, vacancies of clinical psychologists are also not provided for this exact reason. If possible, this function is performed by the attending neurologist.

During the survey , neurologists answered the following questions:

1. Do you think that it is necessary to hide the diagnosis under an ALS-like disease, for example, vertebrogenic myelopathy, so as not to cause depression, suicide, vascular crises in the patient, which can lead to acute disorders of cerebral circulation and acute coronary syndrome, etc., or give them full information about the disease?. 7.4% of doctors answered affirmatively, and 92.6% of the surveyed doctors believe that it is not necessary to conceal the disease from the patient;

2. Do patients with ALS and their relatives require the help of a psychologist? 100% of the respondents answered "Yes".

3. Does your organization have a psychologist?

A survey among doctors of state budgetary and autonomous institutions showed that 48% of these institutions have a psychologist;

4. Does your organization have a local bioethics committee?

The survey showed that only 20% of medical organizations have local bioethics committees;

5. Are you familiar with the contents of the Order of the Ministry of Health of the Russian Federation No. 348n "On approval of the list of medical devices intended to maintain the functions of organs and systems of the human body provided at home" dated May 31, 2019, the list of which includes general-purpose artificial lung ventilation devices provided for use at home. 19% of doctors were not familiar with this order;

6. What measures of medical and social assistance would you recommend?

Neurologists recommend these measures of medical and social assistance:

- supervision by multidisciplinary teams, including home visits – 3.2% of the surveyed doctors;
- management of patients by specially trained neurologists and treatment of patients in specialized ALS clinics– 31,2%;
- creation of medical and social care centers for patients with ALS – 3.2%;
- creation of a unified ALS register – 3.1 %;
- creation of local bioethics committees – 51.1%.
- simplification of the procedure for obtaining at-home-ventilators through Palliative care centers – 11.4%

The method of clinical interview revealed that patients were concerned about the following questions (each question out of 100% of cases):

1. How will ALS affect life expectancy - (92%)?
2. Will they be able to cope with their daily work – (85%)?
3. Is this disease inherited – (65%)?
4. Why did the patient get this disease – (97%)?
5. Is there a search for an effective drug – (86%)?
6. Are there state support measures for patients with ALS (45%)?

Discussion. Observation of patients with ALS revealed bioethical problems at different stages of disease progression. The patient's condition depends on how the diagnosis is presented: will the patient be able to accept the inevitability of a fatal outcome, will he choose a position of humility and non-resistance to the disease or will he find the inner strength to live with this incurable and fatal disease. And this circumstance imposes on the attending physician a great moral responsibility in connection with the need to communicate the diagnosis to the patient and people close to him. When communicating the diagnosis of ALS to the patient and his loved ones, it is necessary to observe the moral norms of medical ethics and deontology.

Bioethical problems in reporting the diagnosis of amyotrophic lateral sclerosis. Guided by paragraph 1 of Article 22, Federal Law No. 323 of 21.11.2011 (ed. of 03.07.2016), the doctor must provide all the necessary information without the intent of concealment. In case of an unfavorable prognosis, the nature of the disease should be reported in a delicate form to a citizen or his spouse, one of the close relatives, unless the patient has forbidden to inform them about it and (or) has not identified another person to whom such information should be disclosed." Due to the lack of a single algorithm of actions in the Russian Federation in such situations, the doctor acts at his discretion and in the way it is customary to act in these cases in this medical institution [1]. When deciding on the diagnosis of "ALS", it is necessary to keep in mind how the patient and his relatives will perceive the diagnosis.

When voicing the diagnosis, the attending physician needs to take into account the characteristics of the patient's character, how close his ties are with family members, whether there are other close people besides relatives. It is important to assess the situation in advance and choose the tactics of voicing the diagnosis: will the patient be able to accept the diagnosis alone or is it preferable to communicate the diagnosis in a circle of relatives or close people. The support of relatives and close friends during the period of informing about a fatal disease is very important[9].

Thus, the doctor must foresee what emotions the news of his diagnosis will cause in the patient and be internally prepared for them in order to be able to react objectively. Sometimes doctors, in order to avoid a difficult moral situation when communicating with a patient, postpone the diagnosis until the day of discharge from the hospital. Such a position on the part of a doctor, on whom patients pin their hopes, is undesirable and unacceptable. We believe that the diagnosis should be reported before discharge from the hospital so that the patient can collect his thoughts and assess the situation with his relatives, as well as discuss what medical and social assistance he can receive after discharge from the hospital.

Bioethical problems when connecting the patient with ALS to a ventilator. For the palliative treatment of ALS, there is a "gold standard" of palliative therapy for ALS, which includes artificial lung ventilation, percutaneous endoscopic gastrostomy, enteral nutrition, the drug riluzol

(not registered in the Russian Federation) and symptomatic pharmaceutical and non-pharmaceutical treatment [8].

Medical personnel should remember that the installation of a ventilator to replace respiratory function requires prior resolution of bioethical problems. Before installing the device, it is necessary to have a conversation with relatives and the patient. It should be noted that the decision to connect a patient to a ventilator primarily falls on his family and is a very important moral step on their part, since the maintenance of the life of a sick family member depends on the care of a patient with a ventilator. In some cases, the patient decides not to continue communicating with doctors and refuses to discuss his diagnosis with loved ones, falls into despondency and refuses to be treated [15]. In such cases, not only the help of close people is needed, but also a psychologist, so that they can help the patient cope with the awareness of the disease and direct him to the path of solving the problem, and not escaping it. The sources describe cases of affective behavior and numerous suicidal attempts in such situations, for example, in Huntington's disease [9]. When choosing to connect to a ventilator, the patient's family or close circle should take into account their financial capabilities, familiarize themselves with the requirements for caring for a patient who is on a ventilator in a hospital or at home. Before connecting the ventilator, it is necessary to obtain informed consent for the installation of the device.

Using a ventilator at home, until June 2019, was an unsolvable problem for most patients with ALS, not only because of the financial difficulties of purchasing equipment for personal use at home, but also the lack of state support. On May 31, 2019, two Orders were issued by the Ministry of Health of the Russian Federation, the Ministry of Labor and Social Protection of the Russian Federation No. 348n "On approval of the list of medical devices intended to maintain the functions of organs and systems of the human body provided at home" [3] and Order No. 345n/372n on approval of the "Regulations on the organization of palliative care, including the procedure for interaction of medical organizations, social service organizations and public associations, other non-profit organizations engaged in their activities in the field of health protection" [4]. In Order No. 348n, the list includes general-purpose artificial lung ventilation devices provided for use at home. These orders are a good state support for patients receiving palliative care at home, about which patients and medical workers are insufficiently informed. Out of all the surveyed neurologists, 88% indicated insufficient provision of ventilators.

Around the world, an interdisciplinary approach to the symptomatic treatment and care of patients with ALS is practiced, which could be adopted and implemented in Russian healthcare at the level of primary medical and social assistance [10, 12, 16].

The preservation of intelligence of patients with ALS, on the one hand, helps them to objectively assess their current situation and independently or jointly with loved ones - solve the issue of gastrostomy and periodically connecting to a ventilator, and on the other hand, they understand and are aware of the fact that palliative care is only a temporary measure. At the same time, the absence of bedsores, replacement respiratory therapy with a ventilator due to good care, as well as the participation of the MND patient's loved ones in solving his issues, make the diagnosis of amyotrophic lateral sclerosis not so fatal. An interdisciplinary, palliative approach can prolong survival and preserve the quality of life [10,13,14]. This requires clarification to relatives that with timely prevention of complications, i.e. adequate patient care, proper ventilation, a patient with ALS can live on a ventilator for an average of up to 1 year or more [2].

In our study, two patients with ALS underwent respiratory replacement therapy with a ventilator at home. This is a woman, 54 years old, Sakha, with a bulbar debut of ALS and a moderate rate of progression of the disease and a man, 54 years old, Russian, with a cervical debut of ALS and a rapid rate of progression. In the first case, before connecting to the ventilator, a preliminary conversation was held with the patient and her husband, informed consent was obtained to connect her to the ventilator at home. Before purchasing a ventilator, the patient was in the intensive care unit. The ventilator was purchased at their own expense. The woman lived for 5 years after connecting the ventilator, nutrition was carried out through a nasogastric probe. The patient was periodically examined by a neurologist, therapist, surgeon, received physical therapy, massage. That is, a multidisciplinary approach was implemented during her care. In the second case, the life expectancy with a ventilator was 2 years. Given the rapid rate of development of the disease, it was decided to inform his wife about the possible imminent death of a patient with ALS, who asked not to inform her husband about it, because the patient himself was in a state of subclinical depression. Since the FVC was less than 50%, the patient had indications for connection to a ventilator. The couple decided to connect to a ventilator. After receiving written informed consent, a ventilator was installed for the patient. In the future, a ventilator for use at home was rented.

Doctors and relatives of the patient should take into account the inevitable emotional and volitional disorders, as well as depression in ALS, which can lead to untimely decisions by the patient regarding treatment methods aimed at prolonging life. It is necessary to convince the patient that the use of a ventilator at home is a method of respiratory support, and not a resuscitation measure, and does not require the patient to stay in the intensive care unit. At the same time, the patient has the opportunity to stay with his family, can travel and even work remotely. Family members should be warned about the reorganization of their everyday life, adjusted for patient care. All patients and their family members need the help of a psychologist and a psychotherapist.

Conclusion. Thus, our study of bioethical issues in the organization of specialized care for patients with amyotrophic lateral sclerosis revealed both the insufficiency of existing measures of medical and social support, and the insufficiency of informing doctors about regulatory documents of state support for patients who need a ventilator, the absence of psychologists and bioethical committees. Psychological support during the entire period of observation of patients, compliance with ethics and deontology, respect for the rights of the patient are the basis for the organization of specialized care for this category of patients. Local ethical committees of medical institutions should help in discussing complex ethical problems that have arisen in clinical practice and need to formulate recommendations on how these problems should be solved. It is also necessary to introduce the experience of interdisciplinary teams in the management of patients with ALS.

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SCREENING OF MALIGNANT NEOPLASMS IN THE POPULATION OF THE REPUBLIC OF SAKHA (YAKUTIA)

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This article describes the incidence of malignant neoplasms in the Sakha Republic's population selected for screening studies and presents an assessment of the results of the Onkopoisk. Sakha (mass screening tests) pilot project. Screening is carried out for six types of malignant neoplasms that are an important problem for regional healthcare. In the course of screening, we carried out 12,826 tests and identified 21 cases of malignant neoplasms and 51 cases of precancerous conditions. The proportion of patients with stage 0-2 malignant processes was 62%.

Preliminary results of Onkopoisk.Sakha demonstrated how it can be useful in screening and early diagnosis of cancer in the Sakha Republic. This screening program using online platforms complements other activities of medical examinations and preventive examinations and is in demand because it meets the requirements of sanitary and epidemiological safety and saves resources.

Keywords: malignant neoplasms, morbidity, mortality, cancer screening, liver cancer, breast cancer, prostate cancer, lung cancer, colorectal cancer, cervical cancer.

Introduction. Cancer screening is an important component of comprehensive cancer control measures aimed at the early detection of asymptomatic malignant tumors or precancerous conditions by inviting patients who correspond to a practically healthy target population but can be classified as risk groups according to certain characteristics [2].

According to Global Burden of Diseases Cancer Collaboration, from 2007 to 2017, the incidence of oncology in the world increased by 33%, and for DALYs oncology has moved from sixth to second place after cardiovascular diseases as the most common cause of death in patients [6]. According to the WHO forecast, by 2050, cancer incidence worldwide will increase to 54 million, and mortality to 16 million cases per year [10]. Global society suffers significant productivity losses due to cancer deaths. Investing in programs targeting high-morbidity cancers that occur in young people can reduce productivity losses for society the most [8, 9].

Introducing new, more effective methods of early diagnosis, treatment, and prevention of cancer in economically developed countries helps reduce mortality and increase life expectancy. For example, in the United States from 1991 to 2017, a continuous decrease in deaths from malignant neoplasms led to an overall decrease in mortality by 29% [3]. Another example demonstrating the possibilities of modern medicine is the widely-adopted 2020 WHO global strategy to accelerate the elimination of cervical cancer as a public health problem [5].

As a process, screening includes a whole system of activities from informing and inviting the target population to providing treatment and evaluating the process to improve it [7]. This process is complex and costly but screening for breast, cervical, and colon cancer is now proven effective in terms of balancing benefit and risk; screening can also be effective for long-term smokers to identify lung cancer, as well as patients with viral hepatitis and cirrhosis of the liver to identify liver cancer, and in people with a positive PSA test to identify prostate cancer.

The Sakha Republic is the largest subject of the Russian Federation in terms of area (3 million sq. km) with a population of 972 thousand people as of early 2020 (population density — 0.31 people per 1 sq. km). There has been a steady increase in the incidence of malignant among the population in recent years. According to Rosstat (Russia's Federal State Statistics Service), for the period from 2010 to 2019 in the Republic, the increase in malignant neoplasms (MN) was 31.3% (from 213.8 to 280.7 per 100,000 people). When implementing the Zdravookhranenie (Healthcare) National Project of the Yakut Republican Oncological Dispensary (Yakutsk, Russia), the project's team developed the Onkopoisk. Sakha online project, and since August 11, 2020, it has been implemented in test mode, aimed at screening and early cancer diagnostics in the Sakha Republic (онкопоисксаха.рф).

Purpose of the Study: providing an up-to-date description of the incidence

of malignant neoplasms (MN) in the Sakha Republic's population selected for screening studies and evaluating the results of the Onkopoisk.Sakha pilot project.

Materials and Research Methods. The study used data published by the P. Herzen Moscow Oncology Research Institute — a branch of the National Medical Research Radiological Center of the Ministry of Health of the Russian Federation [1]. The results of the Onkopoisk. Sakha pilot project for screening MNs based using the database at YROD are provided as of September 15, 2022. The project's questionnaire includes six cancer locations: prostate, lung, breast, liver, colon and rectum, and cervix. By having personal accounts for the patients, we were able to provide convenient registration and store the results in an online database.

During the first stage, the patients use a web app at a convenient time to register their personal account and complete the questionnaire; if there's a high risk of developing oncopathology identified, they are invited to undergo an additional examination at the medical facility. Meanwhile, in patients with an increased risk of developing cancer or signs of cancer, an additional comprehensive examination is performed.

It is important to note that the research did not set the task of completing controlled studies to evaluate the effectiveness of the screening methods or the sensitivity of the tests.

Results and Discussion. Analyzing the average values of age-standardized incidence ratios (SIR) of incidence over a 10-year period showed that, in general, the incidence of malignant neoplasms in the Sakha Republic was lower than the average for the Russian Federation, both in men and women (Table 1). We observe a relatively stable situation in dynamics for men in the Sakha Republic and the Russian Federation as a whole from 2010 to 2019 (+4 and +2.8%, respectively), while for women we see a moderate growth trend (+11.3 and +12, 2%). At the same time, when considering the localizations of MNs included in the screening programs in the population of both the Sakha Republic and the Russian Federation, there are significant differences in the levels of SR depending on gender.

For instance, the incidence of liver cancer (C22) in the Sakha Republic's male population was 3.8 times higher than in the Russian Federation as a whole. There is also a slight excess of lung cancer SIR (C33, 34) by 8.2%. While the dynamics of lung and liver cancer in the Sakha Republic's SIR from 2010 to 2019 are relatively stable, despite their wave-like movement (-8.2% and +3.9%, respectively), in the Russian Federation as a whole, there is both a decrease and increase depending on the localization (-15.9% and +25.6% respectively). Meanwhile, the SIR of prostate cancer (C61) and colorectal cancer (C19-21)

Table 1

Comparison of Standardized Cancer Incidence Rates Per 100,000 Population (World Population Standard) [1]
SR — Sakha Republic, RF — Russian Federation

Localization	Territory	Years										M (95% CI)
		2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	
Men												
All neoplasms (C00-97)	РС (Я)	250.1	255.8	249.0	228.6	248.9	270.9	245.3	255.2	247.4	260.0	251 (243-258)
	РФ	279.6	273.5	270.7	271.3	277.6	284.0	283.1	286.7	286.5	286.8	280 (275-285)
Trachea, bronchi, lung (C33, 34)	РС (Я)	58.7	54.0	49.3	49.2	54.1	50.1	59.3	49.0	48.0	53.9	53 (50-56)
	РФ	54.0	51.1	50.0	49.2	48.8	49.9	48.9	49.0	47.5	45.4	49 (48-51)
Liver and intrahepatic bile ducts (C22)	РС (Я)	18.1	17.6	18.6	20.2	18.2	21.1	18.0	19.8	20.2	18.8	19 (18-20)
	РФ	4.3	4.1	3.9	4.1	4.3	4.8	4.9	5.2	5.1	5.4	5 (4-5)
Prostate gland (C61)	РС (Я)	14.8	13.3	12.9	14.3	18.9	33.7	19.1	24.3	21.8	25.6	20 (15-25)
	РФ	30.6	32.3	32.5	34.6	39.4	40.2	39.0	40.5	41.5	43.5	37 (34-41)
Rectum, rectosigmoid junction, anus (C19-21)	РС (Я)	11.0	12.9	10.9	9.8	10.6	13.6	12.1	9.1	10.0	12.2	11 (10-12)
	РФ	14.6	14.1	14.3	13.9	14.3	14.9	14.9	15.1	15.4	15.8	15 (14-15)
Women												
All neoplasms (C00-97)	РС (Я)	179.6	186.5	183.8	187.1	185.8	207.1	184.7	191.8	194.0	199.9	190 (184-196)
	РФ	209.0	207.9	208.5	210.7	216.9	223.0	225.6	229.6	230.2	234.5	220 (212-227)
Trachea, bronchi, lung (C33, 34)	РС (Я)	15.4	16.7	13.4	13.8	13.1	14.2	14.7	15.0	13.2	15.1	15 (14-15)
	РФ	7.1	7.0	6.8	7.2	7.3	7.7	7.7	8.1	8.3	8.0	8 (7-8)
Liver and intrahepatic bile ducts (C22)	РС (Я)	10.5	10.9	9.9	9.0	10.8	12.0	9.6	7.1	11.4	10.2	10 (9-11)
	РФ	1.9	1.9	1.8	1.9	2.0	2.1	2.1	2.2	2.1	2.1	2 (2-2)
Mammary gland (C50)	РС (Я)	25.2	34.4	35.3	38.9	37.3	38.6	35.5	37.7	34.9	38.9	36 (33-39)
	РФ	45.8	45.2	46.2	47.1	48.9	49.8	50.9	52.0	51.6	53.3	49 (47-51)
Rectum, rectosigmoid junction, anus (C19-21)	РС (Я)	7.5	8.0	9.5	8.6	8.2	8.0	7.5	7.3	10.4	9.2	8 (8-9)
	РФ	8.9	8.9	8.8	8.9	9.1	9.3	9.2	9.2	9.4	9.4	9 (9-9)
Cervix (C53)	РС (Я)	13.4	16.7	16.6	20.3	19.6	19.2	22.9	20.2	21.6	18.4	19 (17-21)
	РФ	13.7	13.7	13.9	14.2	14.5	15.0	15.5	15.8	15.8	15.4	15 (14-15)

Note: 1 — Malignant Neoplasms in Russia in 2007-2019 (Morbidity and Mortality). Moscow: P. Herzen Moscow Oncology Research Institute, Branch of the National Medical Research Radiological Center of the Ministry of Health of the Russian Federation; M (95% CI) — average value for the 2010-2019 period (95% confidence interval).

were lower than in the Russian Federation by 1.9 and 1.4 times, respectively. However, in the dynamics of prostate cancer, the SIR tends to increase both in men in the Sakha Republic by 1.7 times, and by 1.4 times in the Russian Federation. The incidence of colorectal cancer in dynamics is stable with a slight increase (+10.9% in the RS (Y), +8.2% in the Russian Federation as a whole).

In the female population in the Sakha Republic, the SIR of lung cancer (C33, 34) was 1.9 times higher than the national average, liver (C22) — 5 times, and cervical (C53) — 1.3 times. The risk factors for breast cancer (C50) and colorectal cancer (C19-21) were 1.4 times and 12.5% lower than the Russian average, respectively. Additionally, in dynamics over a 10-year period, the SIR of breast cancer in women in the Sakha Republic increased by 1.5 times, colorectal cancer — by 1.2 times, and cervical cancer — by 1.4 times, while lung and liver cancer SIR remained practically unchanged. In women in the Russian Federation, there is a moderate increase in most SIRs covered in this article (with the exception of the practically unchanged SIR of colorectal cancer): breast cancer — increased by 16.4%, lung cancer — by 12.7%, cervix — by 12.4%, liver cancer — by 10.5%, and colorectal cancer — by 5.6%.

Hence, screening for six cancer types has an important place in the fight against cancer in the Republic of Sakha (Yakutia). The high incidence of liver cancer (C22) and lung cancer (C33, 34) among the population of both sexes, cervical cancer (C53) among the female population all with mortality higher than the national average (with the exception of lung cancer in men) should be treated as regional features in the epidemiological situation.

15,521 people who have filled out a total of 18,202 questionnaires (Table 2) took part in the pilot project for screening MNs using the YROD database (as of September 15, 2022). The largest number of completed questionnaires has been for screening of liver cancer (31.3%), as well as lung and breast cancer, which is explained by the greater awareness of the population regarding cancer developing in these localizations. 21 patients had MN of the total completing the questionnaires, that is, the overall detection rate was 1.4 cases per 1,000 respondents.

In the course of screening, we have completed a total of 12,826 studies (Table 3) where we have detected 21 cases of malignant neoplasms and 51 cases of precancerous conditions, including 9 patients with precancer according to Lung-RADS (LDCT control after 1-3-6 months) and 9 more patients with precancer according to BI-RADS (mammography control after 6 months). The screening excluded 12,410 people with 11,059 healthy patients, 73 patients refusing further examination, and 1,207 patients sent

Table 2

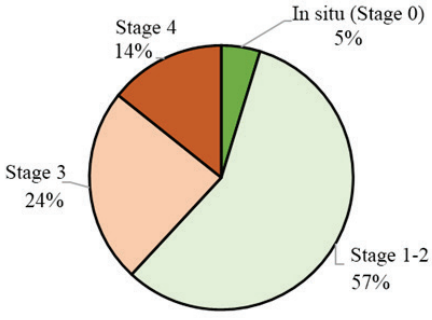
Detection of Malignant Neoplasms Among Screening Patients (n = 15521)

Questionnaires	Total		MN Detected	
	Number	%	People	Per 1000
Number of people who have completed questionnaires	15521	100	21	1.4
Total number of completed questionnaires	18202	100	21	1.2
Including screening for				
Lung cancer	3978	25.6	4	1.0
Liver cancer	4855	31.3	2	0.4
Breast cancer	4380	28.2	5	1.1
Prostate cancer	464	3.0	1	2.2
Colon and rectal cancer	2965	19.1	3	1.0
Cervical cancer	1560	10.1	2	1.3

Table 3

Results of Additional Comprehensive Examinations to Detect Malignant Neoplasms

Examination		Total	Revealed		Continuing examination	Причины отсева		
			MN	Other conditions subject to remote control		No pathology detected	Other pathology identified	Refusing further testing
NDCT	n	2045	4	9	6	1742	282	2
	%	100	0.2	0.4	0.3	85.2	13.8	0.1
Liver ultrasound	n	2200	2	4	11	1450	719	14
	%	100	0.1	0.2	0.5	65.9	32.7	0.6
Mammography (women >40 years old)	n	926	3	0	7	809	106	1
	%	100	0.3	0	0.8	87.4	11.4	0.1
Breast ultrasound (women <40 years)	n	480	2	9	11	357	96	5
	%	100	0.4	1.9	2.3	74.4	20.0	1.0
IHA stool for occult blood	n	1679	3	8	19	1583	37	29
	%	100	0.2	0.5	1.1	94.3	2.2	1.7
Tumor markers	n	4363	3	11	11	4325		13
	%	100	0.1	0.3	0.3	99.1		0.3
PSA for prostate cancer	n	173	1			170		2
	%	100	0.6			98.3		1.2
Cervical screening (smear test)	n	932	2	10	7	600	294	6
	%	100	0.2	1.1	0.8	64.4	31.5	0.6
Thyroid ultrasound	n	28	1			23	3	1
	%	100	3.6			82.1	10.7	3.6
Total	n	12483	21	51	72	11059	1207	73
		100	0.2	0.4	0.6	88.6	9.7	0.6



Malignant neoplasms detection stages during the Onkopoisk.Sakha screenings

to polyclinics at their places of residence with other pathologies. We have examined 511 patients at YROD, with 72 patients continuing with their examinations, while 73 patients have refused further examination.

As a result of screening, patients at risk of having or developing the discussed diseases in the future are singled out from an apparently healthy population for an earlier intervention to improve their health [4]. In the pilot project and out of 21 cases of malignant neoplasms identified during screening studies, 1 case was detected at stage 0 (in situ), 12 — at stages 1-2, 5 cases — at stage 3, and 3 cases — at stage 4 of disease (Fig. 1). The proportion of patients with a malignant process of stage 0-2 was 62%. Additionally, we have taken 51 patients under dispensary observation.

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HYGIENE, SANITATION, EPIDEMIOLOGY AND MEDICAL ECOLOGY

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CHARACTERISTICS OF PATHOLOGICAL LESION OF THE WORKING POPULATION OF THE SOUTHERN ZONE OF YAKUTIA

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A comprehensive survey of the working population of the Aldan district of the Republic of Sakha (Yakutia) was conducted. A total of 175 people of working age of non-indigenous nationality were examined. A high frequency of pathology on the part of the digestive, cardiovascular and endocrine systems, including the first identified, was revealed. Overweight and obesity had a high incidence, being a risk factor for the development of cardiovascular diseases. Every 10 respondents have registered oncopathology, 3 participants of the study were sent for further examination with suspicion of malignant neoplasms. The role of primary health care, including shop doctors, is of great importance in the early detection and prevention of risk factors for the most common chronic non-communicable diseases and timely effective medical care, as well as health schools for patients with hypertension, coronary heart disease, diabetes mellitus, gastritis, cancer, etc.

Keywords: pathological lesion, working population, South Yakutia.

The development of the socio-economic sphere of any country is largely determined by labor resources dependent on the health of the able-bodied population, which directly or indirectly depends on a number of factors. Considering that the Aldan district of the Republic of Sakha (Yakutia) is located on the territory of the Elkon uranium ore industry with a possible technogenic effect of uranium decay products, the relevance of the study of the working and living population is undeniable. The medical and demographic analysis according to the official data of the Federal State Social Service for the Republic of Sakha for 2000-2020 showed high mortality rates of the population of the Aldan district in comparison with the national indicators (13.4-15.3 versus 8.6-9.3 per 1000 people). In 2020, the mortality rate from diseases of the circulatory system (DCS) exceeded the national data by more than 2 times (815.8 vs. 404.9 per 100 thousand people). Mortality from neoplasms in the Aldan district in 2020 also exceeded by more than 1.5 times in comparison with the national data (215.5 and 131.6 per 100 thousand people, respectively). The indicators for the incidence of DCS in this area in 2020 were almost 2 times higher than the national ones (328.1 vs. 186.6 per 1000 people), and malignant neoplasms (MNP) by 32.2% (337.5 vs. 255.2 per 100 thousand people) [1].

Objective: To assess the health status of the working population of the Aldan district of the Republic of Sakha (Yakutia)

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based on the results of a comprehensive medical examination.

Materials and methods of research. From March 27 to April 3 , 2022 , a singe-stage comprehensive medical examination of the working population of the mining cities of Aldan and Tommot of the Aldan district was conducted . The study was conducted by a visiting team of practicing medical specialists of the Yakut Science Centre of Complex Medical Problems which consisted of: a therapist, cardiologist, neurologist, ophthalmologist, oncologist, ultrasound diagnostics doctor and endoscopist. According to the list of employees, every 3rd employee of the institution was invited to the examination of specialists. The response was 75%.

175 residents of the Aldan district of working age of non-indigenous nationality were analyzed, including 66 men and 109 women (37.7% and 62.3%, respectively), whose average age was 43.4±1.08 years for men and 44.7±1.40 years for women.

The research program of the adult population included the following sections: informed consent of the respondent (according to the protocol of the Ethics Committee of the YSC CMP dated 10.03.2022 No.56, decision 3); questionnaire survey to assess the objective condition; anthropometric examination with measurement of height and body weight with calculation of body mass index, clinical examination and instrumental research.

For further analysis, the traditional indicator was used - body mass index (BMI) or Quetelet index, which was calculated by the following formula: BMI (kg / m²) = body weight (kg) / height (m²). Overweight was considered to be a BMI ≥ 25 and <30 kg/m², obesity was determined at a BMI of ≥ 30 kg/m² [National clinical guidelines for the treatment of morbid obesity in adults, 2018, 3rd revision].

Blood pressure (BP) was measured twice with an OMRON M2 Basic automatic tonometer (Japan) in a sitting position with calculation of average blood pressure with a margin of permissible measurement error of ± 3 mm Hg. (ESH/ESC, 2013) according to the instructions for the correct measurement of blood pressure, outlined in the European clinical guidelines for the diagnosis and treatment of hypertension. Hypertension is present at the 140/90 mmHg or taking antihypertensive drugs during the study or stopping them less than 2 weeks before the study (2017 ACC/AHA Guideline).

Nosological diagnosis was made by specialists according to the International Classification of Diseases X revision.

The incidence of disease was calculated by the following formula:

Incidence of disease = *(number of diseases registered in the population)/(number of population)* x100

Statistical analysis was carried out using the SPSS STATISTICS software package (version 26.0). Qualitative variables are described by absolute and relative frequency (percentages), quantitative variables are described using the mean value and the standard error of the mean value. When comparing the groups, Spearman's criteria were used χ2. The statistical significance of the differences (p) was assumed to be equal to 5%.

Research results and discussion. A comprehensive medical examination of the adult population showed that only 23 people (13.1%) were recognized as practically healthy, i.e. not having one or another disease According to the results of the study, disease was detected in 152 out of 175 people (86.9%). The incidence of disease was 270.1 per 100 examined, i.e. 2-3 diseases per person on average. For the first time in their life, the diagnosis was made in 43 people. In the structure of disease incidence, the leading place was occupied by diseases of the digestive organs, which were registered in more than half of the study participants (57.7 per 100 examined, or 21.4% of all diseases) (Table 1). The second ranking position with a small difference was occupied by diseases of the circulatory system (DCS), which were also registered in more than half of the surveyed contingent (57.1 or 21.2%, respectively). Diseases of the endocrine system, eating disorders and metabolic disorders occupied the third position in the structure of diseases (51.4 or 19.1%). The total share of the most common disease among the study participants was more than half of the total. In addition to the above classes of diseases, diseases of the eye and its accessory apparatus, musculoskeletal system, connective tissue and genitourinary system occupied a significant share in the structure of pathological lesions.

The first rank position in terms of the frequency of pathological lesions was occupied by diseases of the digestive organs. The most common pathology from the gastrointestinal tract (GI) in the adult population was chronic cholecystitis - 54 cases, the proportion was 43.5% of the total pathology of the GI, chronic gastritis (n= 47, 37.9%, respectively). Perhaps this is due to poor-quality drinking water, because 35 ppl. or every fifth respondent indicated in the questionnaire that he drinks untreated water from water supply systems extracted from water intake wells, which does not properly undergo complex water treatment, where the presence of radon, a uranium decay product, is also not excluded.

The second most frequent occurrence was occupied by DCS, which were mainly represented by hypertension, coronary heart disease (CHD) and cerebrovascular diseases (CVD). More than half of the respondents had hypertension (AH) (n=98 or 56.0%), including 25 who were diagnosed for the first time, which was 14.3%. AH is mainly represented in the 2nd stage - 45 people, the 1st and 3rd stages are exhibited in 25 and 28 people, respectively. CHD was detected in 25 respondents or 14.4% of all study participants, more than half of them were diagnosed for the first time (n=14, 8.0%, respectively), including the subacute stage of myocardial infarction in 1 patient who was urgently referred for inpatient treatment to the cardiology department of the central district hospital. CVDs were mainly represented by dyscirculatory (hypertensive) encephalopathy and the consequences of ischemic stroke (n=32, 18.3%, respectively).

Endocrine system diseases, eating disorders and metabolic disorders were next in frequency of occurrence among respondents. Taking into account the high frequency of overweight and obesity in the adult population, respectively, exogenous constitutional obesity prevailed among diseases of the endocrine system (77 cases, the proportion was 85.5% of the total pathology of the endocrine system). Type 2 diabetes mellitus was registered in 7 study participants (7.8%).

Among the diseases of the eye and its accessory apparatus, the most frequent pathology is mainly represented by retinal angiopathy (n=38 or 65.5% of the total pathology of the visual organs).

The most frequent pathology among diseases of the genitourinary system is represented by diseases of the female genital organs, among which cervical dysplasia was the leader (n=25 or 53.2% of all existing pathology of the genitourinary system).

Diseases of the musculoskeletal system and connective tissue were mainly represented by osteochondrosis of the spine (n=26), the proportion of all pathology in this structure was 72.2%.

Neoplasms occupied the VII rank position in the structure of pathological lesions, and were found in almost every 10th participant of the study (n=18 or 10.3 per 100 examined). Malignant neoplasms (MNP) were registered in 4 study participants: 2 women with cervical can-

Table 1

The structure of pathology of the adult population of the Aldan district

Class of diseases (ICD – 10)	Rank	Pathological lesions (per 100 examined)	Specific gravity (per 100 diseases)
A00-B99 Infectious and parasitic diseases	XIII	0.6	0.2
C00-D48 Neoplasms	VII	10.3	3.9
D50-D89 Diseases of the blood, hematopoietic organs and individual disorders involving the immune mechanism	XII	1.1	0.4
E00-E90 Diseases of the endocrine system, eating disorders and metabolic disorders	**III**	51.4	19.1
G00-G99 Diseases of the nervous system	VIII	4.0	1.5
H00-H59 Diseases of the eye and its accessory apparatus	IV	33.1	12.3
I00-I99 Diseases of the circulatory system	**II**	57.1	21.2
J00-J99 Respiratory diseases	IX	2.3	1.0
K00-K93 Diseases of digestive organs	**I**	57.7	21.4
M00-M99 Diseases of the musculoskeletal system and connective tissue	VI	20.0	7.4
N00-N99 Diseases of the genitourinary system	V	26.8	9.9
Q00-Q99 Congenital anomalies (malformations), deformities and chromosomal disorders	X	2.3	1.0
S00-T98 Trauma, poisoning and some other consequences of exposure to external causes	XI	1.7	0.7
All diseases		270.1	100.0

Table 2

The structure of pathology of the adult population of the Aldan district depending on gender

Class of diseases (ICD – 10)	Number per 100 men examined	Number per 100 examined women	χ^2	p
A00-B99 Infectious and parasitic diseases	0	0.9	0.609	>0.05
C00-D48 Neoplasms	7.6	11.9	0.843	>0.05
D50-D89 Diseases of the blood. hematopoietic organs and individual disorders involving the immune mechanism	0	1.8	1.225	>0.05
E00-E90 Diseases of the endocrine system. eating disorders and metabolic disorders	50.0	52.3	0.086	>0.05
G00-G99 Diseases of the nervous system	3.0	4.6	0.259	>0.05
H00-H59 Diseases of the eye and its accessory apparatus	27.3	36.7	1.647	>0.05
I00-I99 Diseases of the circulatory system	62.1	54.1	1.072	>0.05
J00-J99 Respiratory diseases	3.0	1.8	0.263	>0.05
K00-K93 Diseases of digestive organs	57.5	57.8	0.008	>0.05
M00-M99 Diseases of the musculoskeletal system and connective tissue	18.1	21.1	0.219	>0.05
N00-N99 Diseases of the genitourinary system	9.1	37.6	17.026	0.000
Q00-Q99 Congenital anomalies (malformations). deformities and chromosomal disorders	0	3.7	2.478	>0.05
S00-T98 Trauma. poisoning and some other consequences of exposure to external causes	1.5	1.8	0.025	>0.05
All diseases	239.2	286.1	0.303	>0.05

cer, 1 man with stomach cancer and 1 man with chronic lymphocytic leukemia. All patients are under the supervision of a therapist and oncologist, have no signs of disease progression. Three study participants with suspected urogenital tract infection were recommended to be examined at the Yakutsk Republican Oncological Dispensary. In the studies of P.M. Ivanov, the leading positions in the prevalence of the digestive and reproductive system in the industrial zone of Yakutia are shown, which causes cancer in patients with precancerous diseases [2; 5]. Other neoplasms are mainly represented by benign uterine fibroids.

For a more detailed analysis, we conducted a gender comparison of disease incidence (Table 2). In a comparative analysis of pathological lesions per 100 examined women, the incidence of one or another pathology was significantly higher compared to men, there were almost three diseases per woman. This is due

to the statistically significantly high incidence of pathology of the genitourinary system, mainly represented by diseases of the female genital organs. In the work of Semenova I.N. [1], the high incidence of genitourinary disease in working women compared with the male population of the mining province of Russia was also confirmed. In the gender comparison, men (n=41 or 62.1 per 100 examined) had significantly more DCS than women (n=57 or 54.1, respectively) (p=0.228). Otherwise, there were no statistically significant differences in pathological lesions. Diseases of the digestive system, endocrine system, eating disorders and metabolic disorders were registered equally often.

According to anthropometric indicators, such as the average BMI, no significant gender differences were obtained, in men it was 28.79 ±0.50 and 28.96±0.57 in women. According to the BMI values, 56 ppl. or 32.2% of the total number of study participants were overweight, obesity was registered in 77 people (44.2%). In a gender comparison, 27 men or 40.9% and 29 women had excess weight (26.9%) (χ^2=3.70 p=0.05). Obesity by BMI was relatively equally common in both men (n=29 or 43.9%) and women (n=48 or 44.4%), and did not differ significantly (χ^2=0.004, p=0.948).

Thus, in the non-indigenous population of the Aldan district of the Republic, overweight and obesity had a high incidence, being a risk factor for the development of cardiovascular diseases. The high pathological incidence of diseases of the digestive tract, cardiovascular system and endocrine pathology may be due to unfavorable exogenous factors, the nature of nutrition and lifestyle. Also, previously conducted comprehensive biomedical studies confirmed the high frequency of these nosologies [3].

Conclusion. Indicators of incidence of disease of the working population of the southern zone of Yakutia, being an additional link of social and hygienic monitoring, presented a real picture of disease prevalence among the population, making it possible to form forecasts of the prevalence of diseases. The high frequency of pathology on the part of the digestive, cardiovascular and endocrine systems in the able-bodied population of South Yakutia shows an unfavorable picture of the state of health, possibly due to the negative influence of both external and internal factors, as evidenced by statistical data on morbidity and mortality of the population of this region. And considering that the surveyed contingent belonged to a non-indigenous or "alien" population, the syndrome of chronic adaptive overstrain can be assumed. The newly identified pathology, including the suspicion of newly diagnosed malignant neoplasms, is alarming. Special attention should be paid to the high frequency of detection of pathology of the cardiovascular system during the comprehensive medical examination, which, if not diagnosed in time, could lead to fatal consequences. Perhaps the reason for this is the low level of medical care, low coverage of medical examinations or the lack of highly qualified specialists in the field.

In this article, we did not detail the influence of lifestyle factors of respondents and their work safety. The role of primary health care, including front-line doctors, is of great importance in the early detection and prevention of risk factors for the most common chronic non-communicable diseases and timely effective medical care, as well as organization of health schools for patients with hypertension, coronary heart disease, diabetes mellitus, gastritis, cancer, etc.

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HYDROCHEMICAL FACTORS AND MALIGNANT NEOPLASMS OF THE URINARY SYSTEM IN RESIDENTS OF THE PRILENSKY ZONE OF YAKUTIA

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In the Republic of Sakha (Yakutia), the incidence of malignant neoplasms of the urinary system from 2001 to 2015 increased by 1.9 times. The quality of the surface waters of the Lena River and its basin is affected by the economic activities of mining, energy, utilities, water transport, oil depots, agriculture with direct discharge of wastewater into them, as well as due to the ingress of pollutants into the adjacent settlements. territories. The purpose of the study is to assess the relationship between the incidence of malignant neoplasms of the urinary system organs and hydrochemical factors polluting the aquatic environment in the Prilensky zone of Yakutia. Correlation analysis was performed on the data on the incidence of MNs in the organs of the urinary system of the population (2001-2015) and the data on the chemical composition of water in the Prilensky zone of Yakutia (1979-1985). The results of the analysis indicate that Yakutia belongs to the territories with a rather pronounced contrast in terms of the incidence of cancer of the urinary system. It can be assumed that the territorial variability in the number of people with cancer of the urinary system organs may well be a reflection of the correlation of the incidence rate, both exogenous and endogenous "risk factors". During the analyzed period, strong direct correlations were established between the parameters of the urinary tract organs with the level of chlorides and synthetic surfactants (surfactants). Correlations of average strength with the level of nitrites, magnesium, transparency, color and mineralization of water in the territories of the Lena zone for 1979-1985 were also revealed.

Keywords: cancer, organs of the urinary system, hydrochemical factors, Republic of Sakha (Yakutia).

Introduction. Urinary tract cancer is common and includes a range of lesions ranging from small benign tumors to aggressive neoplasms with high mortality. The predominant malignant neoplasm (MN) of the urinary tract is bladder cancer [11]. Bladder cancer is the 10th most common cancer in the world and its incidence is steadily increasing worldwide, especially in developed countries [6]. According to GLOBOCAN, 550,000 people were diagnosed with bladder cancer in 2018. This represents approximately 3% of all new cancer diagnoses. The region with the highest rate of bladder cancer among women is Southern Europe (same as among men), where it is estimated that 26.5 men per 100,000 and 5.5 women per 100,000 of the population develop the disease each year [5]. The regions with the lowest incidence of bladder cancer are Central and West Africa, Central America with a below-average Human Development Index, possibly due to lower exposure to industrial chemicals and limited access to tobacco [6]. Urothelial cells lining the bladder and urinary tract are constantly exposed to potentially mutagenic environmental agents that are filtered into the urine by the kidneys [9]. In Russia, bladder cancer ranks 8th in the structure of oncological pathology in men and 18th in women [4]. The increase in the incidence of bladder cancer is associated primarily with the difficult diagnosis of the disease in the initial stages, due to the asymptomatic course, or the similarity of the clinic and symptoms with other diseases of the genitourinary organs. Most cases of bladder cancer are associated with the effect of carcinogenic substances excreted in the urine on the urothelium [1]. In the Republic of Sakha (Yakutia), the incidence of malignant neoplasms of the urinary system from 2001 to 2015 increased by 1.9 times. In particular, this pathology in men was 8.92%, in women 5.57% of all MNs in 2015 (Table 1) [2].

In Yakutia, the quality of drinking water is one of the key problems of preserving the gene pool, improving the healthcare system and promoting a healthy lifestyle for the population. One of the key objectives of research carried out in the RS(Y) is the identification of hydro-, biogeochemical environmental factors in the permafrost zone, which are important in the formation of high incidence rates of malignant neoplasms in the population. On the quality of surface waters of the river. The Lena and its basins are affected by the economic activity of mining, energy, utilities, water transport, oil depots, agriculture with direct discharge of wastewater into them, as well as due to the ingress of pollutants into the territories adjacent to settlements. Taking into account the insufficient knowledge of the role of hydrosphere factors in the pro-

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Table 1

Dynamics of the incidence of malignant neoplasms of the urinary system by five-year periods (2001-2015)

Years	Both sexes		Men		Women	
	n	%	N	%	n	%
2001	95	5.07	61	6.59	34	3.57
2005	106	5.43	72	7.27	34	3.54
2010	118	5.81	70	7.05	48	4.83
2015	181	7.16	107	8.92	74	5.57

cesses of carcinogenesis, we made an attempt to compare the incidence rates of the urinary system organs with the chemical factors of the aquatic environment.

The purpose of the study was to assess the relationship between the incidence of malignant neoplasms of the urinary system organs and hydrochemical factors polluting the aquatic environment in the Prilenskaya zone of Yakutia.

Material and research methods. We analyzed the annual reporting data of the State Budgetary Institution of the Republic of Sakha (Yakutia) "Yakutian Republican Oncological Dispensary" and materials on the chemical composition of the water of the river. Lena "Ministry of Nature Protection of the Republic of Sakha (Yakutia)". Aggregate data on the incidence of malignant neoplasms of the urinary system of the entire population (per 100 thousand) of three subzones (Nizhnelenskaya, Srednelenskaya and Verkhnelenskaya) of the Prilenskaya zone (II) of the Republic of Sakha (Yakutia) for the period 2011-2015 and the concentration of water chemicals in those same subzones of the Prilenskaya zone for the period 1979-1985. Taking into account the data of a number of studies that revealed the relationship of bladder cancer with harmful substances in drinking water, where the duration of their exposure was considered from 30 [7] to 40 years [10], we adopted a period and exposure of pollutants in water up to 30 years.

To determine the strength of the influence of polluting components of drinking water on the incidence of cancer of the urinary organs, we calculated the coefficients of pair correlation. Statistical data processing was carried out using the SPSS Statistics 19 application package. To study the relationships between variables, the procedure of the paired correlation method was used using the Pearson test, where r is the correlation coefficient. The Pearson correlation coefficient (r-Pearson) is used to study the relationship between two variables measured in metric scales on the same sample. The article presents the data of correlation analysis, the critical values of the significance level (p) of which were less than 0.05.

Results and discussion. The results of studies of other medical-geographical zones of Yakutia, taken into account on the basis of the involvement of their territories in the basins of the largest rivers, showed that high levels of growth in the general oncological incidence of the population are found along the riverbed of the Aldan (150%), Amga (116%) and Vilyui 113%. Compared to these zones, the same indicator in the Prilenskaya zone was 105%, in particular, in the Nizhnelenskaya subzone - 102%, Srednelenskaya - 117% and Verkhnelenskaya - 96%. The leaders in the growth rate for MN in the Prilenskaya zone are Megino-Kangalassky (158%), Zhigansky (123%) and Khangalassky (120%) districts. In men, the maximum rate of increase in the incidence of MN is observed in the Zhigansky district (175%), in women - in the Megino-Kangalassky district (154%) (Table 2). In the Russian Federation in 2010, 18.7 thousand patients with newly diagnosed kidney cancer were identified, which accounted for 3.6% of all MNs registered in the same year. For the period from 2001 - 2010. there was a significant increase in the incidence of kidney cancer, both in the male (from 3.6 to 4.3%) and female (from 2.6 to 3.0%) population. In 2010, the "rough" incidence rate of the entire population was 13.2 per 100 thousand people, which is 36.1% higher than in 2001 (10.2), including 15.7 in men, women - 11.1$^0/_{0000}$. The levels of standardized incidence rates for the male and female population were 12.1 and 6.6$^0/_{0000}$, respectively, and the average annual growth rate was 3.1 and 2.1%, respectively. The maximum incidence occurs in the age group of 70 years and older [3, 4].

For the period 2001-2015. in the Prilensky zone, the total number of patients registered with the first-ever diagnosed MN was 5953 patients, of which 467 patients had cancer of the urinary system, which accounted for 7.8% of the total number of registered patients and the overall incidence rate was 18, 8 per 100000 population. The most affected group in both populations was formed by people over 50 years old (73.6%). There were 29 children between the ages of 0 and 14 years old, which accounts for about 0.1% of cases of kidney cancer in the republic on average per year (in the Russian Federation in 2010 - 0.97%). An active increase in the incidence is observed starting from 40-49 years old, reaching a maximum (33.8) in the age group of 50-59 years, due to a high increase in the incidence rate, both in men (38.5%) and in women (30.8%). During the analyzed period, the consolidated incidence of cancer of the urinary system tended to increase (from 18.0 to 22.7$^0/_{0000}$) with an average annual growth rate of 4.75%. Thus, among the 10 administrative territories located along the Lena River, the Verkhnelenskaya subzone of 23.9% (from 7.6 to 22.2$^0/_{0000}$) stands out with the highest average annual rate of increase in incidence, where the gas and oil industries are developed, followed by followed by Srednelenskaya

Table 2

Dynamics of incidence rates of malignant neoplasms in the population of the Prilensky zone for five-year periods (per 100,000 population)

Territories			Both sexes			Men			Women		
Zone (subzone)		District	2001-2005	2011-2015	темп роста	2001-2005	2011-2015	темп роста	2001-2005	2011-2015	темп роста
Prilensky zone	Nizhnelenskaya	Bulunskiy	168.5	135.4	0.80	136.0	100.7	0.74	203.7	173.5	0.85
		Zhigansky	201.8	248.4	1.23	163.3	286.8	1.75	238.6	211.4	0.89
	Srednelenskaya	Gorny	147.2	146.7	1.00	150.3	158.9	1.06	144.3	135.1	0.94
		Kobyaisky	198.1	177.4	0.90	208.2	168.0	0.81	188.2	186.3	0.99
		M-Kangalassky	175.6	227.7	1.58	168.2	273.3	1.62	183.6	283.1	1.54
		Namsky	145.7	176.0	1.21	158.5	179.4	1.13	133.8	172.9	1.29
		Khangalassky	189.1	227.7	1.20	215.3	253.7	1.18	164.1	202.6	1.23
		Yakutsky	214.5	243.5	1.14	215.9	234.4	1.09	213.3	251.8	1.18
	Verkhnelenskaya	Lensky	252.9	207.6	0.82	260.6	187.2	0.72	245.1	228.0	0.93
		Olekminsky	243.6	265.0	1.09	276.0	279.5	1.01	211.8	250.7	1.18

(agriculture) - 3.05% (respectively from 19.8 to 23.0‰) and Nizhnelenskaya - 1.45% (from 14.7 to 15.8‰). One of the key tasks of onco-epidemiological research is to identify risk factors that are important in the formation of high incidence rates of cancer of the urinary system in some selectively taken climatic and geographical zones of the territory of Yakutia. An analysis of the annual indicators of the chemical composition of water in the territories of the Prilensky zone for 1979-1985 indicates a significant mosaic of the hydrochemical characteristics of the aquatic environment of the subzones of the Lena River (tabl.3).

Among the selected medical-geographical territories, the Verkhnelenskaya subzone stands out in terms of the level of pollution of the aquatic environment, belonging to the territories of intensive industrial development with the involvement of shift workers for work, where the infrastructure of diamond, oil, and gas production is predominantly developed. On the territories of the Upper Lena, which is part of the Irkutsk region (Vitim, Ust-Kut), there is the base of the Lena Shipping Company, which is one of the leading transboundary polluters of the Lena River. So, in the zone of the upper reaches of the Lena River (subzone of South Yakutia), the indicators of: bichromate oxidizability, the content of suspended solids and phenol are 4 or more times higher than those of the lower reaches (Arctic subzone) of the Lena River. From 3 to 4 times exceeded the indicators of contamination with oil products, mineral and total phosphorus, water-soluble CO_2 and nitride nitrogen. From 2 to 3 times the content of chlorides, total mineralization, magnesium, synthetic surfactants and from 1.2 to 2 times the content of silicon, calcium, sulfates, carbonates and biochemical oxygen consumption for 5 days. A relatively low content of zinc, water-soluble oxygen, iron and copper was found.

Our correlation analysis of the morbidity of the population of MNs of the urinary system (2011 and 2015) with hydrochemical factors in the subzones of the Prilensky zone (1979-1985) revealed the presence of strong direct relationships (at the level of p≤0.01) with the level of chlorides (r=0.996; p=0.004) and synthetic surfactants (surfactants) (r=0.997; p=0.007). Also, correlations of medium strength (at the level of p≤0.05) with the level of nitrites (r=0.972; p=0.028), magnesium (r=0.983; p=0.017); transparency (r=0.971; p=0.029), color (r=0.956; p=0.044) and mineralization of water (r=0.951; p=0.049).

Table 3

Comparative characteristics of the annual indicators of the chemical composition of water in the territories of the Lena zone for 1979-1985

Chemical factors	Total in the Prilenskaya zone	Subzones:			Ratios indicators	
		A-Nizhnelenskaya	B-Srednelenskaya	C-Verkhnelenskaya	A : B	A : Б
Suspended solids	15,1	5,9	12,8	25,8	4,4:1,0	2,2:1,0
Transparency	61,7	17,2	70,2	80,5	4,7:1,0	4,1:1,0
Acidity	7,26	7,38	7,29	7,11	1,0:1,0	1,0:1,0
Carbon dioxide	9,4	3,4	12,1	10,7	3,1:1,0	13,6:1,0
Oxygen	10,1	11,6	9,2	10,0	0,9:1,0	0,8:1,0
Bichromate oxidizability	34,7	8,7	43,7	49,9	5,7:1,0	5,0:1,0
Five-day biochemical oxygen demand	1,93	1,74	2,01	2,03	1,2:1,0	1,2:1,0
Chroma	35,0	22,8	40,3	38,4	1,7:1,0	1,8:1,0
Oil products	0,191	0,062	0,19	0,22	3,5:1,0	3,1:1,0
Phenols	0,006	0,002	0,004	0,008	4,0:1,0	2,0:1,0
Synthetic surfactants	0,044	0,022	0,049	0,043	2,0:1,0	2,2:1,0
N ammonium	0,13	0,1	0,17	0,1	1,0:1,0	1,7:1,0
N nitride	0,06	0,02	0,07	0,05	3,1:1,0	4,4:1,0
N nitrate	0,02	0,05	0,02	0,01	0,2:1,0	0,4:1,0
N common	0,27	0,29	0,33	0,21	0,7:1,0	1,1:1,0
Phosphorus mineral	0,032	0,005	0,066	0,018	3,6:1,0	13,2:1,0
Phosphorus total	0,051	0,011	0,091	0,037	3,4:1,0	8,3:1,0
Iron	0,15	0,35	0,16	0,13	0,4:1,0	0,5:1,0
Silicon	2,3	1,4	2,8	2,6	1,9:1,0	2,0:1,0
Copper	2,6	5,3	2,8	2,2	0,4:1,0	0,5:1,0
Zinc	12,3	14,1	11,9	12,0	0,9:1,0	0,8:1,0
Carbonate (HCO_3)	75,3	61,8	86,9	82,3	1,3:1,0	1,4:1,0
Sulfates	33,1	21,5	35,7	39,4	1,8:1,0	1,7:1,0
Chlorides	63,76	23,29	71,17	60,1	2,6:1,0	3,1:1,0
Calcium	32,32	19,16	33,13	35,84	1,9:1,0	1,7:1,0
Magnesium	9,32	4,54	9,57	9,64	2,1:1,0	2,1:1,0
Mineralization	276, 0	132,4	271,3	297	2,2:1,0	2,0:1,0
Hardness	1,88	1,33	2,16	2,06	1,5:1,0	1,6:1,0

Our findings of a strong association of chlorides with urinary tract malignancies are consistent with several epidemiological studies that have shown an association between bladder cancer risk and exposure to trihalomethanes, the main disinfection by-products of chlorinated water, with risks increasing significantly at exposure levels above 25 mcg/l and with more than 30 years of exposure to chlorinated water [10]. Surfactants are introduced into the reservoir in large quantities with domestic wastewater, with wastewater from industrial enterprises, with runoff from farmland. Over the past few years, there has been an increase in evidence of possible links between nitrate levels in drinking water and cancer risk. N-nitroso compounds are presumably carcinogens of the human bladder [8]. Assessing the role of individual hydrochemical environmental factors in the occurrence of cancer of the urinary system, it can be noted that under the influence of factors that often have a multidirectional effect on homeostasis, various pathophysiological, pathomorphological changes occur, leading to chronicity of diseases that are considered "precarcinogenic conditions".

Conclusion. The results of the analysis indicate that Yakutia belongs to the territories with a rather pronounced con-

trast in terms of the incidence of cancer of the urinary system. It can be assumed that the territorial variability in the number of patients with cancer of the organs of the urinary system of organs may well be a reflection of the correlation of the incidence rate, both exogenous and endogenous "risk factors".

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BIOCHEMICAL PARAMETERS OF THE BLOOD OF THE POPULATION IN THE ZONE OF INCREASED NATURAL RADIOACTIVITY

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An analysis of the main biochemical parameters of the population living in the territory with unfavorable natural radioactivity of 222Rn revealed changes in the biochemical spectrum of blood serum in the population of the Aldan region, indicating the presence of signs of disadaptation. Lipid profile changes are associated with gender and smoking. The shift of lipid metabolism towards atherogenicity is more pronounced in men due to an increase in the atherogenic fractions of lipids in the blood that are susceptible to peroxidation and a decrease in the level of the antiatherogenic fraction of lipids. The shift of metabolic flows towards catabolism, activation of glycolysis, dyslipidemia indicate the tension of carbohydrate and lipid metabolism, which is a sign of disadaptation of the body and the risk of developing environmentally conditioned diseases, including neoplasms.

Keywords: radon, metabolism, lipids, carbohydrates, disadaptation.

Introduction. One of the environmental risks for human health is the radiation hazard caused by the anomalous content of radioactive elements in nature. On the territory of the Republic of Sakha (Yakutia), the population of the Aldan region is exposed to a similar risk, where there is an increased natural radioactivity of the rocks of the Aldan shield and the close location of the Elkon uranium ore region, in which the main reserves of uranium in Russia are concentrated. From rock dumps, the decay product of uranium isotope radium radon (222Rn) enters water, soil and air. In the sources of drinking water supply and in the air of the premises of Aldan and Tommot, as well as nearby villages, its high content is often noted [1, 3].

Radon is considered a carcinogenic agent and is an element of risk to human health [12]. An indicator of negative environmental impacts on the body is the level of the state of health of the population with such the most objective indicator as mortality. The general death rates of the population of the Aldan region are high compared to the nationwide indicators (respectively 13.4-14.2 versus 8.6-7.8 per 100 thousand population). Mortality from diseases of the circulatory system occupies a leading place and exceeds more than 2 times the data for RS (Y) (741.7 versus 354.0 per 100 thousand population). The second place is occupied by mortality from neoplasms (in 2019 - 214.0 and in 2020 - 215.5 per 100 thousand population), the third - mortality from external causes (in 2019 - 146.9 and in 2020 . - 143.3 per 100 thousand population). In the structure of the general morbidity of adults for 2000-2018. diseases of the respiratory organs, the circulatory system, and the musculoskeletal system are of decisive importance [2].

Thus, in the conditions of unfavorable natural radioactivity in the Aldan region, the state of health of the population is characterized by a steady downward trend. And any shift from health to disease occurs on the basis of a gradual decrease in the adaptive reserves of the body associated with a violation of homeostasis, and the constancy of the internal environment of the body is achieved by biochemical adaptation, which allows maintaining the physiologically normal state of the body [6]. In this regard, the determination of biochemical parameters of blood serum is necessary not only to identify pathological processes occurring in the body, but also to assess the signs of disadaptation.

Target. Assessment of biochemical parameters of the adult population living

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in the territory under the influence of natural sources of radiation to identify signs of disadaptation of the body.

Material and research methods. In the spring of 2022, a comprehensive medical and biological examination of 175 adults in the city of Aldan and the city of Tommot, Aldan district, was carried out, there were 66 men (37.7%), women - 109 (62.3%). The mean age was 44.0 (35.0; 52.0) ME, for men 45.0 (35.0; 53.0), for women 42.0(36.0; 51.0). The study was carried out as part of the research work of the YSC KMP "Regional features of biochemical, immunological and morphological parameters in the indigenous and alien population of the Republic of Sakha (Yakutia) in normal and pathological conditions" (FGWU-2022-0014) and the research work of the Academy of Sciences of the Republic of Sakha (Yakutia) "Evaluation of exposure levels of the population of the Aldan region due to natural sources of exposure and recommendations for the implementation of protective measures to reduce them». The study was conducted with the informed consent of the subjects and was approved by the decision of the Local Ethics Committee at the FSBSI "YSC CMP".

The material of the study was venous blood serum. Blood sampling was carried out from the cubital vein in the morning from 8 to 10 am on an empty stomach after a 12-hour abstinence from food. To assess the state of lipid metabolism, total cholesterol (TC), high-density lipoprotein cholesterol (HDLC), triglycerides (TG) were determined by the enzymatic method on an automatic biochemical analyzer "Labio" using reagents "Analyticon" (Germany). LDL-C and VLDLC were calculated using the formula of Friedewald et al. (1972). Atherogenic coefficients (AC) were calculated according to the formula proposed by A.N. Klimov: AC=(TC - HDLC)/HDLC (Klimov, Nikulcheva, 1999). Enzyme activities were determined: alanine aminotransferase (ALT), aspartate aminotransferase (AST), glutamyl transpeptidase (GGT), alkaline phosphatase (ALP), creatine kinase (CK), lactate dehydrogenase (LDH), blood serum levels of glucose, uric acid, urea, creatinine, total protein and albumin.

To determine the frequency of lipid metabolism disorders, the Russian recommendations of the VII revision of the Russian Society of Cardiology in 2020, compiled taking into account the European recommendations of 2019, were used. The level of total cholesterol ≥5.0 mmol / l (190 mg/dl) was taken for hypercholesterolemia (HCH) taking into account the risk cardiovascular death on the SCORE scale, elevated LDL-C >3.0 mmol/l (115 mg/dl) at low, >2.6 mmol/l at moderate, >1.8 mmol/l at high, >1 .4 mmol/l at very high and extreme risk, reduced HDLC - HDLC ≤1.0 mmol/l (40 mg/dl) in men and 1.2 mmol/l (46 mg/dl) in women . Hypertriglyceridemia (HTG) was defined as a TG level of >1.7 mmol/l (150 mg/dl). Fasting hyperglycemia was established at a glucose level of >5.6 mmol/l. Participants receiving specific medical treatment for these conditions were also classified as having these disorders.

Statistical data processing was carried out using the IBM SPSS Statistics 23 application software package. Standard methods of variation statistics were used: calculation of average values (M), mean error (m). The normality of the distribution was verified by the Kolmogorov-Smirnov method. In the case of a normal distribution of quantitative indicators, the Student's t-test was used to evaluate statistical hypotheses, and in the case of a deviation from the normal distribution, the Mann-Whitney U-test was used. Evaluation of relationships between variables was carried out using a paired correlation method using Pearson's criteria (for metric variables) and Spearman's (for variables measured in a rank scale), where r - is the correlation coefficient, p -is the significance of the result.

Results and discussion. The average values of all biochemical blood parameters of the examined sample did not go beyond normal values, except for a slight excess of total cholesterol (TCH) and triglycerides (TG). Gender comparison showed that ALT and ALP enzymes are significantly active in men, and the state of lipid metabolism turned out to be worse, as evidenced by the increased value of the atherogenic coefficient (AC) (p=0.017), which reflects the degree of risk of developing atherosclerosis and related heart diseases and vessels. The imbalance of the lipid profile in men is caused by a significantly high level of total cholesterol, a relatively low level of the anti-atherogenic lipid fraction (HDLC) (p=0.000) and a relatively high level of the atherogenic lipid fraction (LDLC) (p=0.000). In women, despite the high level of TG, AC was within the normal range due to a compensatory increase in HDLC cholesterol levels. The content of creatinine, uric acid and albumin within the normal range was significantly higher in men, and the content of urea in women (p<0.05) (Table 1).

The correlation analysis was carried out between the activity of enzymes (ALT, AST, GGT) involved in glucose homeostasis showed a close direct correlation at a level above r=0.600 (p=0.000). The activity of LDH (r=0.366; p=0.000), GGT (r=0.211; p=0.006) and CK (r=0.295; p=0.000) positively correlated with the glucose level. In addition, glucose concentration was associated with total protein (r=0.456; p=0.000), albumin (r=0.389; p=0.000), urea (r=0.224; p≤0.004), uric acid (r=0.242 p≤0.001).

Direct correlations of enzyme activity with the content of total cholesterol were obtained: ALT (r=0.261; p=0.001), AST (r=0.341; p=0.000), GGT (r=0.314; p=0.000), ALP (r=0.324; p =0.000).

The close correlations between enzymes and substrates are explained by the fact that the constancy of six biochemical blood parameters (total protein and albumin, glucose and cholesterol, urea and creatinine) is ensured by a numerous system of enzymes. The main enzymes involved in the main transamination flows are AST and ALT, where AST ensures the entry of substrates into the tricarboxylic acid cycle, i.e. supports adequate bioenergetics and thermogenesis, and ALT ensures the operation of the glucose-alanine shunt to maintain a constant blood glucose level. GGT, ALP, LDH and CK are also involved in the same metabolic pathways and are also the main enzymes that maintain homeostasis. All enzymes are involved in the processes of adaptation to new environmental conditions, loads, etc. The conditional general marker of metabolism is the de Ritis coefficient (AST/ALT), where AST shows the level of catabolism, ALT - the level of anabolism, while metabolic equilibrium is achieved at a value equal to 1.5, and fluctuations from 1.2 to 1.6 are taken as adaptive range [4, 5]. In clinical biochemistry, the de Ritis coefficient is used in the case of high enzyme activity to diagnose cardiac (>1.5) or hepatic (<1.5) pathology.

According to our data, the average value of the ratio of AST to ALT within the reference values was 2.18, which indicates an adaptive metabolic shift towards the predominance of the catabolic orientation of metabolic flows and is especially brighter in women (p=0.022).

Despite the average value of biochemical parameters within the normal range, significant differences were found in the frequency of a decrease or increase in certain indicators associated with gender (Table 2).

The frequency of lipid spectrum disorders. The level of total cholesterol (TCH) was high in 54.5% of men and 37% of women. A high value of triglycerides (TG) was found in 42.4% of men and 37.7% of women. Fluctuations in the levels of TCH and TG did not have

a significant association with gender, but violations of TCH are much more often recorded in men, which is confirmed by a significant high average value of total cholesterol (5.65±0.23 mmol/l).

Changes in the content of HDLC, LDLC and VLDLC are significantly associated with gender, which also indicates the dependence of changes in the atherogenic coefficient (AC) on these indicators. HDLC levels were mostly normal or high. Its value within the reference values was 14.5% more common in men, and an elevated level slightly above the upper limit of normal (>2.2 mmol/l) was 2 times more common in women (p=0.000). Among the surveyed, low HDLC was extremely rare - in two men. The content of LDLC in normal values was found in 80.4% of women and 56.1% of men, therefore, an increased level of this atherogenic lipid fraction is more than 2 times more common in men (p=0.001).

The value of AC within the normal range was significantly more common in women (94.4%), in men this figure was 21.7% lower. Accordingly, a high AC value was more often found in men (p=0.000).

Comparison of the average values of biochemical parameters in smokers and non-smokers showed significant differences in the content of lipid profile indicators. In smokers, the level of HDLC is significantly lower, and LDLC and AC are significantly higher. The imbalance of the lipid profile is especially pronounced in smoking men, as evidenced by the AC of smoking men, equal to 4.72, and this is 2 times higher than in non-smoking men (Fig. 1). Correlation analysis also showed a negative relationship of smoking with HDLC (r=-0.233; p=0.003), a direct relationship with LDLC (r=0.156; p=0.047), VLDLC (r=0.164; p=0.038) and AC (r=0.257; p=0.001) (Fig.1).

The frequency of violations of enzyme activity. The average activity of enzymes transaminases ALT, AST, as well as alkaline phosphatase, LDH and CC were within the reference values (table 1). Depending on gender, there was a significant difference in the activity of ALT and ALP within the normal range: in men, the activity of these enzymes was higher (Table 1).

Violations of the activity of enzymes ALT, AST, ALP occurred in isolated cases. Decreased or increased activity of LDH was significantly more common in women (23.6%) (p = 0.000). LDH is an enzyme involved in the final reaction of glycolysis under anaerobic conditions (the conversion of pyrubate to lactate). LDH maintains acid-base balance, and is stable, providing a constant pH level. If we take as the norm the reference indicator of LDH equal to 250 IU/l, then 78% of the population of the Aldan region has an increased activity of this enzyme. Moderately high LDH activity and elevated glucose levels indicate an intensification of anaerobic glycolysis. Glycolysis is a central metabolic pathway, and intermediates are the branching point of other pathways, including amino acid and fat synthesis pathways. The rate of glycolysis in tumor cells is 200 times higher than in normal cells; The source of energy for a rapidly growing tumor in a state of hypoxia is glucose. This phenomenon was described by Otto Warburger (1930) and called the Warburger effect, according to which the primary cause of cancer is dysfunction of mitochondrial metabolism [8]. While the dependence of cancer cells on glycolysis for energy production is well understood, the role of adipocytes and lipid metabolism reprogramming in the energy support of cancer growth is still being studied. Changes in lipid metabolism lead to changes in membrane composition, protein distribution and function, gene expression and cellular functions, and cause the development and progression of many diseases such as inflammation, hypertension, diabetes, liver disease, heart disease, kidney disease, neurological disorders, cystic fibrosis and cancer [10, 14, 15].

In science, correlations of radon with the incidence of lung cancer are known, especially in smokers [11, 13] and, possibly, kidney cancer, melanoma, hematological cancer and primary brain tumors are associated with long-term exposure to high doses of radon [9, 14]. In terms of the severity of the increase in oncological morbidity rates, the Aldan region is in second place (1.50%) after the Ust-Maisky region (1.79%) [2]. In terms of the prevalence of malignant neoplasms among the population of the Aldan region, the first place is occupied by neoplasms of the digestive system (25.9%), then the respiratory system (13.0%) and then the urinary system (6.3%).

The increase in GGT activity did not depend on gender; in both groups, the percentage of excess enzyme activity was 30% each. The Mann-Whitney test revealed a direct relationship between GGT activity and smoking (p=0.043). The average GGT activity in smokers (n=53) was 44.89±4.75U/l, in non-smokers -

Table 1

Indicators of biochemical parameters of blood in residents of the Aldan region

Indicator	Reference	Mean value (M±m)			P
		Total n=173	Men n=66	Women n=107	
Enzymes					
ALT <30 U/l	<30 U/l	15.62±0.66	17.39±0.85	14.53±0.92	0.024
AST	<40 U/l	30.56±1.05	32.62±1.61	29.29±1.38	0.120
AST/ALT	1,2-1,6	2.18±0.05	2.01±0.07	2.28±0.07	0.022
ALP	<258U/l	145.02±4.17	163.21±6.20	133.80±5.29	0.000
GGT <32, m	w.<32, m. <50U/l	39.36±2.97	45.09±3.83	35.82±4.15	0.103
LDH	225-450E/l	335.28±6.72	321.36±9.60	343.86±9.04	0.090
CK	<190U/l	122.62±13.02	117.58±12.91	125.73±19.54	0.728
Lipids					
Total cholesterol	<5,0 mmol/l	5.37±0.10	5.65±0.23	5.20±0.08	0.038
TG	<1,7 mmol/l	1.89±0.10	1.80±0.12	2.02±0.19	0.304
HDLC	>1,0 mmol/l	1.98±0.04	1.70±0.06	2.15±0.05	0.000
LDLC	<3,0 mmol/l	2.53±0.09	3.03±0.19	2.22±1.00	0.000
VLDLC	<1,5 mmol/l	0.86±0.04	0.91±0.09	0.82±0.05	0.396
Atherogenic coefficient	<3,0	2.23±0.33	3.26±0.86	1.60±0.07	0.017
Substrates					
Glucose	3,3-5,5 mmol/l	5.22±0.06	5.14±0.08	5.26±0.10	0.354
Urea	1,7-8,3 mmol/l	3.60±0.13	3.13±0.273	3.89±0.16	0.008
Creatinine<80, m	f. <80, м. <97 nmol/l	90.08±1.22	99.33±1.80	84.55±1.38	0.000
Urine acid	f. <357, м. <488 μmol/l	324.12±6.01	358.48±9.73	302.92±6.92	0.000
Tot. protein	65-85 g/l	78.11±0.58	77.64±0.85	78.39±0.77	0.518
Albumin 34 -48g/l	34 -48 g/l	46.45±0.39	47.45±0.78	45.82±0.40	0.046

Table 2

The frequency of violations of biochemical parameters men and women of Aldan region, n/%

Indicators	Gender		Pearson Chi-square	df	p
	Men	Women			
Total cholesterol (3.6-5.0 mmol/l)					
norm	28/42.4	65/60.7	5.536	2	0.063
<3.6	2/3.1	2/1.9			
>5.0	36/54.5	40/37.4			
Triglycerides (<1.7 mmol/l)					
norm	38/57.6	66/62.3	0.373	1	0.541
>1.7	28/42.4	40/37.7			
HDLC (0.78-2.2 mmol/l)					
norm	55/83.3	64/59.3	18.397	2	0.000
<0.78	2/3.0	0/0.0			
>2.2	9/13.6	44/40.7			
LDLC (<3.0 mmol/l)					
norm	37/56.1	86/80.4	11.548	1	0.001
>3.0	29/43.9	21/19.6			
VLDLC (0.8 - 1.5 mmol/l)					
norm	59/89.4	101/92.5	11.548	1	0.001
>1.5	7/10.6	6/5.6			
Atherogenic coefficient (<3)					
norm	48/72.7	101/94.4	15.741	1	0.000
>3.0	18/27.3	6/5.6			
ALT (<30 E/l)					
norm	64/97.0	104/96.3	0.057	1	0.812
>30	2/3.0	4/3.7			
AST (40 E/l)					
norm	61/91.0	98/90.7	0.005	1	0.946
>40	6/9.0	10/9.3			
De Ritis coefficient (1.2-1.6)					
Норма	17/25.8	23/21.3	2.294	2	0.318
>1.6	49/74.2	83/76.9			
GGT (women - 7-32 E/л; men - 11-50 E/l)					
norm	45/68.2	77/72.0	0.279	1	0.597
> norm	21/31.8	30/28.0			
LDH. E/l					
norm	64/97.0	81/76.4	15.808	2	0.000
<225	1/1.5	14/13.2			
>450	1/1.5	11/10.4			
Glucose (3.3-5.5 mmol/l)					
norm	52/78.8	75/71.4	1.169	1	0.280
>5.5	14/21.2	30/28.6			
Urine acid (women 155-357; men 268-488 µmol/l					
norm	57/86.4	85/78.7	8.296	1	0.004
> norm	4/6.1	23/21.5			
< norm	5/7.6	0/0.0			
Urea (1.7-8.3 mmol/l)					
norm	50/75.8	98/90.7	7.442	2	0.024
> norm	3/ 4.5	3/2.8			
< norm	13/19.7	7/6.5			
Creatinine (men <97; women <80 µmol/l)					
norm	25/39.7	48/44.4	2.359	2	0.307
> norm	38/60.3	58/53.7			
< norm	0/0.0	2/1.9			
Total protein (65-85 g/l)					
norm	58/87.9	78/74.3	8.756	2	0.020
<65.0	4/6.1	4/3.8			
>85.0	4/6.1	23/21.9			
Albumin g/l (34-48 g/l)					
norm	45/68.2	81/76.4	2.184	2	0.336
<34	1/1.5	3/2.8			
>48	20/30.3	22/20.8			

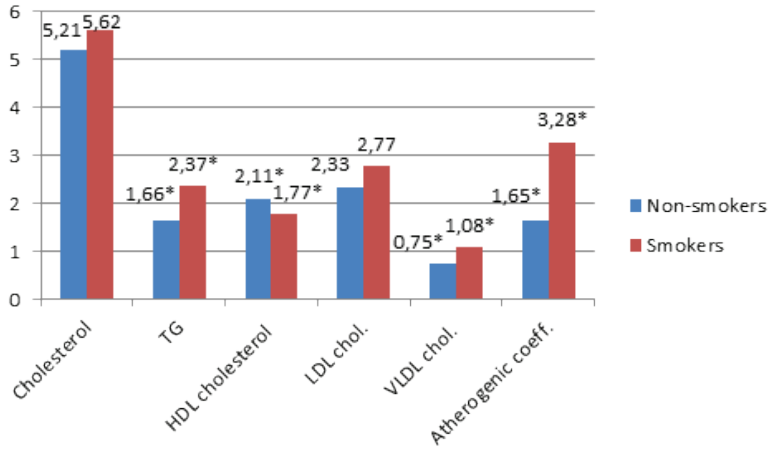
39.20±4.94U/l. GGT is one of the body's detoxifying systems and is a marker of intoxication and allergization. The main localization of HHG is in the liver. A slight activation of the GGT enzyme is an adaptive mechanism caused by a deficiency of amino acids in the blood and opens access to the proteins of internal organs in order to eliminate hypo- and dysproteimemia [4].

Hypoproteinemia among the population of Aldan was found only in 4 men and 4 women, hypoalbuminemia in 1 man and 3 women. Total protein and albumin are constant indicators. Their mean values were within the normal range, and the revealed fluctuations in the level of total protein were significantly associated with gender (p=0.001): in men -12.1%, in women - 26.7%. Elevated levels of total protein were 3.6 times more common in women (21.9%), possibly due to the fact that high levels of total protein are associated with stress. Maintaining the optimal level of total protein is controlled by transaminases ALT, AST and GGT. Residents of the Aldan region showed significant correlation coefficients for the level of total protein and albumin with the level of glucose (0.456; p=0.000 and 0.384; p=0.000, respectively) and LDH activity (0.578; p=0.000 and 0.426; p=0.000, respectively), which shows conjugation of carbohydrate and protein metabolism.

Urea and creatinine - products of nitrogen metabolism in the blood are one of the key indicators of kidney activity. Evaluation of violations of urea and creatinine content showed a gender association of changes in its level. High rates were found in 3 men and 3 women, and low rates were relatively more common, especially in men (Table 2). A decrease in the concentration of urea in the blood occurs during stress and is the result of an increased inclusion of nitrogen in blood proteins in the metabolic mechanisms of the body. The average level of urea in both groups was within the normal range, but significantly higher in women. Determining the level of urea is necessary to assess the intensity of catabolism and depends on the ratio of transaminase activity [5].

Mean creatinine levels were slightly elevated in both men and women (Table 1). The frequency of its moderate increase is not associated with gender. The percentage change in its level was 60.3% for men and 55.6% for women. The slight increase in creatinine may be related to muscle volume.

Glucose abnormalities above the upper limit of normal were not associated with gender (p = 0.280). An increase in



Lipid profile indicators depending on smoking

glucose levels was detected in 14 men and 30 women, which in percentage terms was 21.2% and 28.6%, respectively. In 6 people (3.4%), a high glucose level exceeding more than 10 mmol/l was determined, of which three did not have an established diagnosis. The correlation coefficient of glucose with TG level was r=0.445; p=0.000, with VLDLC - r=0.466; p=0.000.

Uric acid is a low molecular weight antioxidant. Its average value, both in men and women, is within the normal range, and changes in its level are also associated with gender: in women, an increased content of uric acid was 3.5 times significantly more common than in men (p<0.004).

Table 3 shows a comparative analysis of the biochemical parameters of women living in Aldan and Tommot. The average content of all biochemical parameters in both groups is within normal limits, except for TG and glucose in women from Tommot, which slightly exceeded the upper limit of normal (p=0.000). Despite the higher value of total cholesterol in the group of women in the city of Aldan, and TG in the group of women in the city of Tommot in both groups of AC was within the normal range. This is due to the fact that women in the city of Aldan have a higher level of HDLC, and in women in the city of Tommot the value of the antiatherogenic fraction of lipids - LDLC is lower than in women in the city of Aldan (p<0.000). In addition, in the group of women in Tommot, activation of ALT, LDH, and CK enzymes is noticeable. Indicators of carbohydrate and protein metabolism: the level of glucose, urea, uric acid, total protein and albumin were significantly higher than in women from Aldan (p<0.05) (Table 3).

The city of Tommot is located 40 km to the southeast, and the city of Aldan is 50 km east of the Elkon uranium ore region. The population surveyed by us is not employed directly in mining enterprises, so the effect of ionizing radiation is indirect. However, annual measurements of radioactive radiation from drinking water and indoor air show an unfavorable situation. In 2021, in 87 samples (20.4%) from underground sources of drinking water supply in the Aldan region, an excess of radon content (222Rn) was detected, and in 46 rooms of public buildings the value of ERVA of radon exceeded sanitary standards, the maximum recorded value of ERVA of 222Rn was 429 ± 86 Bq /m3 [1]. At low temperatures in rooms with thermal insulation and lack of ventilation, the radon concentration reaches significantly higher values [7,12], and the cold period in Yakutia lasts 6 months. Further studies of the health status of the working contingent of mining enterprises are needed.

Thus, in the population of the Aldan region, changes in the biochemical spectrum of blood serum indicate the presence of signs of disadaptation. Changes in the lipid profile are associated with gender. Violation of lipid metabolism in the direction of atherogenicity is significantly more common in men due to an increase in atherogenic fractions of lipids in the blood, susceptible to peroxidation and a decrease in the level of HDLC, especially in smokers. The observed shift

Table 3

Biochemical blood parameters in women in Aldan and Tommot

Indicator	Reference	Women, Mean value (M±m)			P
		Total n=107	Aldan n=57	Tommot n=50	
Enzymes					
ALT	<30 mmol/l	14.53±0.92	12.61±0.73	16.90±1.73	0.026
AST	<40 mmol/l	29.29±1.38	27.00±1.03	31.62±2.68	0.096
AST/ALT	1.2-1.6	2.26±0.07	2.36±0.09	2.15±0.11	0.160
ALP	<258 E/л	133.80±5.29	149.42±7.54	112.92±6.40	0.055
GGT	f.<32. м.<50 E/л	30.13±2.16	26.82±2.32	33.98±3.77	0.099
LDH	225-450 E/л	343.86±9.04	288.14±9.16	400.02±8.16	0.000
CK	<190 E/л	125.73±19.54	66.36±6.36	152.42±9.43	0.000
Lipids					
Total cholesterol	<5.0 mmol/l	5.20±0.08	5.32±0.13	5.04±0.08	0.094
TG	<1.7 mmol/l	2.02±0.19	1.32±0.13	2.37±0.15	0.000
HDL cholesterol	>1.0 mmol/l	2.15±0.05	2.14±0.07	2.17±0.06	0.789
LDL cholesterol	<3.0 mmol/l	2.22±1.00	2.55±0.12	1.85±0.13	0.000
VLDL cholesterol	<1.5 mmol/l	0.82±0.05	0.60±0.06	1.08±0.07	0.000
Atherogenic coefficient	<3.0	1.60±0.07	1.64±0.105	1.53±0.11	0.487
Substrates					
Glucose	3.3-5.5 mmol/l	5.26±0.10	4.93±0.12	5.83±0.17	0.000
Urea	<8.3 mmol/l	3.97±0.19	2.84±0.14	5.34±0.28	0.000
Creatinine<80, m	<97 µmol/l	84.55±1.38	83.58±1.74	85.54±2.22	0.454
Urine acid	f.<357. м.<488 µmol/l	302.92±6.92	277.72±8.53	331.14±9.77	0.000
Total protein	65-85 g/l	78.39±0.77	73.60±0.90	83.75±0.77	0.000
Albumin	34 -48 g/l	45.82±0.40	43.94±0.55	47.87±0.42	0.000

of metabolic flows towards catabolism, activation of glycolysis, dyslipidemia indicate a decrease in the body's adaptive reserves. Further intensification of enrichment of uranium ores will increase the impact of ionizing radiation on the population, which will require the continuation of biomedical and environmental research to prevent environmentally caused diseases.

Reference

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HIV-INFECTION AS A CAUSE OF DISABILITY OF THE POPULATION

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The article presents an analysis of the data of the state statistical reporting on the disability of the population of the Irkutsk region due to HIV infection and dispensary observation of patients. Changes in the stages of HIV infection, including the stage of secondary manifestations, are shown. The median of the average age of patients by disability groups among men and women was calculated, the structure of the causes of disability was presented. A forecast is given regarding the change in disability groups for 2022. Measures to increase the adherence of citizens with HIV infection to antiretroviral therapy, the prevention of secondary and opportunistic diseases will prevent early disability of the population.

Keywords: HIV infection, stages of HIV infection, disability, causes of disability.

Introduction. The HIV pandemic continues, influencing the main indicators characterizing the state of public health. The Health Development Strategy until 2025 defines HIV infection as a “threat to national security in the field of public health”. As of December 31, 2021, 0.8% of the population in the Russian Federation was living with HIV, including 1.5% of those aged 15–49 years. In 2021, the Irkutsk region was the leader in terms of incidence (99.6 versus 47.8 per 100 thousand in the Russian Federation); in terms of damage, it ranked second after the Kemerovo region with an indicator of 2042.5 per 100 thousand (http://www.hivrussia.info). Currently, HIV infection is classified as a chronic, treatable infection [7]. Thanks to antiretroviral therapy (ART), the life expectancy of patients with HIV infection increases. As a result, the social significance of HIV infection increases, including the disability of the population [6, 8]. General indicators of disability due to HIV infection in the Irkutsk region were studied until 2019 [4, 5]. However, given

the social and economic significance of HIV infection in modern conditions, there is a need for further study of the problem.

The purpose of the study: to study various aspects of the primary disability of the adult population due to HIV infection in 2010-2021.

Materials and research methods. The work used forms No. 7-Sobes, No. 61 for 2010-2021. The analysis of disability groups depending on the stage of HIV infection, the list of secondary diseases, gender distribution was carried out on the basis of an electronic database of examined citizens for 2021 (n=247). Descriptive epidemiology and statistical methods were used. Spearman's correlation coefficient, χ^2 were calculated using the Epi-Info program.

Results and discussion. In 2021, 30,095 HIV-infected patients were registered in the Irkutsk region, of which 29,781 people (98.9%) were under dispensary observation. For the period 2010-2021 there were changes in the number of patients according to the stages of the disease. Thus, the number of persons with stages 2 and 3 of the disease decreased ($\chi2$=42.6 and 7175.2, respectively, p<0.001); with stage 4, on the contrary, increased significantly and amounted to 669.9 per 1000 under observation ($\chi2$=7274.3; p<0.001) (Table 1).

The number of patients with progressive secondary diseases is increasing. The spectrum and frequency of this pathology is presented in [2]. The fourth clinical stage of HIV infection - the stage of secondary manifestations - is classified depending on the clinical symptoms and nosologies into stages 4A, 4B, 4C [1]. The results of the analysis demonstrate an increase in the share of stage 4A to 55.6% and a decrease in the share of stage 4B to 27.2% by 2021. The share of stage 4C at the beginning of the study period was dynamically decreasing, since 2017 it was at the level of 17.0% (Fig. 1). Thus, the proportion of people with progressive HIV infection is increasing.

The spread of HIV infection inevitably affects the disability of the population - the Spearman correlation coefficient was 0.83 (p<0.05).

The dynamics and structure of disability in the population of the Irkutsk region due to HIV infection have been studied previously [4,5]. In 2021, according to the Federal State Institution ITU State Security for the Irkutsk Region, 247 people were recognized as disabled due to HIV infection for the first time, including 166 men (67.2%), 81 women (32.8%). Residents of urban settlements prevailed, the share was 86.2%. The median mean age of men and women was 40 years.

In the structure of primary disability due to HIV infection, the proportion of disabled people of group II significantly prevailed - 58.3% versus 14.6% (group I) and 27.1% (group III), both among men and women. In general, the proportion of disabled people of groups I and II remains at a high level [4], which confirms the severe course of the disease. An analysis of the stages of HIV infection by groups showed that in disabled people of group I, stage 4B prevailed, the share of which was 86.1%, stage 4B in disabled people of groups II and III - 51.7 and 56.1%, respectively. It draws attention to the fact that in women of II disability group 4B stage prevails (56.3%) (Fig. 2).

Thus, there are significant changes in the structure of disability by groups: the proportion of disability group I increased by 1.5 times over the observation period (9.8% in 2010 versus 14.6% in 2021). If this trend continues, the proportion of dis-

Table 1

Stages of HIV infection in persons under dispensary observation for 2010-2021 in comparison according to f. №61 (per 1000 patients)

Stages of HIV infection	2010		2021		$\chi2$; p
	was under supervision	per 1000 registered patients	was under supervision	per 1000 registered patients	
Stage 2 (primary manifestations A, B, C)	64	3.8	29	0.9	42.6; p<0.001
Stage 3 (subclinical)	12212	739.5	9801	329.1	7175.2;p<0.001
Stage 4 (secondary manifestations A, B, C)	4237	256.6	19951	669.9	7274.3; p<0.001
Total	16513	-	29781	-	

Table 2

Structure of primary disability depending on sex and age in the Irkutsk region in 2021 (absolute number / %)

age groups	men						women						total					
	groups (abs.number)			groups (%)			groups (abs.number)			groups (%)			groups (abs.number)			groups (%)		
	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III
up to 29	1	5	1	4.3	5.3	2.1	1	3		7.7	6.3		2	8	1	5.6	5.6	1.5
30-34	1	14	5	4.3	14.6	10.6	4	8	5	30.8	16.7	25.0	5	22	10	13.9	15.3	14.9
35-39	9	23	18	39.1	24.0	38.3	4	8	5	30.8	16.7	25.0	13	31	23	36.1	21.5	34.3
40-44	4	33	16	17.4	34.4	34.0	4	16	6	30.8	33.2	30.0	8	49	22	22.2	34.0	32.8
45-49	4	18	4	17.4	18.8	8.5		8	3		16.7	15.0	4	26	7	11.1	18.1	10.4
50-54	1	3	1	4.3	3.1	2.1		2	1		4.2	5.0	1	5	2	2.8	3.5	3.0
55-59	2		2	8.7		4.3		2			4.2		2	2	2	5.6	1.4	3.0
60+	1			4.3				1			2.1		1	1	0	2.8	0.7	
total	23	96	47	13.9	57.6	28.5	13	48	20	16.0	59.3	24.7	36	144	67	14.6	58.3	27.1

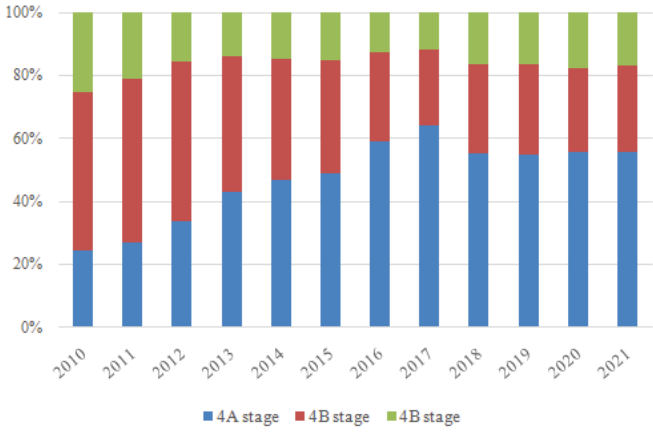

Fig. 1. Structure of the 4th stage of HIV infection depending on secondary manifestations in 2010-2021 (%)

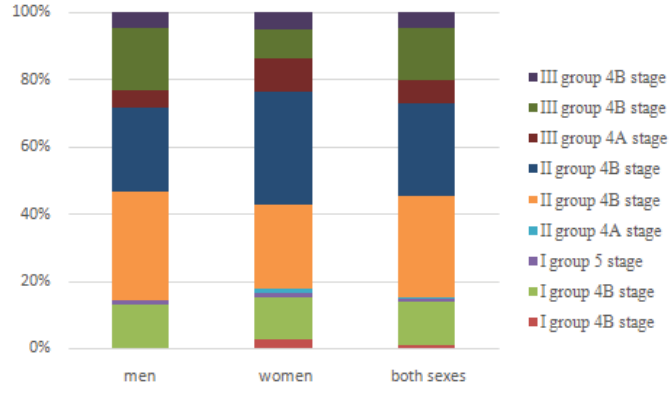

Fig. 2. Share of HIV infection stages by disability groups (%)

ability group I is expected to increase in 2022 to 15.5±2.2%.

Persons of working age predominate in the age structure among men and women (Table 2). The median average age for disability groups (I, II, III) for men was: 40; 41 and 39 years old; in women - 37; 40.5 and 39.5 years respectively.

The progression of HIV infection, the development of secondary and concomitant diseases is determined by a number of factors, including the presence or absence of ART. UNAIDS, one of the main directions in the fight against HIV infection, has set goals according to which by 2030 95% of people living with HIV/AIDS (PLWHA) should have information about their HIV status, 95% of them should have access to ART and 95% should achieve viral load suppression. Today it is considered a proven fact that ART improves the quality of life and increases the life expectancy of patients, which, according to various authors, can be the same as in the general population (without HIV infection) [6]. An important aspect of achieving these goals is adherence to ART [3].

According to the study, during the observation period in the Irkutsk region, the number of people receiving ARVT increased by 4.6 times, in 2021 the therapy coverage rate was 82.2%. However, among persons recognized as disabled, the proportion of patients in the remission phase slightly exceeded the proportion of persons in the progression phase: 56.5% versus 43.5%, and 72.1% of patients were in the progression phase while taking antiretroviral drugs.

In the structure of conditions associated with HIV infection, in accordance with ICD-10, in persons recognized as disabled for the first time, the proportion of infectious and parasitic diseases prevailed (B20, including B20.0-B20.9) - 83.4 %. Among which, the share of mycobacterial infection (B20.0) accounted for 52.0%; HIV with manifestations of multiple infections (B20.7) - 32.8%. The proportion of people with disabilities due to HIV with manifestations of encephalopathy (B22) was 8.1%; HIV, manifested as malignant neoplasms (B21) - 4.0%.

Conclusion. In the context of the continuing growth of the primary incidence and prevalence of HIV infection, there is an increase in the number of patients in the stage of secondary diseases with irreversible health problems. Among the secondary diseases that have served as the causes of disability, infectious and parasitic diseases, mainly tuberculosis, are in the lead. These changes occur against the backdrop of an increase in ART coverage.

Thus, in order to increase the effectiveness of existing national and regional programs for the prevention of HIV infection, it is necessary to strengthen the work on: 1) the formation of patients' adherence to dispensary observation and ART, the achievement of the designated indicators for this criterion; 2) timely prevention and treatment of secondary, opportunistic and concomitant diseases, which will prevent early disability, including patients of working age.

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TOPICAL ISSUE

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PERIPHERAL BLOOD CELLULAR IMMUNITY PARAMETERS IN COVID-19

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This article carried material about the results of the investigation of peripheral blood cellular immunity parameters in the COVID-19 patients with ≤30% lung damage hospitalizated in 2020 year. When infected with SARS-CoV-2 on the background of severe lymphopenia and neutrophilia were revealed the multidirectional changing in a cellular immunity parameters, the severity and dynamics of which can be determined by the initial type of immune system response.

Keywords: COVID-19; new coronavirus infection; immune state, cellular immunity, lymphocytes, SARS–CoV-2.

Introduction. From the very beginning of the COVID-19 pandemic since April 2020, it has been noted that the infection manifests itself in people in different ways: from a simple infection without clinical manifestations to a severe condition with damage to various organs, primarily the lungs. Progressive lung damage was most often the cause of death of patients. Persons over 65 years of age and those with chronic diseases are most susceptible to severe course [7,11,12]. Lymphocytes and their subpopulation structure play an important role in antiviral immune protection [4]. Viral infections lead to dysregulation of the main subpopulations of lymphocytes (T-, B- and natural killer (NK) cells) involved in the humoral and cytotoxic antiviral immune response [6,8]. Studies conducted during 2020 have shown that SARS-CoV-2 has a unique pathological effect on the immune system compared to other coronaviruses [5,10]. A typical characteristic of SARS-CoV-2 infection is a sharp decrease in the level of lymphocytes, shifts in the T-cell link of immunity, including a decrease in the absolute number of CD3+-, CD4+- and CD8+-T-lymphocytes. The severity of changes in the T-cell link of immunity directly depends on the severity of the course of coronavirus infection. It is important to note that the success of the human body's response to SARS-CoV-2 infection, as well as the success of vaccination, largely depends on the state of the immune system [1,2,3]. The study of the development of the response of the immune system of a macroorganism to infection with the SARS-CoV-2 virus is an important factor both for understanding the pathogenesis of the disease and for developing therapeutic strategies and preventing the development of severe conditions caused by COVID-19.

The aim of the study was to identify the characteristics of the cellular immunity in patients with COVID–19.

Material and methods. This study was conducted as part of the implementation of NIOKTR №121051700033-3 "Lung lesion of infectious etiology. Improvement of methods of detection, diagnosis and treatment". The study group consisted of 31 people in the initial period of the COVID-19 pandemic of the 2020 year, the comparison group consisted of 42 people who were not infected with SARS-CoV-2. The study group at the time of admission to the hospital had positive results of a PCR test for SARS-CoV-2, changes on a computed tomogram (CT)≤30%, blood oxygen saturation >95%, antibacterial and hormonal drugs were not taken before hospitalization. The comparison group was of the appropriate gender and age structure, practically healthy, with negative levels of antibodies to the SARS-CoV-2 virus at the time of examination. The examination of the groups included an assessment of anamnesis, complaints, CT of the chest organs, PCR testing for SARS-CoV-2 infection.

The survey data were entered into a standardized questionnaire. All patients underwent a general clinical examination of peripheral blood (PB) using the Medonic M20 hematological analyzer (Boule Medical, Sweden), including determination of the absolute number of leukocytes, platelets and platelet parameters MPV (average platelet volume), PDW (platelet distribution width by volume) and P-LCR (large platelet ratio), lymphocytes, microscopic determination of the leukocyte formula. The percentage and absolute content of T-lymphocyte subpopulations (CD3+, CD3+CD4+-T-helper cells, cytotoxic CD3+CD8+-T-lymphocytes, CD3+16+-T-lymphocytes, CD3+56+-T-lymphocytes, CD3+HLA-DR+), NK subpopulations were determined-cells (CD16+56+, CD3-CD8+), B-lymphocytes (CD19+), HLA-DR+-lymphocytes and CD95+-lymphocytes in PB by flow cytofluorimetry using a FACS Calibur cytometer (BD, USA) and monoclonal antibodies labeled with FITC and phycoerythrin (Sorbent, Russia).

The leukocyte shift index (LSI) according to N.I.Yabuchinsky was determined by the ratio of the number of granulocytes (neutrophils, eosinophils and basophils) to agranulocytes (lymphocytes and monocytes). The leukocyte-T-cell index (LTI) according to A.M.Zemskov was determined by the ratio of the absolute number of leukocytes to that of CD3+-T-lymphocytes. The immunoregulatory index (IRI) was determined by the ratio of the percentage of CD3+CD4+ lymphocytes to the percentage of CD3+CD8+ lymphocytes. To clarify the etiology of clinical manifestations, the levels of IgG antibodies (At) to the recombinant structural protein S1 of the SARS-CoV-2 virus spike in serum were determined using a semi-quantitative enzyme immunoassay system (EIAS) (EUROIMMUN AG, Ger-

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many), the levels of IgM–At and IgG-At to the recombinant SARS-CoV protein-2 – using high-quality EIAS (Vector-Best, Russia).

Statistical processing was carried out using Software SPSS 22.0 (SPSS Inc.). Median (Me), 25th (Q1-quartile) and 75th (Q3-quartile) percentiles, Mann–Whitney rank criterion, Pearson's criterion χ2 were used. The significance level (p) was taken < 0,05.

Results. In the group of patients with COVID-19, 66% of women, 34% of men. The average age is 50,8±15,4 years. In most cases, the average group indicators of the number of white cells and platelets in patients with COVID-19 do not exceed the reference values (Table 1).

In the group of patients with COVID-19, the lymphocytes level was on average lower than in the comparison group, both percentage (by 23%) and absolute (by 26,3%) (Table 1). Severe lymphopenia (<1,1•10^9 cells/l) was in 34,4%. With a higher neutrophil level (by 17,3%), there is also a 1,5-fold increased LSI relative to the comparison group. 67,7% (n=21) showed marked rearrangements in the population structure of white PB cells, among which 57% (n=12) showed an increase in LSI values due to lymphopenia on the background of neutrophilia (Table 1). Microscopic examination of blood smears in patients with COVID-19, the percentage of atypical neutrophils varied from 1 to 16% (on average 8%), atypical lymphocytes – from 2 to 15% (on average 4,1%). No atypical forms were observed in the comparison group.

When assessing the cellular link of immunity, the indicators of T- and B-lymphocytes (CD3+, CD3+CD4+-, CD3+CD8+-, CD16+CD56+-, HLA-DR+- and CD3+HLA-DR+-) in most cases do not go beyond the reference values. In patients with COVID-19, relative to the comparison group, against the background of a decrease in the average group absolute level of CD3+-lymphocytes by 30,8%, there is an increase in LTI by 20%, significantly lower average group values of the absolute level of the studied subpopulations of T- and NK-cells (Table 2). Despite the fact that the average group percentage of CD3+CD4+- and CD3+CD8+-lymphocytes in patients with COVID-19 and in healthy donors did not differ significantly, the analysis of individual immunograms indicates the variability of these lymphocytic parameters. In the COVID-19 group, cases of reduced CD4+-T lymphocytes (<35%) were 1,5 times more common (35,5% (n=11) vs 26,2% (n=11) in the comparison group. Elevated levels of CD19+-B lymphocytes (>17%) are 2,6 times more common in the comparison group (Table 2).

When studying the NK-cell system, the average group of the absolute number of CD16+CD56+ lymphocytes is 29,4% lower than the comparison group and in 50% of cases lower than the reference value – 0,13•10^9 cells/l (Table 2). On average, there were no significant changes in the % level of both CD3+CD16+- and CD3+CD56+-lymphocytes and CD3-CD8+-lymphocytes in the group of patients with COVID-19 relative to the comparison group (Table 2). However, a comparative analysis of the distribution of these subpopulations of lymphocytes showed elevated levels of CD3+CD16+- and CD3-CD8+ - lymphocytes (> 8%) are noted 2 times more often.

In patients with COVID-19, an in-

Table 1

Blood cells counts of patients with COVID-19 (study group) and healthy donors (comparison group)

Parameter	Comparison group (n = 42)		Study group (n = 31)		*p*
Units of measurement [reference value]	*Me*	Q1 – Q3	*Me*	Q1 – Q3	
Leucocytes, •10^9 cells/l [4 – 9]	6	5 – 7.8	6	5 – 7.8	0.5
Lymphocytes, % [20 – 50]	35.1	28.1 – 43.6	27	12.5 – 34	0.003
Lymphocytes, •10^9 cells/l [1,13 – 2]	1.9	1.6 – 2.4	1.4	0.9 – 1.9	0.001
Platellet, •10^9 cells/l [180 – 320]	222	165 – 248.2	204	173 – 298	0.6
MPV, fl. [9,4 – 12,4]	8.8	8 – 9.4	8.6	8 – 9.3	0.9
PDW, fl. [10 – 20]	12	11 – 13	12.1	11.2 – 12.8	0.9
PLCR, % [13 – 43]	19.1	14.6 – 24.3	19.75	15.6 – 23.7	0.8
Neutrophils, % [48,5 – 84]	56	49.2 – 65.2	65.7	58.3 – 81.4	0.004
Monocytes, % [3 – 11]	7.7	6.6 – 8.75	7.2	4.4 – 9	0.3
LSI, unit [1,46 – 2,36]	1.26	0.95 – 1.9	1.9	1.4 – 4	0.001

Table 2

Cellular immunity parameters of patients with COVID-19 (study group) and healthy donors (comparison group)

Parameter	Comparison group (n = 42)		Study group (n = 31)		*p*
Units of measurement [reference values]	*Me*	Q1 – Q3	*Me*	Q1 – Q3	
CD3, % [61-85]	71	64.5 – 75	70	60.5 –- 76	0.5
CD3, •10^9 cells/l [0,94-2,1]	1.3	1 –1.67	0.9	0.5 – 1.4	0.001
CD3+CD4+,% [35-55]	40.9	32.5 – 46.3	39	30.3 – 45.3	0.4
CD3+CD4+,•10^9 cells/l [0,58-1,3]	0.77	0.56 – 0.9	0.5	0.3 – 0.9	0.009
CD3+CD8+,% [19-35]	23.15	17 –34.1	23.4	17.3 – 31.2	0.7
CD3+CD8+,•10^9 cells/l [0,37-1]	0.375	0.3 – 0.7	0.35	0.17– 0.48	0.02
CD3-CD8+,%	4.2	3 – 6.8	5.75	2.8 – 11.1	0.14
CD3-CD8+,•10^9 cells/l	0.09	0.06 – 0.14	0.07	0.03 – 0.1	0.13
CD16+CD56+,% [10-23]	10.25	7.4 – 14.25	9.4	5.1 – 14.4	0.4
CD16+CD56+,•10^9 cells/l[0,13-0,5]	0.17	0.14 – 0.24	0.12	0.06 – 0.19	0.009
CD3+CD16+,% [5-8]	3.2	2 – 5	3	1.6 – 9.6	0.8
CD3+CD56+,% [5-8]	7	3.7 – 12.9	5.7	4 – 8.4	0.3
CD19+,% [7-17]	8	6 – 11.3	9	6.2 – 13.6	0.26
CD19+, •10^9 cells/l [0,1-0,38]	0.15	0.1 – 0.23	0.13	0.07– 0.2	0.12
CD3+HLA-DR+, % [1-6]	7	5 – 11.3	10	7.4 – 14.8	0.025
HLA-DR+,% [7-20]	18.8	15.35– 22.3	21.55	17 – 27.2	0.1
CD95+,% [5-43]	9.3	4.3 – 33.45	29.6	13 – 39.75	0.05
LTI, unit [4-7]	4.5	3.3 – 5	5.4	4.2 – 14.1	0.002
IRI, unit [1,5-2,6]	1.7	1 – 2.6	1.9	0.9– 2.5	0.81

creased (>20%) level of HLA-DR+ lymphocytes was observed in 60% (n=18) vs 33,3% (n=14) of the comparison group ($\chi 2$ = 5, p=0,03). An increased (>6%) level of CD3+HLA-DR+ lymphocytes was observed in 86,7% (n=36) patients vs 58,3% (n=18) of the comparison group. There was an increase in the CD95+ lymphocyte level by 3,2 times on average in the group of patients with COVID-19 relative to the comparison group (p=0,05) (Table 2).

Conclusion. Against the background of severe lymphopenia and neutrophilia during infection with SARS-CoV-2, multidirectional changes in the studied indicators of cellular immunity were revealed, including a decrease in the absolute level of CD3+-, CD3+CD4+–, CD3+CD8+ – and CD16+CD56+-lymphocytes; an increase in the % level of CD3+HLA-DR+ and CD95+ lymphocytes, the severity and dynamics of which can be determined by the initial type of immune system response. It is promising to study the initial "immunological passport" of a person to identify personal predictors of the course of the disease when infected with coronavirus infection.

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EXCESS MORTALITY IN THE REPUBLIC OF SAKHA (YAKUTIA) DURING THE COVID-19 PANDEMIC (2020-2021)

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Excess mortality is defined as an increase in all-cause mortality over expected mortality (historical baseline for previous years). In the context of COVID-19, excess mortality may reflect the overall impact of the pandemic on mortality, including not only the number of confirmed deaths from COVID-19, but also deaths from COVID-19 when they were not correctly diagnosed and reported, and deaths from other diseases due to pandemic-related causes.

The purpose of the study: to assess the indicators of excess mortality during the COVID-19 pandemic (2020-2021) in the Republic of Sakha (Yakutia). For the analysis, data from the Federal State Statistics Service for 2015-2022 were used. For 2 years of the spread of a new coronavirus infection in the Republic of Sakha (Yakutia), 19556 people died. 7.8% of deaths in 2020 and 21.5% in 2021 were related to COVID-19. The number of all deaths was 22% and 44% respectively higher than the expected number of deaths. The proportion of excess deaths in 2020 was 19% of all deaths, in 2021 - 31%. Of the excess deaths, 42% and 69%, respectively, were related to COVID-19. The excess mortality rate reached 333 per 100,000 population in 2021. The high correlation coefficients (0.94-0.95) between COVID-19-related deaths and additional deaths suggest that excess deaths during the period 2020-2021 will largely be due to the spread of COVID-19.

The decline in mortality underreporting in 2021 against the background of an increase in excess mortality reflects improved diagnosis and correct identification of the causes of death. Research into the causes of excess mortality is needed to assess the impact of the pandemic and other factors on various aspects of mortality in the population.

Keywords: new coronavirus infection, COVID-19, pandemic, excess mortality, Republic of Sakha (Yakutia).

Introduction. Excess mortality is defined as an increase in all-cause mortality over expected mortality (historical baseline for previous years). The increase in mortality is associated with the emergence of some new factors, emergencies that affect the health of the population. In the context of COVID-19, excess mortality may reflect the overall impact of the pandemic on mortality, including not only the number of confirmed deaths from COVID-19, but also deaths from

Table 1

Number of deaths from various causes in 2020-2021 compared to expected (Rosstat)

Causes of death	Expected *	2020		2021	
		official	increase / decrease %	official	increase / decrease %
all causes	7340	8956	22.0	10600	44.4
diseases of the circulatory system	3344	3956	18.3	4003	19.7
neoplasms	1396	1286	-7.9	1241	-11.1
external causes	1011	1206	19.3	1098	8.6
respiratory diseases	321	400	24.6	466	45.2
diseases of the digestive system	376	437	16.0	428	13.8

Note: *-calculated based on 2015-2019 years (linear regression).

COVID-19 when they were not correctly diagnosed and reported, and deaths from other diseases due to pandemic-related causes. For example, due to a decrease in the availability and quality of medical care, the impact of stressful factors, the influence of other conditions during the spread of the infection [2, 5, 6].

Karlinsky A. and Kobak D., creators of the World Mortality Dataset database, based on data on deaths from all causes in 103 countries, showed that in some countries (Peru, Ecuador, Bolivia, Mexico), excess mortality during the COVID-19 pandemic exceeded 50% expected annual mortality. While in other countries (Australia, New Zealand), mortality during the pandemic was below normal levels, which may be due to social distancing measures that reduce infectious mortality not associated with COVID-19 [6]. Accounting for COVID-19 deaths is affected by: COVID-19 death case definition, availability of testing, and fairness of reporting.

In this regard, it is of interest to study excess mortality during this period in a region located on a vast territory, where transport distance from the center affects the availability of medical care.

The purpose of the study: to assess the indicators of excess mortality during the COVID-19 pandemic (2020-2021) in the Republic of Sakha (Yakutia).

Materials and methods. For the analysis, data from the Federal State Statistics Service for 2015-2022 were used. [3]. The expected number of deaths for each month of 2020 was calculated using linear regression analysis in IBM SPSS Statistics 26 based on monthly mortality data in 2015-2019. Further, using the actual data of 2020, the excess mortality for the month was calculated as the difference between the observed number of deaths and the predicted value. The final

Table 2

Number of deaths associated with COVID-19 (Rosstat)

Period	COVID-19 – main cause of death			COVID-19 is not the main cause of death, but has had a significant impact on the development of fatal complications of the disease
	total	virus identified	virus not identified	
2020				
March	0	0	0	0
April	0	0	0	0
May	7	7	0	0
June	26	24	2	0
July	47	42	5	0
August	35	31	4	0
September	58	53	5	6
October	98	93	5	9
November	223	206	17	8
December	179	170	9	5
Total in 2020	**673**	626	47	**28**
2021				
January	83	81	2	7
February	57	56	1	11
March	44	40	4	0
April	41	38	3	7
May	90	86	4	3
June	158	156	2	6
July	151	150	1	7
August	190	186	4	9
September	278	269	9	4
October	418	411	7	20
November	439	430	9	18
December	231	223	8	7
Total in 2021	**2180**	2126	54	**99**

estimate of excess mortality for the year was determined as the sum of excess mortality for all months, starting from March 2020. This approach, according to the researchers, considers both seasonal fluctuations in mortality and the annual trend, and is not inferior in efficiency to more complex methods [6]. To avoid further extrapolation, the same expected mortality rate was taken for 2021.

COVID-19-related deaths included cases where COVID-19 was the primary cause (regardless of virus identification), as well as cases where COVID-19 was not the primary cause of death but had a significant impact on the development of deaths. complications of the disease.

The ratio of the number of excess deaths in the study period to the number of registered deaths associated with COVID-19 in the same period was used as the death underreporting coefficient. This index is called by Russian researchers the "Covid mortality multiplier" in view of the fact that the increase in mortality during this period is due not only to mortality from COVID-19, but also to mortality associated with the overload of the medical care system and the stress experienced by the population [2].

Results and discussion. Excess mortality during the pandemic includes direct COVID-19 deaths and non-COVID-19 indirect deaths. The causes of non-COVID-19 deaths are diverse and include behavioral factors, changes in the healthcare delivery system, the negative effects of social restrictions, economic factors, and others [5]. For example, this can be a change in behavior regarding seeking medical care in cases of occurrence or exacerbation of diseases due to the risk of contracting COVID-19, prioritizing cases of COVID-19 in the provision of care by reducing services for people with chronic noncommunicable diseases, and others. WHO, in a survey conducted in 155 countries, showed that 42% of the countries surveyed have partially or completely discontinued services for the treatment of cancer, 49% for the treatment of diabetes and its complications, 31% for urgent cardiovascular diseases. This indicates that the impact of the pandemic on the healthcare system is global [8]. According to the researchers, excess mortality during the COVID-19 pandemic may reflect the following causes of death: deaths directly caused by COVID-19 infection; deaths caused by the collapse of the healthcare system due to the pandemic; excess mortality from other natural causes; excess mortality from external causes; excess mortality from extreme events (wars, natural disasters, etc.) [5].

Retrospective analysis of the situation in the RS(Y) in 2020-2021 showed that during the summer periods in the republic there were a series of forest fires that caused air pollution by combustion products. For example, according to IQAir, as of August 12, 2021, the content of PM2.5 particles in the air was 2473 μg/m3, while the WHO recommended rate is 25 μg/m3. Long-term exposure to PM2.5 has been associated with an increased long-term risk of cardiopulmonary mortality, according to studies in various countries. Particularly vulnerable groups are people with lung or heart disease, the elderly, and children [7, 9].

For the period 2015-2019. in the Republic of Sakha (Yakutia), as in the whole of the Russian Federation, there was a downward trend in mortality. Thus, the indicators of general mortality in the republic over this period decreased from 8.5 to 7.8 per 1000 population (in the Russian Federation from 13.1 to 12.5, respectively).

Since the beginning of the pandemic of a new coronavirus infection, the situation has changed dramatically. In 2020, 8956 people died in the Republic of Sakha (Yakutia), which is 22% higher than the expected number of deaths (Table 1). The overall mortality rate was 9.2 per 1000 population. A pronounced increase in mortality has been observed since July 2020. During the year, the number of deaths from diseases of the circulatory system (by 18%), respiratory diseases (by 25%), diseases of the digestive system (by 16%) and external causes (by 19%) increased significantly against the background of a decrease in deaths from neoplasms (- eight%). Similar trends were noted in other regions of the Russian Federation [1].

In 2021, 10,600 people died, which is 44% more than the expected number of deaths. The overall mortality rate was 10.8 per 1000 population. The largest number of deaths occurred in September-November 2021. The number of deaths from diseases of the circulatory system for the year was 20% more than expected, from diseases of the respiratory system - by 45%, from diseases of the digestive system - by 14%, from external

Table 3

Excess mortality in the Republic of Sakha (Yakutia) in 2020-2021

Period	Expected *	Mortality 2020				Mortality 2021			
		factual	excess	related to COVID-19**	undercount	factual	excess	related to COVID-19**	undercount
January	698	678	-20	0	-	816	118	90	1.3
February	589	593	4	0	-	726	137	68	2.0
March	590	573	-17	0	-	804	214	44	4.9
April	584	594	10	0	-	657	73	48	1.5
May	730	735	5	7	0.7	702	-28	93	-0.3
June	541	508	-33	26	-1.3	831	290	164	1.8
July	661	803	142	47	3.0	835	174	158	1.1
August	632	748	116	35	3.3	890	258	199	1.3
September	592	860	269	64	4.2	1011	420	282	1.5
October	636	876	240	107	2.2	1190	554	438	1.3
November	548	1010	462	231	2.0	1206	658	457	1.4
December	539	978	439	184	2.4	932	393	238	1.7
Total	7340	8956	1682	701	2.4	10600	3288	2279	1.4

Note: *-calculated based on 2015-2019 data; ** - mortality associated with COVID-19 (the main cause of COVID-19, or COVID-19 is not the main cause of death but had a significant impact on the development of fatal complications of the disease).

causes by 8.6%, respectively. The number of deaths from neoplasms decreased by 11% compared to expected.

The increase in mortality from diseases of the circulatory system, respiratory diseases, and digestive diseases probably reflects both the unaccounted-for mortality from COVID-19 and the high vulnerability of people with chronic diseases in a pandemic. Analysis of the causes of deaths from external causes requires a separate study.

In 2020, 673 people died from COVID-19, in 47 (7%) deaths from COVID-19 the virus was not identified (Table 2). In 28 of the deceased, COVID-19 was not the main cause of death but had a significant impact on the development of fatal complications of the disease. Thus, according to official statistics from Rosstat, in 2020, 7.8% (701) of deaths in the republic were associated with a new coronavirus infection, including 7.5% (673) of cases, COVID-19 was the main cause of death. In 0.3% (28) of deaths, COVID-19 had a significant impact on the development of fatal complications of the disease. When calculating the intensive indicator, the mortality rate associated with COVID-19 was 71.8 per 100,000 population.

In 2021, 2180 people died from COVID-19, in 54 (2.5%) cases the virus was not identified (Table 2). In the deaths of 99 people, COVID-19 was not the main cause of death but had a significant impact on the development of fatal complications of the disease. Thus, in 2021, 21.5% (2279) of deaths were related to COVID-19, of which 20.6% (2180) of cases had COVID-19 as the leading cause of death. In 0.9% (99) of cases, COVID-19 had a significant impact on the development of fatal complications of the disease. The death rate associated with COVID-19 was 230.9 per 100,000 population in 2021.

During the 2 years of the pandemic in the republic, the death of 2980 people was associated with COVID-19. In 2021, 3.25 times more people died from this cause than in 2020, which is associated with a more severe course of the infection, with the delta variant of the virus prevailing in this year.

Comparison of the actual number of deaths with expected levels separately by months showed that since July 2020, excess mortality rates have increased sharply, in just 1682 more people died than expected (Table 3). The excess mortality rate was 172 per 100,000 population. Its share in the structure of total mortality was 18.8%. If all deaths reported as related to COVID-19 are considered, then 42% of excess deaths were due to this cause. The number of deaths associated with COVID-19 was positively correlated with the excess mortality rate, Pearson's correlation coefficient was 0.94, $p<0.001$. The undercount rate in September 2020 was 4.2. In general, for the period March-December, the underestimation rate was 2.4. As shown in studies, underreporting values above 1.0 are mainly due to underreporting of deaths from COVID-19 infection [6].

In 2021, excess mortality was 3288 cases (31% in the structure of total mortality), i.e. 333 per 100,000 population. 69% of them were related to COVID-19. Based on an analysis of all-cause mortality reports from 74 countries over the same period (January 1, 2020 to December 31, 2021), the global excess death rate was 120.3 deaths (113.1–129.3) per 100,000 population. In 21 countries, it exceeded 300 deaths per 100,000 population [4]. According to researchers, excess mortality during an epidemic outbreak can be considered as an indicator of mortality from COVID-19 [5].

The correlation coefficient between the number of deaths associated with COVID-19 and excess mortality in the republic was 0.95, $p<0.001$. The underestimation rate was the highest in March (4.9), for the period January-December 2021 it was 1.4.

In general, for 2020-2021. in the Republic of Sakha (Yakutia) 19556 people died, of which 4970 cases were classified as excess mortality. The number of excess deaths in 2021 was 1.95 times the number in 2020.

According to Goroshko N.V. with co-authors in 2020, excess mortality was observed in 82 out of 85 subjects of the Russian Federation. The increase in the number of deaths in 2020 amounted to 288.0 thousand people compared to the average number of deaths in 2015-2019. When compared with the data of 2019 - 340.3 people [1].

Conclusion. Thus, over 2 years of the spread of a new coronavirus infection (2020-2021) in the Republic of Sakha (Yakutia), 19556 people died. 7.8% of deaths in 2020 and 21.5% in 2021 were related to COVID-19. The number of all deaths was 22% and 44% respectively higher than the expected number of deaths. The proportion of excess deaths in 2020 was 19% of all deaths, in 2021 - 31%. Of the excess deaths, 42% and 69%, respectively, were related to COVID-19. Therefore, the causes of the 1990 excess deaths need to be clarified. The excess mortality rate reached 333 per 100,000 population in 2021. The high correlation coefficients (0.94-0.95) between COVID-19-related deaths and additional deaths suggest that excess deaths during the period 2020-2021 will largely be due to the spread of COVID-19. The decline in mortality underreporting in 2021 against the background of an increase in excess mortality reflects improved diagnosis and correct identification of the causes of death. Research into the causes of excess mortality is needed to assess the impact of the pandemic and other factors on various aspects of mortality in the republic's population.

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THE INFLUENCE OF HERPES FAMILY VIRUSES ON THE COURSE OF NOVEL CORONAVIRUS INFECTION

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We have analyzed the impact of herpes virus infection on the course of a new coronavirus infection (NCVI). Infection of the examined contingent with herpes family viruses reached 95.3-100%. An association of NCVI with herpes simplex viruses 1, 2 types (HSV 1, 2 types) was found, but no correlation was found between the positivity coefficient (CP) of HSV 1, type 2 and the severity of NCVI. This can be explained by the fact that the sampling was carried out in the remote period after the transferred NKVI. Considering that both herpes viruses and the SARS-CoV-2 virus cause multiple organ damage and can aggravate each other, the study of co-infection seems to be very relevant.

Keywords: viruses of the herpes family (Herpes viridae), herpes simplex virus 1, 2 types (HSV types 1,2), cytomegalovirus (CMV), Epstein-Barr virus (EBV), new coronavirus infection (NCVI).

Introduction. Among the viruses of the herpes family, 8 of the most common are distinguished: herpes simplex virus types 1 and 2 (HSV-1 and HSV-2); shingles virus, or human herpesvirus type 3 (HHV-3); human herpes virus type 4, or Epstein-Barr virus (EBV); human herpes virus type 5, or cytomegalovirus (CMV); human herpes viruses type 6 and 7 (HHV-6 and HHV-7); and herpes virus type 8 or Kaposi's sarcoma-associated virus (HHV-8). The presence of herpes virus infection is a marker of immunodeficiency [1].

HSV-1 and HSV-2 are widespread human pathogens with worldwide prevalence rates of about 67% and 13%, respectively [20]. Diseases caused by HSV types 1, 2, including labial / nasal herpes, genital herpes, herpetic stromal keratitis, herpetic eczema, disseminated disease in newborns, meningitis and herpes simplex encephalitis. There is evidence that there is a direct link between the occurrence of HSV type 1 and type 2 infection and neurodegenerative diseases. [8, 23, 29, 32, 33].

Human cytomegalovirus (CMV) is widespread and affects 40–100% of the population worldwide [5]. Most infected people remain asymptomatic due to a rapid immune response [18]. Primary infection or reactivation viral can cause severe multiple organ damage in the presence of immunodeficiency [15].

More than 90% of the adult population is latently infected with oncogenic EBV[17, 26]. Chronic or recurrent EBV infection of epithelial cells is associated with systemic lupus erythematosus and Sjogren's syndrome. Chronic/recurrent infection of B cells is associated with rheumatoid arthritis, multiple sclerosis, and other diseases [22].

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a highly contagious and virulent virus, an inflammatory respiratory disease called novel coronavirus infection (NCVI, COVID-19), that is having a global impact on the global public health system[30]. According to WHO data, as of October 7, 2022, there were 617,597,680 confirmed cases of COVID-19 in the world, including 6,532,705 deaths [34]. Possible SARS-CoV-2-associated neurological diseases have been described: Guillain-Bare syndrome [13], encephalomyelitis [27], myalgia, and damage to the neuromuscular synapse [27].

The COVID-19 pandemic has caused numerous adverse consequences for people with neurological disorders and neurodegenerative diseases [25], such as Parkinson's disease, Alzheimer's disease, multiple sclerosis [2, 3, 6, 10, 12, 28].

Since reactivation of varicella-zoster virus, EBV, and CMV has already been reported in patients with COVID-19 [7, 14, 16, 19, 21], of particular interest is the study of the interaction of herpes family viruses and SARS-CoV-2 in terms of studying a possible mutually reinforcing pathogenic influence.

Purpose of the study: to assess the relationship between the carriage of herpes family viruses and a new coronavirus infection.

Tasks:

1. Analyze the carriage of herpesvirus infection in the examined contingent
2. To assess the degree of mutual influence of pathogens of herpesvirus infection and a new coronavirus infection in patients with confirmed COVID-19

Materials and methods. The object of the study were 175 people working and living in the cities Aldan and Tommot of the Aldan region, of which 66 (37.7%) men and 109 (62.3%) women. The study program for the adult population included the following sections: informed consent of the respondent to conduct research; blood donation (according to the protocol of the Ethics Committee of the YSC CMP); questionnaire survey to assess the objective state; blood sampling from the cubital vein in the morning on an empty stomach after a 12-hour abstinence from food for an immunological study.

The study protocol was approved at a meeting of the local committee on biomedical ethics of the Federal State Budgetary Scientific Institution "Yakutsk Scientific Center for Complex Medical Problems" (Minutes No. 56 dated March 10, 2022, decision No. 3).

The subjects were divided into two groups: group 1 (main) consisted of 132 people (132/175, 75.5%) who had undergone NCVI of varying severity (the severity was assessed on a specially developed scale; the form of the disease was taken into account according to hospital discharges and outpatient cards); group 2 (control) included 43 people (43/175, 24.5%) without a history of NCVI. In group 1 there were 49/132 men, 83/132 women; on a racial basis, the distribution

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was as follows: 118/132 people - Caucasians, 14/132 people - Asians; the average age was 43.4±11.3 years. In group 2 17/43 people - men, 26/43 people - women, racially 40/43 - Caucasians, 3/43 - Asians, the average age of the subjects in the control group was 45.3±11.6 years. Both groups were comparable in terms of gender and age characteristics.

Analysis of the level of immunoglobulin IgM and IgG (the analysis took into account PP - the coefficient of positivity) to HSV 1, type 2, CMV, EBV, SARS-CoV-19 was carried out in blood serum by enzyme-linked immunosorbent assay (ELISA) on a Uniplan photometer using standard kits "Vector best" (Russia), according to the manufacturer's instructions. In addition, the level of avidity of antibodies to antigens of HSV 1, type 2, CMV, EBV viruses was determined.

The obtained data were analyzed using the SPSS 22 program (IBM, USA). Descriptive statistics for quantitative data are given as the median and the 25th and 75th quartiles (Me [Q25; Q75]). To compare two independent groups, the analysis was carried out using the Mann-Whitney U-test, for three independent groups - using the Kruskal-Wallis test. When comparing qualitative data, $\chi 2$ and Fisher's exact test were used. The critical level of statistical significance for the two groups was determined at $p \leq 0.05$.

Research results. In 120/175 (68.6 ± 7.9%) of the examined, clinical manifestations of chronic herpes virus infection in history were detected, such as: labial, genital, herpes.

As a result of the study, it was found that IgG to HSV type 1.2 was found in 95.4% (125/132) in group 1 and in 100% (43/43) of the examined - in group 2 without statistically significant differences. The positivity coefficient (PC) in group 1 was 16.12 [15.91; 16.29], in group 2 - 16.06 [15.84; 16.23] c.u., without statistically significant differences between the groups. HSV-IgM was found in 2/132 (1.5%) of those examined in group 1 and in 1/43 (2.3%) in group 2 (Table 1). The median avidity of antibodies to type 2 HSV-1 antigens was 100.0 [97.0; 100.0] and 100.0 [96.5; 100.0] in the first and second groups, respectively (U = 2120, p=0.839).

CMV-IgG were detected in 100% in both examined groups (132/132 in group 1 and 43/43 in group 2). CMV-IgM in group 1 is positive in 5 (3.8%), in group 2 - in 4 (9.3%) people (table 1). The median avidity of antibodies to CMV antigens was 95.0 [82.0; 99.0] and 94.5 [77.5; 99.0] in the first and second groups, respectively (U = 1139.5; p=0.997).

EBV-IgM was positive in 7/132 people (5.7%) in group 1 and in 2/43 people (4.7%) in group 2. EBV-IgG was positive in 129/132 people (98.5%) in group 1 and in 41/43 people (95.3%) in group 2, without a statistically significant difference. The positivity ratio (CP) of EBV-IgG to the nuclear antigen in group 1 was 12.78 [11.93; 13.03], in group 2 - 12.76 [9.5; 13.0] c.u., without statistically significant differences between the groups (table 1). The median avidity of antibodies to EBV antigens was 99.67 [98.21; 100] and 99.32 [98.32; 100.0] in the first and second groups, respectively (U = 960; p=0.888).

Of the 132 people who recovered from the, 63/132 (47.7%) had a mild disease, 53/132 (40.2%) had a moderate degree, 12/132 (9.1%) had a severe degree, the severity of NCVI is unknown in 4/132 people (3.0%). On average, the period from the moment of NCVI was 6.97+6.5 months. 35/132 people (26.5%) had post-COVID syndrome. Of the main complaints in persons with post-covid syndrome, the following were noted: general weakness and increased fatigue - in 15/35 people (42.9%), violation of smell, taste, vision - in 10/35 people (28.6%), memory loss in 5/35 people. (14.3%), myalgia - in 4/35 people (11.4%).

We analyzed the relationship between class M and G antibody titers and the severity of NCVI (Table 2). The CP of antibodies to SARS-CoV-2 in mild infection was at the level of 11.64 [11.31; 11.82] c.u., with a moderate course - 11.66 [11.57; 11.83] c.u., in severe cases – 11.66 [11.13; 11.78] (p = 0.487, H = 1.44).

IgM titers to CMV did not differ in different degrees of severity of NCVI: 0.29 [0.21; 0.38] - with a mild degree, 0.29 [0.21; 0.46] - with an average degree, 0.24 [0.16; 0.29] – in severe course of the disease (p=0.187; H=4.81). The level of IgG to CMV also did not differ depending on the severity of NCVI: 10.93 [9.91; 11.03] with mild course, 10.78 [9.63; 10.96] with moderate and 10.86 [8.7; 11.1] in severe infection (p=0.472; H=2.52). IgM to EBV to the capsid antigen (VCA) in mild NCVI was 0.18 [0.16; 0.26], with moderate NCVI - 0.24 [0.16; 0.32], in severe cases - 0.21 [0.16; 0.29] (p=0.379; H=3.08). IgG to the EBV nucle-

Table 1

The level of antibodies to viruses of the Herpes family in two examined groups

	Main group, N = 132	Control group, N = 43	p-level
HSV-IgM Negative Positively Doubtful	123 (93.9) 2 (1.5) 6 (4.6)	40 (93.1) 1 (2.3) 2 (4.6)	p=0.933; χ
HSV-IgM, CP c.u.	0.32 [0.25; 0.43]	0.3 [0.23; 0.41]	p=0.47; U = 2528.5
HSV 1,2-IgG Negative Positively	6 (4.6) 125 (95.4)	0 43 (100)	p=0.16; χ
HSV 1,2-IgG, CP c.u.	16.12 [15.91; 16.29]	16.06 [15.84; 16.23]	p=0.47; U = 2528.5
Avidity HSV-1,2	100.0 [97.0; 100.0]	100.0 [96.5; 100.0]	p=0.839; U = 2120
CMV-IgM Negative Positively	126 (96.2) 5 (3.8)	39 (90.7) 4 (9.3)	p=0.15; χ
CMV-IgM CP c.u.	0.29 [0.21; 0.37]	0.33 [0.21; 0.66]	p=0.97; U = 2743.5
CMV-IgG Positively	131 (100)	43 (100)	-
CMV-IgG, CP c.u.	10.82 [8.97; 11.0]	10.75 [9.6; 11.0]	p=0.28; U = 2446
CMV avidity	95.0 [82.0; 99.0]	94.5 [77.5; 99.0]	U = 1139.5; p=0.997
EBV-IgG Negative Positively	2 (1.5) 129 (98.5)	2 (4.7) 41 (95.3)	p=0.23; χ
EBV-IgG to nuclear, CP c.u.	12.78 [11.93; 13.03]	12.76 [9.5; 13.0]	p=0.153; U = 2348
EBV-IgM negative Positively Doubtful	114 (93.5) 7 (5.7) 1 (0.8)	41 (95.3) 2 (4.7) 0	p=0.86; χ
EBV-IgM VCA, CP c.u.	0.21 [0.16; 0.29]	0.18 [0.13; 0.26]	p=0.11; U = 1807.5
Avidity VEB	99.67 [98.21; 100]	99.32 [98.32; 100.0]	U = 960; p=0.888

Table 2

Relationship between herpes family viruses and NCVI

	Mild disease of the NCVI	The average severity NCVI	Severe disease NCVI	p-level
HSV1,2 type IgM	0,3 [0,24; 0,42]	0,35 [0,28; 0,47]	0,3 [0,28; 0,43]	p=0,382; H = 3,06
HSV 1,2 type IgG	16,18 [15,96; 16,42]	16,07 [15,83; 16,23]	16,29 [16,12; 16,5]	p=0,025; H = 9,34 p1,2=0,021 U = 1296 p1,3=0,228 U = 276 p2,3=0,008 U = 142,5
CMV IgM	0,29 [0,21; 0,38]	0,29 [0,21; 0,46]	0,24 [0,16; 0,29]	p=0,187; H = 4,81
CMV IgG	10,93 [9,91; 11,03]	10,78 [9,63; 10,96]	10,86 [8,7; 11,1]	p=0,472; H = 2,52
EBV to nuclear IgG	12,91 [12,27; 13,03]	12,74 [9,19; 13,03]	12,86 [12,44; 13,24]	p=0,477; H = 2,49
EBV VCA IgM	0,18 [0,16; 0,26]	0,24 [0,16; 0,32]	0,21 [0,16; 0,29]	p=0,379; H = 3,08

ar antigen in mild NCVI was at the level of 12.91 [12.27; 13.03], with moderate severity - 12.74 [9.19; 13.03], with severe NCVI - 12.86 [12.44; 13.24] (p=0.477; H=2.49)

IgM to HSV 1, type 2 amounted to 0.3 in mild NKVI [0.24; 0.42], with a moderate course - 0.35 [0.28; 0.47], in severe cases - 0.3 [0.28; 0.43] (p=0.382; H=3.06).

The relationship between the titers of class G antibodies to HSV type 1,2 viruses and the severity of NKVI turned out to be statistically significant. Thus, in mild NKVI, the IgG level was 16.18 [15.96; 16.42] c.u., with an average severity of the disease - 16.07 [15.83; 16.23] c.u., in severe course - 16.29 [16.12; 16.5] (p=0.025; H=9.34). At the same time, he draws attention to the fact that the level of titers did not have a noticeable effect on the severity of the course of NCVI, but indicates the activity of herpes virus infection.

The influence of herpes viruses on the development of post-COVID syndrome was assessed. We did not find any statistically significant differences between patients with and without post-COVID syndrome.

Discussion. Thus, the infection of the examined contingent with herpes family viruses reached 100%: HSV 1,2-IgG was positive in 95.4% in group 1 and 100% in group 2; CMV-IgG were detected in 100% of cases in both groups; EBV-IgG was found in 98.5% of cases in the first group and in 95.3% in the second group. A high level of avidity of antibodies to antigens of herpes viruses indicates a long-standing infection process in the main and control groups.

When assessing the level of antibodies to herpes viruses at different degrees of severity of NCVI, a connection was established with HSV types 1, 2, however, we did not find a correlation between the level of CP HSV types 1, 2 and the severity of NCVI. This can be related to the fact that the sampling was carried out in the remote period after the transferred NCVI. In general, our results are consistent with previously published information on the relationship between NCVI and herpesvirus lesions.

So, in the study of Weber S. et al. (2022) found that patients under 60 years of age with severe COVID-19 had a very high prevalence of CMV seropositivity, while the distribution of CMV status in those with mild disease was similar to that in the German population. Predictive models support the hypothesis that CMV serostatus, unlike HSV, may be a strong biomarker for identifying younger individuals at higher risk of developing severe COVID-19, particularly in the absence of other comorbidities [9].

Katz J. et al. (2021) found that the prevalence of herpes simplex virus-1 in the COVID-19 patient population was 2.81% compared to 0.77% in the hospital population with an odds ratio of 5.27 (adjusted for sex, race and age odds were 5.18, 4.48, and 4.61, respectively); the prevalence of varicella zoster virus in patients with COVID-19 was 1.8% compared to 0.43% among inpatients, odds ratios of 5.26 before adjustment and 5.2, 5.47 and 4.76 after adjusting for sex, age and race, respectively [24].

Some studies have reported skin manifestations of COVID-19, such as rashes, petechiae, and urticaria, which are non-specific and may be associated with herpes viruses rather than the SARS-COV-2 strain [4, 19]. Shingles virus may even be an indicator of latent COVID-19 infection [19]. Reactivation of herpes viruses due to an immunosuppressive condition associated with COVID-19 can be potentially life-threatening [11]. Therefore, a number of authors recommend studying the prevalence of these cases and taking them into account in the differential diagnosis of human herpes viruses, even if COVID-19 is confirmed [24]. The prevalence of both HSV-1 and zoster virus was significantly higher in the COVID-19 group [31]. It can be assumed that COVID-19 lowers the threshold for reactivation of herpes viruses, which requires further research in this direction.

Conclusion. Thus, the overlaying of herpesvirus infection on NKVI can probably contribute to the aggravation of the course of COVID-19. It should be remembered that both herpes viruses and SARS-Cov-2 lead to multiple organ damage, as well as to the development of neurodegeneration. Considering that severe forms of herpetic lesions are similar to the COVID-19 clinic, it is important in such cases to include studies on the herpes viral load in the differential diagnosis. Of particular interest is the study of co-infection with herpes viruses and SARS-Cov-2 in the long term, since there is an assumption that herpes viruses lead to the development of latency of the NKVI pathogen. In particular, this is important in assessing the risks of developing neurodegenerative diseases.

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LIPID IMBALANCE IN YAKUTSK RESIDENTS WHEN INFECTED WITH THE SARS-COV-2 VIRUS AND IN THE POST-COVID PERIOD

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A study of lipid metabolism was carried out in 161 residents of Yakutsk aged 20 to 72 years who had a disease with a new coronavirus infection SARS-CoV-2 from 3 to 12 months ago. The aim of the work was to assess the lipid profile after recovery from COVID-19, depending on the post-COVID period and severity of the disease, as well as the level of total cholesterol in inpatients at admission, discharge and in the post-COVID period. According to the results of the study, when infected with the SARS-CoV-2 virus, lipid metabolism is disturbed: in the acute period of infection, the level of total cholesterol decreases, in the post-COVID period, its level significantly increases. The most pronounced shift in the lipid profile towards atherogenicity occurs in patients who recovered from COVID-19 three months ago due to a decrease in the antiatherogenic fraction of lipids. Dyslipidemia is most pronounced in patients who have had an infection with severe lung damage (50-75%) due to an increase in atherogenic lipid fractions that create a risk of atherosclerosis.

Keywords. SARS-CoV-2, COVID-19, lipids, dyslipidemia.

Introduction. SARS-CoV-2 coronavirus infection does not manifest as severe acute respiratory syndrome in all infected people. In most cases, COVID-19 is asymptomatic or leads to a mild form of the disease. Some patients need intensive hospital treatment and respiratory support, especially the elderly, patients with obesity, diabetes, cardiovascular disease and hypertension, as they are at risk of severe infection, often fatal.

Scientific studies have shown that the SARS-CoV-2 virus causes a violation of metabolic processes in the body, including lipid metabolism. Lipids not only constitute the basic structure of membranes, but also play an important role as intercellular signaling agents and energy sources [3]. But it should be noted that lipids also facilitate the penetration of viruses through the host cell membrane. Viruses use and modify lipid metabolism in favor of virus replication [10].

In the acute phase of infection with SARS-CoV-2, a change in cholesterol (CH) metabolism was revealed, which is the cause of a decrease in circulating cholesterol in the blood serum [10]. An analysis of data from 1411 hospitalized patients with COVID-19 showed that low levels of the anti-atherogenic fraction (HDL-C - high-density lipoprotein cholesterol), high levels of triglycerides (TG) before infection and on admission are significant predictors of disease severity [2]. Correlations of these parameters with higher levels of D-dimer and ferritin indicate the role of acute inflammation in lipid metabolism disorders. Another large study found a decrease in low-density lipoprotein cholesterol (LDL-C) levels as the disease progressed [5].

Since lipid metabolism disorders caused by COVID-19 can persist in the post-COVID period, causing the development of cardiovascular complications, the study of the lipid profile during infection with the SARS-CoV-2 virus and after recovery is relevant.

The purpose of the study: to study changes in lipid fractions in patients who underwent COVID-19 depending on the duration and severity of the disease.

Material and methods. The study, conducted at the Clinic of the Yakut Scientific Center for Complex Medical Problems (YSC CMP), included 161 people who had a laboratory-confirmed COVID-19 infection with varying degrees of lung damage. The age of the examined patients ranged from 20 to 72 years. The mean age was Me 53.0 [41.5; 61.7] years, men - 50.5 [40.0; 61.7], women - 53.0 [42.0; 61.5] years. The percentage of men was 37.1% (60 people), women 62.7% (101 people), respectively. The exclusion criterion from the group was patients with signs of acute respiratory viral infections and active COVID-19 infection at the time of the study.

All subjects were divided into 4 groups depending on the time since recovery: group 1 - up to 3 months, group 2 - 3-6 months, group 3 - 6-9 months, group 4 - 12 months ago and the severity of lung damage (Table 1). Another 4 groups were divided according to the severity of lung damage: CT 0 (zero) - no lung damage was detected; CT 1 (mild) - damage to less than 25% of the lung volume, CT 2 (moderate-severe) 25-50%, CT 3 (severe) 50-75% (Table 1).

The study was approved by the decision of the ethical committee of the Federal State Budgetary Scientific Institution "Yakutsk Scientific Center for Complex Medical Problems", Protocol No. 52 dated March 24, 2021, and was performed with the informed consent of the subjects in accordance with the ethical standards of the Declaration of Helsinki (2000). The material of the study was blood serum from the cubital vein, taken on an empty stomach.

All biochemical studies were carried out on a Labio-200 automatic biochemical analyzer (Mindray, China) using Biocon reagents. The determination of total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C) was carried out by the enzymatic method. Low-density lipoprotein cholesterol (LDL-C) and very-low-density lipoprotein cholesterol (VLDL-C) were calculated using the formula of Friedewald et al., the atherogenic coefficient (C_a) was calculated using the formula proposed by A.N. Klimov.

To determine the frequency of lipid metabolism disorders, the Russian recommendations of the 7th revision of 2020, compiled taking into account the European recommendations of 2019, were used. Hypercholesterolemia (HCH) was taken as the level of total cholesterol ≥5.0 mmol/l, taking into account the risk of cardiovascular death according to the SCORE scale, an elevated level of LDL cholesterol >3.0 mmol/l, a reduced level of HDL cholesterol - the level of HDL cholesterol ≤1.0 mmol/l in men and 1.2 mmol/l in women. Hypertriglyceridemia (HTG) was classified as a TG level of >1.7 mmol/l.

In 29 patients who received inpatient treatment, a statistical analysis of the content of only total cholesterol at admission, discharge and in the post-COVID period was carried out, since the assessment of the complete lipid profile in patients at admission and discharge from the hospital was not included in the protocol of mandatory laboratory tests.

Data on the degree of ground-glass lung damage were assessed by the results of X-ray computed tomography (CT).

Statistical processing of the obtained data was carried out using the SPSS Statistics 19 statistical program. The descriptive analysis data are presented in tables as Me (median), Q1 and Q3 (quartiles 25 and 75%). The significance of differences was assessed using Student's t-test and ANOVA, for independent samples with a normal distribution and the Mann-Whitney test with an abnormal distribution. The critical value of the level of statistical significance of differences (p) was taken equal to 5%. The data in the table are presented as M±m, where M is the mean, m is the standard error of the mean. Correlation analysis of the data was carried out according to the method of Pearson and Spearman.

Results and discussion. The results of our study revealed changes in the level of total cholesterol in patients on admission and discharge. Median total cholesterol at admission was 4.10 [3.60; 5.18] mmol/l, at discharge 4.50 [3.70; 4.75] mmol/l. It was found that at admission in men, cholesterol was lower by 11% than in women: Me 3.85 [3.35; 4.87] and 4.30 [3.61; 5.28] mmol/l, respectively.

In the post-COVID period, the value of the median total cholesterol exceeded the reference value, reaching 5.42 [4.70; 6.04] mmol/l and was statistically significantly higher by 32% than at admission (p=0.000) and 20% higher than at discharge (p=0.001) (Fig. 1).

Our analysis of the state of the lipid profile in the blood serum depending on the post-COVID period showed that at all times after recovery from infection with SARS-CoV-2, the level of total cholesterol, LDL cholesterol and C_a value did not correspond to the values recommended by the Russian Society of Cardiology.

The level of TG in all terms after recovery was within the reference values and had no significant differences; over time, there is a slight decrease in indicators. The highest level of total cholesterol was observed in group I (up to 3 months after the infection), exceeding the norm by 9.6% (5.48±0.22 mmol/l). In groups in which the period after COVID-19 was from 3 to 12 months, there was a trend towards a decrease in the level of total cholesterol, but still the indicators remained above the normative values up to 12 months.

The content of HDL cholesterol was

reduced, especially in groups I and III: 0.87±0.04 and 0.98±0.04 mmol/l, respectively. In groups II and IV, the level of HDL cholesterol is close to the minimum limit value. A statistically significant difference was found between HDL cholesterol levels in groups I and II (terms after COVID-19 up to 3 months and 3-6 months) (p = 0.002) (Table 2).

The anti-atherogenic fraction of lipids LDL-C at all times exceeded the norm (<3.0 mmol/l) and its level was higher (3.94±0.19 mmol/l) in group I (in those who recovered 3 months ago).

The average content of LDL cholesterol in the post-COVID period was increased by 20-24% of the norm. A higher rate was noted in group I (in those who recovered 3 months ago), remaining at a high level up to 12 months (Table 1).

As a result of an imbalance in the lipid profile, the value of the atherogenic coefficient (C_a) was the highest in group I (in those who had been ill three months ago) (5.48±0.43), exceeding the norm by 82%. A significant difference in the value of C_a was revealed when comparing the indicators of groups I and II (p=0.047). Over time, there is a downward trend, however, in all groups, C_a remains high, even after 12 months (Table 2).

The results of the analysis of the lipid spectrum, depending on the indicators of the degree of lung damage, showed a tendency to increase the level of total cholesterol, triglycerides, LDL cholesterol, C_a from CT 0 to CT 3, and vice versa, the antiatherogenic fraction of lipids - HDL cholesterol remained at a reduced level regardless of the severity of lung damage. The content of TG at CT 3 increases by 32%, the level of LDL cholesterol increases by 7% compared with CT 0 (Fig. 2). At the same time, the content of HDL cholesterol was reduced by 10% of the standard values. As the severity of the lesion increased, the value of Ka increased and in the group with severe lung damage (CT 3) C_a was the highest - 4.51±0.40.

Correlation analysis showed a direct relationship between the degree of lung damage (CT) and the level of total cholesterol (r=0.192; p=0.015), TG (r=0.258; p=0.001) and VLDL cholesterol (r=0.246; p=0.002). This confirms the dependence of changes in the lipid profile on the severity of lung damage in COVID-19, which indicates the impact of the development of infection on lipid metabolism. The revealed decrease in the level of total cholesterol in patients upon admission to the hospital does not contradict the literature data. During the period of acute SARS-CoV-2 infection, the lev-

Table 1

Distribution of the median of patients who recovered from COVID-19 by age groups depending on the post-COVID period and the severity of the disease, Me [Q25; Q75]

Groups	Post-COVID period	Average age	Severity of lung injury	Mean age
Group I n=15	Up to 3 months	51 (42.0; 62.0)	KT 0. n=27	46 (37.0; 56.0)
Group II n=75	3-6 months	53 (41.0; 62.0)	KT 1. n=60	47 (39.0; 58.7)
Group III n=49	6-9 months	54 (39.0; 62.0)	KT 2. n=42	60 (49.7; 63.0)
IV group n=22	6-12 months	51 (42.0; 61.0)	KT 3. n=32	57(42.2; 64.7)

Note. n is the number of patients.

Table 2

Average lipid values depending on the post-COVID period

Groups by term	Total number of studied	M±m	Standard deviation	95% CI	P
Triglycerides					
I	15	1.36±0.18	1.36	0.96-1.76	-
II	75	1.39±0.11	0.98	1.16-1.61	0.921
III	49	1.14±0.10	0.69	0.94-1.35	0.099
IV	22	1.23±0.14	0.66	0.93-1.50	0.598
Total cholesterol					
I	15	5.48±0.22	0.87	4.99-5.96	
II	75	5.47±0.13	1.21	5.18-5.73	0.976
III	49	5.42±0.14	0.97	5.13-5.69	0.837
IV	22	5.36±0.17	0.83	5.00-5.73	0.645
HDL cholesterol					
I	15	0.87±0.04	0.17	0.07-0.97	
II	75	1.09±0.04	0.40	0.99-1.17	0.002^{1-2}
III	49	0.98±0.04	0.32	0.88-1.07	0.091^{1-3}
IV	22	1.02±0.07	0.34	0.88-1.18	0.098^{1-4}
LDL cholesterol					
I	15	3.94±0.19	0.76	3.52-4.36	
II	75	3.75±0.12	1.05	3.50-3.99	0.418^{1-2}
III	49	3.92±0.12	0.84	3.67-4.16	0.930^{1-2}
IV	22	3.75±0.15	0.76	3.444.10	0.475^{1-4}
VLDL cholesterol					
I	15	0.62±0.08	0.33	0.43-0.80	
II	75	0.63±0.05	0.45	0.52-0.73	0.937^{1-2}
III	49	0.51±0.04	0.31	0.43-0.61	0.296^{1-3}
IV	22	0.56±0.06	0.30	0.42-0.68	0.599^{1-4}
C_a					
I	15	5.48±0.34	1.34	4.73-6.22	
II	77	4.59±0.25	2.20	4.10-5.10	0.047^{1-2}
III	48	4.94±0.25	1.81	4.45-5.50	0.228^{1-3}
IV	22	4.86±0.49	2.29	3.81-5.79	0.320^{1-4}

Note. Group I - recovered from COVID-19 3 months ago; Group II - recovered from COVID-19 6 months ago; Group III - those who recovered from COVID-19 9 months ago; Group IV - recovered from COVID-19 12 months ago.

el of circulating cholesterol in the blood decreases [11]. Perhaps this is also due to alimentary reasons, depletion of the body and a number of other factors. In a study by N.J. Wierdsma et al., one in five patients admitted to the hospital with COVID-19 suffered severe drastic weight loss, and 73% were at high risk of sarcopenia. Moreover, almost all patients had one or more nutritional complaints. Of these complaints, the predominant nutritional complaints were decreased appetite, feeling full, dyspnea, altered taste, and loss of taste. These symptoms have serious implications for nutritional status [9].

The SARS-CoV-2 coronavirus, like other enveloped viruses, enters host cells via endocytosis, interacting with lipid rafts within the cell membrane. Lipid rafts are subdivisions of the cell membrane rich in glycosphingolipids and cholesterol. They contain many molecules such as dynamin, caveolin, and clathrin, which may be important for viral infiltration [4, 8]. Cholesterol levels directly affect the permeability of lipid rafts; therefore, a higher concentration of cholesterol in the membrane promotes endocytosis. Conversely, lower levels of membrane-bound cholesterol reduce virus entry. Perhaps this is a protective reaction of the body from the destruction of cell membranes by the virus. At the same time, according to other researchers, low cholesterol is a risk factor for poor prognosis not only in COVID-19, but also in other critical illnesses, such as sepsis. In a study by H. Chen et al., patients with sepsis had significantly lower levels of cholesterol, HDL-C and LDL-C compared with patients without sepsis [10].

HDL cholesterol is known to exhibit anti-atherosclerotic, anti-inflammatory, anti-apoptotic, and anti-thrombotic properties [1]. The pleiotropic effects of HDL cholesterol are directly related to the immune system and host defense mechanisms against pathogens. HDL cholesterol transports acute phase proteins associated with inflammation and regulation of the component system. HDL-C particles interact with key cells of the immune system, such as macrophages, dendritic cells, megakaryocytes, T-cells and B-cells, and therefore regulate immune signaling [6].

LDL cholesterol transports most of the cholesterol contained in the circulatory system [7]. They are considered more proatherogenic due to their long retention in circulation in the blood, easy penetration into arterial walls, and greater susceptibility to oxidation. Anti-inflammatory cytokines induced by viral infection

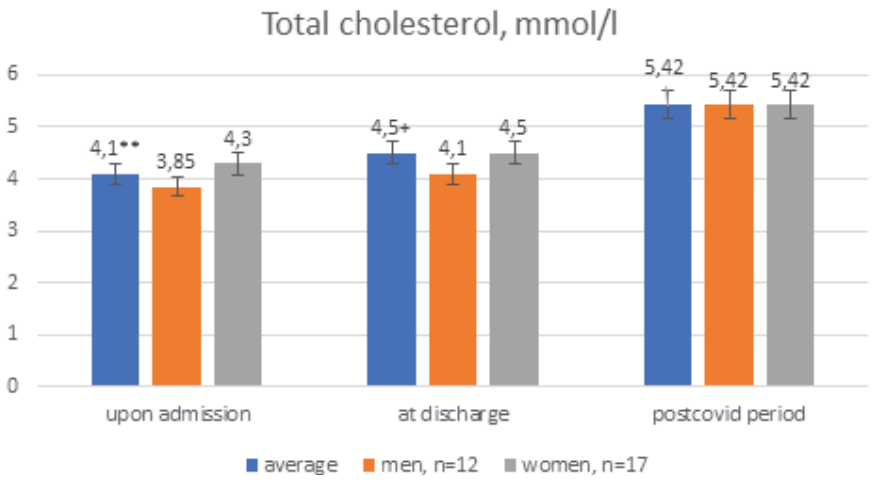

Fig.1. Median total cholesterol in blood serum in patients with COVID-19 during different periods of the disease and after recovery

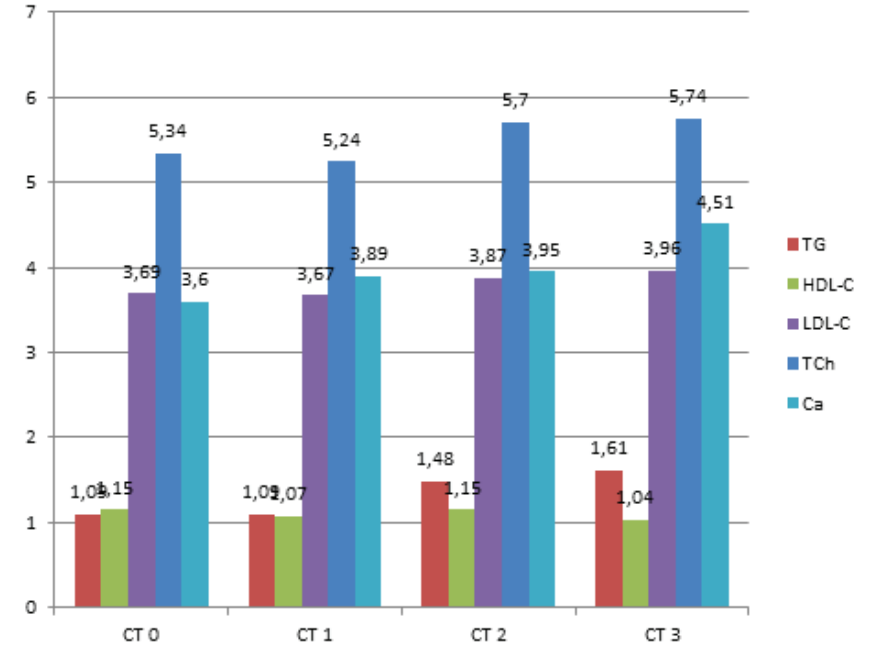

Fig.2. Lipid profile of patients with COVID-19 in blood serum depending on the severity of CT

modulate lipid metabolism, including the oxidation of LDL-C by reactive oxygen species released to facilitate clearance of LDL-C [2]. This indicates the prevalence of pro-oxidative processes after the disease for 12 months.

Thus, those who recovered from COVID-19 three months ago are at the highest risk of developing cardiovascular complications. The risk of developing cardiovascular complications persists for up to a year.

Conclusion. In patients with COVID-19 who received treatment in a hospital during the acute period of the disease, there is a decrease in total cholesterol and its increase in the post-COVID period. Patients who recovered from COVID-19 three months ago have a high risk of developing cardiovascular complications, as evidenced by a high atherogenic coefficient, which is 1.8 times higher than the normal value. The imbalance of the lipid profile increases depending on the severity of lung damage, which manifests itself in an increase in the level of total cholesterol, triglycerides, low-density lipoprotein cholesterol, which persists for up to 12 months. A pronounced shift in the lipid profile with a significant increase in the value of Ka is observed in the group of patients with severe damage to the lung volume (CT 3), which requires closer long-term medical and biological monitoring of these patients in order to prevent and prevent cardiovascular complications, including myocardial infarctions and strokes.

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NEUROSTEROID HORMONES AND PSYCHO-EMOTIONAL STATE OF INDIGENOUS MEN (YAKUTIA)

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A survey of 56 practically healthy men from among the indigenous inhabitants of Yakutia (yakuts-34, evenks-22) was carried out. The average age of men was 40.1±1.58 years. The purpose of this study was to assess the psycho-emotional state and the level of neurosteroid hormones in men of the indigenous population of the Republic of Sakha (Yakutia). The results of a psycho-emotional study of men showed that good psychological adaptation was noted in 64.3% (37), of which the «health» level was 28.6% (16) and optimal adaptation was 35.7% (20).

Non-pathological maladaptation was detected in 16.1% (9), and severe pathological maladaptation - in 19.6% (11) (pathological mental maladjustment - 8.9% (5) and probably a disease state - 10.7% (6)). Depression (D) was absent in 35.7% (20), mild depression was noted in 17.8% (10), moderate in 28.6% (16) and severe in 17.8% (10), severe depression not identified. An analysis of the degree of aggression showed that in 42.9% (24) of the surveyed, the aggression index (IA) was normal, in 53.6% (30) it was low and in 3.6% (2) it was high. An increase in the level of neurosteroid hormones in indigenous men is a protective reaction of the body in ensuring homeostasis and adaptation to the conditions of the North. The concentration of steroid hormones in men decreases with the deterioration of the psycho-emotional state.

Keywords: testosterone, cortisol, dehydroepiandrosterone sulfate, serotonin, depression, neuropsychological adaptation, Yakutia.

The process of adaptation and maintenance of homeostasis in residents living in extreme climatic conditions of the North, under the influence of man-made, socio-economic factors is accompanied by activation of metabolism (metabolism), changes in the endocrine and nervous systems [8; 7; 6]. Constant exposure to stress-limiting factors can lead to depletion of the body's reserve capabilities, disrupt homeostasis and provoke «oxidative stress», thereby increasing morbidity and mortality of the working-age population [13; 4, 1, 16]. Manifestations of stress reactions in residents of Northern latitudes in more than 60% of practically healthy people are expressed in psychoemotional and endocrine changes [5, 15]. There was an increase in psychoemotional tension by 19.4% and the level of the stress hormone cortisol by two times, compared with healthy residents of the middle latitudes [14]. A relatively frequent occurrence of anxiety-depressive states was noted in residents of Southern Yakutia (Neryungri) [12].

Hormones of the hypothalamic-pituitary-adrenal system regulate not only neuroendocrine function, but also affect behavior, thinking, sleep cycle regulation, memory, depression, anxiety and aggression [21]. Aggression is one of the most common ways to solve problems that arise in complex and difficult (frustrating) situations that cause mental tension. It is essential that aggressive actions used to overcome difficulties and relieve tension are not always adequate to the situation. Aggressive actions act as: 1) means of achieving some significant goal; 2) methods of mental discharge, replacement of satisfaction of blocked needs and switching activities; 3) satisfaction of the need for self-realization and self-affirmation.

Sex steroids are involved in the formation of cognitive functions, reduce the clinical manifestations of depression and other mental disorders. Understanding the effect of nonsteroidal hormones on the development and functioning of the central nervous system in different periods of men's lives is extremely rele-

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vant and can be used to correct various dishormonal disorders in men.

The purpose of this study was to assess the psychoemotional state and the level of steroid hormones in men of the indigenous inhabitants of the Republic of Sakha (Yakutia).

Material and methods of researc- During the field expeditions, in the spring (March), 56 practically healthy men from among the indigenous inhabitants of Yakutia (yakuts-34, evenks-22) (Megino-Kangalassky, Vilyuysky and Ust-Maysky districts) were examined. The average age of men was 40.1±1.58 years. Blood sampling was carried out from the ulnar vein in the morning from 8 to 11, on an empty stomach, in a state of muscular rest.

Determination of the concentration of cortisol, testosterone, dehydroepiandrosterone sulfate (DHEA-S) was carried out in blood serum using sets «Alkor-Bio» (Russia), dehydroepiandrosterone (DHEA) from DBC (Canada), serotonin - LDN (Germany) by three-phase enzyme immunoassay on a «Uniplan» photometer (Russia) on the basis of the Laboratory of Immunology of the FSBSI «YSC CMP», Yakutsk.

The psychological examination was conducted using the validated Bass-Darkey questionnaire (for aggression research), the Beck depression scale and the neuropsychic Adaptation Test (NAT) (for categorical assessment of mental health). The study was approved by the decision of the Local ethics committee at the FSBSI «YSC CMP» and was carried out in accordance with the ethical standards of the Helsinki Declaration (2000), after receiving the informed consent of the participants to use the materials in scientific generalizations.

Statistical processing of the results was carried out using the package of applied statistical programs SPSS Statistics 26. To identify the relationship between the studied indicators, the method of correlation analysis of data with the calculation of coefficients and Spearman rank correlation was used. The reliability of the differences was determined by ANOVA for independent groups. The critical value of the level of statistical significance of differences (p) was assumed to be 5%.

Results and discussion. The results of a psychoemotional study of men showed that 64.3% (37) had good psychological adaptation, of which 28.6% (16) had the level of «health» and 35.7% (20) had optimal adaptation. Non-pathological maladaptation was detected in 16.1% (9), and pronounced pathological maladaptation - in 19.6% (11) (pathological mental maladaptation - 8.9% (5) and probably a painful condition - 10.7% (6). Depression (D) was absent in 35.7% (20), mild depression was noted in 17.8% (10), moderate in 28.6% (16) and pronounced in 17.8% (10), severe depression was not detected.

According to the results of the study, Alekseeva Z.N. et al. (2018) in men of rural residents of Yakutia, the severity of depression and neuropsychiatric maladaptation is less pronounced than in women, while the severity of depression among men was 17.4% (moderate - 12.9%, pronounced - 3.8% and severe - 0.8%), and the NAT of the «painful state» was 28.8% [10].

The analysis of the degree of aggression showed that in 42.9% (24) of the surveyed, the aggression index (AI) is normal, in 53.6% (30) it is low and in 3.6% (2) it is high. The predominance of individuals with a low level of aggression requires further study. According to the definition of the methodology itself, the norm of aggression is an average indicator, and deviation from the norm to a low or high level is considered as destructive changes. A low level of aggression can be regarded as the suppression of aggression, which is more often regarded as a predictor of psychosomatization [3].

Correlation analysis showed that the level of NAT had a direct relationship with the level of depression (0.376; p=0.004) and AI (0.383; p=0.04), the main stress hormone - cortisol had an inverse correlation with indicators of psychoemotional state: with a degree of NAT (-0.326; p=0.018), with depression (-0.329; p=0.013) and with AI (-324; p=0.015).

The average level of cortisol in men was 566.21±21 (nmol/L), while an increased level of cortisol was noted in 25% of respondents, which is comparable to the data of studies of northerners, who had an increase in cortisol compared with healthy residents of the middle latitudes [14].

In groups with high psychological maladaptation (Table 1), with a higher level of depression (Table 2) and aggression (Table 3) a lower cortisol content was noted in comparison with groups with normal and low levels of psychological indicators (Tables 1, 2), which may be one of the disadaptation signs.

According to literature data, in two groups with unidirectional hormonal, psychoemotional manifestations of the severity of northern stress, practically healthy newcomers with an increased level of psychoemotional stress and a low concentration of cortisol showed more pronounced functional disorders of the cardiovascular system, gastrointestinal tract and painful meteoreaction [14]. When exposed to physical or psychological stressors, the brain sends a signal to the adrenal glands, and cortisol is released. Under the influence of which concentration of attention improves, blood circulation and glucose synthesis increases — this helps the body to release

Table 1

The content of neurosteroid hormones in men with different levels of NAT

Indicators	NAT level				
	1 n=16	2 n=20	3 n=9	4 n=5	5 n=6
Cortisol, 150-660 (nmol/L)	607.07±31.57	617.24±39.04	509.39±51.29	460.94±43.63 p_{2-4}=0.047	459.28±71.4 p_{1-5}=0.049; p_{2-5}=0.031
Testosterone, 12.1-38. (nmol/L)	22.21±1.37	25.32±1.64	20.91±2.61	21.48±2.61	23.47±3.40
Serotonin 40-400 (ng/ml)	321.72±20.41	336.21±23.56	315.91±36.72	293.50±37.10	314.56±36.45
DHEA, 3-11(mcg/ml)	5.72±0.75	6.50±0.66	6.54±1.27	4.21±0.64	6.87±2.66
DHEA-S, 1.0-4.2 (mcg/ml)	2.81±0.38	3.39±0.38	3.75±0.64	2.25±0.49	1.92±0.36 p_{2-5}=0.053 p_{3-5}=0.035
T/C, u.e	0.037±0.002	0.044±0.004	0.045±0.007	0.046±0.003	0.054±0.008
DHEA/C, u.e	0.96±0.14	1.24±0.24	1.49±0.43	0.94±0.16	0.32±0.40 p_{1-5}=0.049
DHEA-S/C, u.e	0.46±0.52	0.63±0.11	0.77±0.13	0.48±0.13	0.46±0.09

Note: 1-health; 2-optimal adaptation; 3- non-pathological mental maladaptation; 4-pathological mental maladaptation; 5-probable painful condition.

additional energy in order to overcome stress more effectively.

The testosterone level in all groups varied within the reference values, and had no significance from the level of NAT and depression (Tables 1, 2). Its significant dependence was revealed on the level of aggression, in the group with a low aggression index, a higher testosterone content was noted by 20.27% ($p<0.05$), in comparison with the group with a normal aggression index, (Table 3). Correlation analysis showed an inverse relationship of testosterone levels with the aggression index (-0.349; p=0.008) and a direct relationship with the cortisol level (0.421; p=0.004), DHEA (0.361; p=0.006) and the T/C index (0.396; p=0.003).

Studies by scientists from the Netherlands and South Africa have shown that aggression (in its everyday manifestation) has a connection not only with testosterone, but also with cortisol, as well as serotonin — a substance that nerve cells use to exchange signals. These studies suggested that testosterone increases aggressiveness mainly when there is little cortisol or receptors responsible for interacting with serotonin, and serotonin receptors partially prevent impulsive aggression, outbursts of rage. [24]. Thus, a strong positive correlation between testosterone and aggression was found in male offenders with low cortisol levels. In male offenders with high cortisol levels, the relationship between testosterone and aggression was not observed [17]. In addition, recent studies have revealed a similar relationship between testosterone, cortisol and aggressive behavior in clinical groups of children with behavioral disorder and adults with psychopathy, as well as in healthy people [19].

Recently, it has been suggested that a low level of serotonin combined with a high ratio of the T/K index contributes, in particular, to an impulsive subtype of aggression [25; 23].

The average level of serotonin in all groups varied within the normal range and did not differ depending on the indicators of the psycho-emotional state (Table 1.2), however, in the group with an increased level of aggression it tended to decrease (Table 3). Serotonin is the most important neurotransmitter involved in the regulation of cognitive, behavioral and other mental functions in humans, the neurobiological profile of low cortisol and high testosterone levels, together with low serotonin levels, predisposes to impulsive aggression.

The average level of DHEA hormone having an anabolic effect was 6.12 ± 0.46 (pg / ml) and, depending on the indicators of psychoemotional state, did not differ, which is consistent with the literature data. The level of DHEA in patients with hysterical symptoms did not differ from the indicators of healthy individuals [9]. In the work of Nakhodkin S.S. et al. (2018), the dependence of hormone levels on smoking and the type of character was revealed: in smoking young yakut men, the level of cortisol increases, and the level of DHEA tends to decrease, the dependence of the content of DHEA in the blood serum of smoking men on extroversion indicators is also shown (r=0.47; p=0.02). [2]. DHEA is a natural anti-glucocorticoid hormone that opposes the key stress hormone cortisol [21]. For adequate protection of the body from stress, DHEA, which is characterized by a protective effect against the central ner-

Table 2

The content of neurosteroid hormones in men with different levels of depression

Indicators	The level of depression			
	1 n=20	2 n=10	3 n=16	4 n=11
Cortisol, 150-660 (nmol/L)	657.39±31.92	527.34±49.91 p_{1-2}=0.028	484.37±30.30 p_{1-3}=0.001	553.64±58.53
Testosterone, 12.1-38.3(nmol/L)	23.23±1.44	24.57±2.24	22.57±1.83	22.69±2.11
Serotonin 40-400 (ng/ml)	314.87±27.39	329.77±14.49	338.05±23.55	306.65±20.97
DHEA, 3-11(mcg/ml)	5.45±0.56	7.97±1.12 p_{1-2}=0.065	6.02±1.17	5.77±.83
DHEA-S, 1.0-4.2 (mcg/ml)	3.14±0.41	3.17±0.49	2.48±0.34	3.5±0.53
T/C, u.e	0.035±0.001	0.049±0.006 p_{1-2}=0.043	0.047±0.009 p_{1-3}=0.045	0.048±0.008 p_{1-4}=0.066
DHEA/C, u.e	0.80±0.05	1.68±0.36 p_{1-2}=0.018	1.20±0.68	1.42±0.48
DHEA-S/C, u.e	0.48±0.05	0.66±0.13	0.53±0.06	0.76±0.02 p_{1-4}=0.065

Note: 1-absent; 2-mild; 3-moderate; 4-pronounced.

Table 3

The content of neurosteroid hormones in men with different levels of aggression

Indicators	Aggression Index		
	1 n =24	2 n=30	3 n=2
Cortisol, 150-660 (nmol/L)	613.41±27.33	518.21±32.39 p_{1-2}=0.029	443±147.88
Testosterone, 12.1-38.3(nmol/L)	25.41±1.15	20.26±1.31 p_{1-2}=0.004	24.86±1.95
Serotonin 40-400 (ng/ml)	326.57±18.21	326.97±17.27	213.09±55.35
DHEA, 3-11(mcg/ml)	6.10±0.56	6.26±0.84	4.88±2.01
DHEA-S, 1.0-4.2 (mcg/ml)	3.11±0.26	2.825±0.36	4.06±2.28
T/C, u.e	0.043±0.003	0.042±0.003	0.066±0.027 p_{1-3}=0.088; p_{2-3}=0.063
DHEA/C, u.e	1.09±0.15	1.30±0.22	1.09±0.09
DHEA-S/C, u.e	0.53±0.48	0.60±0.11	0.85±0.23

Note: 1-low; 2-normal; 3-high

vous system, should always prevail over cortisol [18, 20]. According to some studies, a low level of DHEA in the blood is one of the causes of insufficient stress resistance, increasing depression with age in adolescents and the elderly, increased suicide rates, and a decrease in the level of circulating DHEA and a decrease in the cortisol–DHEA ratio are associated with accelerated aging, depression, memory disorders, chronic fatigue syndrome, Alzheimer's disease. [18, 20].

In men of indigenous nationality, the average concentration of DHEA-S was equal to 3.02±0.21 (mcg/ml). The level of DHEA-S tended to decrease depending on the deterioration of the NAT. Changes in the concentration of DHEA-S were detected only depending on the level of NAT, in the group of people with a likely painful condition, the content of DHEA-S was reduced by 20%, compared with the «health» group and by 33.6% with optimal adaptation (Table 1). The DHEA-S content positively correlated with the DHEA level (0.427; p=0.001) and the DHEA/C index (0.313; p=0.019). DHEA-S has a weak androgenic effect, but in the process of its metabolism, testosterone and DHEA are formed in peripheral tissues.

Stressful situations lead to a violation of the central nervous system, according to most authors, they lead to a decrease in DHEA and DHEA-S. DHEA, unlike DHEA-S, can be metabolized in the brain, and affect mental processes.

The testosterone/cortisol index (T/C) had a direct relationship with NAT and depression (0.334; p=0.012 and 0.307; p=0.021), which indicates the activation of adaptive processes in the body of people with stress of a psychoemotional state. The ratio of DHEA to cortisol, which characterizes the anabolic-catabolic balance, is used as a marker of stress resistance in patients with mental disorders [9]. The DHEA/C index and DHEA-S/C had an inverse relationship with cortisol and a direct relationship with the level of DHEA and DHEA-S, respectively.

Thus, 35.7% of the surveyed indigenous men (yakuts, evenks) of the Republic of Sakha (Yakutia) have a stress of the psycho-emotional state according to the level of NAT. 46.4% of men were found to have depressive states. Low level of aggression is found in 53.6% (30), high in 3.6%. An increase in the level of neurosteroid hormones in indigenous men is a protective reaction of the body in ensuring homeostasis and adaptation to the conditions of the North. The concentration of steroid hormones in men decreases with the deterioration of the psycho-emotional state.

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ARCTIC MEDICINE

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FEATURES OF CELLULAR AND HUMORAL IMMUNE REACTIONS IN THE INHABITANTS OF THE EUROPEAN NORTH AND THE ARCTIC

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The features of cellular and humoral immune responses in the inhabitants of the European North and the Arctic have been studied. It has been established that a parallel increase in the frequency of registration of erythrocytosis, thrombocytosis, elevated hemoglobin, hematocrit and thrombocrit in residents of the Murmansk region is a mechanism for adaptation to an increased need for O2 in the Arctic. The examined individuals showed a high frequency of registration of leukocytosis, lymphocytosis, neutrophilia, monocytosis, eosinophilia and basophilia against the background of a significant level of deficiency of active phagocytes. A feature of the immunological reactivity of the inhabitants of the polar village is the predominance of reactions of cell-mediated and antibody-dependent cytotoxicity against the background of an increase in pro-inflammatory cytokines IL-6, IFN-γ, reagins, intercellular adhesion molecules sCD54 and sCD62L.

Keywords: erythrocytes, platelets, hematocrit, thrombocrit, phagocytosis, NK cells, cytokines, IgE, intercellular adhesion molecules, Arctic.

Introduction. The complex of natural and climatic factors typical for the Arctic poses a significant risk to the health of residents exposed to them [4; 15]. The cumulative effect of all unfavorable climatic and ecological conditions of these territories enhances their negative impact on the human body, causing a more intense reaction of adaptation to constantly changing living conditions, with increased energy consumption and the use of not always economical options for regulating and maintaining homeostasis [2; 6]. In people living in the northern climate, there is a decrease in immune protection [5; 6].

According to the integrated map of the impact of natural conditions on the territory of the Russian Federation on the living conditions of the population, the Arkhangelsk region belongs to a relatively unfavorable natural zone. The settlements of the Murmansk region belong to the regions of the Far North of the Russian Federation, are located beyond the Arctic Circle and are located in the area of residence with a pronounced effect of uncomfortable conditions on people, causing stress on the body's adaptation systems [1; 3; 11].

The aim of our study was to identify the characteristics of cellular and humoral immune responses in residents of the European North and the Arctic.

Material and methods. 315 currently practically healthy residents of the village were examined. Revda, Murmansk region, 237 women and 78 men, aged 21 to 50 years. The comparison group consisted of 181 practically healthy people of the Arkhangelsk region of the same age range, 138 women and 43 men.

All studies were carried out with the consent of the volunteers and in accordance with the requirements of the document "Declaration of Helsinki of the World Medical Association. Ethical principles for conducting medical research involving a person as a subject" (1964, as amended and supplemented in 2013), and also approved and approved Commission on Biomedical Ethics at the IFPA FGBUN FITSKIA Ural Branch of the Russian Academy of Sciences (protocol No. 5 dated February 11, 2022).

The complex of immunological examination included the study of hemogram, phagocytic activity of neutrophilic leukocytes in peripheral blood. The number and ratio of hemogram cells were counted in blood smears stained by the Romanovsky–Giemsa method. The phagocytic activity of neutrophilic granulocytes was determined using the Reacomplex test kit (Russia). On the hematological analyzer XS-500i (Sysmex, Japan), in the peripheral venous blood of the examined, WBC (total leukocyte count), RBC (total erythrocyte count), HGB (hemoglobin concentration), HCT (hematocrit - the proportion of blood volume occupied by erythrocytes), PLT (total platelet count), PCT (thrombocrit - the proportion of platelets in the total blood volume.).Lymphocyte phenotypes (CD3+, CD4+, CD8+, CD10+, CD16+, CD71+, CD25+, HLADRII, CD23+, CD95+, CD19+, CD54+, CD56+) were studied by indirect immunoperoxidase reaction using monoclonal antibodies (Sorbent, Moscow) and flow cytometry using an Epics XL apparatus from Beckman Coulter (USA) with reagents from Immunotech a Beckman Coulter Company (France). The content of pro-inflammatory cytokines IL-6, IFNγ, anti-inflammatory cytokine IL-10 immunoglobulin E, free intercellular adhesion molecules sCD54 and sCD62L with reagents from Bender Medsystems (Austria) in blood serum was studied by enzyme immunoassay on an automatic enzyme immunoassay analyzer Evolis manufactured by Bio-RAD "(Germany).

The obtained data were statistically processed using the Statistica 21.0 software package (StatSoft, USA). The results are presented as the arithmetic

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mean and error of the mean (M±m). An independent sample t-test was used for comparison between groups. For bivariate normal distribution data, the Pearson correlation coefficient was calculated. The critical significance level (p) in the work was taken equal to 0.05.

Results and discussions. It was found that the residents of the village Revda has a higher content of erythrocytes, hemoglobin, platelets (table 1), which is confirmed by an increase in the frequency of registration of erythrocytosis by 1.6 times (51.43±0.23 versus 31.49±0.31%, respectively), an increased content of hemoglobin by 3, 2 times (54.92±0.23 and 17.13±0.23%) and thrombocytosis by 2.5 times (21.90±0.15 and 8.84±0.16%).

In parallel with the increase in the content of erythrocytes and platelets, the levels of hematocrit (43.42±0.40 and 40.23±0.39%, p<0.001) and thrombocrit (0.27±0.012 and 0.23±0.007%, p= 0.028). Low air temperatures have a significant impact on the function of oxygen transport in the body, exposing it to the development of pathological processes. [7]. It is known that the inhabitants of the North have a low life expectancy of erythrocytes, the average content of hemoglobin in them. Changes in the shape of red blood cells and thickening of their cell wall reduce the activity of providing oxygen to tissues [8; 10]. A decrease in blood flow velocity and an increase in the content of erythrocytes and platelets contributes to the activation of the process of erythrocyte aggregation in microvessels, causing hypoxia. Previously, we have shown that the activity of aggregation of erythrocytes, platelets and leukocytes of peripheral venous blood in residents of the Arctic is 1.5–2.5 times higher than that in people living in the European North of the Russian Federation [20]. The mechanism for compensating for impaired oxygen supply to tissues is the increased formation of hemoglobin.Most likely, a parallel increase in the content of erythrocytes, platelets, hematocrit and thrombocrit levels, hemoglobin concentration can be considered as a mechanism for adaptation to an increased need for O_2 in the Arctic. An increase in hematocrit leads to a slowdown in blood flow velocity, creating favorable conditions for aggregation of erythrocytes in microvessels and adhesion of leukocytes on vessel walls [9]. It has been shown that during short-term general cooling at t-25°C for 5 minutes, an increase in hematocrit is associated with an increase in the concentration of endothelin-1 and irisin, which indicates the activation of vasoconstriction and heat production [12].

In the surveyed residents of the Murmansk region, the total content of leukocytes in the blood is higher due to lymphocytes, neutrophils, incl. stab and segmented forms, respectively, monocytes, eosinophils and basophils (table 2).

It is noteworthy that the residents of the polar village, compared with the comparison group, have a 7 times higher frequency of registration of leukocytosis, 9 times higher - lymphocytosis, 5.5 times - neutrophilosis, 4 times - monocytosis, 13 times - eosinophilia and 8 times - basophilia (Fig. 1.).

In the examined persons of the Murmansk region, an increase in the content of stab neutrophils was recorded in the blood, which indicates a shift in the leukocyte formula to the left. Along with increased concentrations of blood cells in the examined individuals, almost the same level of leukopenia was recorded, but the frequency of registration of lymphopenia in residents of the Arkhangelsk region was 3 times higher, neutropenia - 2 times and monocytopenia - 3 times (Fig. 2.).

Phagocytosis activates the body's immune defense reactions, ensuring the duration and activity of the immune response. The phagocytic activity of neutrophils is on average lower in residents of the Murmansk region (51.46±0.92 and 65.42±1.10, p<0.001) without changing the phagocytic number (5.46±0.10 and 5.52±0,25 pcs.) with a 4-fold higher frequency of active phagocyte deficiency (47.62% versus 12.71%). The lack of phagocytic protection may be associated with the predominance of immature neutrophils.

An increase in the total content of lymphocytes in residents of the village. Revda occurs mainly due to cytotoxic lymphocytes CD8+, natural killers CD16+ and CD56+ (Fig. 3.), which is confirmed by the high frequency of registration of these cells (respectively in 34.92%, 52.7% and 16.51% of cases) and indicates activation of cell-mediated and antibody-dependent cytotoxicity.

It is known that the mechanism of the cytotoxic action of natural killers and cytotoxic T-lymphocytes is associated with the content of perforin and granzyme granules, which have lytic activity with subsequent DNA degradation and programmed cell death [21].

In residents of the Murmansk region, the concentration of the pro-inflammatory cytokine IL-6 is significantly higher (13.79±0.80 pg/ml versus 3.05±0.28 pg/ml, p<0.001), however, it is within the physiological limits of the content. Ac-

Table 1

Erythrocyte and platelet parameters of venous blood in residents of the Arkhangelsk and Murmansk regions (M±m)

Indicator	Residents of the Arkhangelsk region (n=181)	Residents of the Murmansk region (n=315)
Erythrocytes. 10^9 cells/l	4.58±0.05	4.83±0.05***
Platelets. 10^9 cells/l	215±7.53	283.38±6.13***
Hemoglobin. g/l	215±7.53	283.38±6.13***

Note: *** p<0.001 - significance of differences when comparing results

Table 2

Indicators of the cellular composition of venous blood in residents of the Arkhangelsk and Murmansk regions (M±m)

Indicator, 10^9 cells/l	Residents of the Arkhangelsk region (n=181)	Residents of the Murmansk region (n=315)
Leukocytes	5.32±0.12	7.50±0.34***
Lymphocytes	2.10±0.06	2.70±0.08***
Stick-nuclear neutrophils	0.22±0.01	0.59±0.04***
Segmented neutrophils	2.50±0.08	2.88±0.12***
Neutrophils	2.73±0.08	3.79±0.21***
Monocytes	0.36±0.02	0.68±0.03***
Eosinophils	0.12±0.01	0.26±0.02***
Basophils	0.04±0.01	0.15±0.02***

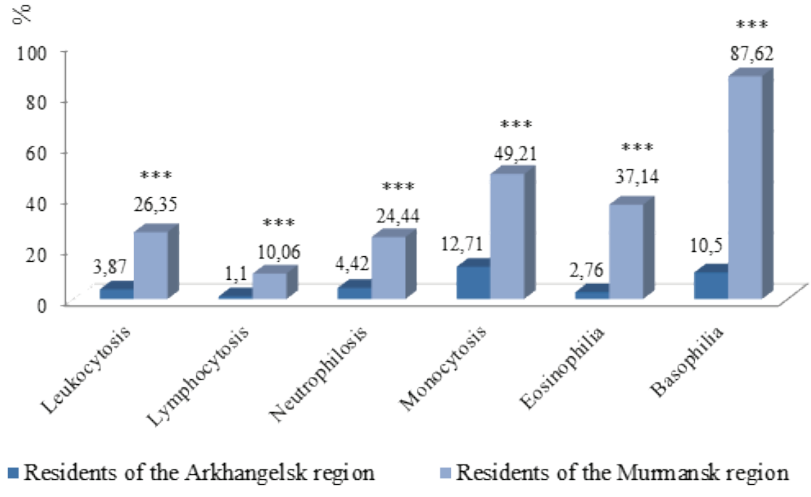

Fig. 1. The frequency of registration of elevated concentrations of blood cells in residents of the Arkhangelsk and Murmansk regions. Note: *** p<0.001 - significance of differences when comparing results.

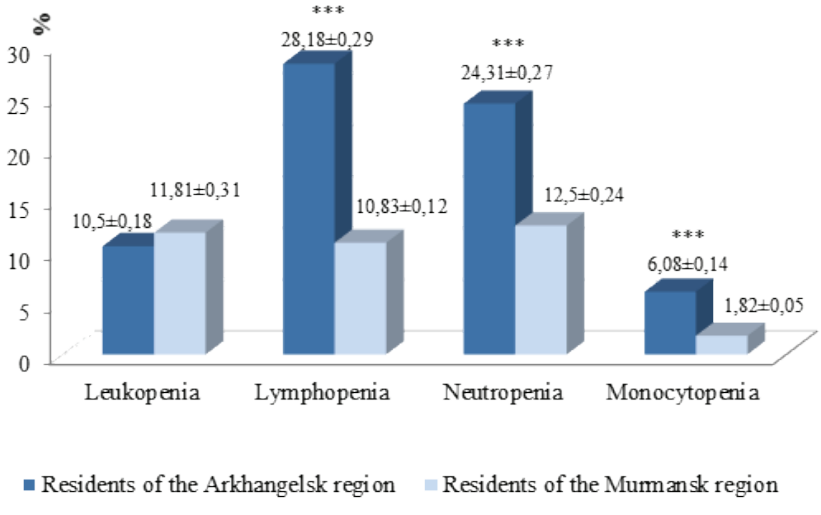

Fig. 2. The frequency of registration of low concentrations of blood cells in residents of the Arkhangelsk and Murmansk regions. Note: *** p<0.001 - significance of differences when comparing results.

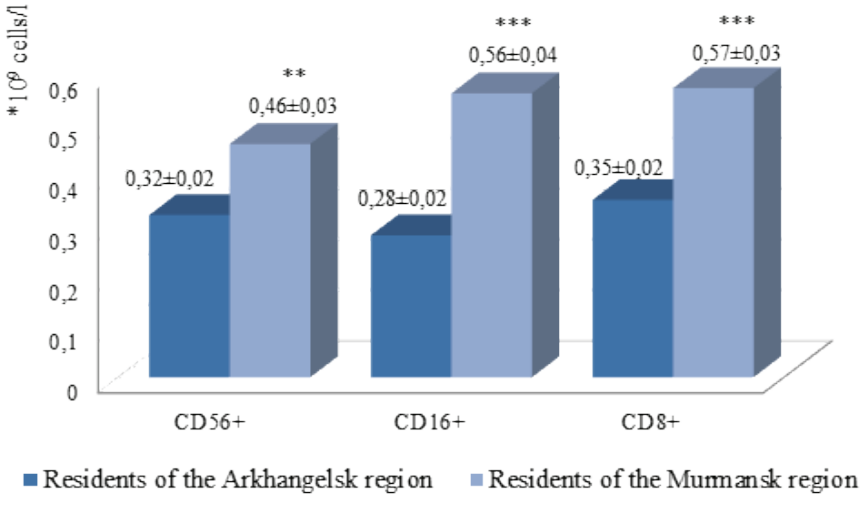

Fig. 3. The concentration of cytotoxic lymphocytes and natural killers in the blood of residents of the Arkhangelsk and Murmansk regions. Note: ** p<0.01, *** p<0.001 – significance of differences when comparing results.

cording to the available data, a sharp increase in the content of the pro-inflammatory cytokine IL-6 was found during hypoxia [14]. IL-6 is the main stimulator of acute phase reactions, accompanied by increased blood viscosity and an increase in the number of active platelets, promotes inflammation of vascular smooth muscle cells and activation of endothelial cells, inducing the expression of chemoattractant proteins and adhesion molecules [19].Indeed, the concentration of free intercellular adhesion molecules sCD54 (202.96±6.11 and 173.92±13.18 ng/ml, p<0.001) and sCD62L (8.44±0.76 and 4, 38±0.62 ng/ml, p<0.001), which are capable of forming the formation of cell conglomerates, autorosettes and clusters [13]. The concentration of pro-inflammatory IFN-γ is 6 times higher in residents of the Arctic (74.74±6.77 versus 12.14±0.22, pg/ml p<0.001) and is confirmed by an increased registration rate of 72.38±0.27% cases.

In 14.60% of the inhabitants of the village. Revda revealed increased concentrations of reagins (79.72±11.23 versus 55.32±10.16 IU/ml, p<0.01), which corresponds to a higher content of T-lymphocytes with the Fc IgE receptor (0.53± 0.04 versus 0.30±0.02X109 cells/l; p<0.001) in 32.06% of cases. IgE binds to lymphocytes, macrophages, monocytes, eosinophils, mast cells and basophils. The Fc receptor for CD23 reagins is involved in the regulation of the response involving immunoglobulins E [18]. The ability for antibody-dependent cytotoxicity of macrophages and eosinophils is carried out through IgE binding. The inhabitants of the village Revda, an increase in the concentration of IgE is interconnected with an increase in the concentration of eosinophils (r=0.87). Eosinophil peroxidase binds to mast cell granules without losing its activity [18]. On the surface of eosinophils, there are IgG, IgE, C3b, C4, CIs, C3a, C5a receptors, the combination of which with the antigen provides a cytotoxic effect, while the cytotoxic effect of eosinophils is sharply enhanced by mast cell secretion products [17].

Conclusion. So, in comparison with people living in more favorable conditions, residents of the Murmansk region have a higher frequency of registration of erythrocytosis, elevated hemoglobin, thrombocytosis, as well as hematocrit and thrombocrit. A parallel increase in the content of erythrocytes, platelets, hematocrit and thrombocrit levels, and hemoglobin concentrations can be considered as a mechanism for adaptation to an increased need for O2 in the Arctic. A high frequency of registration of leuko-

cytosis by 7 times, lymphocytosis by 9 times, neutrophilia by 5.5 times, monocytosis by 4 times, eosinophilia by 13 times and basophilia by 8 times was revealed against the background of a significant level of deficiency of active phagocytes (47.62% vs. 71%), which indicates an increased need for immunocompetent cells in tissues. A feature of the immunological reactivity of the inhabitants of the polar village is the predominance of reactions of cell-mediated and antibody-dependent cytotoxicity against the background of an increase in pro-inflammatory cytokines IL-6, IFN-γ, reagins, intercellular adhesion molecules sCD54 and sCD62L.

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A LOCAL FOCUS OF ACCUMULATION OF THE MITOCHONDRIAL FORM OF HEARING LOSS IN EVENO-BYTANTAISKY DISCTRICT OF YAKUTIA

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УДК 612.017.1(470.21)

Previously, the m.1555A>G mutation in the MT-RNR1 gene associated with the mitochondrial form of hearing loss was detected in one patient from Eveno-Bytantaisky district of Yakutia. The aim of this work is to study the mitochondrial form of hearing loss in this region of Yakutia, which probably has a local focus of accumulation of the m.1555A>G mutation in the MT-RNR1 gene. In the work, a clinical-genealogical, clinical-audiological and molecular-genetic examination of 72 residents of Eveno-Bytantaisky district was carried out for the presence of the m.1555A>G mutation in the MT-RNR1 gene of mitochondrial DNA. As a result of molecular genetic analysis, among the examined individuals, the m.1555A>G mutation was found in 6 people. Clinical and genealogical analysis, carried out up to the fifth generation, revealed that these six individuals belong to three families (including 25 deaf people). In the examined individuals with the m.1555A>G mutation of the MT-RNR1 gene, clinical phenotype variability was revealed - from normal hearing to bilateral hearing loss of III degree, with a late debut (onset from 30 to 60 years). The revealed variability is probably due to incomplete penetrance and requires further extensive research aimed at searching for genes that modulate nuclear or mitochondrial genomes.

Keywords: a mitochondrial form of hearing loss, mutation m.1555A>G, MT-RNR1 gene, Eveno-Bytantaisky national dictrict, Yakutia.

Introduction. In the world, congenital deafness is registered on average 1 per 1000 newborns and is one of the most common diseases among children in the world [3]. Both environmental and genetic factors are thought to contribute to the etiology of hearing loss. It is assumed that about half of all cases of congenital or early childhood deafness are due to hereditary causes [4]. For most hereditary diseases associated with the hearing loss, a large number of genes and mutations have been identified that cause their development; shows regional and ethnic differences in the spectrum and frequencies of detected mutations [Hereditary Hearing Loss Homepage: https://hereditaryhearinggloss.org/]. Most inherited forms of hearing loss are transmitted in a number of generations in an autosomal recessive manner, autosomal dominant and X-linked recessive and mitochondrial forms of hearing loss are less common [5].

Although mitochondrial forms of hearing loss are much less common than nuclear genome-related forms of deafness, they were among the first to be described. Thus, in 1993, Prezant et al [6] described a family case of hearing loss associated with m.1555A>G of the *MT-RNR1* gene (OMIM 561000) [6]. At the same time, the family members in whom this substitution was found showed incomplete penetrance - the hearing phenotype varied from profound hearing loss to completely normal hearing. There is a hypothesis that incomplete penetrance during the m.1555A>G substitution in the *MT-RNR1* gene of mitochondrial DNA is probably due to the use of aminoglycoside antibiotics [6-8]. This hypothesis is based on the fact that when adenine is replaced by guanine at position 1555 in the A site of human 12S rRNA, C–G pairing occurs, which leads to similarity with the A site of bacterial 16S rRNA, which is a target for aminoglycoside drugs [7]. Currently, most antibiotics from this series are used only for the treatment of severe infections [9]. However, in many developing countries, they are often used as broad-spectrum drugs [10-12]. In addition, modifier genes most likely located in the nuclear genome can influence the phenotypic manifestation of the mutation [13], it is less likely that the mitochondrial background can have a modulating effect, since this mutation was found on various mtDNA haplotypes [14, 16].

It is now known that the frequency of the m.1555A>G mutation of the *MT-RNR1* gene among patients with hearing impairments in different regions of the world varies widely, on average from 0.27% in Australia to 4.42% in Asia [2] . However, the worldwide maximum occurrence of the m.1555A>G mutation among patients was registered in Spain - 20% [8, 17], and a relatively high mutation frequency was shown in Morocco - 3.6% [18], China - 5, 1% [19], Indonesia - 5.3% [20] and Japan - 5.4% [21]. Among Russian patients, the m.1555A>G mutation of the *MT-RNR1* gene was previously registered only in a sample from St. Petersburg with a frequency of 0.8% (one Russian patient), and in a sample from Yakutia (one patient from the Eveno-Bytantai region), with a frequency of 0.57% and was not found among the examined deaf people from the Republic of Altai and the Volga-Ural region [1]. In another study, among the examined 108 individuals with hearing impairments in Yakutia, the m.1555A>G mutation of the *MT-RNR1* gene was not found (0/108) [2].

Due to the fact that m.1555A>G of the *MT-RNR1* gene most likely has a local distribution, the aim of this study was to study the mitochondrial form of hearing loss in Eveno-Bytantaisky district of Yakutia.

Materials and methods. ***Sample.*** To study cases with hearing loss of unknown etiology, a survey was conducted among residents of the Eveno-Bytantaisky district. The sample consisted of 72 individuals (68 from the village of Batagay-Alyta, 4 from the village of Kustur). Of these, males accounted for 34.7% (n=25), females - 65.2% (n=47). Average age - 44±17.21 years. Ethnic composition of the sample: Evens - 48 people. (66.6%), Yakuts - 22 people. (30.5%), Evenk - 1 person. (1.4%), mixed ethnicity (Even / Yakut) - 1 person. (1.4%).

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Clinical and audiological analysis. Assesment of level of hearing impairment was performed using threshold tone audiometry using a MAICO ST 20 audiometer (Germany) for air conduction at frequencies of 0.25, 0.5, 1.0, 2.0, 4.0, 8.0 kHz and for bone conduction at frequencies of 0.25, 0.5, 1.0, 4.0 kHz step 5.0 dB. The degree of hearing loss was assessed by the average threshold of hearing in $PTA_{0.5.1.0.2.0.4.0}$ kHz according to the WHO classification: I degree - 26.0-40.0 dB, II degree - 41.0-55.0 dB, III degree - 56.0-70.0 dB, IV degree - 71.0-90.0 dB, deafness -> 91.0 dB.

Clinical and genealogical analysis. To conduct a clinical and genealogical analysis, the collection of anamnestic data was carried out according to the individual card developed by us, which included information about the main ENT diagnosis, the probable cause of hearing loss, age at the onset of hearing loss, the presence or absence of hereditary burden and concomitant diseases. After collecting the necessary information about the proband (a study participant with hearing loss), data about the siblings and parents of the proband, information about relatives on the mother's side and on the father's side, a pedigree was compiled. To confirm the hereditary nature of hearing loss and clarify the type of inheritance, segregation analysis (SF) was performed using the Weinberg proband method [22-24]. The following formulas were used for calculations:

To calculate the probability of registering a trait in families (Fisher's method):

$$\pi = \sum n/\sum r, \quad (1)$$

where: π - probability of registration; n is the number of all probands in all properties; r - the number of affected in all sibstv;

To calculate the expected segregation frequency of a trait in families:

$$SF = \sum r - n/\sum s - n, \quad (2)$$

where: SF - expected segregation frequency, r - number of affected in all sibs, n - number of all probands in all sibs, s - total number of sibs in sibs;

To calculate standard deviation:

$$\sigma = \sqrt{SF\ (1 - SF)/\sum s - n}, \quad (3)$$

where: σ - standard deviation, SF - expected segregation frequency, s - total number of sibs in sibs; n is the number of all probands in all properties;

To test the hypothesis about the type of inheritance:

$$t = SF_0 - SF/\sigma, \quad (4)$$

where: t - Student's t-test, SF_0 - theoretically expected segregation frequency, SF - expected segregation frequency, σ - standard deviation.

Molecular genetic analysis. Genomic DNA was isolated from venous blood using a standard method using phenol-chloroform extraction followed by enzymatic digestion with proteinase K. To detect the m.1555A>G mutation of the *MT-RNR1* gene (mitochondrially encoded 12S RNA; NCBI, Gene ID: 4549; NC_012920.1) the PCR-RFLP method was applied. The sequences of oligonucleotide primers for gene fragment amplification included forward primer F5'GCTCAGCCTATATACCGCCATCTTCAGCAA3', and reverse mismatch primer R5'TTTCCAGTACACTTACCATGTTACGACTGG3'; creating a restriction site for the *Hae*III endonuclease. Visualization of the results of PCR-RFLP analysis was carried out using electrophoretic separation of restriction products in a 3% agarose gel with ethidium bromide in UV light.

Ethical control. The surveys included in the scope of this research work were conducted after the informed written consent of the participants. The research work was approved by the local committee on biomedical ethics at the YSC CMP in 2019 (Yakutsk, protocol No. 7 dated August 27, 2019).

Results and discussion. Clinical genealogical, clinical audiological and molecular genetics analyzes were performed in 72 people for the presence of the m.1555A>G mutation in the *MT-RNR1* gene of mitochondrial DNA. As a result of molecular genetic analysis, the studied mutation was found in 6 out of 72 examined people. Clinical and audiological analysis showed that the hearing loss was clinically significant in 4 people, and in two people the hearing thresholds were within the normal range (Table 1).

As a result of clinical and genealogical analysis, carried out up to the fifth generation, it was found that these six individuals belong to three families, including 25 affected people. Fragments of pedigree families with the m.1555A>G mutation of the *MT-RNR1* gene are shown in Figure.

Since only family members available at the time of the study were tested for the m.1555A>G mutation in the *MT-RNR1* gene, as well as the fact that hereditary forms of hearing loss are characterized by extremely high heterogeneity, it was important to confirm that the identified cases are associated with the mitochondrial form hearing loss. In this regard, a segregation analysis was carried out. For the correctness of the analysis (calculation is carried out only among siblings), out of 25 individuals with hearing loss, 4 people were excluded: family 1, IV-1 was not taken into account, since he was not biologically related (husband IV-2); family 2, I-2 no data on sibs, II:1 half-sibs; family 3, proband II-1 adopted (Figure). Data used for segregation analysis is presented in Table 2.

When establishing the hereditary nature of a pathological trait (deafness/hearing loss) in families, the probability of registration (π) of the trait (probability probability) according to the Fisher method (1) was:

$$\pi = 9/21 = 0.43$$

The resulting probability of registering a trait (π = 0.43) indicates its hereditary nature and corresponds to multiple incomplete registration, where $0 < \pi \leq 1$ [23, 24].

Table 1

Clinical and audiological characteristics of individuals with the m.1555A>G mutation of the *MT-RNR1* gene

Family	Cipher	Sex	Ethnicity	Age	Hearing status	Age of onset of HL	Case of HL
1	III-11	Female	Yakut	56 years	Normal	-	-
	III-25	Female	Even	62 years	Bilateral sensorineural HL (of IV degree (Moderate)	-	-
	IV-8	Female	Yakut	42 years	Bilateral sensorineural HL of III degree (Moderate)	30 years	Hereditary
	V-17	Female	Even	4 years	Normal	-	-
2	II-7	Female	Even	54 years	Bilateral sensorineural HL of III degree (Moderate)	48 years	-
3	II-1	Female	Even	62 years	Bilateral mixed HL of I degree (Mild)	60 years	Age-related

Note: "-" - no answer or data not available.

The segregation frequency (SF) (2) or the expected proportion affected for all sings (3) was:

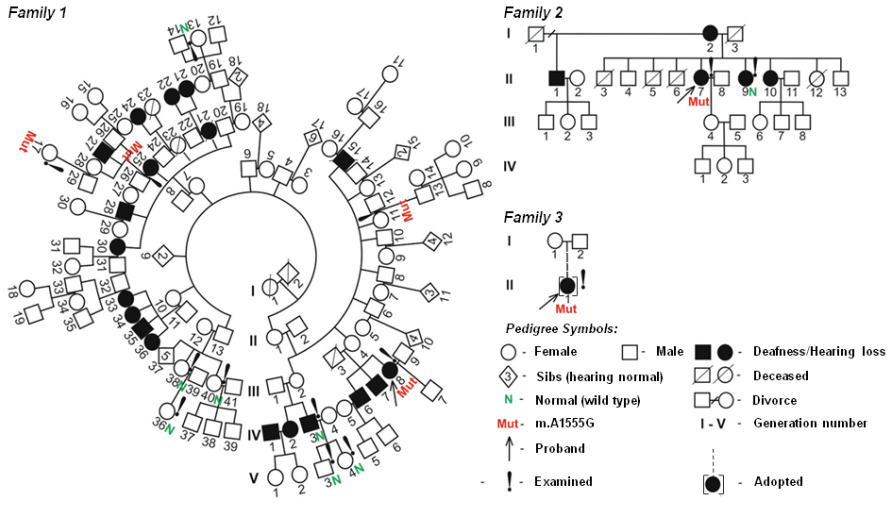
SF = 21 - 9/40 - 9 = 12/31 = 0.39

σ = √0.39 x (1 - 0.39)/40 - 9 = 0.1

The calculated segregation frequency, SF = 0.39, was higher in autosomal recessive inheritance (SF_0 = 0.25), since all probands had hearing parents, except for three nuclear families with one affected parent. Further, the obtained frequency was compared with the theoretically expected one for different types of inheritance (SF_0 = 0.25 - autosomal recessive (AR), SF_0 = 0.50 - autosomal dominant (AD)) using Student's t-test (4). As a result, negative values were obtained (t = 0,25 - √0,39/0,1 = -1,7; t = 0,50 - √0,39/0,1 = -1,4.), which refute these types of inheritance.

In general, given the results of segregation analysis, which did not confirm the autosomal dominant and autosomal recessive types of inheritance, it can be assumed that the most likely type of transmission of the disease in a number of generations is the mitochondrial type of inheritance. The obtained results testify in favor of a local focus of accumulation of hearing impairment in Eveno-Bytantaisky district of Yakutia, associated with the mitochondrial form of hearing loss associated with m.1555A>G of the *MT-RNR1* gene.

Family 1 in family 1, 11 people were examined, according to which a clinical and genealogical analysis was carried out up to the fifth generation, as a result of which it was possible to obtain information about 19 people affected by deafness who belonged to this family (Figure). In this family, testing for the presence of the mutation was carried out in 11 family members (9 nuclear families), of which four people had the m.1555A>G mutation in the mitochondrial DNA *MT-RNR1* gene. Despite the results of segregation analysis that the type of transmission of the disease in a number of generations does not contradict the mitochondrial type of inheritance, in family 1, cases of "slippage" of the pathological trait were identified, which are likely due to incomplete penetrance or incomplete information about the hearing status of members of this family in the first two - three generations (Figure). According to the results of the clinical and audiological analysis, two family members had a clinically significant hearing loss (III:25 and IV:8), and two (III:11 and V:17) had hearing thresholds within the normal range (Table 1). Thus, in this family, the m.1555A>G mutation of the *MT-RNR1* gene was found among two hearing family members. The revealed variability in the manifestation of the phenotype may be due to incomplete penetrance associated with the use of the aminoglycoside group of drugs, which, as is commonly believed, can be the main triggers of this form of hearing loss [6, 7, 11]. However, according to the results of the survey, it turned out that the affected individuals did not associate hearing loss with taking medications, and the onset of signs of hearing loss occurs at a fairly mature age (from 30 to 60 years) (Table 1). In addition, it is known that gene variants of the nuclear or mitochondrial genomes may have a modulating effect, or this may be associated with a different level of heteroplasmy. Thus, in one member of this family with hearing loss (IV-3), the m.1555A>G mutation in the *MT-RNR1* gene of mitochondrial DNA was not identified (Figure).



Pedigrees of families with the m.1555A>G mutation of the *MT-RNR1* gene

Table 1

Segregation analysis in families with deafness cases from the Eveno-Bytantai region

Sibling size	Number of nuclear family/probands (n)	Number of siblings with affected children				Total number of children		
		1	2	3	4	Affected	Non-affected	Total
						(r)	-	(s)
2	3	1	2	-	-	5	1	6
3	2	1	-	1	-	4	2	6
4	1	-	-	-	1	4	-	4
7	1	-	-	-	1	4	3	7
8	1	1	-	-	-	1	7	8
9	1	-	-	1	-	3	6	9
Total	9	3	4	6	8	21	19	40

Family 2 in family 2, two sisters with hearing loss were examined (II:7 and II:9), according to which, in this family there are three more family members (siblings and mother) with signs of hearing loss. As a result of the clinical and genealogical analysis carried out in this family, it was possible to draw a pedigree line up to the fourth generation. It should be noted that as a result of molecular genetic analysis, the m.1555A>G mutation was found only in one of the sisters (II:7), while this mutation was not detected in the other (II:9) (Figure). In this case, as in family 1, where no mutation was identified in one of the affected members of family 1 (IV:3), there may be several explanations for the lack of segregation. One of the most likely reasons may be different levels of mtDNA heteroplasmy [25] in different organs and tissues, which

makes it difficult to detect this mutation from DNA samples isolated from venous blood. Another likely reason may be the lack of maternal biological relationship between the examined individuals. However, given the common pathological phenotype present in both sisters, this variant is less likely.

Family 3 in family 3, only a 62-year-old female proband (II-1) was examined, in which the m.1555A>G mutation of the *MT-RNR1* gene was found (Figure). The patient's hearing loss was characterized as bilateral mixed hearing loss of the first degree (Table 1), and the onset of the disease was at 60 years of age. Since the proband was an adopted child, it was not possible to conduct a clinical and genealogical analysis in this family.

Conclusions

1) As a result of molecular genetic analysis, among the examined 72 individuals from the Eveno-Bytantaisky national region, the m.1555A>G mutation of the *MT-RNR1* gene was found in 6 people. At the same time, hearing loss was clinically significant in 4 people, and in two people, hearing thresholds were within normal limits. The revealed clinical variability of the phenotype in individuals with the m.1555A>G mutation of the *MT-RNR1* gene - from normal hearing to bilateral hearing loss of the III degree, is probably due to incomplete penetrance and requires further extensive research aimed at searching for modulator genes of the nuclear or mitochondrial genomes.

2) As a result of clinical and genealogical analysis, it was found that these six individuals belong to three families, including 25 people affected by deafness, in which hearing loss segregated according to the mitochondrial type of inheritance. Thus, the study revealed a local focus of accumulation of hearing impairment in Eveno-Bytantaisky district of Yakutia, associated with the mitochondrial form of hearing loss associated with m.1555A>G of the *MT-RNR1* gene. The revealed absence of the m.1555A>G mutation of the *MT-RNR1* gene in two deaf people from two different nuclear families, in which the mitochondrial type of inheritance was traced, may indicate a different level of mtDNA heteroplasmy.

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SCIENTIFIC REVIEWS AND LECTURES

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THE ROLE OF ASSORTATIVE MARRIDGES AMONG DEAF IN THE PREVALENCE OF HEREDITARY HEARING LOSS

The article presents an overview of the role of assortative deafness marriages in the spread of hereditary hearing loss. In 1883, Alexander Graham Bell, the famous inventor of the telephone, first suggested that frequent marriages between deaf people could lead to an increase in the incidence of deafness, but this hypothesis was not recognized by his contemporaries. In the 2000s, with the development of molecular genetic studies, which made it possible to identify one of the most common forms of hearing loss due to gene mutations GJB2, connexin 26, which encodes the interstitial contact protein, has been reinterpreted by Walter Nance. In the series of studies, he and his coauthors were able to show that the reproductive capabilities of the deaf have increased, and marriages between the deaf occur not by chance, but by the principle of assortativity, which in total could lead to an increase in the prevalence of one of the most frequent "connexin" forms of hearing loss.

Keywords: hereditary deafness, sign language, assortative marriages.

The emergence of systematic sign language education for deaf people. The earliest known case of education for the deaf dates back to XVIth century. The lord of the city of Onya in the province of Burgos in Spain, Juan de Velasco, sent his two deaf sons, Francisco and Pedro, to the monastery of San Salvador [29]. They were taught by the priest Pedro Ponce de Leon. In teaching the brothers, he applied more than 360 gestures which were used during the vow of silence in the monastery in various everyday situations. It is also assumed that additional gestures were used, which the brothers developed at home with their two deaf sisters. In total, de Velasco had 9 children, four of whom were deaf. His wife, Juana Enrique de Riviera, was related by blood to him, which was a common practice for preserving wealth within the family at that time. The father of the deaf boys wanted to give them the opportunity to inherit the property in the event of the death of the eldest hearing son and received permission from the emperor to do so. In this regard, he provided his sons with a high level of education, as a result of which they were able to learn to read and write in Spanish, Greek and Latin. De Leon used a mixed approach that included writing, the sign (dactyl) alphabet, monastic gestures, and the" home" gestures of the brothers. He later published his experience as a manuscript *Doctrina para los mudos sordo,* the original and copies of which are now considered lost [29].

In 1615, Manuel Ramirez de Carrion was invited to Madrid to teach Luis Fernandez de Velasco, the great-nephew of de Leon's first students. De Carrion taught Luis using the dactyl alphabet. De Carrion was forced to leave and Luis de Velasco was trained by Jean Pablo Bonet, who learned methods of de Carrion. In 1620, he published *Reduction de las letras y arte para enseñar a ablar los mudos* (trans.: "Letter reduction and the art of teaching the mute to speak"), which later became very important, being the first book on ways of teaching the deaf. Luis de Velasco himself also played an important role in showing how highly educated a deaf person can be [29].

The recognition of sign language as a distinct language, as well as the development and implementation of a curriculum for its training, begins with Charles-Michel de l'Épée. l'Épée was born to a wealthy family in Versailles and was studied to be a priest, but was forbidden to preach. He found his vocation by chance when he met two deaf girls who were taught from pictures. He believed that faith and salvation of the soul should not depend on hearing and can be achieved through gestures. Using his father's house and his own funds, l'Épée founded the first free school for the deaf in 1760. His first publication appeared in 1774, in which he defined and published the syntax of sign language [1]. At the same time, the first school for the deaf in Germany was opened in 1778. Its founder was Samuel Geinicke, who began teaching the deaf in 1754. He considered the sound method and spoken language necessary for a full-fledged education. In turn, it was based on the writings of the Dutch physician Johann Conrad Ammann, who left two works "*Surdus Loquens*" (Amsterdam, 1692) and "*Dissertatio de loquela*"(Amsterdam, 1700). These two works were reprinted many times (7th edition in 1740) and were translated into French and German. They served as a basis for subsequent teachers of the deaf and dumb, especially Geinicke, in their further research.

Alexander Bell's hypothesis on the relationship between congenital forms of deafness and marriages between deaf people. In 1883, Alexander Graham Bell, the famous inventor of the telephone, in his speech at the National Academy of Sciences of the United States for the first time suggested that frequent marriages between deaf people can lead to an increase in the incidence of deafness [3]. Bell himself was quite familiar with the problems of the hard of hearing and the deaf, as his mother began to lose her hearing when he was 12 years old [12]. To communicate with her and help her understand others Bell learned sign language [17]. Alexander Bell at the beginning of his career followed in his father's footsteps, a linguist who developed the so-called "visible speech" system. Visible speech is a phonetic alphabet and writing system, the main feature of which is a visual representation of the position of the organs of the articulatory apparatus in the pronunciation of phonemes [5]. Alexander Bell significantly improved the system of visible speech. In 1871, Alexander Bell was invited to the school for the deaf and dumb in Boston (USA) to teach their teachers this system. In 1872, he opened the" School of Voice Physiology and Speech Mechanics" in Boston, which attracted a large number of deaf students.

Like many scientists of the time, Bell was very interested in the science of heredity, which had become popular since the publication of Charles Darwin's work. On his estate, he conducted long-term experiments in breeding [5]. Bell's observations on deafness showed that the proportion of deaf children born to deaf parents is many times higher than the

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proportion of deaf children born in the general population. He published his observations and reflections in a report of 1883, which caused heated discussions. But at the end, he was unable to develop any theory to explain his assumptions and observations [19].

Bell's initiative was continued by Edward Allen Fay, Vice President of Gallaudet College for the Deaf (Gaulladet) and the editor of "American Annals of the Deaf" journal [18, 19]. Bell gave him all the pedigrees he had collected. Over a six-year period, Fay and his assistants were able to collect information on more than 8,500 people from an analysis of the pedigrees of 4,471 marriages among Gallaudet College graduates and graduates of other boarding schools for the deaf across the United States, from 1803 to 1894 [13]. Their findings did not support Bell's arguments, but neither did they refute his critics. As Bell had suspected, there was a link between deafness and heredity. For example, among the sample of children with one or both deaf parents, 9% were also deaf, compared to the incidence of hearing loss in the general population, which was approximately 1 per 1000 newborns. However, a smaller percentage of deaf offspring were born to couples in which both parents were deaf than to deaf-hearing couples. At the same time, 76% of the analyzed marriages were between two deaf people. The presence of deaf relatives and consanguinity of parents were the factors most strongly increasing the probability of having a deaf child [13], but these studies were not continued.

Confirmation of the hypothesis by Walter Nance. Later the scientific community usually ignored Bell's suggestion, given the assumed large number of genes associated with deafness. So, James Crow and Joseph Felsenstein, based on the classic works of Ronald Fischer and Sewell Wright [14, 31], showed that assortative marriages (marriages based on similarity of traits), in the absence of selection pressure, affect only genotype frequencies, and not gene frequencies [31]. Regarding deafness, they concluded that if the phenotype is due to genes with the similar frequency in 35 loci (as was then considered [11]), then even intensive assortative marriages, in the absence of selection, will give only a 2-3% increase in the incidence of deafness [31].

In the 1970s, a comparative analysis of the work of Edward Fay was published [13] with up-to-date data from the annual study of children with hearing loss at Gallaudet University, which included information on 12,665 cases [27;28]. In the works of Susan Rose, for the first time, the concept of *complementary* and *noncomplementary marriages* is used when analyzing assortative marriages of deaf people based on the state of hearing of their offspring. *Complementary* marriages are defined as marriages between deaf married partners with different etiologies of hearing loss (acquired hearing loss in one of the partners or mutations in various genes associated with hearing loss); in such marriages, there may be only hearing or, in some cases, both deaf and hearing children. *Noncomplementary* marriages are marriages between deaf people who share the same genetic cause of hearing loss – the presence of biallelic recessive mutations of the same gene. All children of such a married couple will also be deaf and have the same genetic etiology of hearing loss as their parents (Fig. 1).

The results of Susan Rose show that between XIX and XX centuries, the proportion of children with one or two deaf parents has increased by 38% from 0.064 to 0.089. Among assortative marriages, the proportion of noncomplementary marriages also increased by 23% from 0.29 to 0.36. In these two large-scale samples, Susan Rose conducted a segregation analysis and concluded that 49% of cases of deafness were sporadic. Among inherited forms, 12% - 14% were classified as autosomal dominant with incomplete penetrance, and 86% - 88% of cases were identified as autosomal recessive. It was assumed that they are caused by genes in 10 independent loci, probably distributed with the same frequency [27;28].

In the end of the XXth century, ideas about the high heterogeneity of hereditary hearing loss have changed dramatically. It became clear that hereditary deafness associated with the DFNB1 locus (autosomal recessive deafness type 1A), in which the *GJB2* (Cx26) gene was mapped, is the most frequent one [7;16]. Mutations in this gene have been found to be a major cause of autosomal recessive nonsyndromic congenital hearing loss in many populations [6-9;20;22;23;26]. New molecular genetic data suggesting that up to half of all cases of inherited hearing loss are caused by mutations in a single gene – *GJB2*, became a big discovery [16 – 23], and subsequently led to a rethinking of many previously existing concepts about the extremely high heterogeneity of this pathology.

The very fact of identifying one major form of hearing loss provided an opportunity to rethink Alexander Bell's hypothesis that assortative marriages between deaf people can contribute to an increase in their number due to an increase in the proportion of noncomplementary marriages in which hearing loss in both spouses is due to the same genetic cause. So, in 2000, Walter Nance suggested that in all noncomplementary marriages from the Edward Fay data sets [13] and Susan Rose [27;28], hearing loss may have been caused by mutations in the gene *GJB2* ("connexin deafness"). In this case, it is possible to indirectly estimate the contribution of these mutations by the proportion of noncomplementary marriages. The proportion of such marriages will be equal to the fourth power of the frequency of the corresponding mutant allele in a given population, i.e. $q^2 \times q^2$, where q^2 it is the proportion of "connexin deafness" in the population. Rose's data showed that among 1,299 fertile assortative marriages, 4.2% were noncomplementary, and then the contribution of connexin form of hearing loss to XIX it was approximately equal to $q^2 = \sqrt{0,042} = 0,204$ (20.4%) [24]. Walter Nance and his colleagues noted a significant increase in the proportion of "connexin deafness" in the United States between XIX and XX centuries, by comparing the 20.4% with modern, at that time, data - 35.6% [4].

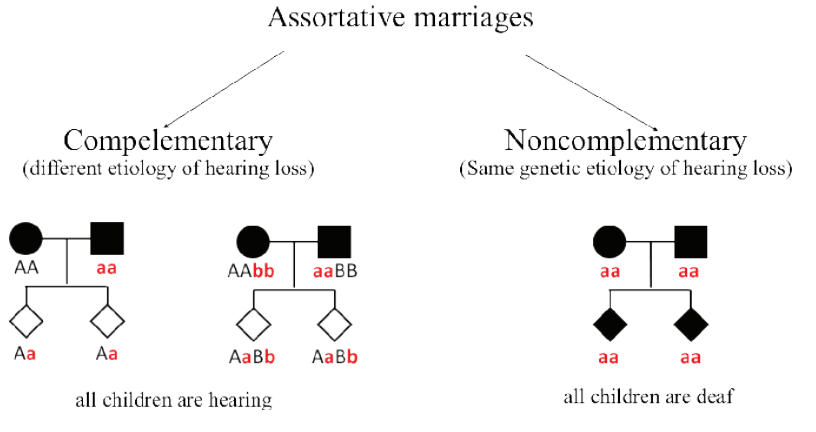

Fig. 1. Types of assortative marriages for deafness

Based on this, it was hypothesized that frequent assortative marriages between deaf individuals, combined with their increased fitness (genetic fitness), could lead to an increase in the incidence of hereditary hearing loss in the United States [24;25]. Walter Nance and his colleagues suggested that in the previous millennium, the fitness of individuals with congenital deafness was very low, and that the frequencies of deafness genes were in balance [25]. Introduction of sign language in Europe ~300 years ago [29] is one of the key events that significantly improved the social and economic conditions of the deaf, as well as their fitness. Genetic fitness of the deaf, in the works of Walter Nance, is measured by relative fertility, which is defined as the ratio of the average number of children in a sample of deaf and hearing individuals. An example of calculating relative fertility is shown in the fig.2.

In many countries, schools for the deaf and hard of hearing have been established, which has facilitated the choice of a marriage partner based on sign language skills, i.e. linguistic homogamy. The increased fitness of deaf individuals, in turn, can be interpreted as a relaxation of the selection pressure directed against deafness. In this connection, it has been suggested that the combination of "relaxed selection" and assortative marriages should give an advantage to the most common form of autosomal recessive deafness in the population [24], and may also be relevant to the evolutionary hypothesis about the mechanism of speech gene fixation in *Homo Sapiens* [25].

Computer modeling of the prevalence of hereditary hearing loss with relaxed selection. To test their hypothesis, Walter Nance and his colleagues conducted computer simulations aimed at assessing the impact of assortative marriages and reducing deafness selection pressure on the prevalence of autosomal recessive deafness [25]. The results of modeling showed a change in the frequency of deafness since the beginning of attenuation of selection, both in the presence and absence of assortative marriages [25]. In addition, it is known that assortative marriages increase the phenotypic expression of alleles, then they modulate the effect of selection pressure on these alleles, therefore, increased fitness will contribute to an increase in the number of deaf individuals [25]. At the same time, the increase in the occurrence of deafness was accompanied by an increase in the frequency of the recessive allele and significantly accelerated in the presence of assortative marriages, which may explain the doubling of the occurrence of the "connexin" form of deafness in the United States within 200 years [25]. At the same time, it was shown that the effect of assortative marriages is limited to the form of recessive deafness that was most common at the beginning [25].

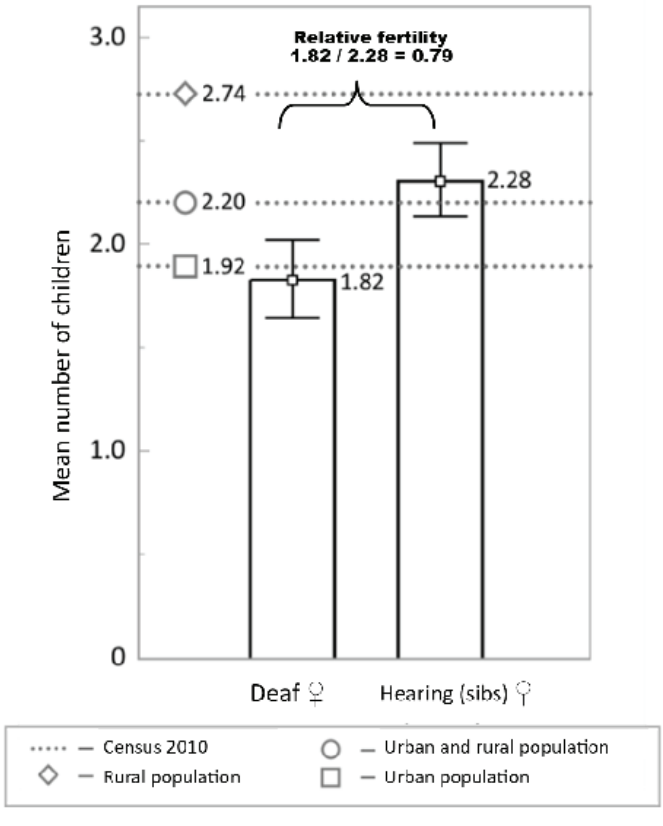

Fig. 2. Relative fertility (genetic fitness). Note. The figure shows the average number of children of deaf and control women (hearing siblings) aged 35–69 in Yakutia. "♀" - women. Confidence intervals at the 95% significance level [27]

The next study devoted to this topic was the analysis of modern data on 311 marriages of graduates of Gallaudet University (Gallaudet University) in comparison with the data of Edward Fay [13], which revealed a more than 5-fold increase in the proportion of noncomplementary marriages: from 4.2% to 23% [1]. From these data, we can estimate the increased contribution of the "connexin" form of hearing loss to the etiology of deafness in the United States. The proportion of noncomplementary deafness marriages, equal to 4.2% in XIX century [8] and 23% at the beginning XX centuries [28], approximately correspond to 20.5% and 47.95% of the contribution *GJB2* due to hearing loss, respectively (√0,042 = 0,2049, √0,23 = 0,4795). As a result, the share of connexin deafness increased by 134% over 100-200 years in the United States [1;13]. At the same time, it was shown that this growth is associated with linguistic homogamy [1].

Later, up-to-date data on reproduction and marital structure in deaf individuals were published based on a sample of Gallaudet University graduates [15]. The average number of children who were deaf was lower than that of their hearing Siblings, and the relative fertility rate was 0.88 [15]. However, it was higher than the figures of the US Census of deaf people thirty years ago – 0.74 [30], which indicated increased fitness (fitness) deaf people and reducing the selection pressure for deafness [15]. The proportion of assortative marriages was 0.79, and an analysis of fertility rates after stratification by type of marriage showed that more children are born in assortative marriages (2.11) than in marriages between deaf and hearing individuals (1.85), suggesting the influence of many factors on the fertility of deaf people [15]. Thus, in the presented series of works [1;15;24;25]. Walter Nance and his colleagues were able to provide evidence for the hypothesis that frequent marriages between deaf people, combined with a relaxed selection pressure, may indeed have led to an increase of the "connexin" deafness in the United States since XIX century (fig. 3).

Another group of researchers from Gallaudet University also studied the effect of assortative marriages between deaf people on the prevalence of auto-

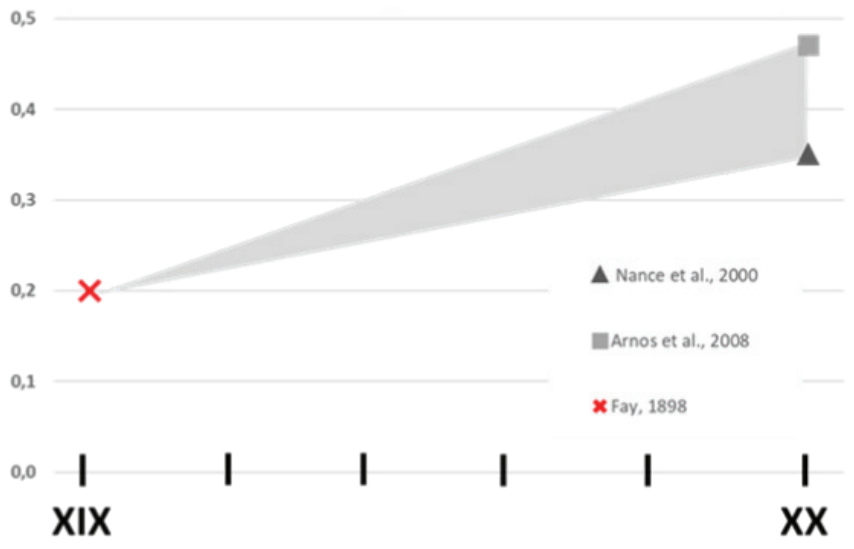

Fig. 3. *GJB2* mutation frequency increase in the USA over 200 years [1;13;24]

somal recessive deafness and the frequency of corresponding alleles to double-check Walter Nance's hypothesis based on Alexander Bell's assumption [12]. Using data from the modeling work of Nance and Kearsey [25], Derek Brown et al. conducted computational experiments using an agent-based model. They showed that the proportion of recessive homozygotes was 23% higher in the population with 90% assortative deafness marriages (q^2 = 0.022%) than in the population without assortative marriages (q^2 = 0.017%) when modeling over 20 generations [12]. It was shown that the increase in the occurrence of autosomal recessive deafness is limited to the first three generations, which also corresponded to calculations performed according to the theoretical calculations of Crow and Felsenstein (1968) [10]. Additionally, modeling was performed with different values of the average number of children in deaf people, as a result of which it was shown that the frequency of the recessive allele increased with a relative fertility value of 1.5 times or higher, when combined with assortative marriages [12]. The conclusions drawn in the work of Brown and his colleagues are generally consistent with the data obtained by Nance [25], adding some clarifications regarding the impact of reduced reproduction and the proportion of non-hereditary forms of hearing loss [12].

In conclusion, it should be noted that the simulation models proposed by Nance [25] and Braun [12], are based on retrospective data from the XIX century. These studies have been aimed at confirming the role of assortative marriages by deafness in increasing the incidence of the "connexin" deafness that has occurred since the emergence of permanent communities of deaf people more than 200 years ago. Currently, there are no models to assess the prevalence of inherited forms of hearing loss in the future, taking into account the changed social environment and current trends in society aimed at improving social equality. Development and growing availability of modern medical technologies, such as cochlear implantation and various social rehabilitation programs for the deaf, leads to their greater inclusion into society and, as a result, unreduced reproductive capabilities. In such conditions, predicting the prevalence of inherited forms of deafness can be used from a practical point of view to plan the amount of necessary medical and social care. In this regard, it is relevant to develop mathematical models that predict the dynamics of hereditary deafness under the influence of relaxed or complete absence of selection pressure for deafness using modern data.

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THE ROLE OF NEUROPILIN-1 (NRP1) IN THE DEVELOPMENT OF SARS-COV-2 INFECTION

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A review of the literature on the role of neuropilin-1 in the development of SARS-CoV-2 infection and a search for probable links between polymorphic variants of the *NRP1* gene and SARS-CoV-2 are presented. This review presents the characteristics of polymorphic variants of the *NRP1* gene, which demonstrate the possibility of their association with the course of SARS-CoV-2 infection.

Keywords: SARS-CoV-2 infection, neuropilin-1, polymorphic variants of the *NRP1* gene.

Introduction. With the onset of the SARS-CoV-2 coronavirus infection pandemic, it was necessary to understand the mechanisms of penetration of this pathogen into the cell and the mechanisms of their interaction as early as possible. In 2020, it was found that the furin-cleaved S1 fragment of the SARS-CoV-2 spike protein directly binds to cell surface neuropilin-1 [6].

Neuropilin-1 (NRP1) is a transmembrane glycoprotein. The neuropilin-1 receptor plays a key role in the development of the nervous and vascular systems, as neuropilins mediate VEGF (vascular endothelial growth factor) dependent angiogenesis and semaphorin-dependent axonal growth direction. In addition, the participation of neuropilins in a wide variety of signaling and adhesive functions has been studied, which indicates their high role as pleiotropic coreceptors [12].

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NRP1 consists of 923 amino acids and has a massive extracellular portion that includes two tandem CUB domains (a1/a2), two tandem domains homologous to coagulation factors V/VIII (b1/b2), a linker sequence, and one MAM domain (C) that supports dimerization and multimerization of neuropilin molecules and promotes the formation of signal receptor complexes [27]. The cytoplasmic domain, which includes 44 amino acid residues, contains a sequence of three C-terminal amino acid residues (SEA-COOH) and demonstrates high phylogenetic conservatism [30].

Neuropilin-1 promotes the breakdown of the spike protein. Cleavage of the SARS-CoV-2 S protein at the S1-S2 site results in the C-terminal sequence TQTNSPRRAR-OH. AgNP nanoparticles coated with the TQTNSPRRAR-OH peptide sequence were efficiently taken up by the neuropilin-positive cell culture. Intensive uptake of AgNP-TQTNSPRRAR-OH by the olfactory epithelium, neurons, and blood vessels of the cerebral cortex has also been shown [6]. NRP1 can modulate SARS-CoV-2 infection by stimulating the separation of the S1 and S2 subunits. Additional sites of interaction between neuropilin-1 and the spike protein, which function as additional points of connection with the lipid bilayer of the infected cell, play a significant role [21]. In turn, the results of isothermal titration calorimetry demonstrate a direct relationship between the b1 domain of NRP1 and the synthetic S1 peptide (679-NSPRRAR-685) with an affinity of 20.3 μM at pH 7.5, and this crystal structure showed significant similarity [7] with the crystal structure b1 domain of NRP1 in complex with its endogenous VEGF-A ligand [28].

Functional and structural diversity of binding sites for neuropilin-1 and spike protein. The analysis of interaction sites between SARS-CoV-2 S-protein and human neuropilin-1 deserves special attention: amino acid residues GLN280, ASP289, TYR322, ARG323, TRP325, GLN327, ASP329, LYS359, ASP361 have been identified as potential binding sites in the b1 domain of NRP1. Relationships are also observed between GLN3, ILE8, PHE29, ALA30 RBD of SARS-CoV-2 S-protein domain and ARG402, ARG405, LYS407 of NRP1 b1 domain [2]. The overlap of SARS-CoV-2 RBD checkpoints with the VEGF-associated NRP1 site is confirmed, and interaction with GLN280 can serve as an example [18]. In turn, the amino acid residues TYR322, ARG323, TRP325, GLN327, ASP329, LYS359, ASP361 are structurally close to the VEGF-binding site of NRP1; moreover, TYR297, ASP320, SER346, THR349, TYR353 play a leading role in its structure [33]. All this indi-

cates that the binding of the SARS-CoV-2 S protein to the b1 domain of NRP1 suppresses the binding of VEGF to neuropilin-1, which was shown by blocking the VEGF-mediated increase in the activation of calcium and sodium channels [24]. Interestingly, the SARS-CoV-2 S-protein RBD-domain (RBD) binds to LYS359 and ASP361 [2], located in the sequence of the neuropilin-1 adhesion site (347-364) [33]. It should be noted that the receptor-binding domain of the SARS-CoV-2 S-protein binds to the positively charged conserved amino acid LYS/ARG359 [2], which is necessary for the binding of NRP1 and heparin [33], which plays a significant role in the course and therapy of COVID-19. [fifteen]. The amino acid residues of THR316, PRO317, and ASP320, as well as SER346, THR349, and TYR353 of NRP1, form relatively strong H-bonds with the positively charged guanidine group and the negatively charged carboxyl portion S1 of the SARS-CoV-2 protein. The guanidine group of ARG685 forms an electrostatic bond with the negatively charged ASP320 and forms hydrogen bonds with the THR316, PRO317, and ASP320 residues of neuropilin-1, while its C-terminal carboxyl group forms hydrogen bonds with SER346, THR349, and TYR353 of NRP1 [20].

It is important to note that the conditional boundary between the S1 and S2 domains runs between amino acid residues 685 (S1) and 686 (S2) of the SARS-CoV-2 spike protein [21], while the main site of interaction between the S1 protein and neuropilin-1 begins at 678 /682 [7; 24] position and ends at position 685 [7]. Moreover, it is arginine at position 685 that is critical for SARS-CoV-2 S1 association with neuropilin-1 through electrostatic interactions with the negatively charged aspartic acid at position 320 and forms hydrogen bonds with THR316, PRO317, and ASP320 amino acids [20]. This destabilizes several basic interactions between S1 and S2. The destabilization of the link between the RBD domain of the S1 protein and the 686-1146 sequence of the S2 protein is mediated by the interaction of RBD with ACE2. However, the cleavage site at position 685/686 is still provided by S1 and S2 binding. However, binding of the 682-RRAR-685 motif to NRP1 at the cleavage site provides accelerated S2 separation, increasing virus infectivity [21].

Peculiarities of NRP1 expression during SARS-CoV-2 infection. Although NRP1 does not by itself mediate infection in cell culture, its co-expression with ACE2 and TMPRSS2 markedly enhances infectivity. With isolated expression of *NRP1*, lower levels of symptomatic infection load were observed [6].

A post-mortem study of 2 patients with anosmia showed focal atrophy of the olfactory epithelium, leukocyte infiltration of the *lamina propria*, and signs of axonal damage to the olfactory nerve fibers [19]. Thus, the extensive role of NRP1 in the immunosuppressive function of regulatory T cells [5], extensive damage to the lungs, olfactory epithelial cells, and olfactory sensory neurons may be related [13].

The lungs of COVID-19 patients show characteristic vascular features. Histological analysis of pulmonary vessels in patients with COVID-19 showed severe thrombosis with microangiopathy. For example, microthrombi in the alveolar capillaries were 9 times more common in patients with COVID-19 than in patients with influenza, and in the lungs of patients with COVID-19, the number of new vessels growing mainly through invagination angiogenesis was 2.7 times higher than in the lungs of patients. influenza [1]. By controlling endothelial adhesion and permeability, NRP1 may be involved in pathological coagulation. By binding the b1 domain of NRP1 and thereby blocking traditional angiogenic ligands, SARS-CoV-2 may contribute to vascular dysfunction and coagulation throughout the body [25].

It should be emphasized that *ACE2* and *TMPRSS2* have a relatively lower level of expression in the CNS [14], therefore, most of the symptoms of COVID-19 in the CNS are usually attributed to the consequences of damage to the peripheral systems of the body [3]. However, convincing evidence has been presented that the virus can infect cells via neuropilin-1 [6;7]. Several studies have shown that infected vascular endothelial cells mediate the spread of SARS-CoV-2 to glial cells in the central nervous system. Thus, neuropilin-1 has been proposed as a key factor in a wide range of neurological manifestations of COVID-19 by increasing the penetration of SARS-CoV-2 into the brain [4].

Significance of neuropilin-1 for the immune response to SARS-CoV-2. Neuropilin-1 has a strong effect on virus-induced production of INF-α by dendritic cells [31] a twofold lower virus-induced production of IFN-α was shown in anti-NRP1-treated dendritic cells compared to untreated dendritic cells [11]. Thus, NRP1 may increase the susceptibility of dendritic cells to SARS-CoV-2 infection by mediating the internalization of the virus into uninfected cells, followed by the production and secretion of cytokines, which can lead to a cytokine storm and an increased risk of complications [31].

The researchers highlight the ability of the CD25+ CD4+ Foxp3+ subset of regulatory T cells to significantly influence the immunological balance in COVID-19. SARS-CoV-2 infection induces the transcription of IL-2, which binds to soluble CD25 in the blood, leading to CD28+ CD4+ mediated release of pro-inflammatory cytokines [9].

Association of polymorphic variants of the *NRP1* gene with multifactorial diseases: the search for probable links with SARS-CoV-2. Based on the above data, we can assume the functional significance of polymorphic variants of the *NRP1* gene for the development, course, and outcome of SARS-CoV-2 infection in humans. For example, it has been reported that the rs10080 G>A polymorphism is associated with reduced *NRP1* expression, and individuals carrying the *G* allele may express lower levels of neuropilin-1 in target cells, which may influence the neuropathogenesis associated with COVID-19 disease [16].

An association of the polymorphic variant *rs2506142* (minor allele *G*) of the *NRP1* gene with the risk of developing standard and menstrual migraine has been demonstrated [29]. In the development of migraine, the regulation of the concentration of cytosolic calcium ions is of particular importance [35], and the VEGF-A mediated effect of NRP1 on nociceptive activation is expressed precisely in an increase in the total number of sodium and calcium channels in the neurons of the spinal ganglia. In turn, the SARS-CoV-2 S protein inhibits pronociceptive VEGF-A/NRP-1 signaling and has an analgesic effect in chronic neuropathic pain in rats [24].

Several studies reveal a significant role of neuropilin-1 in the pathogenesis of malignant neoplasms. Relatively high levels of *NRP1* expression were observed in squamous cell carcinoma of the kidney, hepatocellular liver cancer, thyroid cancer, and gastric adenocarcinoma; however, researchers note the involvement of neuropilin-1 in pathological angiogenesis as the leading mechanism of pathogenesis [22]. In this context, it is important to recall that in the lungs of patients with COVID-19, there is an active growth of new vessels, mainly through the mechanism of invagination angiogenesis [1].

The *rs2228638* polymorphic variant is of interest in the context of its influence on the relationship between neuropilin-1 and SARS-CoV-2, since the SARS-

CoV-2 S protein competes with VEGF-A for interaction with neuropilin-1 [17; 24]. It has been shown that this polymorphic variant is associated with several cardiovascular anomalies, and the main reason for this association is a decrease in the activity of NRP1 as a coreceptor in VEGF intermolecular signaling [8].

The *GA* and *AA* genotypes of the *rs2070296* polymorphic locus are associated with a weaker response to antiangiogenic therapy via ranibizumab blockade of VEGF-A [23]. It is also important to report the ability of the *rs3750733 C/T* polymorphic variant of the *NRP1* gene to modulate VEGF-dependent angiogenesis [10].

For the group of polymorphic variants of the *NRP1* gene: *rs750880625 c.676C>T* p. R226C; *rs180868035 c.A418C* p.I140L; *rs1178713109 c.A1274T* p.K425M; *rs117525057 c.C1571T* p.S524L; *rs143124682 c.C1676T* p.T559M; *rs767902777 c.2200G>A* p.G734S; *rs548175518 c.2596G>A* p.A866T and *rs566437913 c.T2633C* p.V878A showed an association with idiopathic hypogonadotropic hypogonadism (IHH; English Idiopathic hypogonadotropic hypogonadism) associated with impaired sense of smell (Kallman syndrome) [26]. Neuropilin-1 has also been shown to be significantly more expressed than ACE2 in the olfactory epithelium [6] and may play a central role in olfactory dysfunction during SARS-CoV-2 infection [13]. *NRP1* is expressed along the vomeronasal/terminal nerve pathway and is involved in the migration of gonadotropin releasing neurons (GnRH neurons) [26].

These data are of extremely high interest in the context of the impact of SARS-CoV-2 infection on the reproductive capacity of men. For example, a significant pathological effect of coronavirus infection on reproductive ability, mediated by the development of orchitis, has been shown. However, no traces of the presence of the virus were found in the testicles [34]. Changes in sperm parameters and the level of sex hormones were also shown, in turn, disturbances in the homeostasis of hormones of the pituitary-testicular axis are isolated as one of the possible pathological mechanisms of impaired fertility during SARS-CoV-2 infection [32].

Conclusion. Neuropilin-1 has been shown to modulate SARS-CoV-2 infection by playing a leading role in separating the S1 and S2 subunits of the spike protein. Several studies demonstrate the significant role of neuropilin-1 in the immune response. Researchers note its significant role in pathological phenomena from the vascular system and the central nervous system. All this points to the need for further research on the role of NRP1 in the development of SARS-CoV-2 infection.

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POINT OF VIEW

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DETECTION OF BETA-GLOBIN GENES IN THE ERYTHROCYTE FRACTION IN A PATIENT WITH CERVICAL CANCER

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The results of detection of DNA fragments encoding human beta-globin and human papillomavirus (HPV) L1 protein in cervical cancer patient's plasma and erythrocyte samples using real-time polymerase chain reaction (qPCR) are presented. Amplification of a DNA fragment encoding human beta-globin was found only in a sample of erythrocytes, which may indirectly indicate the presence of extracellular DNA. The results of qPCR for the presence of a DNA fragment encoding HPV L1 were negative, which did not allow us to confirm that the isolated DNA belonged to the tumor DNA. The origin of the identified in the erythrocyte fraction DNA fragment encoding human beta-globin requires further research.

Keywords: tumor DNA, extracellular vesicles, neutrophil extracellular traps.

Beta-globin is a part of hemoglobin - an iron-containing metalloprotein. In mammals hemoglobin is found in the largest amount in red blood cells, its main function is to transport oxygen from the lungs to various body tissues. Hemoglobin also interacts with other gases such as carbon dioxide, carbon monoxide, and nitric oxide. Hemoglobin is a tetramer composed of two beta-globin chains and also of two alpha-globin chains. Heme is attached to each of these chains, and thus each chain can transport oxygen. Hemoglobin is expressed by erythroid cells as well as by nonerythroid cells, such as epithelial cells, including epithelial cells of the cervix uteri [22].

It has been established that in normal cells of the cervical and vaginal mucosa alpha-globin and beta-globin can act as endogenous antimicrobial protective proteins against infection [21]. Hemoglobin and mRNA are also found in human cervical carcinoma cell lines (SiHa and CaSki), and the expression of alpha-globin and beta-globin genes in them is significantly higher than in normal cells of the cervix uteri [14]. Expression of these genes improves the viability of tumor cells by suppressing oxidative stress [28].

Alpha-globin and beta-globin are encoded by genes located on chromosomes 16 and 11, respectively. The beta-globin gene cluster is packed into inactive heterochromatin in non-erythroid cells, while the alpha-globin genes are built into open chromatin conformations in all cell types [9]. Transcriptionally inactive heterochromatin plays a vital role in maintaining a stable structure of specialized chromosomal regions with repetitive DNA; loss of integrity in these regions of chromosomes can contribute to the cancer development [7].

DNA, including genomic DNA, is found in blood fractions purified from cells - in plasma and serum. Both normal and tumor cells release DNA into the circulation, but it is present in increased amounts in cancer patients [23]. It is assumed that the detection of tumor DNA in the blood can significantly improve the detection of a tumor at an early stage, determine its progression and prognosis, and also help in targeted therapy [5]. Detection of tumor DNA is carried out mainly in blood plasma, but the complexity of the determination is associated with low concentration of DNA in plasma [11].

Human genomic DNA is also found in the erythrocyte fraction of blood, even after 25 years of storage [4]. Genomic DNA, both of human and infectious, can be associated with the surface of erythrocytes through receptors, since it is known that erythrocytes express receptors on their surface, which, at least bind bacterial and mitochondrial DNA in vitro [13].

We assumed that in cervical cancer (CC) patient genomic DNA is contained in the blood in an increased amount, and the presence of beta-globin genes in genomic DNA can be an indicator of carcinogenesis, so the goal of the study was to isolate DNA from the patient's blood fractions in which there are no nuclear cells - from plasma and erythrocyte fraction, as well as to detect a DNA fragment encoding human beta-globin. In order to determine, let indirectly, whether the isolated DNA belongs to a tumor DNA, it was decided to detect a DNA fragment encoding the L1 protein region of the human papillomavirus (HPV). Since it is believed, that in CC patient circulating extracellular DNA containing the HPV genome originates from tumor cells [8].

Materials and methods. 4 types of biological samples were prepared from venous blood taken by venipuncture in vacuum blood collection tubes-K3 EDTA: plasma samples (sample 1); a suspen-

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The main results of the pilot study

	Sample 1 (plasma)	Sample 2 (erythrocyte suspension)	Sample 3 (kontrol - erythrocyte suspension, trypsin-treated)	Sample 4 (phosphate buffer precipitate)
DNA Isolation	(+)	(+)	(+)	(-)
For DNA fragment encoding human beta-globin:				
Amplification of a gene fragment encoding human beta-globin using qPCR	(-)	(+)	(-)	Not carried out
Verification of amplification by gel electrophoresis	(-)	(+)	(-)	Not carried out
Conclusion	**DNA fragment encoding human beta-globin found in erythrocyte suspension**			
For DNA fragment encoding human papillomavirus protein L1:				
Amplification of a gene fragment encoding human papillomavirus protein L1 using qPCR	(+/-) questionable	(-)	(-)	Not carried out
Verification of amplification by gel electrophoresis	(-)	(-)	(-)	Not carried out
Conclusion	**DNA fragment encoding HPV L1 protein was not found in any of the samples**			

sion of erythrocytes washed in phosphate buffer (sample 2); a suspension of trypsin-treated and then washed also in phosphate buffer erythrocytes (sample 3). The sample 3 served as a control for the absence of admixture of cells containing nuclei in the suspension of erythrocytes. In addition, the treatment of erythrocytes with trypsin was necessary to confirm the presence of the detected DNA fragments on the surface of erythrocytes, as much as it is known, that trypsin destroys cell surface receptors by proteolysis [10]. The phosphate buffer precipitate (sample 4), which was used to wash trypsin-treated erythrocytes, was also used for DNA detection.

The blood was centrifuged at 1600 rpm for 10 minutes to separate into fractions. Then, after collection and removal of the mononuclear cell plasma samples and erythrocyte fraction were isolated. The erythrocyte fraction was washed three times with phosphate buffer to obtain an erythrocyte suspension. In half of the erythrocyte suspension trypsin - 0.25% in a ratio of 1:1was added, and incubated at a temperature of 37°C for 10 minutes, after incubation was centrifuged for obtain a precipitation of erythrocytes; then the erythrocytes were washed three times with phosphate buffer.

All samples were stored in a freezer at –20°C before DNA extraction and real-time PCR.

DNA was isolated by phenol-chloroform extraction. The concentration and quality of the isolated DNA were determined using NanoPhotometer Pearl (Implen).

Real-time PCR (qPCR) was carried out on CFX96 Touch Real-Time PCR Detection System using qPCRmix-HS SYBR+LowROX reaction mixture (Evrogen).

The primers PC03/04 (5'-ACACAACTGTGTTCACTAGC-3'/5'-CAACTTCATCCACGTTCACC-3') were used to detect DNA fragments encoding human beta globin.

Primers MY09/11 (5'-CGTCCMARRGGAWACTGATC-3'/5'-GCMCAGGGWCATAAYAATGG-3') were used to detect DNA fragments encoding the HPV L1 protein.

The length of the amplicons was determined in an agarose gel.

The study was approved by the Local Committee on Biomedical Ethics of the North-Eastern Federal University named after M.K. Ammosov (Yakutsk, Sakha (Yakutia) Republic, Russia) in accordance with protocol No. 13 dated April 4, 2018, Decision No. 2. Patient B., a resident of Yakutia, with newly diagnosed cervical cancer, gave written informed consent to the study.

Results and discussion. The main results are presented in Table 1.

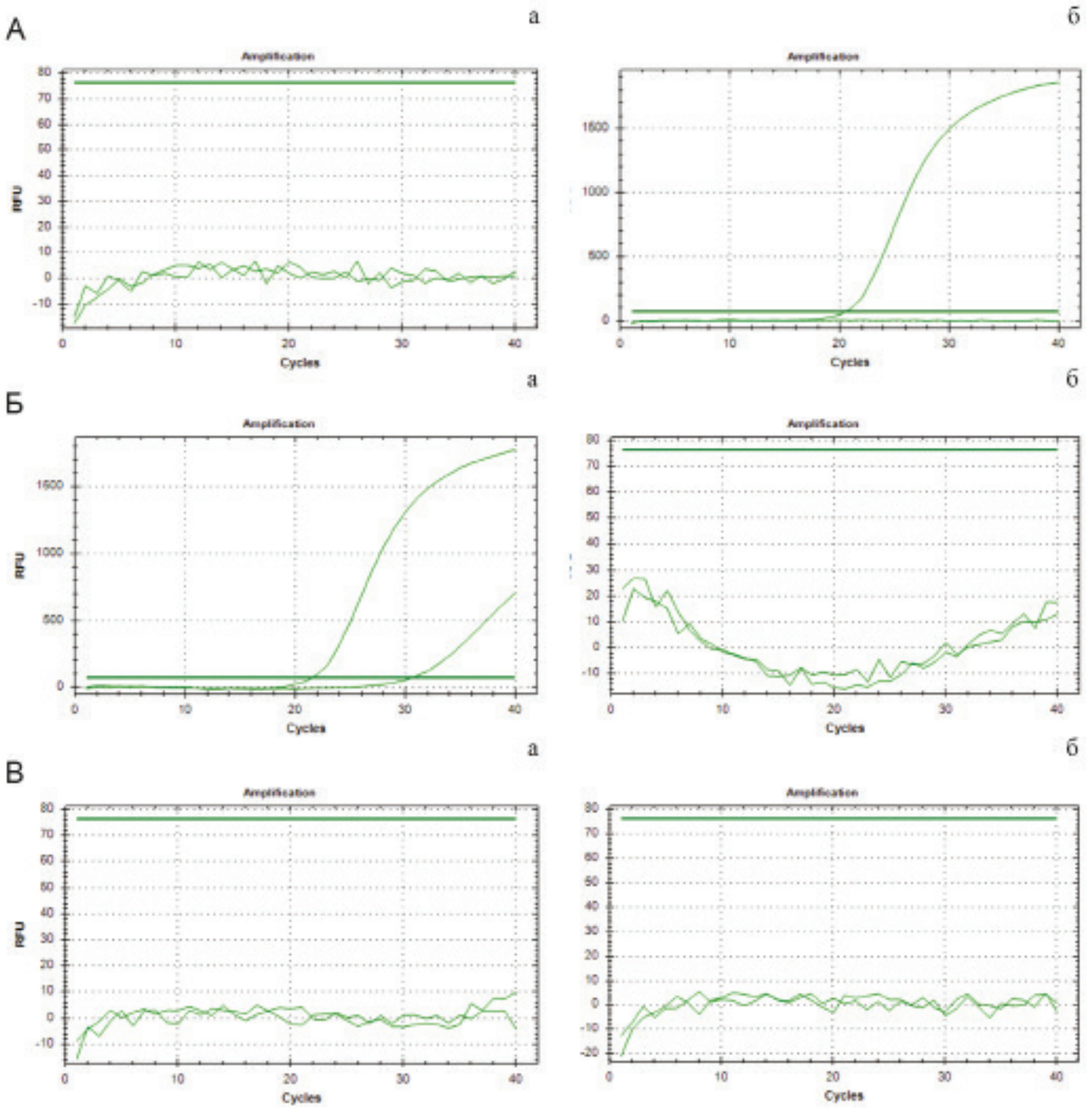
DNA was isolated from three samples - from plasma, a suspension of washed erythrocytes and erythrocytes treated with trypsin. DNA concentration in all of these samples exceeded the upper sensitivity limit of the spectrophotometer - 18750 ng/μl. In the fourth sample, a precipitate of phosphate buffer, which was used to wash erythrocytes treated with trypsin, DNA could not be isolated.

Sample 1 study. qPCR using primers PC03/04 and MY09/11 for DNA isolated from a plasma sample showed the absence of a fragment encoding human beta-globin, and a questionable result for a fragment encoding HPV L1 protein (Fig. 1). The electrophoresis confirmed the absence of amplification products of the DNA fragment encoding human beta-globin, and also showed the absence of amplification products of the DNA fragment encoding HPV L1 protein.

Sample 2 study. qPCR with DNA isolated from a sample of erythrocyte suspension revealed the presence of a fragment encoding human beta-globin, and the absence of a fragment encoding HPV L1 protein (Fig. 2). Electrophoresis confirmed the presence of amplification products for the DNA fragment encoding human beta-globin and the absence of amplification products for the DNA fragment encoding HPV L1 protein.

Sample 3 study. As for the DNA isolated from a suspension of erythrocytes treated with trypsin, using real-time PCR no detections of fragments encoding human beta-globin and encoding HPV L1 protein were (Fig. 3). Gel electrophoresis showed consistency with these results - showed the absence of amplification products for both DNA fragments.

Thus, in this case, DNA was found in blood fractions in which there are no cells containing nuclei - in plasma and erythrocytes. DNA containing a fragment encoding human beta-globin, was found only in the erythrocyte fraction of blood. The presence of this DNA in the erythrocyte fraction cannot be explained by poor-quality isolation of erythrocytes, that is, the presence of cells containing nuclei - reticulocytes and leukocytes in the erythrocyte fraction. Since the detection of DNA containing a fragment encoding human beta-globin did not occur after treating erythrocytes with trypsin. This also suggests that, most likely, this DNA is associated with the surface of erythrocytes through receptors, since trypsin is able to destroy by proteolysis receptor bonds. The absence of DNA fragments encoding HPV L1 in the erythrocyte fraction reduces the likelihood of DNA origi-



The fluorescent signal accumulation of the amplification of DNA fragments isolated from plasma: a - amplification of a DNA fragment encoding human beta-globin, b - encoding HPV L1 protein

nating from cervical tumor cells, although, of course, this statement is not obvious. It is possible that the isolated DNA belongs to neutrophil extracellular traps (neutrophil extracellular trap, NET), which play a unique role in carcinogenesis [17, 25]. And, perhaps, exactly neutrophil extracellular traps can be determined in the erythrocyte fraction earlier than in plasma and serum. Currently, researchers have shown that plasma and serum levels of neutrophil extracellular traps correlate with the progression of cancer - colorectal [26], breast [20], and stomach [27]. Endogenous extracellular particles that were visualized on the surface of erythrocytes [15], including those in CC patients [16], can also be a possible source of isolating DNA containing a fragment encoding human beta-globin. For example, it has been established in pancreatic cancer patients that endogenous extracellular particles, such as, serum exosomes, contain human genomic DNA [6].

The question is the origin of DNA, which does not contain the sought for fragments, isolated from plasma and erythrocyte suspension treated with trypsin. It is not excluded that DNA isolated from plasma may be of infectious origin. Since CC patients often have positive molecular genetic tests for the presence of other infections, for example, sexually transmitted ones [1, 2, 24]. In cervical samples, even with negative results of HPV DNA detection, the results of tests

for herpes virus DNA can be positive [3], and herpes virus DNA is also detected in plasma [12].

The infectious origin of DNA isolated from a suspension of erythrocytes treated with trypsin also is not excluded. Perhaps, its detection in this sample indicates the presence of DNA-containing immune complexes on the erythrocyte surface. It has been established that trypsin destroys not all receptor bonds, for example, surface cell receptors for IgG are resistant to its action [18], probably due to the fact that the proteolytic activity of trypsin is still specific [19].

Conclusion. In CC patient genomic DNA was isolated and detected in increased amounts in plasma and erythrocyte fraction, including after treating with trypsin. DNA containing a fragment encoding human beta-globin, in the absence of its detection in plasma, was detected in the erythrocyte fraction, probably in a bound state to the surface of erythrocytes by receptors. The question of whether the isolated DNA belongs to the tumor DNA is debatable, especially since the DNA fragment encoding HPV L1 protein was not found in any of the fractions. The origin of the isolated DNA should be studied in the future.

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S.K. Kononova

ISSUES OF TRANSLATIONAL MEDICINE IN THE FIELD OF MOLECULAR GENETIC RESEARCH IN YAKUTIA

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The issues of translational medicine in implementation of the results of molecular genetic research into the practical medicine of Yakutia are discussed. The process of gradual implementation of its main directions is noted: 'Discovery', 'Population studies', 'DNA diagnostics', 'Bioethical research', 'State programs for the prevention of hereditary diseases'. The translation of scientific achievements into clinical practice is a long process, as it turned out, in the conditions of the Sakha Republic, it lasted for about 20 years. On the one hand, the use of new technologies has raised the healthcare of the Republic of Sakha (Yakutia) to a qualitatively new level, on the other hand, it has brought many intractable problems of an ethical, legal and informational nature. It is necessary to raise the issue of the importance of implementing state programs for the prevention and treatment of hereditary diseases in the Republic of Sakha (Yakutia).

Keywords: translational medicine, molecular genetics, DNA diagnostics, bioethics.

Introduction. The concept of translational medicine (TM) implies the implementation of the latest achievements of fundamental science and biotechnology into clinical practice. In many cases, this is due to the use of modern methods in the field of genomics, gene therapy and transcriptomics, bioinformatics and proteomics, the introduction of biobanks and repositories of large clinical databases. It is an integration between researchers, clinicians and health care providers. At the same time, the "old" models of medical care are being improved in areas such as oncology, surgery, therapy, obstetrics and gynecology, pediatrics, etc. The patient receives standard or experimental therapy, the boundaries between experiment and treatment are blurred [9.10, 28.32].

Particularly important and breakthrough discoveries have occurred in the field of molecular genetics and molecular medicine, and research already touched upon the subtle regulatory mechanisms of genes, for example, the study of the effect of variation in the number of copies of DNA sites and methylation of regulatory sites of genes, as well as the search for approaches to identify new regulators and mechanisms in the cellular nuclear organization, regulation of gene transcription, spatio-temporal genome development and assembly [29, 35,36].

Despite the impressive discoveries of recent years, experts note a number of significant problems and disappointments from the expectations of translational medicine. The main ones are the speed of introduction of scientific achievements into clinical practice, bioethical problems and the need for significant financial support not only for research, but also for the process of effective application of scientific achievements by the healthcare system of states and, ultimately, improving the life and health of the patient. The study by Ioannidis et al, 2004 analyzed the publications of fundamental works from 1979 to 1983 in leading journals (Science, Nature, Cell, Journal of Biological Chemistry, Journal of Experimental Medicine and Journal of Clinical Investigation). He showed that at least 101 articles made clear promises of clinical application of the results, but only after 20 years 5 of these published results were licensed. The authors also conclude that the development of simpler, more practical and safer interventions may be an equally important goal for translational research, and the profit motive is unlikely to be sufficient to advance biomedical research to genuine progress [13,18].

The purpose of the article is to discuss the problems of translational medicine in implementation of the results of molecular genetic research in the practical medicine of Yakutia.

Let's consider our proposed scheme of translational medicine in the field of research of hereditary diseases (HD) and describe some aspects of the practical implementation of its stages in Yakutia Fig. 1.

I. The first stage of translational medicine is "Discovery". For example, the discovery of a mutation of the gene responsible for the development of the disease or polymorphisms of the genome regions responsible for the high risk of developing the disease. The clinical and translational discovery turns the decoding of the sequence, structure and function of DNA into a clinical application for predicting and diagnosing specific symptoms of human diseases.

Molecular genetic research in Yakutia began in 1993 as part of an international scientific program to study Vilyuisk encephalomyelitis and spinocerebellar ataxia type 1 (SCA1), the most common autosomal dominant disease in the Yakut population [20]. This was the first discovery in the field of molecular genetics and quite naturally, SCA1 became the most studied hereditary pathology and the first genomic technology introduced into clinical practice[2]. Over the next 20 years, a number of studies were carried out to detect genes that are the cause of other frequent hereditary diseases in Yakuts Table 1.

II. The second stage of TM molecular research includes the study of the population characteristics of HD and the frequency of heterozygous carriage of autosomal recessive diseases, which is of great importance for the organization of preventive measures in medical practice.

As is known, established populations are characterized by differences in the total burden of hereditary diseases, the sum of the frequencies of autosomal dominant, autosomal recessive and X-linked diseases can vary from 1.5 to 3.5 per 1000 people. For example, the number of patients with phenylketonuria and cystic fibrosis, frequent for the European population, is significantly lower in Yakutia [1,4]. The frequency of certain forms of hereditary diseases due to population reasons may vary more in cases of ethnospecific diseases [3,6]. Such diseases in Yakutia as SCA1, myotonic dystrophy, 3 M syndrome, etc. are ten times more frequently diagnosed than around the world. The accumulation of some nosologies of HD in Yakutia occurred for population reasons, the main of which

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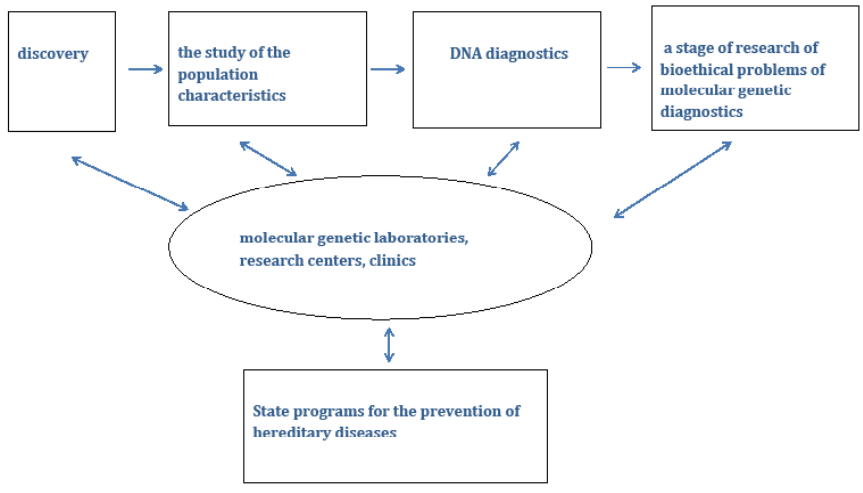
are the founder effect, gene drift and the passage of the population through the "bottleneck" due to wars, epidemics, famine [7,25].

III. The third stage of TM is the development of algorithms for DNA diagnostics of HD based on molecular genetic studies and the introduction of DNA testing of patients from burdened families into practical medicine. Currently, more than 30 different nosologies of hereditary diseases are available for DNA diagnostics in the Republic of Sakha (Yakutia).

Molecular genetic methods (DNA diagnostics) are a diverse group of methods that allow detecting violations and/or variations in the structure of a DNA molecule, up to the decoding of its nucleotide sequence (sequencing). The simplest to perform in routine clinical practice are direct and indirect (indirect) methods of DNA diagnostics based on the search for pathological mutations leading to the disease or on the analysis of polymorphic markers closely linked to the pathological gene. Direct methods of DNA diagnostics are the most informative, can be used to confirm a clinical diagnosis, for asymptomatic and differential diagnosis. In the Republic of Sakha (Yakutia), direct DNA diagnostics is used for spinocerebellar ataxia type 1, Charcot-Marie-Tutt type 1A neural amyotrophy, Duchene-Becker myodystrophy, oculopharyngeal myodystrophy, etc.; direct method using PCR-RFLP is used for DNA diagnostics of 3M syndrome, autosomal recessive deafness type 1A, a congenital autosomal recessive form of cataract. Indirect DNA diagnostics based on marker alleles linked to a damaged gene is used for such hereditary diseases as hemophilia A, B [2,11,12,19,21,22].



The fluorescent signal accumulation of the amplification of DNA fragments isolated from plasma: a - amplification of a DNA fragment encoding human beta-globin, b - encoding HPV L1 protein

The main problems of using DNA diagnostics of HD as a routine analysis in clinical practice are:

- confidence in the clinical diagnosis and a clear justification for the molecular genetic diagnosis of HD, since the search for a mutation without a diagnosis can turn into a useless procedure with the expense of costly reagents;
- availability of the necessary family members for analysis, and, as a rule, proband genetic material is required for research;
- increasing the personal responsibility of persons involved in the process of molecular genetic diagnosis of HD, responsible for the reliability of the results of the examination, as well as the qualification of a laboratory assistant performing the actual analysis;

IV. Translational medicine provides a stage of research of bioethical problems of molecular genetic diagnostics of HD.

Differences in the manifestation of phenogenotypic signs of HD cause differences in the medical and social consequences of HD. Ethical issues are resolved through the adoption of laws and regulations concerning the use of genetic information of an individual/family. For example, presymptomatic testing for incurable NC raises a number of ethical and psychosocial problems, including possible discrimination of carriers of a pathological gene, deterioration of the quality of life of a person who received such information, etc. The most acute of the existing problems is the possibility of severe emotional trauma with a positive result of DNA testing at the preclinical stage without psychological preparation of the patient. This means that a person may be doomed to an incurable disease that gradually destroys his personality, leading to deep disability and inevitable death in 10-15 years from its onset [8].

Frequent hereditary diseases in the Yakut population

Hereditary disease	frequency in the Yakut population	Gene (OMIM)	Frequency of heterozygous carrier	citation
* Spinocerebellar ataxia Type 1	38,6 : 100000	*SCA1* (164400)	* Autosomal dominant	Lunkes A., Goldfarb L.G., Platonov F.A. et al.,1994 [20]
Short stature syndrome 3 M syndrome	12,72 : 100000	*CUL7* (273750)	3%	Maksimova N, Hara K, Miyashia A et al.,2007 [21]
Short stature syndrome characterised by optic nerve atrophy and Pelger-Huët anomaly (SCOP)	9,95 : 100000	*NAG* (614800)	1%	Maksimova N, Hara K, Nikolaeva I et al.,2010 [22]
Autosomal recessive deafness 1A (DFNB1A)	16,2 : 100000	*GJB2* (220290)	11,7%	Barashkov NA, Dzhemileva LU, Fedorova SA et al., 2011 [11]
A new type of mucopolysaccharidosis with severe systemic symptoms	8,3 : 100000	*VPS33A* (617303)	2,1%	Kondo H, Maksimova N, Otomo T et al.,2017 [19]
Autosomal recessive cataract (CTRCT18)	3,0 : 100000	*FYCO1* (610019)	7,9%	Barashkov NA, Konovalov FA, Borisova TV et al.,2021[12]

The articles describe cases of affective behavior and suicidal attempts in similar situations [15,33]. For example, Huntington's chorea (HC), as well as SCA1, refers to HD with dynamic mutations and late onset of clinical signs. Studies of the issue of patients' attitude to the presymptomatic and prenatal DNA testing of Huntington's Chorea showed a low percentage (5-20%) of those wishing to undergo such a survey [23,24,34].

No less controversial ethical issues are contained in the prenatal diagnosis (PD) of late-manifesting diseases, such as HC and SCA1. There is no consensus on which hereditary disease should be considered serious enough for PD. Some experts believe that the late onset of the disease after a long period of healthy and fulfilling life may make it possible to attribute the condition to frivolous for the decision to terminate the pregnancy of a fetus with a mutation [14,26].

Organizational and bioethical approaches to the prevention of socially significant hereditary diseases remain undeveloped in the healthcare system of Yakutia. Approaches to solving ethical problems in different countries are related to the cultural and religious characteristics of peoples, there are differences in the regulatory documents of the organization of medical and genetic services. The generalized nature of international guidelines on ethical problems of medical and genetic counseling requires concretization taking into account national characteristics [5].

Bioethical problems associated with the latest methods of correction of hearing defects are widely developed by foreign authors. In particular, the dilemma of the acceptability of the modern method of cochlear implantation (a new generation of hearing aids implanted in early childhood) for hearing correction is discussed. The main bioethical problem in this case is obtaining the consent of both parents, because cases are described in families when both parents who are deaf do not want their child to be able to hear. This fact is associated by many researchers with a well-developed social security system and a sufficiently developed system of public organizations for hearing-impaired people in Western countries who do not consider lack of hearing a serious defect and are ready to raise a deaf child [16,17,30,31]. In Russia, the attitude of persons with impaired sound perception to cochlear implantation and DNA diagnostics has not been studied. Molecular genetic studies of hereditary non-syndromic forms of deafness in Russia are at an early stage, the needs of the population for DNA testing have not been determined. In Yakutia, with a high frequency of this disease, there is a gap between the results of scientific developments and their application in practice due to extremely low awareness of the population about genetic technologies [27].

When developing the ethical aspects of TM for the prevention of HD, it is necessary to emphasize the following aspects:

- late -manifesting character;
- vital prognosis depending on nosology;
-methods of introducing genetic analysis and the consequences of using DNA testing;
- public perception of applied genetic technologies;
- the need of burdened families for prenatal diagnosis;
- ethnic characteristics in the area of the study, etc.

V. The fifth stage of TM is the involvement of the state in the process of introducing and applying scientific achievements in practical medicine to improve the quality of medical care provided to the population with the greatest coverage of all those in need and a fair distribution of financial resources to improve the health and quality of life of patients. As a rule, this is the adoption of state programs for the prevention of diseases. For example, genetic screening programs are implemented by government programs taking into account the needs of populations in need of a particular type of screening. The main evaluation of screening is the effectiveness of the proposed methods of prevention and treatment of hereditary diseases.

An example of the successful implementation of the Republican target Program "Development of human genodiagnostics in the Republic of Sakha (Yakutia) for 2001-2005, adopted by the Decree of the Government of the Republic of Sakha (Yakutia) dated May 11, 2001 No. 277, is the introduction of the results of molecular genetic studies and prenatal diagnostics of HD into practical healthcare of the Republic, as well as the expansion of the scope of complex preventive measures for the diagnosis of hereditary diseases in general. During the implementation of this program, the material and technical base of the first molecular genetic laboratory was modernized, specialists in molecular genetics for the Republic of Sakha (Yakutia) were trained in leading federal genetic centers.

Conclusion. In the Republic of Sakha (Yakutia), a new direction in science - translational medicine - has been developed in the field of molecular genetics research of hereditary diseases. The process of gradual implementation of its main directions is noted: "Discovery", "Population studies", "DNA diagnostics", "Bioethical research", "State programs for the prevention of hereditary diseases". The translation of scientific achievements into clinical practice is a long process, as it turned out, in the conditions of the Republic, it lasted for about 20 years. On the one hand, the use of new technologies has raised the healthcare of the Republic of Sakha (Yakutia) to a qualitatively new level, on the other hand, it has brought many intractable problems of an ethical, legal and informational nature. The main problems of TM are medical, social, organizational and financial. In the field of medical and social problems, bioethical ones stand out, since with the existing differences in the clinical and social effects of hereditary diseases common in Yakutia, differentiated approaches are required in the development of ethical rules for DNA testing and the study of public opinion on the genomic technologies used. Organizational problems include the issues of training laboratory doctors for molecular genetic laboratories, equipping laboratories with equipment and reagents for genetic research. Economic problems are specific to our republic, due to the remoteness of the region from Central Russia, so it is necessary to raise the issue of the importance of implementing state programs for the prevention and treatment of hereditary diseases in the Republic of Sakha (Yakutia).

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RELATIONSHIP OF PERSONALITY TRAITS AND STRESS WITH FUNCTIONING OF THE HYPOTHALAMIC-PITUITARY-ADRENAL AXIS

The aim of this study is to test the hypothesis that personality traits (neuroticism, extraversion/introversion) and stressful life events in childhood could be related to HPA axis reactivity. We studied 121 healthy adult men of Yakut ethnicity aged 18–25 years. High neuroticism and introversion have been found to be associated with lower blood cortisol. Stressful life events have been positively associated with neuroticism, but we failed to detect a significant correlation with ACTH and serum cortisol levels in young people.

Keywords: personality traits, cortisol, adrenocorticotropin (ACTH), HPA-axis, neuroticism, extraversion, introversion, stressful events in childhood, Yakuts.

Introduction. The neurobiological response to stress is coordinated by the hypothalamic-pituitary-adrenal (HPA) axis through changes in cortisol levels. HPA axis reactivity represents a potential marker of sensitivity for various neuropsychiatric disorders. The prospective studies of individuals without depression have demonstrated that such personality trait as neuroticism, which can be characterized by a tendency to experience negative emotions, is a risk factor for the subsequent development of depression [1; 19]. Moreover, the results of behavioral and molecular genetic studies showed that neuroticism and depression had common genetic risk factors [2; 6; 8; 29]. However, it is still poorly understood whether there is a relationship between personality trait measures (neuroticism, extraversion/introversion) and the level of HPA hormones. The results of several studies attempted to identify associations between these variables are conflicting [9; 12; 17; 20; 23; 24; 26 - 28].

It is known that HPA axis functioning is affected by several factors, including stress at an early age [5; 11; 14; 15; 31; 33], as well as anxiety disorders, but these potential predictors have been rarely systematically assessed or controlled for. However, there is some evidence that stress and trauma at an early age can be associated with hypocortisolemia and a weakened cortisol response to stress [5; 14; 15; 33]. Stressful life experiences, apparently, can trigger the development of depression, anxiety and mental disorders [4; 18]. Animal and human studies showed that early exposure to stress could cause persistent changes in HPA axis functioning that reflect some of the neuroendocrine abnormalities commonly associated with depression and various post-traumatic stress disorders [16; 31].

The aim of the present study is to test the hypothesis that personality traits (neuroticism, extraversion/introversion) and childhood stressful events are associated with HPA axis reactivity.

Materials and methods. The study sample consisted of 121 apparently healthy young men (21.5±2.25 years), the Yakuts from the Republic Sakha (Yakutia). All individuals were students or employees of Universities without a hereditary burden of mental illness. To form the sample, we have considered the fact that the hormonal background in men is more stable than in women. Hormonal disruptions in men often occur when the level of sex hormones falls after the age of 30, so young men under the age of 30 were included in the sample to minimize this factor. The ethnicity of the subjects was determined on the basis of a questionnaire survey. At the same time, ethnic self-identification was taken into account mainly up to the third generation.

Hormonal levels were assessed by determining the levels of serum cortisol and adrenocorticotropic hormone (ACTH) in EDTA-plasma. Blood sampling was carried out in the morning on an empty stomach, in the appropriate vacutainers. The analysis was carried out by enzyme-linked immunosorbent assay (ELISA) using standard kits from Diagnostics Biochem Canada Inc. (Canada) and Biomerica (USA) on the Victor X5 multifunctional plate analyzer.

The Eysenk Personality Inventory (EPI) was used to diagnose extraversion/introversion and neuroticism. EPI is one of the most common tests for assessing the basic personality traits, proposed by G. Eysenck and S. Eysenck in 1964. Despite the fact that the questionnaire evaluates personality traits, actually they comprise of temperament traits, since in foreign psychological studies the concept of personality includes the concept of temperament. The EPI contains 57 questions, 24 of which assess extraversion/introversion, the other 24 - evaluate

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emotional stability/instability (or neuroticism), the remaining 9 form a control group of questions designed to assess the sincerity of the subject, his attitude to the examination and the reliability of the results. Diagnostics according to G. Eysenck is a classic technique and serves as a reliable tool in modern psychology.

The Life Events Checklist (LEC) is a short questionnaire used to identify potentially traumatic events (PTEs) such as spouse's death, parental divorce, substance use by a spouse, accidents, relatively low levels of family income or poor living conditions, disasters, sexual or physical abuse, or other effects associated with the impact on the nervous system [3]. The original English version was recently tested for reliability and validity and showed good psychometric properties and is therefore recommended for use in trauma assessment. Our study used a modified version of the original Blake et al. [3]. Our modified LEC consists of 16 items, and each item reflects a potentially traumatic event.

Statistical analysis and data processing were performed using the STATISTICA software package (version 10.0, StatSoft, Inc., USA, 2011). To identify the significance of intergroup differences in personality traits and hormone levels, the nonparametric Mann-Whitney U test and the Kolmogorov-Smirnov test were used. For the quantitative indicators of the G. Eysenck questionnaire, a comparative analysis of the mean group values was carried out, the mean rank values were derived using the Student's t-test. The analysis of the relationship of individual personality traits (extraversion, neuroticism) was carried out accounting for the results obtained on the level of cortisol and ACTH via correlation analysis with linear correlation and correlation ratio algorithms. Direct and inverse relationships were taken into account determining weak, medium, and strong correlations. Correlation coefficients have been calculated via Spearman rank correlation coefficient - a measure of linear relationship between random variables.

The study was conducted in compliance with the principle of informed consent. All participants were informed about the plans, methods and aims of the study and gave written consent to participate in it. The design of the study was approved by the local committee on biomedical ethics of the YSC CMP (Protocol No. 41 of November 12, 2015).

Results. The average level of plasma ACTH and serum cortisol in the total sample of young Yakut men was 31.92 pg/ml and 10.01 μg/dl, which corre-

Table 1

ACTH and cortisol levels in the groups of individuals with high and low neuroticism

Hormones	Average levels of hormones in the group		p	
	Low neuroticism (n=46)	High neuroticism (n=75)	Mann-Whitney test	Kolmogorov-Smirnov test
ACTH, pg/ml	34.09	30.52	0.09	< 0.10
Cortisol, μg/dl	11.04	9.02	**0.05**	**<0.01**

Table 2

ACTH and cortisol levels in the groups of individuals with high extraversion/ introversion

Hormones	Average levels of hormones in the group		p	
	Extraverts (n=63)	Introverts (n=58)	Mann-Whitney test	Kolmogorov-Smirnov test
ACTH, pg/ml	32.42	32.15	0.80	>0.10
Cortisol, μg/dl	11.20	9.01	**0.04**	**<0.05**

Table 3

Levels of ACTH, cortisol, extraversion and neuroticism in groups of individuals with high and low LEC scores

	Group with low LEC levels (n=78)	Group with high LEC levels (n=43)	p	
			Mann-Whitney test	Kolmogorov-Smirnov test
ACTH	32.24	31.35	0.69	> 0.10
Cortisol	9.97	10.35	0.66	> 0.10
Neuroticism	11.7	13.8	**0.024**	> 0.10
Extraversion	11.96	12.51	0.41	> 0.10

Table 4

Correlations of ACTH and cortisol levels with personality traits (extraversion and neuroticism) and stressful life events (LEC)

	Extraversion(n=121)	Neuroticism (n=121)	LEC
ACTH	r= 0.01 p>0.05	r= -0.14 p>0.05	r= -0.03 p>0.05
Cortisol	r= **0.19*** **p<0.05**	r= -0.07 p>0.05	r= 0.04 p>0.05

Table 5

Correlations of ACTH and cortisol levels between subscales (levels) of extraversion/ introversion and neuroticism

	Extraverts (n=63)	Introverts (n=58)	High Neuroticism (n=75)	Low Neuroticism (n=46)
	Neuroticism		Extraversion	
ACTH	r= **-0.42*** **p<0.05**	r= 0.05 p>0.05	r= -0.19 p>0.05	r= **0.23*** **p<0.05**
Cortisol	r= -0.08 p>0.05	r= -0.07 p>0.05	r= 0.07 p>0.05	r= **0.30*** **p<0.05**

sponds to the manufacturer's indicated average high ACTH levels and average low cortisol level. The concentrations of ACTH and cortisol depending on neuroticism and extraversion / introversion are presented in tables 1 and 2.

When examining the level of ACTH in the subjects with low neuroticism, we observed a tendency for high plasma ACTH level compared with individuals with high neuroticism (p=0.09) (Table 1). In the group of men with low neuroticism, the average cortisol level was 11.04 μg/dl, while in the group of men with higher neuroticism, the level of serum cortisol was significantly lower - 9.02 μg/dl (p = 0.05)

There were no significant differences in mean ACTH levels between the extravert and introvert groups (p=0.80) (Table 2). When conducting a comparative analysis of cortisol level, it was found that the average level of cortisol in the blood serum was 11.20 μg/dL in the group of extraverts, while it was significantly lower - 9.01 μg/dL (p=0.04) in the group of introverts.

Table 3 demonstrates a comparative analysis of individuals with high and low levels of stressful life events (based on LEC). As a result of comparing hormonal levels, no significant differences in both cortisol and ACTH between low- and high-LEC groups were revealed. When comparing mean neuroticism scores, it was detected that in low-LEC group they were statistically significantly lower than in high-LEC group (11.7 and 13.8, respectively, p=0.024) (Table 3). This observation allows to characterize the men from high-LEC group as emotionally unstable than from low-LEC group.

When conducting a correlation analysis of ACTH and cortisol levels with extraversion, neuroticism and the number of stressful life events, we observed no correlation between cortisol level in the total sample of Yakut men and neuroticism; however, a weak correlation was determined with extraversion (r = 0.19, p<0.05) (Table 4). According to ACTH level, no significant correlations with these indicators were found.

When searching for correlations in the subgroups of extraverts and introverts, a significant persistent negative correlation between ACTH and neuroticism was characteristic for extraverts (r=-0.42, p<0.05), whereas no such correlation was reported for introverts (Table 5). In low neuroticism scoring individuals, a positive correlation was found between cortisol and ACTH levels and extraversion (r=0.30, r=0.23, p<0.05) (Table 5).

Discussion. Neuroticism reflects a tendency to experience negative emotions, and people with emotional instability show increased levels of anxiety, apprehension, and negative emotions [22]. Previous studies report contradictory findings on the relationship between neuroticism and cortisol levels. In some studies of young subjects separated into high or low neuroticism groups measured with the Eysenck Personality Inventory, it was reported that high levels of neuroticism predicted lower cortisol levels [12; 17; 20; 26]. In other studies neuroticism has been frequently associated with elevated cortisol levels, or a lack of association between these variables was shown [9; 23; 24; 28; 27]. In our sample a negative relationship was observed between neuroticism and cortisol (Table 1), which is consistent with the results of the first group of researchers [12; 17; 20; 26].

The neuroendocrine correlates of extraversion/introversion have not been systematically examined, but it has been suggested that HPA axis hormones are involved. Some studies indicated that higher extraversion was associated with increased cortisol levels [20; 23; 25]. According to Oswald et al., lower extraversion in men is associated with a reduced cortisol response to stress, and introverts can be expected to cope better with stress and, therefore, have lower activation thresholds for their HPA axis [25]. Oppositely, extraverted individuals may have higher thresholds for physiological arousal. This effect may be mediated by aggressive interactions causing peer rejection, which can represent a powerful social stressor [13]. Several other studies reported an opposite tendence (higher extraversion associated with lower cortisol levels) [33], or the absence of associations [28]. The underlying reason for such discrepancy between the results of various studies remains unclear, but our result is consistent with previous suggestion that extraverts are characterized by higher levels of cortisol (Table 2), and there is a weak positive correlation between cortisol levels and extraversion (Table 4).

To assess a degree of experienced stress we used a modified version of the LEC questionnaire. The Life Events Checklist (LEC) was added to the list of Potentially Traumatic Events (PTEs) [7] and Post Traumatic Stress Disorder (PTSD) [35] after Gray et al. [10] showed that it was reasonably reliable. The LEC is the most widely used adult self-report tool to evaluate PTEs and is usually administered prior to a structured interview with the CAPS scale, which is the "golden standard" to diagnose PTSD. The results of our study indicate that severe adverse life events are significantly associated with neuroticism level; however, they insignificantly affect ACTH and cortisol levels (Table 3). Thus, it can be argued that such personality trait as neuroticism, which is traditionally considered as a risk factor for depression, depends on stressful events in the past.

On the other hand, within the framework of the present study, correlations between the level of HPA-axis hormones and personality traits were found only in extraverts and in emotionally stable individuals. Interesting data on the presence of a correlation between personality traits and the level of HPA-axis hormones were obtained depending on individual's specificity of sports activity. In particular, a positive correlation of cortisol levels with extraversion was observed only in bowmen, while such a relationship was not found for helmsmen [21]. It is known that the differentiation of athletes' roles in a team, among other things, is determined by a combination of certain personality traits: bowmen are characterized by high activity, socially oriented behavioral style and purposefulness, while helmsmen are characterized by a high degree of introversion [21]. Thus, our data indicating a positive correlation of cortisol (and ACTH) levels with extraversion, observed only for emotionally stable Yakut men (with low neuroticism) to a certain extent are consistent with the results of differential correlation in athletes. At the same time, similar findings were obtained without reference to sports activities: high cortisol levels positively correlated with self-esteem, endurance and emotional stability, as well as with reduced neuroticism and reduced depression in men [32]. Previous studies also indicated a positive correlation of extraversion with the level of pro-inflammatory cytokines (interleukin-6) [34], which may also indicate a general relationship between hyperactivity of the HPA axis and inflammatory response system in individuals searching for social contacts (i.e., extraverts). Another interesting finding of the present study is the negative correlation between ACTH levels and neuroticism, which was only significant in extraverts. This observation is congruent with the results of another research group, which demonstrated a decrease in adrenocorticotropic activity in individuals with a reduced sense of control (to some extent coinciding with an increase in neuroticism) [12]. At the same time, literature data indicate a difference in daily fluctuations in cortisol levels in extraverts and introverts. In particular, extraverts

are characterized by higher levels of cortisol during the day, while introverts are characterized by higher levels of cortisol during the nocturnal phase [30], which may to some extent explain the presence of a statistically significant correlation between the level of HPA-axis hormones and neuroticism only in extraverts.

This study is limited by a single-point assessment design, which does not allow to make conclusions regarding the stability of these associations. It should be noted that our sample is relatively homogeneous (it includes young individuals, Asians, high-school students), therefore, the results obtained may not reflect the processes occurring in adults and elderly, which limits the possibility of generalizing our results to a wider population. Despite these limitations, our study is the first one examining the associations of HPA axis activity with personality traits in more than a hundred informants using the stressful life events questionnaire. We also used a relatively large sample to detect associations with cortisol levels compared to other publications to more reliably assess HPA axis activity.

Conclusions. The results of this study confirm the link between two important personality traits (neuroticism, extraversion/introversion) and the activity of the HPA axis. Personality traits traditionally have been associated with greater risk for developing depression (high neuroticism) and introversion have been found to be associated with lower blood cortisol levels. It has been demonstrated that a greater number of experienced stressful events was associated with increased emotional instability (neuroticism), but insignificantly affected ACTH and serum cortisol levels in young individuals.

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CLINICAL CASE

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A CASE OF ULTRASOUND DIAGNOSTICS OF NON-COMPACT MYOCARDIUM

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The article describes a case of ultrasound diagnostics of non-compact left ventricular myocardium. Echocardiography allowed establishing a rare congenital pathology of the left ventricular myocardium.

Keywords: non-compact myocardium, echocardiography, diagnostic criteria.

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Non-compact, or spongy, left ventricular myocardium (NLVM) is a diagnosis introduced into clinical practice relatively recently in connection with the improvement of methods for examining the heart and, therefore, is of great interest for clinicians, ultrasound diagnostics, MRI. Currently, this disease remains little known to a wide range of clinicians and causes difficulties in the correct diagnosis.

According to the literature, the prevalence of this pathology among adults is 0.014% [10]. In pediatric practice, the proportion of this pathology is 9.2% of all cases of diagnosed cardiomyopathies, ranking third after hypertrophic cardiomyopathy and dilated cardiomyopathy [9]. At the same time, experts note that real figures may significantly exceed official data.

Embryogenesis: between the 5th and 8th weeks of embryonic development, the heart muscle is organized - the fiber network is thickened and the intertrabecular lacunae are narrowed. At the same time, the formation of coronary circulation occurs, the intertrabecular spaces are reduced to the size of capillaries. In case of a violation of the normal course of this process, zones of non-compact myocardium with increased trabecularity (more than three trabeculae) remain in the heart of the newborn. In this case, deep intertrabecular spaces are formed - lacunae or sinusoids [1].

The American Heart Association classifies the non-compact LV myocardium as a primary genetic cardiomyopathy, and the WHO identifies it as an unclassified cardiomyopathy [3]. According to Benjamin et al., The non-compactness of the left ventricular myocardium is a "spongy" myocardium formed as a result of a violation of the intrauterine myocardium, characterized by a thin compact epicardial layer and a thick non-compact endocardial layer with pronounced trabecularity and slit-like spaces communicating with the cavity rather than the left ventricle coronary blood flow [4]. There is evidence of the hereditary nature of this disease. Recent studies indicate the presence of mutations in the G4.5 gene of the Xq28 locus [7].

Echocardiography is currently the method of choice for diagnosing this disease. According to the literature, there are a number of criteria that suggest that a patient has a violation of the structure of the left ventricular myocardium during ultrasound examination. The first diagnostic criteria were proposed by Chin et al., Who calculated the ratio between the distance from the epicardium to the base of the trabeculae (X) and the distance from the epicardium to the apex of the trabeculae (Y), measured at the end of diastole. At the same time, a progressive decrease in the X / Y<0.5 ratio was observed in patients with noncompact cardiomyopathy [5]. The criteria of Jenni et al are most often used in clinical practice: the ratio of the thickness of the non-compact layer to the thickness of the compact layer >2.0, measured at the end of systole, in the projection of the short axis, in the absence of other cardiac anomalies [8]. Stollberger C. et al. included in the criteria the presence of at least 3 separate trabeculae, as well as the ratio of the thickness of the compact layer and the non-compact layer 2.0 [11]. Currently, for the correct diagnosis, it is recommended to use the following diagnostic criteria: the appearance of 4 prominent trabeculae and deep intertrabecular spaces; the appearance of blood flow from the LV cavity into the intertrabecular spaces; segments of unconsolidated myocardium mainly include the apex, as well as the middle third of the inferior lateral wall of the LV; clear two-layer structure of the myocardium; the thickness of the unconsolidated subendocardial layer at the end of systole is more than twice the thickness of the compacted subepicardial layer [6].

Currently, the "gold standard" for diagnosing disorders of the left ventricular myocardium is magnetic resonance imaging of the heart, which allows visualizing the bilayer structure of the myocar-

dium with a higher spatial resolution than routine echocardiography [3]. Below is a clinical case of ultrasound diagnostics of non-compact left ventricular myocardium.

Patient B., born May 29, 2019, Sakha. The child was born from 6 pregnancies, from a 28-year-old mother with a burdened obstetric and gynecological history: the first pregnancy ended in spontaneous miscarriage at 6 weeks of gestation; a boy from 3 pregnancies died 2 days after birth.

This pregnancy proceeded with acute respiratory infections in the 1st half without fever. Ultrasound examination at 19 weeks showed the patient to have low placentation, at 27-28 weeks - complete placenta previa. Ultrasound examination of the fetus at the 31st week of pregnancy revealed cardiomegaly due to expansion of the right heart, regurgitation on the tricuspid valve, displacement of the fibrous ring of the tricuspid valve with symptoms of "atrialization" of the right ventricle. According to the ultrasound examination, pathology of the heart was suspected and the conclusion was made: the echographic picture may correspond to dysplasia of the tricuspid valve, Ebstein's anomaly. Partial abnormal drainage of the pulmonary veins, hypoplasia of the aortic arch cannot be ruled out.

The third childbirth is planned, operational for a period of 35 weeks. The birth weight of the child is 55 cm, the body length is 47 cm. The assessment of the newborn according to the Apgar scale is 8/8. With dynamic observation of the child during the first day, the condition was regarded as severe, due to cardiac pathology, prematurity, general neurological symptoms, with negative dynamics. The child was feeding through a tube, spitting up curdled milk, did not cry on examination, general pastiness was noted, spontaneous motor activity was reduced.

On the first day after birth in the ward of the Department of Intensive Care, Anesthesiology and Reanimation of Newborns, a comprehensive ultrasound examination of the heart was carried out using the “Acuson X300” apparatus manufactured by Siemens. Muscle tone and reflexes were drastically reduced. The skin is pale pink with a cyanotic tinge. Respiration rate 50 / min. SpO2 -98%. phased sensor P8-4. The study revealed: a ductus arteriosus with a diameter of 0.31 cm, an atrial septal aneurysm with blood discharge from left to right 0.36 cm. Expansion of the cavities of the right ventricle up to 1.52 cm and of the right atrium up to 2.29 cm was noted; revealed regurgitation on the tricuspid valve of grade 3,

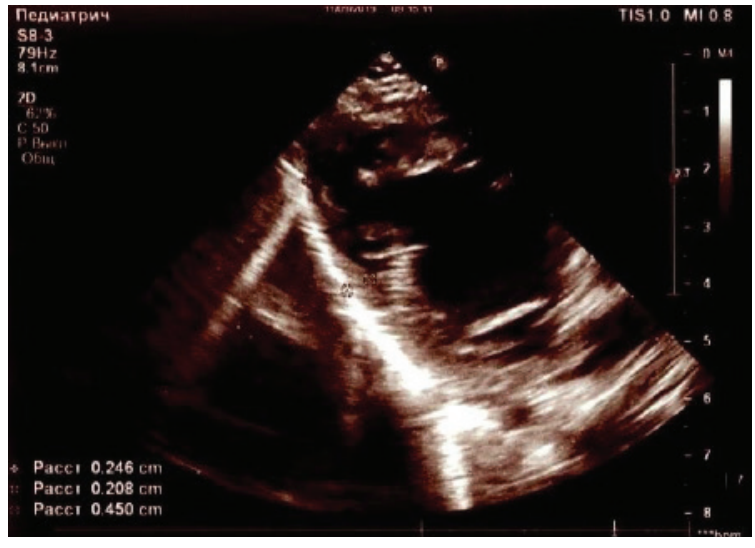

Fig. 1. Areas of myocardial thinning in the apex and areas of increased trabecularity of the left ventricle are visible

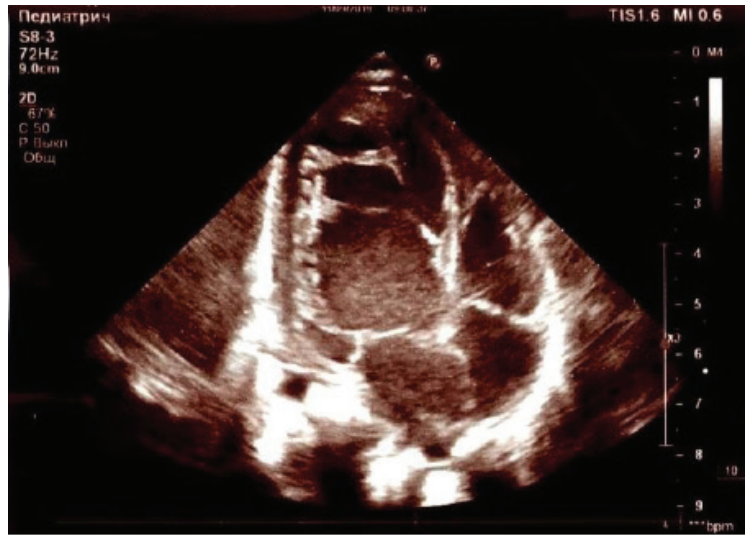

Fig. 2. Two-layer structure of the left ventricular myocardium. Dilation of the left ventricular cavity

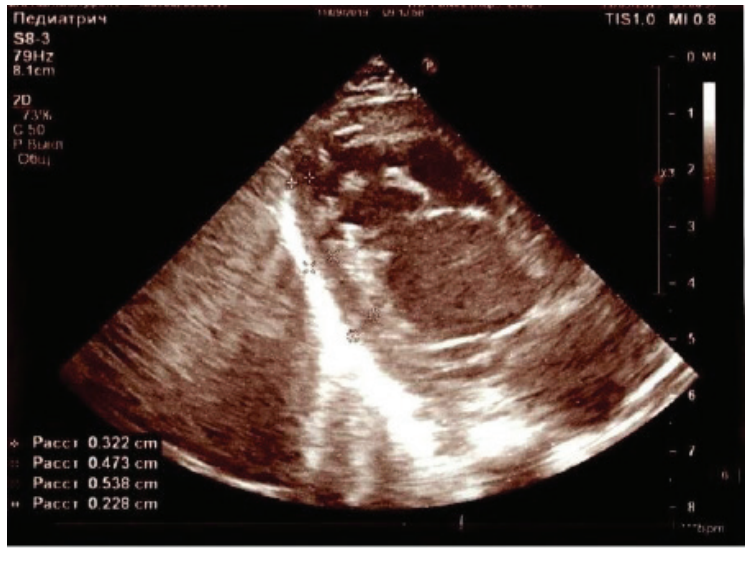

Fig. 3. Trabeculae protruding into the cavity of the left ventricle with deep intertrabecular spaces

on the mitral valve - grade 2. The child was diagnosed with grade 2 pulmonary hypertension, right ventricular myocardial hypertrophy, and expansion of the pulmonary artery trunk. At the same time, there was a decrease in the systolic function of the left ventricular myocardium, ejection fraction of 53.7%. Of course - the diastolic size of the left ventricle was within the age norm. It should be noted that during the ultrasound examination, difficulties were noted associated with tachycardia (up to 183 beats per minute) and anxiety of the child.

The child received treatment in the Department of Intensive Care and Reanimation of Newborns and the Department of Neonatal Pathology for two months, then was discharged home. Clinically, the disease proceeded according to all signs of cardiomyopathy.

Subsequently, the child was repeatedly admitted to the hospital due to the deterioration of his condition. Ultrasound examinations of the heart showed a progressive expansion of the left ventricular cavity, and increased trabecularity of the left ventricle was noted. With the passage of time, there was a change in the configuration of the heart in the form of a spherical shape.

In the fourth month of the child's life, an expert ultrasound examination of the heart was carried out using an Epiq 7 apparatus, manufactured by Phillips, with a phased transducer S8-3. From the study protocol: the left atrium is dilated to 1.9 cm.The left ventricle is dilated, the end-diastolic size is 3.5 cm.The spherical shape of the left ventricle with areas of increased trabecularity and areas of myocardial thinning in the apex area up to 0.2 cm.

A two-layer structure of the myocardium with a thinned compact and thickened non-compact layer was found, identified from the parasternal position. The N / C ratio was more than 2, where N is the non-compact layer of the myocardium, C is the compact layer of the myocardium.

The presence of numerous, excessively protruding into the cavity of the left ventricle trabeculae with deep intertrabecular spaces was noted. The right parts of the heart were not dilated. The calculated pressure in the right ventricle was 50-60 mm Hg. Attention was drawn to a diffuse decrease in the contractility of the left ventricle with areas of aki, dyskinesis in the apical segments of the left ventricle. At the time of the study, no additional echo structures were found in the cardiac cavities.

Based on the data obtained, it was concluded: Increased trabecularity of the left ventricle with areas of thinning. This echo pattern may correspond to a non-compact myocardium. Pronounced diffuse decrease in left ventricular contractility, apex dyskinesis. EF 30-35%. Expansion of the left ventricular cavity, end-diastolic size - 3.5 cm. Insufficiency of the mitral valve of the 3rd degree, probably of a relative nature. Regurgitation on the tricuspid valve of the 2nd degree. Pulmonary hypertension 2 degrees.

Subsequently, the child was consulted in absentia at the Federal State Budgetary Institution "National Medical Research Center named after Academician E.N. Meshalkin" of the Ministry of Health of the Russian Federation, where he was diagnosed with: Cardiomyopathy I42.0 - Dilated cardiomyopathy: Non-compact myocardium; Insufficiency of the mitral valve of the 2nd degree. Insufficiency of the tricuspid valve 2-3 degrees. Pulmonary hypertension 1-2 degrees. Congestive heart failure: grade 2A chronic heart failure. FC 4, threatened by the development of life-threatening arrhythmias. Threatened by sudden cardiac death syndrome. Perinatal CNS lesion of hypoxic genesis. Delayed static-motor development. Solution: An examination by a geneticist is recommended, it is necessary to take a blood test on the panel for "hereditary heart disease" to exclude microdelicia and monogenic pathology.

The parents refused to pass the analysis on the panel for "hereditary heart disease".

At the 10th month of life, the child with a worsening condition was urgently hospitalized in the Department of Intensive Care, Anesthesiology and Reanimation of the Pediatric Center. On admission, an ultrasound examination of the heart was performed. Attention was drawn to the pronounced trabecularity of the left ventricle - additional trabeculae, multiple and thickened. There was a significant expansion of the cavities of the left ventricle (EDC 3.0-3.69 cm), left atrium (3.0 cm), right ventricle (2.2 cm), right atrium (4.0 cm), pulmonary artery trunk (1 , 4-1.5 cm). The total contractility of the left ventricular myocardium was significantly reduced. EF 22.5-26%. In the cavity of the left ventricle - hyperechoic heterogeneous with uneven, indistinct contours of the echo structure - thrombi: closer to the apex, along the IVS, measuring 1.27 * 0.49 cm; in the area of the apex measuring 0.36 * 0.31 cm. Insufficiency of the tricuspid valve of the 4th degree, the mitral valve of the 2-3 degree, the pulmonary valve of the 1st degree. Pulmonary hypertension 2 degrees. Right ventricular myocardial hypertrophy.

On the X-ray imaging of the brain revealed: a large area of acute ischemia in the right parietal-temporal region, expansion of the ventricular system, convexital subarachnoid spaces on both sides. Three main clinical syndromes play a leading role in the pathogenesis of non-compact left ventricular myocardium: heart failure (73%), arrhythmic syndrome (40%), thromboembolic syndrome (33%). Patient B. had heart failure 2B, FC IV, WPW syndrome, which is most common in children with LVNM, transient ischemic attacks.

Despite the ongoing therapy, the child died three days after admission to the hospital. The diagnosis was confirmed by postmortem examination. Autopsy: left ventricular wall thickness 1.2 cm with thinning areas 0.2 cm. The myocardium is pale brown; deep intertrabecular spaces are noted in the right and left ventricles. Trabeculae are whitish, thickened, stony density. In the left ventricle in the region of the apex in the intertrabecular cavity there is a parietal red thrombus with a diameter of 0.3 cm. Histological examination: heart: stromal edema, moderate hypertrophy of cardiomyocytes, muscle fibers with foci of hyalinosis, sclerosis, fibrosis, fragmentation of muscle fibers is noted. In the left ventricle, there are sections of a thinned wall, consisting of a thickened endocardium and epicardium, which are represented by connective and adipose tissue. When examining the brain: perivascular, pericellular edema. In the tissue of the left and right hemispheres, areas with necrosis and hemorrhages are noted. Vessels along the periphery of the site are full-blooded with symptoms of erythrocyte stasis, leukocyte stasis, leukodiapedesis sites. In some of the vessels, mixed blood clots are noted. The given clinical case is presented for review, to help practitioners of ultrasound diagnostics for the correct interpretation of the echocardiographic picture of such a rare pathology as non-compact left ventricular myocardium, since literature data indicate that the actual frequency of this disease may be higher than the official data.

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THROMBOTIC COMPLICATIONS ASSOCIATED WITH THE NOVEL CORONAVIRUS INFECTION COVID-19

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We present a clinical case of a fatal thrombotic complication in a patient with coronavirus disease 2019 (COVID-19) in the recuperative period. A retrospective analysis of the patient's medical records with the chronology of laboratory and instrumental examinations, clinical course and intensive therapy was performed. The algorithm of pulmonary embolism (PE) diagnosis, validity and decision-making concerning thrombolytic therapy and extracorporeal membrane oxygenation are shown. The results of autopsy are also presented, which confirmed the diagnosis of PE and the appropriateness of medical interventions.

Keywords: COVID-19, recuperation period, myocarditis, thrombotic complications, pulmonary embolism, acute respiratory failure, artificial ventilation, extracorporeal membrane oxygenation.

Introduction. Covid-associated severe thrombotic complications develop in 17% of patients with coronavirus disease 2019 (COVID-19) and are one of the main causes of patient's death [1, 2].

Thrombosis is observed more often in people with severe course of the disease, with large area of lung lesions [4], in elderly patients, as well as in patients with severe comorbid conditions [7]. At the same time, the probability of thrombotic complications persists in patients for several months after COVID-19, which is a serious threat and explains the need to control the hemostasis system and to continue anticoagulant therapy after the patient is discharged from the hospital.

We present a clinical case of COVID-associated pulmonary embolism (PE) in the recuperative period in a young female patient without comorbid conditions, and 16% of lungs affected by COVID-19.

The **aim** of the investigation was to analyze the clinical course of and intensive therapy interventions in severe thrombotic complication in a patient with COVID-19.

Clinical observation. Patient M., female, 36 years old. On February 24, 2022 at 18:40 she was delivered by the ambulance to the Republican Hospital №1-National Center of Medicine (RHN№1-NCM) with the referral diagnosis: "Viral myocarditis? Opulent pericarditis. Bilateral polysegmental pneumonia. COVID-19 reconvalescent since February 19, 2022."

The patient complained of generalized weakness and palpitations up to 112 beats/min, elevated blood pressure (BP) up to 150/80 mmHg, dyspnea on minimal physical exertion, bilateral lower legs edema. On physical examination: height 164 cm, weight 74 kg (body mass index 27.5). General condition of the patient was severe. Patient was alert (15 points on the Glasgow Coma Scale (GCS)) and oriented. The skin was of normal color, normal moisture, turgor was preserved. Visible mucous membranes of normal color and moisture. Body temperature was 36.1˚C. Breathing unlabored, without the involvement of auxiliary muscles. On auscultation – breathing was conducted in all pulmonary fields, with increased intensity, symmetrical on both sides, no rales. Respiratory rate (RR) 18-20 breaths/min, SpO_2 - 89% on room air and 94% when insufflating 5 L/min of humidified oxygen. Cardiac tones muffled, rhythmic. BP -121/69 mmHg, heart rate (HR) 101/min. The tongue was clean and moist. The abdomen was not swollen, symmetrical, soft and painless on palpation. The liver and spleen were not enlarged. Intestinal peristalsis was active, uniform. No meteorism. No peripheral edemas. Diuresis, according to the patient, was sufficient.

It is known from the anamnesis that she considers herself sick since January 29, 2022, when she began to feel generalized weakness, vomiting, liquid stools up to 5 times a day. The patient

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called a doctor, PCR result was negative for COVID-19. Taking into account the epidemiological situation, antiviral medications (umifenovir, grippferon) were prescribed, which was a recommended treatment plan according to the 14th version (relevant at the time of presentation) of the Provisional Guidelines of Prevention, Diagnosis and Treatment of Novel Coronavirus Infection (COVID-19) released by Ministry of Health of Russian Federation [1]. The patient's condition remained without improvement. On 31.01.2022, a PCR test returned negative for COVID-19. At the same time, an enzyme immunoassay for COVID-19 showed the presence of IgG 3.0 BAU/ml, with no IgM. All this time the patient's body temperature remained up to 37.2°C. On February 7, 2022, computed tomography (CT) of the chest revealed multiple foci of ground glass opacities in both lungs with up to 10% affected on the right and up to 3% on the left and a high probability of bilateral COVID-19-associated interstitial pneumonia (CT-1). The patient was admitted to the infectious disease unit on February 08, 2022, where she was treated until February 18, 2022. According to the patient, after her discharge she had palpitations, shortness of breath, BP increase to 160/80 mmHg. The patient is known to have had several episodes of syncope on 20 February 2022, and did not seek medical help. On February 24, 22, the patient consulted her general practitioner with the above complaints. Echocardiography revealed 3.0 cm dilated right ventricle (RV), pressure in RV was 42 mmHg, 1st degree regurgitation on mitral valve (MV), 1st degree on tricuspid valve (TV), 1st degree on pulmonary artery valve. End-diastolic dimension (EDD) 4.0 cm, end-systolic dimension (ESD) 2.4 cm, ejection fraction (EF) 67%. Traces of fluid in the pericardial cavity: along the anterior wall of the RV up to 0.4 cm, in the area of the apex 0.8 cm. No local hypo- and akinesis were detected. Patient was referred to the Republican Hospital №1-National Center of Medicine (RHN№1-NCM) immediately.

Diagnosis was made following initial survey: "Subacute viral diffuse myocarditis. Exudative pericarditis. Chronic heart failure with preserved ejection fraction (66%), Functional class 2 by New York Heart Association (NYHA) classification. Bilateral multisegmental pneumonia, moderate severity, 1st degree respiratory failure. Post-COVID syndrome. COVID-19 reconvalescent since 19th February 2022."

Taking into account the severity of her condition due to respiratory failure, the need for oxygen therapy, hemodynamic control, the patient was immediately transferred from the Emergency department to the Cardiac Intensive Care Unit (CICU), where treatment for myocarditis and multisegmental pneumonia was initiated. Antibiotic therapy, anticoagulant, anti-inflammatory, cardioprotective therapy and correction of electrolyte disorders were prescribed.

On February 25, 2022, the patient's condition stabilized, clinical improvement was observed: complaints of dyspnea, palpitation, generalized weakness disappeared. Normal mental status. Skin and visible mucous membranes were of natural color, BP – 18-20/min, SpO_2 on room air - 94-96%. Hemodynamic parameters were stable: HR 95/min, BP 110/90 mmHg. Survey didn't reveal active course of coronavirus infection (the virus was not identified). Cardiospecific enzymes and D-dimer did not exceed reference values. By the evening of the same day, the patient's condition was assessed as moderate severity, and she was transferred from the CICU to the Cardiology department. After transfer from the ICU to the general ward, dyspnea at rest did not bother her, the patient noted it only during physical exertion. Visible mucous membranes and skin were normal. On auscultation breathing was conducted in all fields, weakening in the lower regions, no rales. BP 19-21/min, SpO_2 99% with insufflation of humidified oxygen. Heart tones muffled, rhythmic, HR 88/min, BP 90/60 mmHg (adapted BP, according to the patient).

From February 27 to March 1, 2022, the complaints of moderate dyspnea on physical exertion, dry cough persisted. Hemodynamic parameters were stable, BP within 17-18 /min. SpO_2 98% against the background of humidified oxygen supply (5-10 l/min). BP within 100/65 mmHg, HR 86 per min.

On 02.03.2022 at 07:45 a.m., the patient got up from bed, after which her condition sharply worsened, the patient had severe dyspnea at rest up to 26-30 breaths/min, SpO_2 on room air was 84%, on oxygen supply (10-15 l/min) 92%, BP 50/00 mmHg, heart rate 120/min. After the consultation of the intensive care physician the patient was urgently transferred to the CICU.

At 08:04 a.m., clinical signs of pulmonary embolism (PE) were revealed: significant increase of D-dimer >5.0 mcg/ml (the norm - 0.046-0.708), signs of right heart overload on ECG, compared with the initial record (Fig.1), as well as on echocardiography (dilated right heart, pressure in the right ventricle 101 mmHg, separation of pericardial sheets 0.3 cm).

General condition was extremely severe, caused by acute cardiovascular and respiratory failure, with signs of obstructive shock due to PE. Skin and visible mucous membranes were sharply pale, acrocyanosis. Body temperature 36.1°C. The patient was lethargic and restless (GCS 13 points), could only answer questions in short phrases. Breathing was unassisted, weakened in the lower portions on both sides, single dry

Laboratory data

Index	Reference	24.02.	25.02.	27.02.	01.03.	02.03.	03.03.
Ph venous blood	7.32 – 7.42	7.43	7.36			7.25	7.16
$PaCO_2$, mmHg	95-110	35				55	43
PaO_2, mmHg	42.0-55.0	95				68	205
SvO_2, %	94-98	71	70			68	65
Lac, mmol/l	0.2-1.8	3.1	2.1		5	6	10
Glu, mmol/l	3.9-5.3	5.6	4.8	4.5	4.5	13.1	9.8
BE, mmol/l	-2.5 – 2.5	-2	2		-3.5	-4	-6
RBC, 10^{12}/l	3.74-5.31	4.6	4.4	4.1	4.7	4.1	2.2
Hb, g/l	118-165	146	141	133	147	131	73
PLT 10^9/l	141-389	136	135	107	118	68	76
WBC, 10^9/l	3.89-9.23	10.6	10.7	12.2	15.7	18.8	28.5
NT-proBNP, pg/ml	0.0 – 125.0	1201			2303		
Troponine, ng/l	<29.000	0.01			0.1	1.1	2.5
CRP, mg/l	0 - 5	154		91	141	130	199
ESR, mm/h	<15	15	13	10	60	65	66
D-dimer, mkg/ml	0.047-0.7	0.7	0.5	0.5	>5.0	>5.0	>5.0
APTT, sec	25.5-33.5	20.8	39	38	77	79	88
ALT, unit/l	0.0-31.0	76.6	73.4	77.9	69.8	179	1438
AST, unit/l	0.0-31.0	48.2	47.0	40,9	38,4	146	1248
Creatinine, umol/l	53-97	89.3	85.7	75.6	75.4	90.6	309.2
Procalcitonin, ng/ml	0.0-0.05	0.05			0.07	0.5	7.1

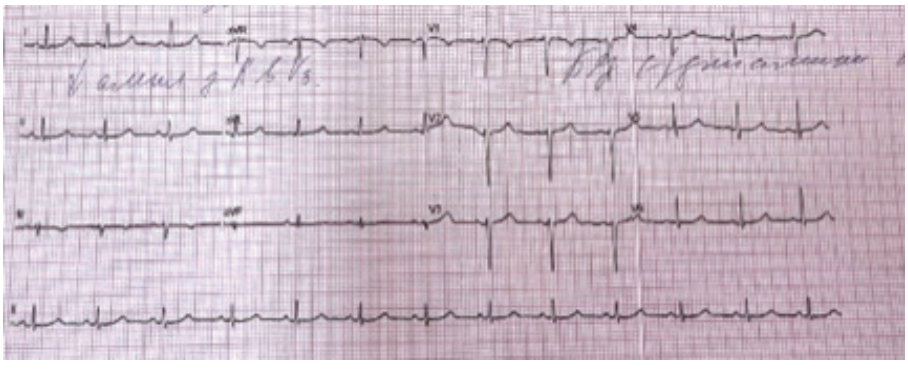
rales, BP 34 per min, SpO_2 60% on 20 L/min with humidified oxygen, auxiliary muscles were noted. Cardiac tones were muffled, rhythmic, heart rate 130 per min, systemic BP 50/00 mmHg, despite intravenous infusion of dopamine 10 mcg/kg/min given by pump. The abdomen was not swollen. Intestinal peristalsis was sluggish. The liver was enlarged by 2 cm at the edge of the right rib arch. Edema of the feet and shins.

At 08:05 a.m. the patient was urgently transferred to artificial lung ventilation (ALV) by Puritan-Bennet 840 machine due to increasing signs of acute respiratory failure and shock with following settings: Control Motion Ventilation (CMV) mode, minute volume (MV) - 7.7 L/min, tidal volume (TV) - 510 ml, FiO_2 - 100%, positive end-expiratory pressure (PEEP) - 5 cm H_2O, pressure airway - 27 cm-H_2O. Despite the used "hard" ventilation pattern, peripheral saturation remained below 88%, and at blood gas study, respiratory index (PaO_2/FiO_2) was 84 mmHg with decompensated respiratory acidosis. Due to the patient's critical condition, chest CT with contrast was not possible at the time. Based on the clinical picture of massive PE and the results of survey, it was decided to administer thrombolysis therapy with Urokinase in a starting dose of 4400 units/kg and a maintenance dose of 4400 units/kg/h for 24 h (according to the recommendations of the European Society of Cardiology, 2019) [6].

At 08:07 a.m. asystole was registered on the monitor. A complex of cardiopulmonary resuscitation (CPR) was performed, including external cardiac massage and ALV in the ratio of 30:2, intravenous injection of adrenaline hydrochloride solution 0.1%-1.0 ml with successful recovery of cardiac activity after 3 min of resuscitation, ECG showed sinus tachycardia of 130 per min. Cardiotonic and vasopressor drugs with stepwise titration of norepinephrine at a dose of 0.5 μg/kg/min, dobutamine at a dose of 5 μg/kg/min were started, and arterial pressure of 115/75 mmHg and central venous pressure (CVP) of 9 mmHg were achieved. A low saturation level of below 82% remained.

At 4:50 p.m. her condition was evaluated as critical with dramatically negative course: SOFA - 19 points (80% probability of death), LIS (Lung Injury Score) - 2.25, RESP (Respiratory ECMO Survival Prediction) - 6 points with a survival rate of 36%. Decision to use veno-venous extracorporeal membrane oxygenation (VV-ECMO) was made after failure of standard therapy to improve hypoxemia despite AVL, the increase of decompensated respiratory acidosis, increasing blood lactate - 4 mmol/l, increasing $PaCO_2$ >55 mmHg, respiratory index (PaO_2/FiO_2) <85, SvO_2 <65%. VV-ECMO was performed on a Maquet Rotaflow device using a Medtronic Bio - Medicus 21 Fr cannula. V. femoralis dextra was used for blood sampling, the Medtronic

a



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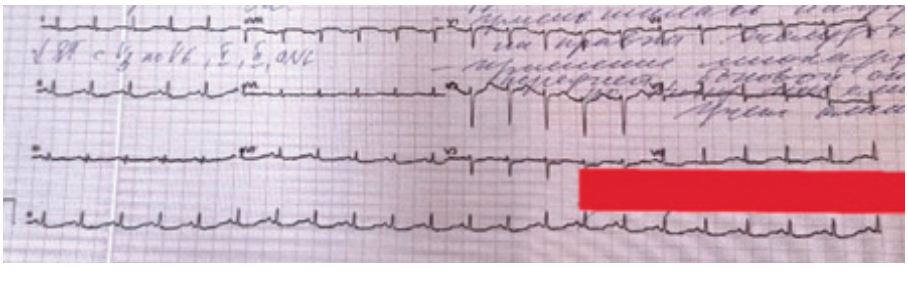

Fig. 1. ECG of patient M.: a - admittance day 24.02.22, b - day of deterioration 02.03.22

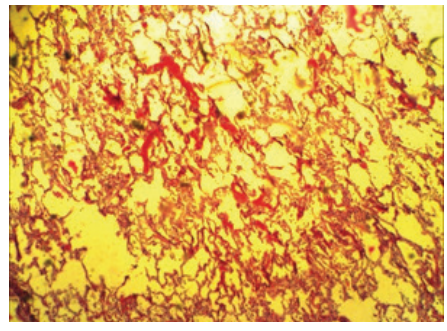

Fig. 2. Fragment of the right lung: thickened interalveolar septa due to fibrosis. Erythrocytes, single- and double-nucleated macrophages, lymphocytes, leukocytes, traces of protein fluid are observed in lumens of alveoli. Desquamated respiratory epithelium can be seen in some alveoli. (Fig.2-4 preparations stained with hematoxylin and eosin, x100)

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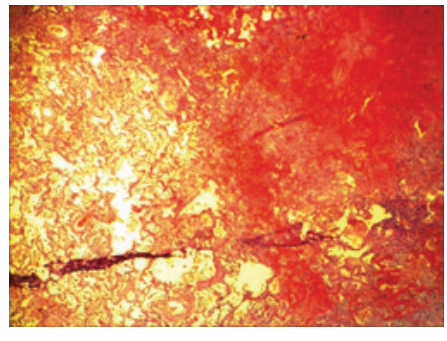

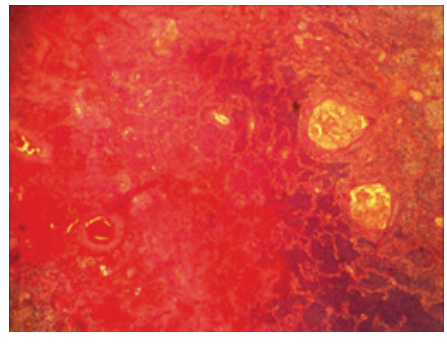

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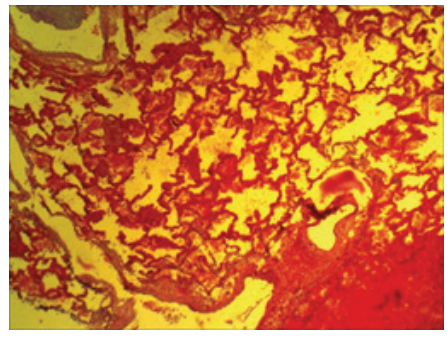

Fig. 3. Fragments of the right lung, infarction zone: a) in fragments from the lower lobe of the lung there are extensive hemorrhage fields with foci of necrosis of the underlying tissue, b) the border of the lung tissue and the infarction zone displayed in the sample

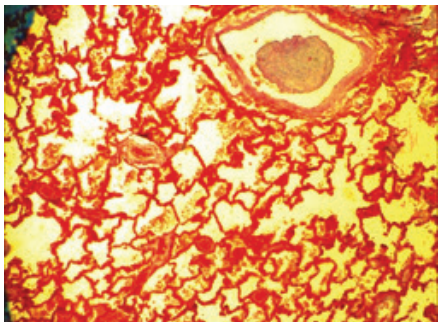

Fig. 4. Fragment of the right lung: there is a mixed thrombus in the vascular lumen.

Bio-Medicus 19 Fr return cannula was placed in v. jugularis interna dextra. Venous catheterization was performed using puncture ultrasound navigation under total intravenous anesthesia. The volume perfusion rate of VV-ECMO was 2.5 l/min, FiO_2 = 90%, and AVL was continued with saturation at 94% to maintain airiness of the lung tissue. Anticoagulant therapy was performed by titrating the dose of unfractionated heparin under constant laboratory control in order to prevent massive blood loss due to hypocoagulation. Activated clotting time was maintained in the range of 380-420 s. The conducted VV-ECMO allowed to improve the parameters of gas exchange in arterial blood (PaO_2 = 125 mmHg, $PaCO_2$ = 43 mmHg), while mixed decompensated lactacidosis persisted (ph 7.16) (Table).

On March 3, 2022, at 11:10 a.m., despite the ongoing measures, clinical death happened due to progressive hypoxemia and multiorgan failure. CPR for 30 minutes without effect and at 11:40 min patient was pronounced dead.

Autopsy established the following diagnosis: Main diagnosis: U07.2 Novel coronavirus infection, not laboratory confirmed (virus not identified). Community-acquired bilateral viral pneumonia, in the process of resolution, with organized thrombi in the pulmonary vessels.

Complications of the main diagnosis: Subacute myocarditis, with foci of necrosis. Disseminated and microfocal cardiosclerosis. Cardiovascular insufficiency (NT-proBNP from 01.03.2022 - 2308 pg/ml). Thromboembolism of small branches of the pulmonary artery. S4, 5, 8 infarctions of the right lung.

The diagnosis was confirmed by the results of pathomorphological study (Fig.2-4).

Discussion. The presented clinical case shows that in the patient during the recuperative period of COVID-19 the symptoms of myocarditis prevailed, which were successfully managed at the initial stage of treatment. However, another complication in the form of thromboembolism of small branches of the pulmonary artery led to acute respiratory and cardiovascular failure with the development of clinical death. Due to the severity of the condition, PE was not verified by computed tomography with pulmonary artery contrast. The diagnosis and decision to perform systemic thrombolysis were justified by the specific presentation and laboratory data (D-dimer >5.0 μg/mL, NT-proBNP - 2303 pg/mL). The results of pathological examination confirmed PE and indicated that the patient had multiple "old" intravascular thrombi of small branches, which probably began to form in the acute period of the disease and had few symptoms. That is why the thrombi were lysed only partially and thrombolytic therapy was not effective.

At present, the mechanism of hypercoagulation triggering and thrombosis development after COVID-19 is not fully understood and requires further investigation. The main theory remains the development of endothelial dysfunction due to SARS-CoV-2 lesion and inflammatory reaction at the contact site [10]. The target of the virus is angiotensin-converting enzyme-2 (ACE-2), which is expressed in endothelial cells of arterial, venous and cardiomyocyte smooth muscle and alveolar lung epithelial cells [3, 5, 8]. The SARS-CoV-2 virus also contacts cells with the major protease Mpro with high affinity, binding to the receptor (ACE-2) and transmembrane serine protease-2 (TMPRSS-2) [9]. The active site of Mpro in SARS-CoV-2 has structural similarities to the coagulation factors Xa and thrombin and can therefore activate the clotting system, triggering a reaction cascade with thrombosis.

This case demonstrates that the process of intravascular thrombosis can develop in cases of mild to moderate COVID-19, in the recuperative period of the disease, in the absence of obvious risks of venous thromboembolic complications and up to a certain point proceed without evident laboratory and clinical manifestations.

Conclusion. Thus, high suspicion for thromboembolic complications should remain in any patient who has had COVID-19. The presented clinical case demonstrates that the risk of fatal thromboembolic complications during the convalescent period persists not only in the severe form of the COVID-19 course, but also in patients who have undergone this disease in mild and moderate severity. If the prophylactic use of low molecular weight or unfractionated heparin is mandatory during the entire inpatient period of treatment of patients with COVID-19, then the issues of choosing anticoagulant drugs, their dose and timing of administration after discharge remain unresolved. Early stratification of patients according to the risk of thrombotic complications, the correct choice of anticoagulant therapy at all stages of treatment of patients with COVID-19 will help to avoid or reduce the development of severe thrombotic complications.

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EARLY ULTRASOUND PRENATAL DIAGNOSIS OF LOWER OCCIPITAL CRANIOCEREBRAL HERNIA, AT 13/3 WEEKS OF PREGNANCY

DOI 10.25789/YMJ.2022.79.28

УДК 611.019

We describe a case of early ultrasound prenatal diagnosis of a rare disabling malformation of the fetal central nervous system, lower occipital encephalocele, at 13/3 weeks of pregnancy. The article describes the characteristic ultrasound markers, stages of prenatal ultrasound diagnostics. The prenatal diagnosis was established on the basis of the signs characteristic of this pathology of the central nervous system. The pregnancy was terminated at 17 weeks by the decision of the family. Pathomorphological examination of abortus confirmed the echographic changes detected in the prenatal period.

Keywords: fetus, lower occipital encephalocele, ultrasound prenatal diagnosis, pathomorphological examination.

Introduction. Craniocerebral hernia (encephalocele) is the formation of a hernial sac, with various anatomical contents, localized in the area of a defect in the vault of the skull bones, mainly in the cranial sutures. Frequency -1.13: 10,000 newborns [1, 2].

Encephalocele is associated with a disorder of the early embryonic development of the cranial vault, during the laying of the brain plate and its closure into the neural tube [3].

By localization, craniocerebral hernias (hereinafter CCH) are divided into anterior (frontal), nasopharyngeal, basal and posterior (occipital). Depending on the location above or below the occipital protuberance, upper or lower CCH is distinguished [2].

According to the contents of the hernial sac, a cranial hernia is divided into meningocele, encephalocele and encephalocystocele. In the first case, a craniocerebral hernia contains dura mater covered with skin; large defects, when the hernial sac includes brain tissue with arachnoid (arachnoidea encephali) and soft (pia mater) membranes, is an encephalocele. Encephalocystocele is a type of extremely pronounced form of CCH, when a part of the ventricular system of the brain also enters the hernia. There are also "detached" cerebral hernias, the most favorable form of CCH, when during the development period the hernia loses its connection with the cranial cavity.[4]

The most frequent (up to 80-90%) and prognostically unfavorable are occipital hernias [1]

An echographic marker of CCH is the detection of a defect in the bones of the cranial vault and paracranial formation of various localization and contents, depending on which brain tissue enters the hernial sac [5,6] Early prenatal diagnosis of CCH in the first trimester of pregnancy is possible after 11 weeks of pregnancy, when the ossification of the bones of the cranial vault is completed [1]

The fetal MRI method can supplement and refine the ultrasound data, which is important for choosing tactics for further pregnancy management and long-term prognoses. In 80% of cases of CCH, various combined anomalies are observed: microcephaly, ventriculomegaly, agenesis of the corpus callosum, holoprosencephaly, congenital heart defects and abnormalities of the musculoskeletal system. During prenatal examination, it is also important to take into account that CCH can be included in a number of syndromic conditions, such as Meckel-Gruber syndrome, amniotic cords syndrome, Walker-Warburg syndrome and frontonasal dysplasia [1] Treatment of CCH hernias is operative, using plastic surgery methods, especially with anterior localization. Postoperative losses in CCH reach 44%. Intellectual deficiency cases in surviving children – vary from 40 to 91% [7] Fetal surgery experience around the world shows the possibility of intrauterine surgical treatment of CCH in the period from 24 to 26 weeks of gestation, with minimal neurological symptoms in the postpartum catamnesis [8,9]

Materials and methods. Patient K., 25 years old. Healthy. This pregnancy is the second, the first ended less than a year ago with premature operative delivery at 33 weeks. The first pregnancy proceeded with severe anemia, weight deficiency, chronic fetoplacental insufficiency, fetal growth retardation syndrome of the 1st degree. The child died at the 6th week of life due to fungal bacterial sepsis.

With the current pregnancy, the patient registered with the hospital for regular screening at 6-7 weeks of pregnancy. This pregnancy proceeded with early toxicosis of moderate severity, for which she received treatment in a hospital. Chronic nicotine intoxication is noted, smoking experience is 2 years before the onset of the first pregnancy. The effect of other teratogenic factors are denied by the patient. Civil marriage, not related. Husband is 27 years old, smokes. Spouses have no industrial hazards at work. The genealogical anamnesis of the husband is not burdened. The first planned screening examination of the fetus was carried out at the time of 13/3 weeks of pregnancy on an Accuvix A-30 ultrasound device, Samsung Medison, with sensors: volumetric 4-8 Mhz and convex 4-9 Mhz.

Results. Fetometrial data during the screening study at 13/3 weeks of pregnancy corresponded to the gestational norm (Fig.1) When examining the bones of the fetal cranial vault, in the lower part of the occipital region, on the left, a thin-walled hernial formation, rounded in shape, measuring 7 x 6 mm was determined paracranially (Fig.2) The echostructure of the hernial formation is heterogeneous (most likely the brain membranes in the cerebrospinal fluid) (Fig.3)

In the occipital region of the fetal head, a defect of the skull bones with a width of 1.7 mm was clearly defined in the arch. In the CDU (Color Doppler Ultrasound) mode – avascular. There were no other echographic markers of chromosomal

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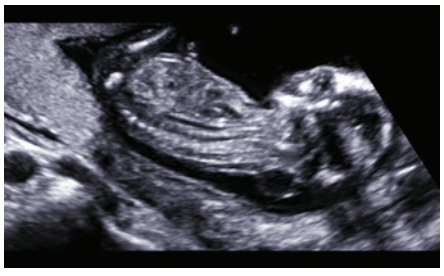

Fig.1. Sagittal section of the fetus

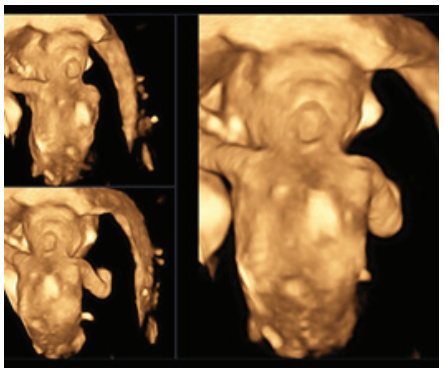

Fig.2. 3D- fetal reconstruction

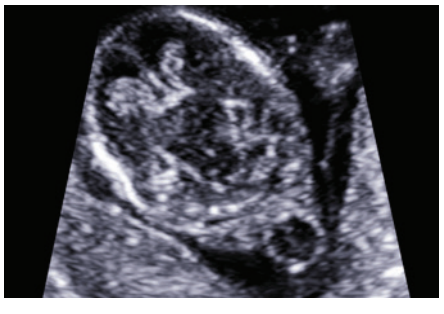

Fig.2. 3D- fetal reconstruction

abnormality and markers pathognomonic for the syndromic diagnosis. The patient was referred to the second stage of examination at the MGC, where the diagnosis was fully confirmed. Prenatal karyotyping was not performed. At the age of 17 weeks, with the consent of the family and following the results of the collegial conclusion of specialists, a medical termination of pregnancy was performed.

A pathoanatomic examination of the fetus revealed: female abortus, weighing 250 g, height 21 cm. Examination of the cervical-occipital region revealed a hernial protrusion with a diameter of 15 mm. The contents of the hernial sac are edematous membranes of the brain, a hernial defect of 6 mm. The cerebellar hemisphere was closely attached to the bottom of the hernial opening. The internal organs are formed correctly, without visible malformations.

Discussion. The described case of early prenatal diagnosis of occipital encephalocele at 13/3 weeks of gestation is the first of its kind in Yakutia, in which all stages of prenatal examination, counseling and postmortem verification are observed. Conducting the first prenatal screening study at more than 11 weeks of gestation made it possible to timely identify a disabling malformation and, with less moral and material losses, prevent the birth of a child with a severe, disabling pathology of the central nervous system.

Around the world, in specialized clinics, most cases of encephalocele are diagnosed during screening ultrasound studies [2]

In the described case, the cause of isolated folate-dependent malformation of the central nervous system in the fetus is probably multifactorial: recent pregnancy, hormonal discorrelation, chronic hypoxia, malnutrition and weight deficiency (the woman's BMI when registering with the hospital - 17.3 kg / m^2)

Taking into account the latter fact, in order to reduce the number of folate-dependent malformations, in women's consultations - it is advisable to work more actively in the family planning office with maternity hospitals with unfavorable pregnancy outcomes, prescribing folic acid, patronage of families in the first year after the loss of a child, offering psychological counseling and regular preventive talks.

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A CLINICAL CASE OF PARTIAL RED CELL APLASIA IN A 2-YEAR-OLD CHILD

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Currently, no more than 300 patients with pure red cell aplasia have been described. A rare case of pure red cell aplasia in a Sakha child is presented in the article.

Keywords: anemia, erythrocytes, red cell aplasia, treatment, sequencing, Sakha, children, Yakutia.

Introduction. Pure red cell aplasia (PRCA) is a rare form of congenital hematopoiesis resulting from apoptosis of erythroid precursors in the bone marrow due to a defect in ribosome biosynthesis [1-4]. PRCA was first described by Kaznelson in 1922. Subsequently, a number of cases of this disease were described, with thymoma tumor of the thymus gland (thymoma) identified in a significant proportion of patients [2].

Currently, most of the genetically deciphered cases of PRCA are the result of haplotypic deficiency of genes encoding small or large ribosome subunit proteins; single cases of PRCA resulting from mutations of GATA1, FLVCR1, and TFR2 have also been identified. Congenital forms of PRCA with a debut at 2 years of age have been described. The course of the disease is chronic, and in some cases remission is achieved [1-3]. This article presents an interesting case of a Sakha patient with pure red cell aplasia (PRCA).

Clinical example. Child, name I., Sakha, 2 years old. Anamnesis of life - child from the 1st pregnancy, which proceeded in the first half with toxemia, in the 2nd half proceeded without features according to the mother's speech. Delivery 1, in term, 41 weeks, natural. Weight at birth is 3020 grams, height is 51 cm. Was breastfed on the first day. Apgar score 8/9b. Discharged home from the nursery on the fifth day. Breast-feeding up to the age of 1 month. Psychomotor development up to the age of 1 year of age corresponded: holds his head after 2 months, rolls over by 4 months, sits after 6 months, crawls after 7 months, walks after 10 months.

Mother's diseases are anemia, allergic diseases. Heredity, according to the mother, was not aggravated.

Preventive vaccinations according to the national vaccination calendar.

Past illnesses: frequent acute respiratory infections, COVID 19, bronchitis, bronchial asthma.

Allergic history: citrus, dust.

There were no operations carried out.

Past medical history: since January 2021, the child has been bothered by frequent acute respiratory infections, with a prolonged cough, pallor, lethargy and weakness. In February 2021, the child was referred to the central regional hospital for treatment. Complaints on admission: frequent acute respiratory infections and prolonged cough, pale skin, weakness, lethargy, cough. Discharged in March 2021 with improvement.

On 29.09.21, with complaints of prolonged cough, pale skin, weakness, lethargy, cough, the child was referred to the pulmonology department of RH #1-NCM.

According to the results of the examination: general analysis and biochemical analysis of blood contained no features. Tests for infections were taken: CMV DNA was positive. Blood PCR for CMV was negative, herpes simplex virus 1.2 was negative. Urine PCR for CMV was positive. Clinical diagnosis: Obstructive bronchitis. Lingering course. Combined etiology. CMV infection with bronchopulmonary involvement.

The child was treated with Viferon 150,000 IU 2 times a day, azithromycin 10 mg/kg/day (120mg) once a day for 5 days, hofitol 0.5 ml 3 times a day, linex 1 capsule 3 times a day, ursodeoxycholic acid 50 mg 2 times a day, valganciclovir 16 mg/kg 2 times a day. On 14.10.22 the child was discharged in satisfactory condition.

From 31.01.22 to 26.02.22, the child had a coronavirus infection. Since 17.02.22 his condition worsened: weakness, lethargy, pronounced pallor. On February 17, 2022, the child was hospitalized in the infectious diseases department of RH#1-NCM.

Paraclinically: General blood test of 16.02.22.: white blood cells (WBC) $11x10^9$/l (RI: 7.5 - 11x10 ^{9}/l), red blood cells (RBC) - 1.72 $x10^{12}$/l (RI: 4-4.9$x10^{12}$/l), hemoglobin (HGB) - 44 g/l (RI: 110-5=132x10 g/l), stabular neutrophils - 6% (RI: 1-5%), segmented neutrophils - 21% (RI: 35-55%), lymphocytes - 69% (RI: 35-55%), monocytes - 4% (RI:4-6%), eosinophils - 4%(RI: 0-5%), reticulocytes 0. 2% (RI: 0.4- 1.3%), COE 37 mm/hour (RI: 1-15 mm/hour). Conclusion: Hypochromic anemia of the 2nd-3rd degree.

Biochemical blood test of 2/16/22: Alanine aminotransferase (ALT) 43.43 U/L (RI:00-40.00 U/L), aspartate aminotransferase (AST) 39.4 U/L (RI: 00-40.0 U/L), blood glucose 4 mmol/L (RI: 3.3-5.60mmol/L), total protein 59.5 g/L (RI: 51.00-73. 00 g/l), albumin 46.5 g/l (RI: 35.0-50.0 g/l), creatinine 25.5 µmol/l (RI: 35.00-110. 00mmol/l), urea 6.8 mmol/l (RI: 3.3-5.8 mmol/l), serum iron 62.9 g/l (RI: 9.0- 30.4 g/l), total bilirubin 4 µmol/l (RI: 3.4- 17.10 µmol/l). Conclusion: elevated serum iron level.

On February 17, 2022 the child was urgently transferred to the oncohematological department of PC RH#1-NCM.

Myelogram from 18.02.22: granulocytic growth with delayed myelocyte stage with absence of erythroid growth indicators. The morphological picture is typical for pure red cell aplasia.

The patient was consulted with the Federal State Budgetary Institution "Research Institute of the Russian Academy of Medical Sciences named after Dmitri Rogachev", and an examination to clarify

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the diagnosis was recommended. Molecular genetic studies confirmed the presence of mutations in ribosomal genes (RPS19, RPS10, RPS24, RPS26, RPL5, RPL11, RPL35a, RPS7, RPS17), indicating pure red cell aplasia.

L-lycine was prescribed, hemotransfusion #1 - 30 ml.

On discharge - general blood test dated 30.12. 2021 - white blood cells (WBC) - 4.4*10^9/l (RI: 7.5 - 11x10^9/l), red blood cells (RBC) - 4.3x10^{12}/l (RI: 4-4.9x10^{12}/l), hemoglobin(HGB) - 120.2 g/l (RI: 110-132x10 g/l), stab neutrophils - 3% (RI: 1-5%), segmented neutrophils - 27% (RI: 35 - 55%), lymphocytes - 55%(RI: 35-55%), monocytes - 5% (RI:4-6%), reticulocytes 0.4 (RI: 0.4 - 1.3%), COE 3 mm/hr(RI: 1-15 mm/h). Conclusion: There was an improvement in the laboratory indexes.

Clinical diagnosis: Pure red cell aplasia. Concomitant diagnosis: Atopic dermatitis. Limited form. Subacute course. Congenital heart defect. Grade 2 mitral valve insufficiency. Left supplementary coronary artery. E66.0 Grade 1 paratrophy. Hypoplasia of the right kidney. Physiological phimosis.

The sick child was discharged from the hospital with recommendations: Prednisolone at a dose of 1.5 tablets per day, divided into 3 doses. Re-admission to the hospital in 1 month.

In the period from 23.05.22 to 7.06.22 he was twice hospitalized to the oncohematology department of PC RH №1-NCM on emergency indications due to worsening of his condition and changes in blood tests.

Examination on admission: Objective examination on admission: Height - 85 cm, weight - 16 kg, temperature - 36.1, heart rate - 24 per minute, heart rate up to 96 per minute. Condition was moderate in relation to the underlying disease. His well-being was not impaired. His appetite was not disturbed. Sleep was calm. Clear consciousness. The build was correct. Subcutaneous fatty tissue; distributed evenly. The pharynx was not hyperemic. The mucous membranes of the mouth and pharynx were clear, pale in color. Nasal breathing was free. The bones and joints system had no features. Peripheral lymphatic system: lymph nodes were not enlarged. The thorax was regular in shape. On percussion of the chest - clear pulmonary sound. On auscultation of the chest, vesicular breathing was heard, no rales. The heart tones were clear and rhythmic. The abdomen was soft and painless. The liver and spleen were not enlarged. Urination was free and painless. There were no peripheral edemas.

Paraclinically: general blood test of 14.06.22 - White blood cells (WBC) - 4.23x10^9/l (RI: 7.5 - 11x10^9/l), red blood cells (RBC) - 2.3*10^{12}/l (RI: 4-4.91012/l), hemoglobin(HGB)- 60.0 g/l (RI: 110-132x10 g/l), stab neutrophils - 1% (RI6 1-5%), segmented neutrophils - 22% (RI: 35-55%), lymphocytes - 54%(RI 35-55%), monocytes - 9% (RI:1-5%), reticulocytes 4 (RI: 4-6%), COE 3 mm/hr (RI: 1-15 mm/h). Conclusion Decreased hemoglobin, erythrocytes.

The child was clinically diagnosed with: Primary disease: Pure red cell aplasia.

Treatment was prescribed: Table #15, L-leucine 1000 mg/sq.m. - 540mg. Three times a day for 3 months, replacement therapy by transfusion of washed red blood cells.

Against the background of the therapy the condition and blood parameters improved. At present the child is receiving treatment at the place of residence, periodically comes to the oncohematology department of RH №1-NCM for replacement therapy with washed red blood cells.

Conclusion: Pure red cell aplasia (PRCA) is a rare syndrome characterized by a reduced number of erythroid cell precursors in the bone marrow. In pure red cell aplasia, a careful examination of the patient is necessary to exclude a neoplastic process, differential diagnosis with sideroblastic anemia and acute leukemia, transient erythroblastopenia, congenital hypoplastic anemia and Pierson syndrome. NGS sequencing and telomere length determination are used in the diagnosis. The main treatment for pure red cell aplasia is red cell transfusion and administration of L-leucine and glucocorticosteroids.

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DIFFICULTIES IN DIAGNOSING PARKINSON'S DISEASE WITH DEMENTIA AND DEMENTIA WITH LEWY BODIES IN CLINICAL PRACTICE

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Dementia is a chronic cognitive decline affecting all domains of cognition with an unfavorable outcome, observed in both dementia with Lewy bodies (DLB) and Parkinson's disease (PD). These two conditions belong to the group of alpha-synucleinopathies. In DLB and PD, attention, goal-directed activity, visual-spatial orientation, visual-constructive ability, and memory are affected. The similarity of the profile of cognitive impairment in PD with dementia and DTL leads to some difficulties in the diagnosing of these two diseases. Using their own observations, the authors have demonstrated that an important aspect in making the correct diagnosis is objective information from relatives and analysis of the available medical records.

Keywords: dementia, dementia with Lewy bodies (DLB), Parkinson's disease (PD), cognitive impairment, visual hallucinations.

Introduction. Parkinson's disease (PD) and dementia with Lewy bodies (DLB), or diffuse Lewy bodies disease, are associated neurodegenerative diseases that belong to the group of alpha-synucleinopathies and have similar cognitive, behavioral, and autonomic disturbances [1, 2]. Alpha-synuclein is a protein consisting of 140 amino acids, which can aggregate under pathological conditions to form intracellular inclusions - Lewy bodies [7].

According to the Braak theory, the neurodegenerative process in PD undergoes 6 stages and starts from the nuclei of the vagus nerve and the olfactory bulb. Classical motor symptoms appear at stage 3 of the degenerative process, when the Lewy bodies are found in the neurons of the substantia nigra of the midbrain. Starting from stage 4, the process involves the cortex neurons, which causes the development of cognitive disorders and their progression up to the dementia level [8]. In DLB, the neurodegenerative process is multifocal, and Lewy bodies are found in the cerebral cortex from the very early stages, which may explain the early onset of cognitive impairment. However, in DLB brainstem lesions may be less severe than in PD, resulting in a less prominent presentation of parkinsonism in patients [9].

In 2017, McKeith and colleagues published updated criteria for the diagnosis of DLB, in which dementia is a core symptom of the disease. Additionally, cognitive fluctuations, visual hallucinations, REM sleep disorder, and signs of parkinsonism are considered to be main features. Symptoms such as hypersensitivity to neuroleptics, falls, severe autonomic dysfunction, hyposmia, anxiety, and apathy are attributed to supportive (but not obligatory) criteria [5]. Thus, dementia is an obligatory feature of DLB.

Regarding PD, at the early stages 20% of patients have mild to moderate cognitive impairment [3]. After 3.5 years, 57% of patients have moderate cognitive impairment, and in 10% of cases, cognitive impairment already reaches the degree of dementia; after 17 years of the disease, dementia develops in almost 80% of patients with PD. Risk factors for the development of dementia in PD includes: age, akinetic-rigid form of the disease, reduced semantic speech, genetic factors, low level of education, and postural instability [10]. Thus, dementia in PD develops later, while in DLB it is already present from the disease onset. In this regard, it is useful to apply the "first-year rule" in clinical practice: if dementia presents in the background of PD after at least 1 year from the onset of motor symptoms, it is regarded as PD with dementia; if dementia develops within the first year from the onset of parkinsonism or even precedes or occurs simultaneously with the development of motor symptoms, these cases are classified as DLB [11].

Table 1 lists the overlapping features and distinguishing features of dementia in PD and DLB [6].

Hence, the cognitive impairment profile in PD with dementia and DLB is nearly identical. When patients are approached without obtaining objective information from relatives or medical records, there may be difficulties in establishing a correct diagnosis.

Further, we present two clinical cases of PD with dementia and DLB.

Clinical case №1. Patient E., 60 years old, was referred to the neurology department with complaints of slowness and depletion of movements, unsteadiness and freezes when walking, acceleration during forward movement, urinary incontinence, persistent constipation, loss of sense of smell, change of handwriting, feelings of sadness, anxiety, sleep disorders, non-intimidating visual hallucinations.

Patient had been ill during 7 years. First symptoms of disease in the form of slowness of movements were noticed by her colleagues. Slowness of movements and shuffling when walking appeared next year. Neurologist diagnosed Parkinson's disease and prescribed 50 mg of piribedil 3 times a day. During treatment, the patient began to show positive dynamics. On the third year of the disease, she had mild urinary incontinence, worsened motor symptoms such as difficulties with standing up from the chair, tremor and increased slowness of movements. At the fourth year of the disease the patient started therapy with levodopa (levodopa/carbidopa 250/25 mg 3 times a day) with a slight effect in the form of reduction of stiffness. From the fifth year of illness, when she lived alone at home, she began to invite strangers home from the street,

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Similarities and differences of PD with dementia and DLB (according to Jellinger K. и Korczyn A., 2018; modified by authors)

Sign	PD with dementia	Lewy bodies dementia
Паркинсонизм	Obligatory	Less pronounced than in PD May absent in 25% of patients Tremor less frequent
Cognitive impairment	Dysregulation	Dysregulation with greater impairment of memory and attention than in PD
Visual-spatial impairment	Typical	Typical
Visual hallucinations	Frequently caused by dopaminergic therapy, but can also occur spontaneously	More frequent, spontaneous, not associated with visual-spatial and gnosis impairment
Anxiety, depression	Typical	Typical
Rapid eye movement phase sleep disorder	Yes, can precede motor symptoms	Yes, can precede dementia
Sensitivity to neuroleptics	+	+++
Autonomic failure	+	++

called them her close friends and offered them food. At the same time, relatives began to notice that the patient periodically began to babble, talking to non-existent people, saw them in the room and in the window. Sometimes she was critical of her own visions, mood swings with pronounced anxiety and restlessness were noted.

At the admission, the patient was taking levodopa/carbidopa 250/25 mg 3 times daily, pramipexole 1 mg once daily and levodopa/benserazide 200/50 mg 3 times daily. The duration of this medication combination is unknown, due to cognitive impairment and lack of discharge.

In the neurologic status: anosmia, hypomimia, bradylalia, positive oral automatism reflexes. Muscle tone in the arms was elevated by the "cogwheel" type, predominantly on the left; in the legs, elevated by the "lead tube" type, more on the left. Tremor of the jaw. Severe oligobradykinesia. Pull-test is positive. Shuffling gait. Bending posture. Drug-induced dyskinesias in the trunk and limbs. She scored 73 on part 3 of the UPDRS scale.

The patient was awake and appeared untidy. While examining her, keeps quietly singing along to the motive of the song, says she "spins in her head", so she feels better. She interacts, self-criticism was reduced. She follows instructions, easily exhausted.

Attention is unfocused, easily distracting. Mnestic impairments such as difficulty with the delayed reproduction, cannot remember with the help of cues. Reduced volume of auditory verbal memory. Reduced phonetic speech activity (7 words per minute, while normal is 11 words). Difficulties in assimilation of motor series in the dynamic praxis test, substitutions for stereotypies were noted. She failed the clock drawing test (Fig. 1A). Serial counting impairment also was noted. Therefore, taking into account the findings of the examination, we can suggest the presence of cognitive disorders of a dysregulatory neurodynamic character associated with cortical frontal dysfunction.

The MoCA test was performed - 13/30 points, the Hospital Anxiety and Depression Rating Scale (HADS) scored 15 points for anxiety and 11 points for depression.

Brain MRI revealed moderate atrophic changes of the cerebral hemispheres, cerebellum, slight atrophy of midbrain.

Based on the medical history (she has been observed with PD for 7 years, symptoms of cognitive decline have been present since the 5th year of the disease) and clinical presentation (parkinsonism, dementia, visual-spatial disturbances, visual hallucinations, autonomic dysfunction, drug-induced dyskinesias) the patient was diagnosed with PD with dementia.

Treatment correction was carried out: one drug of levodopa was withdrawn, leaving only levodopa/carbidopa in a dose of 250/25 mg 3 times a day, pramipexole was withdrawn, and memantine was added with titration up to 20 mg/day and atypical neuroleptic quetiapine 12.5 mg before bedtime. After 3 weeks, as a result of treatment correction, drug-induced dyskinesias had resolved, the severity of hypokinesia and muscle rigidity had decreased, walking became better (scored 41 points on Part 3 of the UPDRS), visual hallucinations had resolved, and her clock drawing test score had improved (Figure 1B), with a score of 20/30 points on the MoCA.

Clinical case №2. Patient K., 69 years old, came to the consultation with her daughter, with complaints of recurrent visual hallucinations, memory loss, forgetfulness, general weakness, trembling of the right limbs, muscle stiffness, more in the right limbs, slowness of movement, walking freezes.

The patient has been observed since

a b

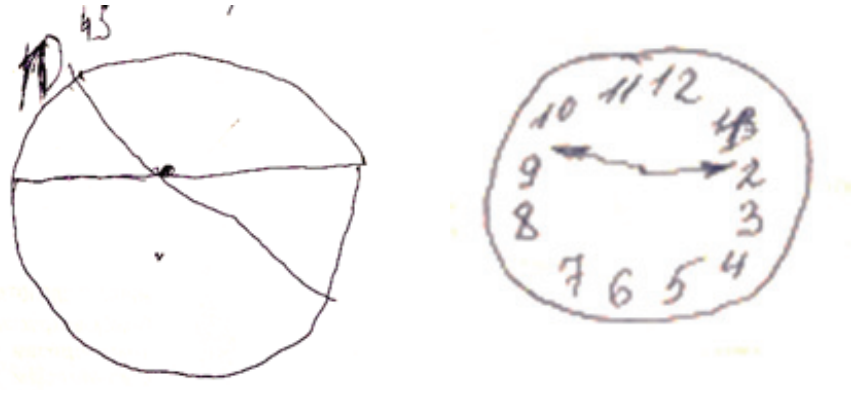

Fig. 1. Result of patient E's clock drawing test: A - at admission: the face and arrows are missing, sectoral pattern determined; B - during therapy correction: slight displacement of digits on the face, equal size of the arrows.

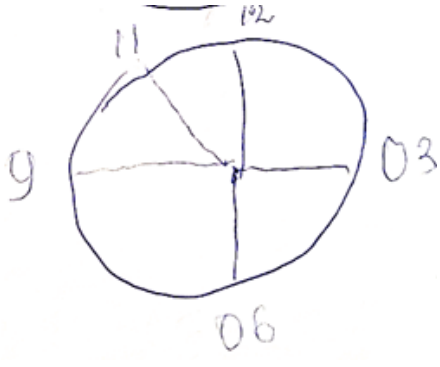

Fig. 2. K.'s clock drawing test results.

the onset of the disease. At the age of 65 years, right leg trembling and shuffling when walking appeared. Initially, the patient did not notice the symptoms, because they were explained as a result of a food poisoning. Over the next six months, however, her right hand began to tremble and her memory started to decline. At the same year the patient came to us for treatment. Examination revealed hemiparkinsonism in the right side, rest tremor in the right extremities, with a score of 38 on UPDRS Part 3. In addition, extracampine hallucinations in the form of false presence were revealed, she was not able to perform the clock drawing test (Fig. 2), her verbal activity was reduced (phonetically associated words - 4, semantically associated words - 2), visual memory was reduced (spontaneous reproduction of 2 pictures out of 12), but recognition was preserved (she named 11 pictures out of 12 with hints). An MRI scan of the brain detected moderate convexital atrophy of the frontal regions. On account of the equal severity and simultaneous development of parkinsonism and cognitive impairment, the diagnosis of diffuse Lewy bodies disease was established. She was prescribed levodopa/benserazide titrated up to 100 mg/25 mg 3 times daily, acetylcholinesterase inhibitor donepezil titrated up to 10 mg daily (however, she took only 5 mg daily because of fear of adverse effects). During therapy, however, motor activity improved and extracampine phenomena were eliminated.

The year following, visual hallucinations commenced, she began to see her children. During examination there was no deterioration of motor symptoms, she was not able to perform the clock drawing test, she drew sectors, there was a deterioration of visual memory: she was able to reproduce only 4 pictures with hints, 5 false memories were detected. For relieving hallucinations, the atypical neuroleptic drug quetiapine in a dose of 12.5 mg at night was recommended, and the dose of donezepil was increased up to 10 mg. There was significant improvement during treatment: hallucinations were eliminated, the patient became able to take care of herself better, and could do her own domestic routine work.

After 6 months, the patient continued to worsen: she began to speak to herself, and the slowness of her movements became more pronounced. Examination revealed a significant increase in parkinsonian symptoms (she scored 62 on UPDRS Part 3). Due to increase of motor symptoms the dose of levodopa/benserazide was increased up to 150/37.5 mg 3 times a day with positive effect. Relatives began to notice fluctuations in severity of cognitive impairment: at evening hours, the patient became clearer, more adequate.

For the next two years (2020-2021), the patient was at home due to COVID-19 restrictive measures. During this time, the cognitive status significantly worsened, visual hallucinations became more frequent, but there was no rapid increase in the symptoms of parkinsonism.

Neurological examination showed: hyposmia, hypomimia, oral automatism reflexes, muscle tone of extremities moderately increased by lead tube type, S>>D; strength in extremities sufficient, without any paresis; a resting tremor in left arm, right leg; moderate hypokinesia, D<S; pull-test is positive; shuffling, achyrokinesis on right side (she scored 37 on UPDRS scale, part 3).

She was unable to complete the clock drawing test, cube copying test; verbal activity decreased (phonetic - 1 word per min, semantic - 2 words per min). She scored 9/30 on the MoCA scale.

Discussion. In summary, the cognitive profile of both patients was nearly identical at the time of examination. The only thing that cardinally distinguished the patients was time of cognitive impairment onset. Patient E.'s dementia developed along with the long-lasting PD. It is highly likely that her visual hallucinations were associated with excessively high doses of levodopa medications (she was taking 1350 mg/day) and intake of dopamine receptor agonist pramipexole, a group of drugs which more frequently than levodopa cause development of hallucinations. We abstained from prescribing acetylcholinesterase inhibitors due to bradycardia. When the hallucinations steadily resolved, the atypical neuroleptic can be completely withdrawn. In contrast, in patient K., the onset of cognitive impairment was almost simultaneous with the development of parkinsonian symptoms. We also observe visual-spatial impairment and visual hallucinations. Additionally, fluctuations of the cognitive status were detected. All these factors combined made the diagnosis of DLB possible.

Conclusion. PD and DLB are clinically and pathomorphologically similar diseases; both relate to disorders with accumulation of Lewy bodies. The difference is in the more widespread neurodegenerative process in DLB and, consequently, in the early development of cognitive impairment. Treatment of DLB and PD with dementia has similarities: to reduce the severity of parkinsonism there is a preference for levodopa drugs, regardless of the age of the patient; to treat cognitive impairment, acetylcholinesterase inhibitors and the NMDA-receptor antagonist memantine are prescribed; to relieve hallucinations, atypical neuroleptics are cautiously prescribed.

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