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

3(71) `2020

YAKUT MEDICAL JOURNAL

YAKUT MEDICAL JOURNAL

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

Quarterly

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

Registration number PI No.TU 14-00475

Subscription index: 78781
Free price

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

The journal is included in the international directory system under periodic and proceeding editions "Ulrich's International Periodicals Directory"

The founder
The Yakut Science Centre of Complex Medical Problems

Address:
Sergelyakhskoe Highway, 4, Yakutsk Republic Sakha (Yakutia) Russian Federation 677019
Fax: +7 (4112) 32-19-81;
Phone: +7 (4112) 39-55-52
yscredactor@mail.ru
e-mail: ymj-red@mail.ru
http: // www.ymj.mednauka.com

The articles are given in the authors' translation

CONTENTS

КОЛОНКА ГЛАВНОГО РЕДАКТОРА

Dear readers!

The editorial board of the “Yakut Medical Journal” is glad to welcome you on the pages of the 71st issue!

The "Yakut Medical Journal" is a professional, scientific and practical medical publication, presenting to the attention of readers the latest achievements of scientists and specialists in the field of medical science and practical health care from different regions of Russia.

One of the journal's priority scientific directions remains coverage of genetic research data and Arctic medicine issues.

In the current issue you will find all the main headings of the journal, and I want to believe that reading will be interesting and informative.

In particular, there is a review of the genetic mechanisms and clinical manifestations of various subtypes of hereditary spastic paraplegia, differential diagnostics and modern therapeutic possibilities (A.M. Tappakhov et al.), data from the research of markers of the degree of tension of functional state in adaptation to extreme conditions of the North-East of Russia (I.V. Averyanova).The diagnostic value of determining the concentrations of markers of bone remodeling in the blood serum of patients with rheumatoid arthritis by constructing ROC curves for predicting the development of osteoporosis has been studied (Yu.R. Akhverdyan et al.). The results of effective application of antioxidant therapy and hyperbaric oxygenation in complex treatment of patients with 2nd degree frostbite (O.S. Olifirova et al.) are presented. Data on the high prevalence of the R-allele of the Q192R polymorphism of the PON1 gene, which determines the increase in paraoxonase 1 activity, in representatives of indigenous peoples of North Asia: Buryat, Tofalar and Evenk (T.A. Bairova et al.) are presented. The data on high prevalence of R-allele of the Q192R polymorphism of the PON1 gene determining the increase of paraoxonase 1 activity in representatives of indigenous peoples of North Asia: Buryat, Tofalar and Evenk (T.A. Bairova et al.) are presented. In the article by A.A. Shvaikovskaya et al. the biological role and diagnostic significance in clinical practice of brain neurotrophic factor (BDNF), which is one of the key indicators of neurogenesis and neuroplasticity, are considered. The article by A.A. Shvaykovskaya et al. discusses the biological role and diagnostic significance in clinical practice of neurotrophic brain factor (BDNF), which is one of the key indicators of neurogenesis and neuroplasticity.

Dear readers and authors! May the coming autumn bring to your life optimism and harmony, creative inspiration, implementation of plans, success in scientific and practical activities! See you in the next issue of the journal.

Editor -in - chief **Anna Romanova**

ORIGINAL RESEARCH

A.A. Tappakhov, T.E. Popova

HEREDITARY SPASTIC PARAPLEGIA: CLASSIFICATION, CLINICAL AND GENETIC CHARACTERISTICS

DOI 10.25789/YMJ.2020.71.01

УДК 616-009.12

Hereditary spastic paraplegia (HSP) is clinically and genetically heterogeneous group of neurodegenerative diseases involving lesions corticospinal tract. Scientific discoveries have allowed to go beyond the classical phenotype of the disease, which includes spastic paraparesis, impaired vibration sensitivity and urination disorder. It is now known that the disease may be accompanied by the development of cerebellar ataxia, cognitive impairment, visual disturbances and other neurological and non-neurological symptoms. Such clinical manifestations form an extensive group of "complex" ("combined") HSP.

There are 82 genetic subtypes of the disease that can have autosomal dominant, autosomal recessive, and X-linked inheritance types.

The article reviews the genetic basis and clinical manifestations of various subtypes of HSP, discusses differential diagnosis and modern treatment options.

Keywords: hereditary spastic paraplegia, Strumpel disease, *SPG* gene, thin corpus callosum, motor neuron diseases, spasticity, ataxia.

Introduction. Hereditary spastic paraplegia (HSP) is a genetically and clinically heterogeneous group of neurodegenerative diseases,characterised by progressive spasticity of the lower limbs due the degeneration of the pyramidal tracts [5, 16].

The disease was first described by a German neurologist Adolf von Strümpel. In the 1880s, he observed a family in which a father and two brothers suffered spasticity and weakness in the lower extremities [7]. To date, 82 genetic forms of HSP have been registered in the OMIM catalog. They can have an autosomal dominant, autosomal recessive, or X-linked inheritance type, designated as SPG (spastic paraplegia genes) and numbered in gene mapping order (1-82). In addition, 1-2% of patients with HSP may have a mitochondrial inheritance type [6].

This review is devoted to the general issues of the clinical picture, genetic basis, diagnostic methods and therapeutic possibilities of HSP.

Prevalence. In a large meta-analysis that included 22 studies and covered more than 14,000 patients from 16 countries, the average prevalence of all subtypes of HSP was 1.8 per 100,000 population. The most common autosomal dominant HSP subtypes were SPG4 and SPG3A, while SPG11 and SPG15 prevailed in frequency among autosomal recessive HSP subtypes [27]. It should be noted that along with widespread forms, there are intra-family cases, which often, in addition to classical symptoms, have additional plus signs [6]. Genetic diagnosis of the latter became possible mainly with the introduction of next generation sequencing.

Pathophysiology. A wide range of molecular genetic subtypes of HSP is due to the long length of the cortico-spinal tract. The leading importance in the development of HSP belongs to mutations in the genes responsible for mitochondrial metabolism, axonal transport, endoplasmic reticulum, lysosomal autophagy, etc. [5, 9]. Figure 1 presents the pathophysiological mechanisms of the development of this disease.

Clinical picture. The disease can debut at almost any age: from the first year to 80 years. Autosomal dominant forms begin earlier, unlike forms with an autosomal recessive type of inheritance [1].

A classic manifestation of most cases of HSP is a progressive gait disturbance. In the early stages of the disease, spasticity occurs in the absence of limb weakness and is only demonstrable on walking. As the disease progresses, spasticity is also observed at rest, paresis joins. In the upper limbs, hyperreflexion without spasticity is often determined [1, 23].

Urinary symptoms related to detrusor instability or detrusor sphincter dyssynergia are frequent, usually occurring later in the disease course [12].

In patients with HSP, impairment of vibration sensitivity, which is explained by degeneration of the dorsal columns of the spinal cord is common [22].

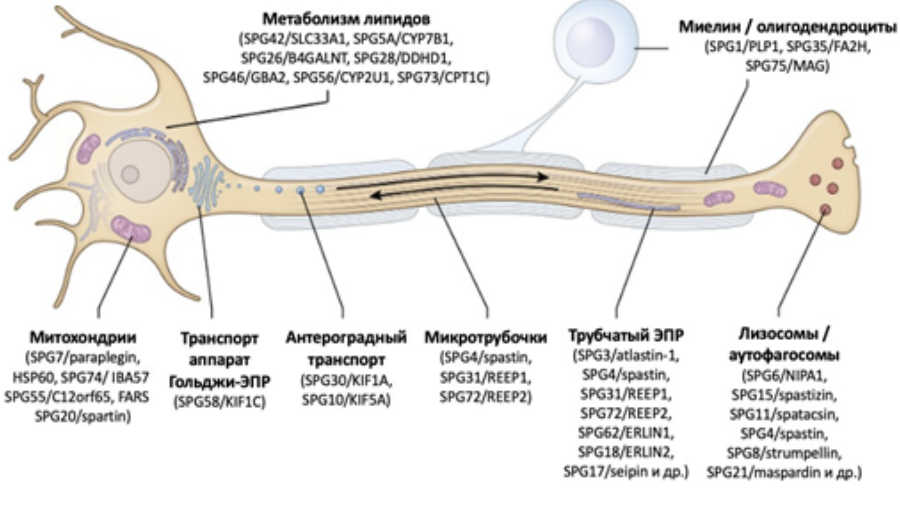


Fig 1. Mechanisms for the development of hereditary spastic paraplegia (according to Blackstone C., 2019)

TAPPAKHOV Alexey A. – Candidate of Sciences in Medicine, Associate Professor at the Department of Neurology and Psychiatry of the Medical Institute (North-Eastern Federal University), Head Researcher of the Laboratory of Neuropsychophysiological Researches (Medical Clinic of NEFU); Senior Researcher, Center for Neurodegenerative Diseases, Scientific Center for Complex. Medical Problems (Yakutsk). Add: 36, Kulavsky str., Yakutsk, 677000, Republic of Sakha (Yakutia). 8(4112)49-67-00, e-mail: dralex89@ mail.ru, **POPOVA Tatyana E.** – Grand PhD in Medical Sciences, deputy Director for Science of Scientific Center for Complex Medical Problems (Yakutsk). Professor at the Department of Neurology and Psychiatry of the Medical Institute (North-Eastern Federal University), Add: 4, Sergellahskoe shosse, str, Yakutsk, 677019, Republic of Sakha (Yakutia). 8(4112)321981; e-mail: tata2504@yandex.ru.

According to the clinical phenotype, HSP are classified into "pure" ("simple", "uncomplicated") and "complex" ("complex", "complicated", SPG-plus syndromes) forms. "Pure" HSP are characterized by the presence of a pyramidal syndrome (para- or tetraparesis, spasticity, hyperreflexia, pathological extension signs), which can be combined with impaired sphincter function and loss of vibration sensitivity [17]. "Complex" HSP include, in addition to spastic paraplegia, other neurological and non-neurological signs (Table 1) [7, 17, 18].

Table 1

Clinical manifestation of "complex" HSP

Group of symptoms	Clinical picture
Neurological symptoms	
Cerebellar signs	Ataxia, tremor, nystagmus
Peripheral nerves lesion	Axonal or demyelinating neuropathy
Cognitive impairment	Dementia
Myopathy	External ophthalmoplegia, ptosis
Extrapyramidal signs	Parkinsonism, chorea, dystonia
Paroxysmal disorders	Seizures
Neuroimaging signs	Leukodystrophy, thin corpus callosum, spinal cord atrophy, iron accumulation, cerebellar atrophy
Non-neurological symptoms	
Visual impairment	Cataract, optic atrophy, retinitis, degeneration of the corpus luteum
Dysmorphia	Microcephalus and macrocephaly, facial dysmorphisms, short stature
Orthopedic anomalies	Scoliosis, hip dislocation, foot deformities

"Pure" HSP are more often associated with an autosomal dominant type of inheritance. "Combined" HSP occurs mainly in families with an autosomal recessive type of inheritance and is detected in countries with a high level of blood marriage, where the prevalence can reach 5.75 per 100,000 population [3, 13, 16].

Autosomal-dominant HSP. About 70–80% of autosomal-dominant patients have "pure" HSP; of these, over 40% have mutation in the SPAST gene (SPG4) [13]. In Russia, according to the results of a study of 90 families with HSP, the proportion of SPG4 was 56%, in the subgroup of dominant forms – 68% [4].

The *SPAST* gene is located on the 2nd chromosome (locus 2p22.3), consists of 90 kb, contains 17 exons and encodes the spastin. Spastin in a member of AAA protein family (ATPase associated with various cellular activities). Two isoforms of spastin were found: isoform M87, the expression of which was detected both in the spinal cord and in the brain; isoform M1, the expression of which is determined only in the spinal cord. The function of the enzyme is to divide longer microtubules into shorter ones. Short microtubules are able to quickly and consistently move inside the axon, ensuring its growth and the formation of new axonal branches [10, 24].

The mean age of onset of SPG4 is 31,7 years, and presents with isolated lower limb spasticity, with or without bladder or sensory dysfunction. Ataxia or peripheral motor involvement were seen in 5–10% of cases; cognitive and extrapyramidal involvements, dysarthria, or dysphagia were seen in fewer than 5% of cases [19, 22].

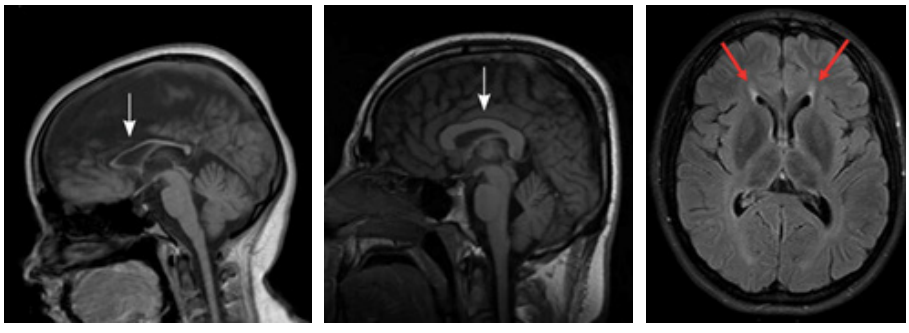
The SPG3A subtype is the second most common autosomal dominant HSP (5-10%) and associated with mutations in *ATL1* gene. The ATL1 gene is located on the 14th chromosome (locus 14q22.1) and encodes a protein atlastin-1, which is a member of membrane-bound, multimeric, ER-localized GTPases. Atlastin is involved in membrane transport, axon growth, morphogenesis of the endoplasmic reticulum (ER) and Golgi apparatus. SPG3A clinically characterized by "pure" spastic paraplegia, however, unlike SPG4, it has a significantly earlier age of onset of the disease (5.6 years versus 31.7 years) and a more frequent development of axonal motor neuropathy (in 25% of cases) [11, 19].

The SPG31 subtype is the third most common autosomal dominant HSP and is associated with mutations in *REEP1* gene. The REEP1 gene is located on the 2nd chromosome (locus 2p11.2), encodes protein 1, which increases the expression of the receptor. The function of this protein is not well understood but is probably involved in the functioning of microtubules and ER. SPG31 has two peak: in the first and fourth decades. It is also clinically manifested by "pure" spastic paraplegia, but the frequency of axonal neuropathy reaches 50% [19, 23].

Autosomal-recessive HSP. The main subtypes of autosomal recessive HSP are SPG11 and SPG15. SPG11 develops due to a mutation in the *KIAA1840* gene (SPG11), which is located on the 15th chromosome (locus 15q21.1) and encodes spatacsin, which is involved with neuronal axonal growth, function, and intracellular cargo trafficking. It presents between the ages of 4 years and 36 years. Most cases present with cognitive impairment or learning difficulties with lower limb spasticity emerging later, typically in the second decade. More than 50% of patients develop dysarthria, ataxia, axonal motor neuropathy, or prominent urinary symptom. Progression to upper limb involvement is common and levodopa-responsive parkinsonism, oromandibular dystonia, seizures, and visual failure have also been reported [7, 16, 23]. Neuroimaging studies disclose the typical ears-of-the-lynx sign, markedly thin corpus callosum (mainly in the rostral portion), mild ventricular dilatation, cortical atrophy, and mild symmetric periventricular white matter changes (Fig. 2) [20, 21, 27].

The SPG15 subtype is associated with mutations in ZFYVE26 gene, which located on the 14th chromosome (locus 14q24.1) and encoded spastizin. This protein is involved in the axonal growth of spinal neurons and lysosomal autophagy [11]. Clinically, SPG15 is similar to SPG11, although axonal neuropathy may be more pronounced, and parkinsonism is more common [23].

The SPG7 subtype is associated with mutations in *SPG7/PGN* gene (locus



a b c

Fig 1. Mechanisms for the development of hereditary spastic paraplegia (according to Blackstone C., 2019)

16q24.3). Paraplegin is mitochondrial protease involved in degradation of misfolded proteins and regulation of ribosomes association [9]. This subtype is characterized by a later onset (average age – 41.7 years), manifested by spasticity of the lower extremities in combination with cerebellar ataxia, which is observed in 57-90% of patients. In addition, there may be the presence of proximal myopathy and progressive external ophthalmoplegia. Although most cases are of an autosomal recessive type, families with an autosomal dominant inheritance type are also rare [23].

Diagnostics. To confirm the molecular diagnosis of HSP, a genetic analysis is necessary. At the same time, the key to the diagnosis is given by the data of the clinical picture, in particular the presence of spasticity, which develops with or without the absence of muscle weakness.

MRI in most patients with HSP reveals thinning of the spinal cord. Hyperintensity of the spinal cord in T2-weighted images should lead to the search for other acquired causes of spastic paraparesis. Cerebellar atrophy is more often detected with SPG7. Thin corpus callosum and periventricular white matter hyperintensity are more often observed with SPG11 and SPG15 [20, 23]. Table 2 presents the changes in MRI for various subtypes of HSP.

According to Table 2, a thin corpus callosum is one of the common MRI signs in autosomal recessive HSP and can aim for a correct diagnosis [1, 14]. We summarized in Table 3 the clinical manifestations of HSP characterized by a thin corpus callosum.

Electromyography identified distal axonal motor neuropathy in the most SPG7, SPG11, and SPG15 cases. Central motor conduction times were absent or delayed in the lower limbs [7, 23].

Table 4 presents the differential diagnosis of HSP with other diseases.

Prognosis. All subtypes of HSP have a progressive character, at the same time, there is wide variation in the rate of increase of the symptoms. A high degree of disability and a fast rate of progression are characteristic of SPG11. Patients with “pure” HSP rarely lose mobility. Gait is impaired due to spasticity with mild muscle weakness or in the absence of it, in these patients periods of stabilization are possible. A correlation was found between the age of the debut and the rate of progression of HSP: patients with early onset have a “mild” course, while manifestation at a late age is accompanied by rapid progression [1, 23].

Treatment. Currently, treatment of HSP remains symptomatic and the stiffness, cramps, spasms, and deformities can be addressed. Use of orthotics, such as anklefoot orthoses and heel raises, is also valuable for mobility. Botulin toxin with physical rehabilitation is recommended [1, 15, 23]. Oral antispasmodics (baclofen and tizanidine) are used to reduce spasticity, although most patients do not notice any effect. This is due to the fact that these drugs poorly penetrate the blood-brain barrier, therefore, in order to obtain a therapeutic effect, it is necessary to take large doses, which will inevitably lead to the development of side effects. At the same time, a good antispastic effect can be obtained with intrathecal baclofen therapy (ITB) [2, 23]. There is evidence of the successful use of functional electrical stimulation of the common peroneal nerves on both sides and chronic spinal cord stimulation to improve walking [8, 25, 26].

Gene therapy of the disease has not been developed, which is associated with numerous pathophysiological mechanisms, the rarity of certain genetic subtypes of HSP, as well as a slow course in most cases. However, the research to investigate specific treatments for genetic HSP subtypes has been increasing, including treatments attempting to address pathophysiological mechanisms and gene therapy [23].

Conclusion. HSP represent an extensive group of diseases with a wide clinical and genetic predisposition. The diagnosis of autosomal recessive subtypes, which are characterized by the development of plus symptoms, is still difficult. The introduction of diagnostic panels into clinical practice significantly helps, at the same time, the unavailability of research in regions remote from Central Russia and the relatively high cost create certain problems. The authors sincerely hope that the information presented in this article will increase the alertness of neuroscientists, pediatricians, geneticists, and general practitioners regarding HSP and help to conduct timely differential diagnosis to exclude other causes of spastic paraplegia and improve patient management, considering also the possibility of botulinum therapy and implantation of a baclofen pump.

Table 2

Neuroimaging changes in HSP (по F. Da Graca et al., 2019)

Sign	HSP subtypes
Thin corpus callosum	SPG4, SPG7, SPG11, SPG15, SPG18, SPG21, SPG35, SPG46, SPG47, SPG49, SPG50, SPG54
Spinal cord atrophy	SPG4, SPG6, SPG8
“Eye-of-lynx” sign	SPG11, SPG15
Enlarged ventricles/hydrocephalus	SPG1, SPG4 (rare)
White matter T2 hyperintensities	SPG2, SPG11, SPG5, SPG35
Bilateral T2 hyposignal of the globus pallidus	SPG28, SPG35, SPG43
Thoracic spinal cord hydromelia	SPG56

Table 3

Clinical polymorphism of autosomal recessive HSP with a thin corpus callosum

Subtypes (gene)	Locus / product	Age of onset	Plus-sign	Neuroimaging picture
SPG7	*16q24.3 / paraplegin*	Childhood, adult	CA, EO, SGP, scoliosis, ON atrophy	Diffuse atrophy of the cortex and cerebellum, CWMB
SPG11	*15q21.1 / spatacsin*	Childhood, adult	CA, CI, amyotrophy, dysphagia, neuropathy, MPG	"Ears-of-the-lynx", Enlarged ventricles, cortex atrophy
SPG15	*14q24.1 / spastizin*	Juvenile, adult	Distal amyotrophy of upper limbs, dysarthria, MPG, hearing loss, rare – neuropathy, psychosis, CA, parkinsonism, seizures, pes cavus	Cortex atrophy, mild CWMB
SPG21 (mast syndrome)	*15q22.31 / maspardin*	Juvenile, adult	CI, extrapyramidal signs, CI, bulbar syndrome	Atrophy of the frontotemporal regions
SPG44*	*1q42.13 / connexin*	Juvenile, adult	CA, CI, hearing loss, febrile seizures, scoliosis	Leukodystrophy
SPG46*	*9p13.3 / glucosyl-ceramidase*	Childhood	CI, kyphoscoliosis, pes cavus, oligophrenia, head tremor, dysarthria, CI, testicular underdevelopment, infertility, cataract	Variable atrophy of the cortex and cerebellum
SPG50*	*7q22.1 / AP4M1*	First years of life	Spastic tetraparesis, severe oligophrenia, pseudobulbar syndrome, impaired sphincter function, recurrent respiratory infections, dysmorphisms, central apnea, convulsions	Ventriculomegaly, cerebellar atrophy
SPG55*	*12q24.31/ C12ORF65*	Childhood, juvenile	Progressive vision loss, ON atrophy, strabismus, distal neuropathy, CI, facial dysmorphisms	Thin corpus callosum
SPG56*	*4q2 / CYP2U1*	Childhood	Axonal neuropathy, dystonia, CI	CWMB, calcification of the basal ganglia
SPG63*	*1p13.3 / AMPD2*	Childhood	Short stature	CWMB
SPG71*	*5p13.3 / ZFR*	Neonatal	Spastic paraparesis	Thin corpus callosum

Notes. CA – cerebellar ataxia, EO - external ophthalmoplegia, SGP – supranuclear gaze palsy, ON – optic nerve, CI – cognitive impairment, MPG – macular pigment degeneration, CWMB – changes in the white matter of the brain, * - described in single families.

Table 4

Differential diagnosis HSP with other diseases with spastic paraparesis

Disease	Clinical features
Adrenoleukodystrophy	X-linked recessive inheritance (exclusively male patients), an increase in the level of very long-chain fatty acids in the blood, signs of adrenal insufficiency, foci of demyelination in the brain and spinal cord (on MRI), detection of a mutation in the ABCD1 gene
Peliceus-Merzbacher disease	The adult form of the disease makes its debut in 20-30 years, characterized by spastic paresis, intellectual impairment and impaired coordination. On MRI of the head, diffuse lesion of the white matter of the cerebral hemispheres and cortico-spinal tracts is detected. Mutations in the PLP-1 gene is established
Amyotrophic lateral sclerosis	The prevalence of paresis over spasticity, the presence of signs of lesion the peripheral motor neuron, a progressive course, the absence of a family history, the absence of disturbances in vibration sensitivity, the development of bulbar and respiratory disorders, EMG data (anterior-horn lesion)
Cerebral palsy, spastic diplegia (Little's disease)	The presence of damaging factors in the perinatal period, non-progressive course, neurological status compensation is possible
Spinocerebellar ataxia	The prevalence of ataxia (SCA3 may begin with paresis), the prevalence of paresis over spasticity, the presence of extra-neural lesions (heart, pancreas, eyes and skin in Friedreich disease), molecular genetic analysis
Arteriovenous malformations of the spinal cord	Stepped or subacute onset; confirmed by MRI or CT with contrast
Paraneoplastic myelopathy	Subacute onset, combination with cerebellar ataxia, polyneuropathy, accelerated erythrocyte sedimentation, detection of antineuronal antibodies (most often GAD-65 antibodies)
B12-deficient myelopathy	Risk factors (atrophic gastritis, gastrectomy, resection of the stomach, etc.), macrocytic anemia, hyperintensive T2 signal from the posterior columns of the spinal cord on MRI, low levels of vitamin B12, reverse development with the appointment of vitamin B12
HIV myelopathy	HIV positive test
Syringomyelia	Dissociated sensitivity disorder, most often at the cervicothoracic level, combined with status disraphicus or Chiari malformation, syringomyelitis cyst on MRI
Multiple Sclerosis, Devic's Disease	The prevalence of paresis over spasticity, decreased abdominal reflexes, visual disturbances, remitting course, the presence of foci of demyelination in the brain and spinal cord on MRI

References

1. Белоусова Е.Д. Наследственные спастические параплегии. *Российский вестник перинатологии и педиатрии*. 2010;1:89-95. [Belousova ED. Hereditary spastic paraplegia. *Russian Bulletin of Perinatology and Pediatrics*. 2010; 1:89-95 (in Russ).]

2. Филатов Е.В., Биктимиров А.Р., Симатов А.И. [и др.]. Интратекальная баклофеновая терапия в России: результаты годового применения национального регистра. *Современные технологии в медицине*. 2020;12(1):79-83. [Filatov EV, Biktimirov AR, Simatov AI [et al.]. Intrathecal baclofen therapy in Russia: results of the annual application of the national register. *Modern technologies in medicine*. 2020; 12(1):79-83 (in Russ).] DOI:10.17691/stm2020.12.1.10

3. Майорова Н.Г., Павлов В.А. Случай редкого фенотипа наследственной спастической параплегии. *Вестник Смоленской государственной медицинской академии*. 2014;1(4):71-74. [Mayorova NG, Pavlov VA. The case of a rare phenotype of hereditary spastic paraplegia. *Bulletin of the Smolensk State Medical Academy*. 2014; 1(4):71-74 (in Russ).]

4. Руденская Г.Е., Кадникова В.А., Сидоров О.П. [и др.]. Наследственная спастическая параплегия 4-го типа у российских больных. *Журнал неврологии и психиатрии им. С.С. Корсакова*. 2019;119(11):11-20. [Rudenskaya GE, Kadnikova VA, Sidorov OP [et al.]. Hereditary spastic paraplegia type 4 in Russian patients. *S.S. Korsakov's Journal of Neurology and Psychiatry*. 2019; 119(11):11-20 (in Russ).] DOI:10.17116/jnevro201911911111

5. Иллариошкин С.Н., Руденская Г.Е., Иванова-Смоленская И.А. [и др.]. Наследственные атаксии и параплегии. М.: Медпресс-информ; 2006: 416. [Illarioshkin SN, Rudenskaya GE, Ivanova-Smolenskaya IA [et al.]. Hereditary ataxia and paraplegiaю Moscow: MEDPress-inform; 2006: 416 p (in Russ).]

6. Руденская Г.Е., Кадникова В.А., Коновалов Ф.А. [и др.]. Наследственные спастические параплегии и генокопии: современные возможности диагностики. Болезнь Паркинсона и расстройства движений руководство для врачей по материалам IV Национального конгресса. 2017:263-266. [Rudenskaya GE, Kadnikova VA, Konovalov FV [et al.]. Hereditary spastic paraplegia and genocopy: modern diagnostic capabilities. Parkinson's disease and movement disorders: a guide for physicians based on the materials of the IV National Congress. Moscow, 2017: 263-266 (in Russ).]

7. Москалева П.В., Вайман Е.Э., Шнайдер Н.А. [и др.]. Трудности диагностики дебюта спастической параплегии 11-го типа у ребенка. *Трудный пациент*. 2019;17(1-2):42-48. [Moskaleva PV, Wyman EE, Schnaider NA [et al.]. Difficulties in diagnosing the debut of spastic paraplegia type 11 in a child. *Difficult patient*. 2019; 17(1-2):42-48 (in Russ).] DOI:10.24411/2074-1995-2019-10007

8. Гуща О.А., Кащеев А.А., Карабанов А.В. [и др.]. Хроническая стимуляция спинного мозга при болезни Штрюмпеля: первое клиническое наблюдение. *Неврологический журнал*. 2015;20(6):22-27. [Gushcha OA, Kashcheev AA, Karabanov AV [et al.]. Chronic spinal cord stimulation in Strumpel disease: the first clinical observation. *Neurological journal*. 2015; 20(6):22-27 (in Russ).] DOI:10.18821/1560-9545-2015-20-6-22-27

9. Aleem AKA. Hereditary spastic paraplegias. Clinical spectrum and growing list of genes. 2017:32. URL: www.smgebooks.com

10. Baas PW, Vidya Nadar C, Myers KA. Axonal Transport of Microtubules: the Long and Short of It. Traffic. 2006;7(5):490-498. DOI:10.1111/j.1600-0854.2006.00392.x

11. Blackstone C. Hereditary Spastic Paraplegia. *Handbook of clinical neurology*. 2018: 148:633-652. DOI:10.1016/B978-0-444-64076-5.00041-7

12. Fourtassi M, Jacquin-Courtois S, Scheiber-Nogueira MC [et al.]. Bladder dysfunction in hereditary spastic paraplegia: a clinical and urodynamic evaluation. *Spinal Cord*. 2012;50(7):558-562. DOI:10.1038/sc.2011.193

13. Boukhris A, Stevanin G, Feki I [et al.]. Tunisian hereditary spastic paraplegias: clinical variability supported by genetic heterogeneity. *Clin Genet*. 2009;75(6):527-536. DOI:10.1111/j.1399-0004.2009.01176.x

14. Aleem AA, Abu-Shahba N, Swistun D [et al.]. Expanding the clinical spectrum of SPG11 gene mutations in recessive hereditary spastic paraplegia with thin corpus callosum. *Eur J Med Genet*. 2011;54(1):82-85. DOI:10.1016/j.ejmg.2010.10.006

15. Niet M, Bot S, Warrenburg B [et al.]. Functional effects of botulinum toxin type-A treatment and subsequent stretching of spastic calf muscles: A study in patients with hereditary spastic paraplegia. *J Rehabil Med*. 2015;47(2):147-153. DOI:10.2340/16501977-1909

16. Souza P, Rezende W, Rezende G [et al.]. Hereditary Spastic Paraplegia: Clinical and Genetic Hallmarks. *Cerebellum*. 2017;16(2):525-551. DOI:10.1007/s12311-016-0803-z

17. Giudice T, Lombardi F, Santorelli FM [et al.]. Hereditary spastic paraplegia: clinical-genetic characteristics and evolving molecular mechanisms. *Exp Neurol*. 2014;261:518-539. DOI:10.1016/j.expneurol.2014.06.011

18. Klebe S, Stevanin G, Depienne C. Clinical and genetic heterogeneity in hereditary spastic paraplegias: from SPG1 to SPG72 and still counting. *Rev Neurol (Paris)*. 2015;171(6-7):505-530. DOI:10.1016/j.neurol.2015.02.017

19. Loureiro JL, Brandão E, Ruano L [et al.]. Autosomal dominant spastic paraplegias: A review of 89 families resulting from a Portuguese survey. *JAMA Neurol*. 2013;70(4):481-487. DOI:10.1001/jamaneurol.2013.1956

20. Graça FF, Rezende T, Vasconcellos LFR [et al.]. Neuroimaging in hereditary spastic paraplegias: Current use and future perspectives. *Front Neurol*. 2019;10:1-10. DOI:10.3389/fneur.2018.01117

21. Rajakulendran S, Paisán-Ruiz C, Houlden H. Thinning of the corpus callosum and cerebellar atrophy is correlated with phenotypic severity in a family with spastic paraplegia type 11. *J Clin Neurol*. 2011;7(2):102-104. DOI:10.3988/jcn.2011.7.2.102

22. Schüle R, Wiethoff S, Martus P [et al.]. Hereditary spastic paraplegia: Clinicogenetic lessons from 608 patients. *Ann Neurol*. 2016;79(4):646-658. DOI:10.1002/ana.24611

23. Shribman S, Reid E, Crosby AH [et al.]. Hereditary spastic paraplegia: from diagnosis to emerging therapeutic approaches. *Lancet Neurol*. 2019;18(12):1136-1146. DOI:10.1016/S1474-4422(19)30235-2

24. Solowska JM, Baas PW. Hereditary spastic paraplegia SPG4: What is known and not known about the disease. *Brain*. 2015;138(9):2471-2484. DOI:10.1093/brain/awv178

25. Ardolino G, Bocci T, Nigro M [et al.]. Spinal direct current stimulation (tsDCS) in hereditary spastic paraplegias (HSP): A sham-controlled crossover study. *J Spinal Cord Med*. 2018. DOI:10.1080/10790268.2018.1543926

26. Marsden J, Stevenson V, McFadden C [et al.]. The Effects of Functional Electrical Stimulation on Walking in Hereditary and Spontaneous Spastic Paraparesis. *Neuromodulation Technol Neural Interface*. 2013;16(3):256-260. DOI:10.1111/j.1525-1403.2012.00494.x

27. Ruano L, Melo C, Silva MC [et al.]. The global epidemiology of hereditary ataxia and spastic paraplegia: A systematic review of prevalence studies. *Neuroepidemiology*. 2014; 42(3):174-183. DOI:10.1159/000358801

N.A. Solovieva, A.T. Dyakonova, N.I. Pavlova, Kh.A. Kurtanov

ASSOCIATION OF POLYMORPHISM OF DOPAMINTRANSPORT PROTEIN *SLC6A3/DAT1* WITH A RISK OF LOW ADAPTIVE POTENTIAL IN YAKUT POPULATION

DOI 10.25789/YMJ.2020.71.02

УДК 575.22

The article presents the results of the first molecular genetic study of the association of the polymorphic variant rs27072 of the dopamine transport protein gene *SLC6A3 / DAT1* with a risk of low adaptive potential in the Yakut population.

We analyzed 240 DNA samples from a population sample whose average age was 44.59 ± 17.99 years. All participants in the study were selected by the method of "random" sampling, lived in the Republic of Sakha (Yakutia), were yakuts and did not have any kindred ties between themselves. Ethnicity of the study participants was taken into account until the third generation. The study was conducted with the written consent of each participant.

It was found that most of the study participants needed additional sources of positive emotions to restore their psychoemotional state after stress, which suggests a lower level of adaptive reserves in the population.

Molecular genetic analysis of the frequency distribution of genotypes and alleles convincingly demonstrated that among individuals in need of additional correction of their psychoemotional state, carriers of the *GG* and *G* allele prevailed, while individuals who did not use additional measures to overcome stress were characterized by the predominance of the heterozygous *AG* genotype and allele *A*.

Thus, the search for the association of the rs27072 polymorphism of the *SLC6A3 / DAT1* gene with the risk of developing low adaptive potential revealed that the *G* allele can be used as a genetic factor that determines a lower level of adaptive potential in the yakut population, and in turn, *A* allele can be used as protective factor contributing to a higher adaptive potential.

Keywords: *SLC6A3 / DAT1* gene, polymorphism, dopamine transport, adaptive potential, stress, Yakuts.

The adaptation of a person to the surrounding conditions ensures his normal life. For the first time the phenomenon of adaptation was described in more detail in the works of physiologists G. Selye, I. Pavlov, I. Sechenov [5]. They considered adaptation as a physiological process of maintaining homeostasis and adapting the body to external changing conditions. Professor of psychophysiology F. Berezin understood adaptation to mean the process of preserving not only physiological but also mental homeostasis; optimal constant human interaction with the environment and the installation of the most effective correspondence between physiological and psychological factors, in the process of forming relatively stable psychophysiological relationships [1]. According to the theory of professor A. Maklakov, adaptation is not only a process, but also a property of a living organism [2].

Thus, adaptation is a broad concept that includes the adaptation of the body to changing or, in other words, "stressful" conditions at all levels of the body: physiological, psychophysiological and psychological, and is understood not only as a process, but also as a personality trait.

For the first time, the concept of stress in medicine was introduced by the W. Kenon. He drew attention to the fact that the body reacts to external influences in a special way and this affects the constancy of its internal environment.

Later, the physiologist, endocrinologist H. Selye used the concept of stress in the concept of the adaptation syndrome. According to his theory, stress is a non-specific reaction of adaptation (adaptation) of an organism to changed or extreme conditions. Nonspecificity lies in the fact that the body is indifferent to what stimulus acts on it: heat or cold, joy or grief - the response is the same. Selye assigned the leading role to the endocrine system. Under stress, the hypothalamic-pituitary-adrenal system is activated. Through a chain from the higher endocrine organs (hypothalamus, pituitary gland) located in the brain, the command goes to two small glands located above the kidneys - the adrenal glands. They produce and release stress hormones into the bloodstream: glucocorticoids (the main one being cortisol) and catecholamines (adrenaline and norepinephrine). Later it became clear that not only the endocrine, but also the nervous system is involved in the stress response [5].

Due to the individual (genetic) characteristics of the nervous system, each person has his own level of resistance to stress. Different people react to the same situation in different ways, it all depends on the inner state of the person. One of the reasons for low stress resistance, and, consequently, low adaptive potential, may be not only a primary decrease in the level of such a neurotransmitter as dopamine, but also its secondary deficiency, mediated by a small number of dopamine receptors. Dopamine is a neurotransmitter related to the brain's "reward system" that transmits signals from one neuron to another, capable of producing feelings of pleasure or satisfaction. It is known that people with such a deficiency are more prone to addictions, including "stress seizure"; it is more difficult for them to overcome stressful situations on their own, without resorting to additional stimulants. It is assumed that this is due to the fact that the deficiency of the neurotransmitter causes, firstly, the initial low emotional background, and secondly, such people need a stronger (pronounced) motivation to get positive emotions [6]. They are forced to make up for the lack of positive emotions in all ways available to them, only in this way they manage to adapt to changing conditions.

In addition, an important role in the development of pathological processes associated with a low adaptive potential belongs to the dopamine carrier protein,

SOLOVIEVA Natalya Alekseevna – Candidate of medical sciences senior researcher, laboratory of ecological and medical biochemistry, biotechnology and radiobiology, Institute for Biological Problems of Cryolithozone, Federal Research Center YSC SB RAS, E-mail: sonata608@yandex.ru; YSC CMP: **DYAKONOVA Aleksandra Timofeevna** – junior researcher of the laboratory of heritable pathology, Yakut Science Centre of complex medical problems, E-mail: dyakonovaa@bk.ru, **PAVLOVA Nadezhda Ivanovna** – Candidate of Biological Sciences, leading researcher, Head of hereditary pathology laboratory, Yakut Science Centre of complex medical problems. E-mail: solnishko_84@ inbox, **KURTANOV Khariton Alekseevich** – Candidate of medical sciences, chief researcher – Head of department of molecular genetics, Yakut Science Centre of complex medical problems E-mail: hariton_kurtanov@mail.ru.

which carries out the transmembrane transfer of dopamine from the synaptic cleft back to neurons for reuse. It is its activity that determines the amount and duration of dopamine stay in the synaptic cleft, which, in turn, makes this protein the most important regulator of dopamine signaling in the brain [7].

The work of the dopamine transporter is encoded by the gene for the dopamine transport protein *SLC6A3 / DAT1*. In humans, the dopamine transporter gene *SLC6A3 / DAT1* is located on chromosome 5 in the p15.3 region, contains 12 exons and has a length of 4.2 thousand np [3, 4]. The mechanism of dopamine transmission is shown in Fig. 1.

Taking into account that in the modern

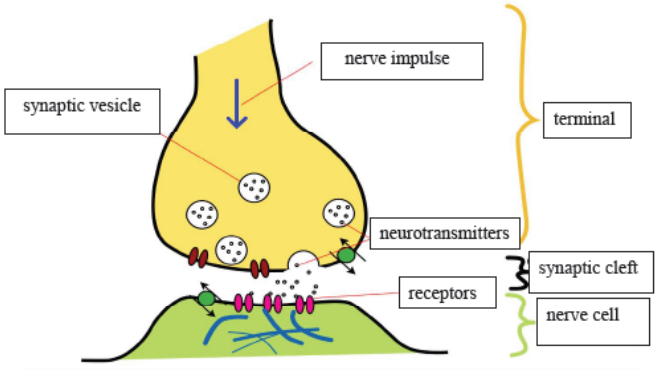


Fig. 1 Dopamine transmission mechanism

world, the problem of the appearance of stress becomes especially relevant, and the success of a person's adaptation is determined by the geno-phenotypic characteristics of his body, the **aim** of this study was to search for an association of the rs27072 polymorphic variant of the *SLC6A3 / DAT1* dopamine transport protein gene with the risk of developing a low adaptive potential in the Yakut population.

Materials and methods. In total, the study included 240 individuals, of which 177 (73.75%) female and 63 (26.25%) male. The average age of the study participants was 44.59 ± 17.99 years, the average age of males was 44.29 ± 17.92 (from 20 to 84 years), of females - 44.71 ± 18.04 years (from 20 to 81 years old). All study participants were selected by the method of “random” sampling, lived in the Republic of Sakha (Yakutia), were Yakut and had no family ties. Ethnicity was considered until the third generation. The study was conducted with the written informed consent of each participant.

The analysis of personal data concerning the psychophysiological characteristics of individuals was carried out in the laboratory of ecological and medical biochemistry, biotechnology and radiobiology of the Institute for Biological Problems of Cryolithozone of the Federal Research Center of YSC SB RAS The work on genotyping the rs27072 polymorphism of the *SLC6A3 / DAT1* gene was carried out in the laboratory of hereditary pathology of the department of molecular genetics of the Yakut Science Centre of Complex Medical Problems.

All study participants, depending on the need for additional sources to overcome stress and their type, were divided into 4 groups (group 1 - additional food intake, group 2 - smokers, group 3 - went in for sports, group 4 - individuals who did not feel the need for additional sources positive emotions).

Molecular genetic analysis was carried out by the method of polymerase chain reaction with subsequent analysis of restriction fragments, the reaction conditions are presented in Table 1. Analysis of the polymorphism of restriction fragment lengths was carried out using the endonuclease MspI (SibEnzyme, Russia) according to the instructions (Table 2).

The amplification results were fractionated in a 2% agarose gel with ethidium bromide, at a voltage of 120-300 V, for 30-45 minutes. Documentation and visualization of the PCR amplification was carried out by photographing in UV light using a VilberLourmat gel documenting device (Fig. 2). Bands were assessed using the Pus19 DNA marker (Thermo Fisher Scientific, USA).

Statistical data processing was performed using the Statistica 13 for Windows program. The distribution of genotypes was checked for compliance with the Hardy-Weinberg equilibrium using Fisher's exact test. To compare the frequency of alleles between different groups, the $\chi 2$ test with Yates' correction for continuity was used. Differences were accepted at a statistical significance of $p < 0.05$.

Results and discussion. The survey showed that about 80% of respondents experienced stressful situations more than 4 times within 7 days. Most often, according to the respondents, they were associated with increased psycho-emotional and physical stress at work, as well as conflict situations in the family (at work). When asked how the study participants recovered their psycho-emotional state, 63.75% of the respondents answered that they ate more meat and cakes; 22.92% smoked; 5% went in for sports; the remaining 8.33% did not feel the need for additional sources of positive emotions.

Analysis of age depending on gen-

Table 1

Condition for AS-PCR

Step	t, °C	Time	Cycle
Initial DNA denaturation	95°C	5 min	1
DNA denaturation	94°C	30 sec	35
Annealing	62°C	30 sec	
Elongation	72°C	30 sec	
Final elongation	72°C	7 min	1

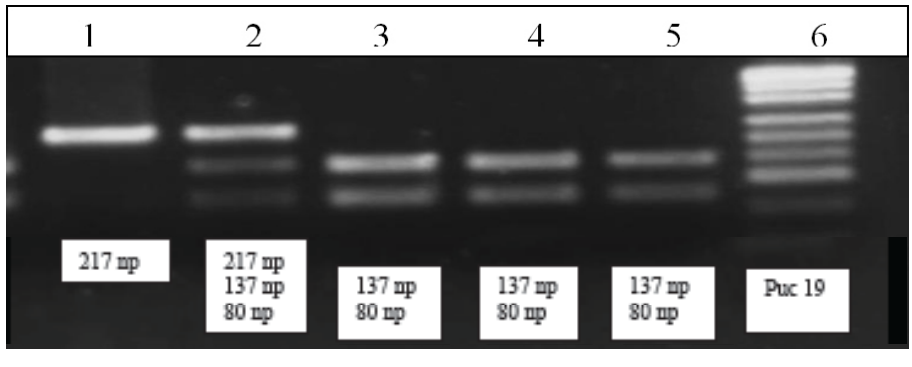


Fig. 2. Electrophoretogram of the rs27072 amplification product of the SLC6A3 / DAT1 gene in 2% agarose gel: np. - nucleotide pairs; 1 - AA genotype (217 np); 2 - AG genotype (217, 137, 80 np); 3, 4, 5 - GG genotype (137, 80 np). Internal control: 6 - DNA marker "Puc19".

Table 2

Length characterization of rs27072 restriction fragments of the *SLC6A3/DAT1* gene

Allele	Fragment length, n.p.
*DAT1*A*	217
*DAT1*G*	80 и 137
AA	217
AG	217, 137, 80
GG	137, 80

der and method of recovery of the psycho-emotional state did not reveal significant differences. The average age of individuals using food / smoking (groups 1 and 2) and playing sports (group 3) was 44.1 ± 17.15 and 43.77 ± 17.06 years, respectively, for individuals not using sources of additional emotions (group 4), - 44, 19 ± 16.44 years.

The results of a comparative analysis of the distribution of genotypes and alleles of the studied polymorphism in groups depending on the method of restoring the psychoemotional state are presented in Table. 3. The analysis showed that there were no significant differences in the distribution of the frequency of genotypes and alleles when comparing the indicators of groups 1, 2, and 3, most often they contained the homozygous genotype *GG* (from 71.1 to 75.0%). Individuals from group 4 were characterized by the predominance of carriers of the heterozygous *AG* genotype (45%). Significant differences in the distribution of the frequency of genotypes and alleles

Table 3

Distribution of genotypes and alleles of rs27072 polymorphism of the *SLC6A3/DAT1* gene depending on the method of restoring the psychoemotional state. absolute number (%)

Genotype	Joint groups 1 и 2 n=208	Group 3 n=12	Group 4 n=20
AA	35 (16.83)	1 (8.33)	6 (30)
AG	25 (12.02)	2 (16.67)	9 (45)
GG	148 (71.15)	9 (75)	5 (25)

were obtained when comparing the combined data of groups 1, 2, 3 with the data of group 4 ($p \leq 0.001$). Thus, individuals from the first 3 groups were characterized by a predominant carriage of the *G* allele (0.775), and individuals from group 4 were more often carriers of the *A* allele (0.525). The high frequency of carriage of the *G* allele in groups of individuals who need additional sources of positive emotions to restore their psycho-emotional state may indicate its negative effect on the work of the dopamine transporter, while the presence of the *A* allele most likely performs a protective function in the work of this transporter.

Conclusion. As a result of the study of the association of the rs27072 polymorphism of the *SLC6A3 / DAT1* gene with the risk of low adaptive potential, it was found that the majority of the study participants used additional sources of positive emotions, which suggests a reduced level of adaptive reserves in the population. The methods of its correction also testify to the lowered level of adaptive potential: about 70% chose additional consumption of high-calorie food or smoking, and only 5% of the respondents chose sports loads. Molecular genetic analysis of the distribution of the frequency of genotypes and alleles showed that among individuals requiring additional correction of their psychoemotional state, carriers of the *GG* genotype and the *G* allele prevailed. Individuals who did not use additional measures to overcome stress were characterized by the predominance of the heterozygous *AG* genotype and the *A* allele.

Thus, the search for an association of the rs27072 polymorphism of the *SLC6A3 / DAT1* gene with the risk of developing a low adaptive potential revealed that the G allele can serve as a genetic risk factor that determines a lower level of adaptive potential in individuals of the yakut population, and can be used as a marker for justified personalized correction of the adaptive potential by means of the use of biological products from plant and animal tissues in Yakutia. Allele *A* can be used as a marker characteristic of individuals with a higher adaptive potential.

The research was carried out within the framework of project VI.62.1.8. "Development of biological products from tissues of plants and animals of Yakutia on the basis of studying the characteristics of their biochemical composition and mechanisms of adaptation to the conditions of the North" (No. 0376-2019-0005 registration number AAAA-A17-117020110055-3) and research of the Yakutsk Scientific Center for Complex Medical Problems "Study of the genetic structure and burden of hereditary pathology of populations of the Republic of Sakha (Yakutia)".

References

1. Березин Ф. Б. Психологическая и психофизическая адаптация человека. Л.: Наука; 1988. [Berezin FB. Psychological and psychophysical adaptation of man. L.: Nauka: 1988].

2. Маклаков А.Г. Личностный адаптационный потенциал: его мобилизация и прогнозирование в экстремальных условиях. *Психологический журнал*. 2001; 22(1):16–24. [Maklakov AG. Personal adaptive potential: its mobilization and forecasting in extreme conditions. *Psikhologicheskiy zhurnal*. 2001; 22 (1): 16-24. (In Russ.).]

3. Марусин А.В. Ассоциация генов подверженности к алкоголизму, шизофрении и болезни Альцгеймера с психодиагностическими признаками в популяции русских. *Бюл. сибирской медицины*. 2016; 15 (5): 83–96. [Marusin AV. Association of genes for susceptibility to alcoholism, schizophrenia, and Alzheimer's disease with psychodiagnostic traits in the Russian population. *Byul. sibirskoy meditsiny.* 2016; 15 (5): 83–96. (In Russ.).] DOI: 10.20538/1682-0363-2016-5-83-96

4. Новикова Е.А. Связь тандемного повтора *48BP VNTR* гена *DRD4 с* избыточной массой тела. *ACTA BIOMEDICA SCIENTIFICA*. 2017; 2 (5): 63-68. [Novikova EA. The relationship of the tandem repeat 48BP VNTR of the DRD4 gene with overweight. *ACTA BIOMEDICA SCIENTIFICA*. 2017; 2 (5): 63-68. (In Russ.).] DOI: 10.12737article_59e85bc95a4e77.95444674

5. Сороко С. И. Индивидуальные стратегии адаптации человека в экстремальных условиях. *Физиология человека*. 2012; 38 (6): 78–86. [Soroko SI. Individual strategies of human adaptation in extreme conditions. *Fiziologiya cheloveka*. 2012; 38 (6): 78–86. (In Russ.).]

6. Dalley J. Nucleus Accumbens D2/3 Receptors Predict Trait Impulsivity and Cocaine Reinforcement. Science. 2007; 315: 1267-1270.

7. Ferreri L, Mas-Herrero E, Zatorre R. Dopamine modulates the reward experiences elicited by music. PNAS February 26, 2019; 9:3793-3798.

E.A. Alekseeva, L.N. Shantanova, S.M. Nikolaev,
I.Yu. Malyshev, I.E. Matkhanov

THE ROLE OF HSP70 AND NITROGEN OXIDE IN REALIZATION OF ADAPTOGENIC REACTIONS ON THE MODEL OF ACUTE EMOTIONAL STRESS

DOI 10.25789/YMJ.2020.71.03

УДК 615.27:616-009

The role of Hsp70 and nitrogen oxide in realization of adaptogenic reactions and adaptogenic effect of the extract from *Astragalus membranaceus* (Fischer) Bunge containing such active substances as isoflavones (28-49 mg%) and triterpenes (52-98 mg%) has been studied on the model of acute emotional stress. The acute emotional stress was simulated by water immersion method. In the control group, the acute emotional stress caused erosive injury of the stomach mucosa, hypertrophy of the adrenal glands and involution of thymus followed by 47% increase of nitrate/nitrite concentration in the blood plasma, 15% decrease of the basal level of Hsp70 in thymus *in vivo*, 4.5 times increase of the Hsp70 level in leucocytes induced by heat shock *in vitro* ($p \leq 0.05$). The course preventive administration of the extract from *Astragalus membranaceus* in experimental-therapeutic dose 50 mg/kg against the background of the acute emotional stress prevents the development of the "Selye's triad": involution of thymus (by 34%), erosive injury of the stomach mucosa (by 2.8 times) and hypertrophy of the adrenal glands (by 20%) ($p \leq 0.05$). The increased resistance to emotional stress against the background of the course administration of the extract from *Astragalus membranaceus* is followed by activation of Hsp70 expression in thymus by 2.6 times ($p \leq 0.05$) that is revealed *in vivo*. In leucocytes undergone the heat shock *in vitro*, the synthesis of Hsp70 is 4.5 times increased and against the background of the course administration of the *Astragalus membranaceus* extract, the induction is 66% decreased ($p \leq 0.05$). The concentration of NO metabolites in the blood plasma of the experimental group was 44% decreased against the background of the course administration of the *Astragalus membranaceus* extract. The administration of the *Astragalus membranaceus* extract was followed by activation of NO system (by 54%) ($p \leq 0.05$). We suppose that the activation of Hsp70 synthesis and prevention of stress-induced NO generation is the molecular-cell mechanism of adaptogenic effect of *Astragalus membranaceus* due to the presence of flavonoids in the extract: isoflavones – calycosin, calycosin-7-O-glycoside and formononetin capable to activate the expression of heat shock proteins, also they have the direct and indirect radical scavenging effect.

Keywords: acute emotional stress, Hsp70, NO, adaptogenes, flavonoids.

The activation of the Hsp70 synthesis plays an important role in adaptation to various disturbing factors being the substantial mechanism of anti-stress protection of cells. Stress-proteins (inducible, Hsp_i) synthesized in stress conditions are molecular chaperones capable to bind with hydrophobic sites of denaturated or mutant proteins injured by stress promoting disaggregation of abnormal protein molecules, utilization of injured proteins and preventing the apoptosis process [1]. During the adaptation of the body to such factors as hypoxia, stress and physical exertion the synthesis of Hsp70 activates under the NO influence [4]. Recent studies have shown that NO being the universal regulator engaged in all physiological processes running both in the central and peripheral nervous system, is involved in the formation of urgent and long-term adaptation and has the marked protective properties in stress impacts. In addition, there is information that stress-induced generation of NO radical suppresses mitochondrial respiration inhibiting cytochrome P-450 and glycolysis resulting in the drain on energy resources, disturbance of protein functions, development of fatigue and injuries in cell structures [2]. The dry extract derived from *Astragalus membranaceus* (Fischer) Bunge has a marked stress-protective activity increasing non-specific resistance of the body to various impacts [5]. In this connection, the role of heat shock proteins and NO in realization of adaptive reactions and adaptogenic effect of the phytoextract is of great interest.

The aim of the work was to determine the role of Hsp70 and NO in realization of adaptive reactions and adaptogenic effect of the phytoextract in the conditions of the acute emotional stress.

Materials and methods. The experiments were carried out on *Wistar* male and female rats weighing 160-180 g. The arrangements for research and animal care in the conditions of the certified vivarium at the Institute of General and Experimental Biology SB RAS (IGEB SB RAS) were compliant with the "Rules of Laboratory Practice" (GLP) and the Order of the Russian Health Ministry "On approval of Rules for Laboratory Practice" (No. 199N, 01.04.2016). The randomization of animals was carried out with due account of sex, age and mass. The experimental work followed the "European Convention for the protection of vertebrate animals used for experimental and other scientific purposes", ETS N 123, 18.03.1986 (Strasburg, 1986). The design of the study and research report was approved by the Ethics Committee at IGEB SB RAS (Protocol N 1 dated 15.01.2016). The acute emotional stress was simulated by the method of water immersion. The animals were put on one occasion into the cage with water (22°C) for 30 minutes. Two hours after stress impact the animals were decapitated under light ether narcosis and the level of hypertrophy of renal glands and number of ulcers in stomach mucosa were determined, also, the thymus mass was determined and then it was refrigerated

ALEKSEEVA Elvira Alekseevna – candidate of Medical Sciences, docent, head of the Department of Anatomy and Physiology, Medical Institute, Buryat State University, 670000 Ulan-Ude, ul. Smolin, 24a, tel. 89833379404, E-mail: alecseevaelvira@mail.ru https://orcid.org/0000-0001-8709-6524, **SHANTANOVA L.N.** – Doctor of biological sciences, professor, head of Laboratory of Safety of Biologically Active Substances, Institute of General and Experimental Biology of SB RAS, 670047 Ulan-Ude, ul. Sakhyanova, 6. Phone 3012-433713, E-mail: shantanova@mail.ru https://orcid.org/0000-0002-4199-1635, **NIKOLAEV S.M.** – Doctor of medical sciences, professor, Chief Researcher of Laboratory of Experimental Pharmakology, Institute of General and Experimental Biology of SB RAS, 670047 Ulan-Ude, ul. Sakhyanova, 6. Phone 3012-433713 MALYSHEV I.Yu. Doctor of Medical Sciences, professor, head of Laboratory of Stress and Adaptation, Institute of General Pathology and Pathophysiology, 125315 Moscow, Baltiyskaya street, 8. Phone (495) 6012248, **MATKHANOV I.E.** – candidate of Medical Sciences, Laboratory of Safety of Biologically Active Substances, Institute of General and Experimental Biology of SB RAS, 670047 Ulan-Ude, ul. Sakhyanova, 6. Phone 3012-433713

and kept at –70^{0}C before electrophoresis. The synthesis of Hsp70 was estimated in the thymus and leucocytes (induced by the heat shock) by the method of Western blot test using antibodies to Hsp70, reagents and equipment from Bio-Rad firm (USA). The quantitative processing of immunoblots was performed with the use of Photoshop computer program. The activity of the NO system was estimated according to the concentration of NO metabolites nitrates/nitrites with the use of special reducer for reduction of nitrates to nitrites [3]. The concentration of nitrites was estimated with the use of the Griess reaction succeeded by spectrometric analysis. The animals of the 0 and 1st experimental groups received the water solution of the *Astragalus* extract *per os* in the dose of 50 mg/kg 1 hour before feeding for 7 days before experiment. The animals of the 2nd experimental group received the preparation of comparison – dealcoholized extract of *Eleuterococcus* in the dose of 5 ml/kg according to the same scheme. The control group received distilled water by analogous scheme. The last dose was administered 1 hour before stress simulation. The dry extract was derived from the roots of *Astragalus membranaceus* (Fischer) Bunge (DEAM) by double extraction with 60% ethanol and ultrasonic treatment succeeded by concentration and vacuum drying. The data of HPLC and HPTLC revealed the presence of triterpenes: astragalosides I, II, III, IV, isoflavones – calycosin, calycosin-7-O-glycoside and formononetin. Standardization of the extract was carried out according to the content of isoflavones (28-49 mg%) and triterpenes (52-98 mg%) [5]. The data obtained were statistically processed with the use of Student t-test. Differences were considered significant when p ≤ 0.05.

Results of the study. It was established that the acute emotional stress caused erosive injury of the stomach mucosa, hypertrophy of the adrenal glands and involution of thymus in the control animals followed by 47% increase of nitrate/nitrite concentration in the blood plasma and 15% decrease of the basal level of Hsp70 in thymus as compared to the same data in intact animals. Besides, the Hsp70 level increased by 4.5 times in leucocytes induced by heat shock *in vitro* (p≤0.05) in the control group. It has been found that the course preventive administration of the extract from *Astragalus membranaceus* increases the resistance to emotional stress as evidenced by 20%decrease of hypertrophy of the adrenal glands, 34% increase in thymus mass and 2.8 times decrease in the number of erosions in the stomach mucosa as compared to the same indices in the animals of the control group (Table 1).

It has been also established that the course administration of the *Astragalus membranaceus* extract inhibits the NO forming: concentration of NO metabolites in the blood plasma is 44% decreased as compared to the indices of the animals in the control group. Interestingly, the course administration of the *Astragalus membranaceus* extract to intact animals induces the NO synthesis thus increasing concentration of NO metabolites in the blood plasma by 54% (Table 2).

The Hsp70 level in thymus increased by 45% (p<0.05) against the background of the course administration of *Astragalus membranaceus* to intact animals, more significant increase of Hsp70 by 2.6 times was noted when the tested remedy was introduced to animals exposed to stress.

The Hsp70 level in leucocytes induced by heat shock in intact animals receiving *Astragalus* was increased by 33%; at the same time, the Hsp70 level was 66% decreased in leucocytes of animals exposed to emotional stress against the background of the course administration of the *Astragalus* as compared to the control.

Thus, the data obtained demonstrated that the acute emotional stress caused erosive injury of the stomach mucosa, hypertrophy of the adrenal glands and involution of thymus followed by the increase of nitrate/nitrite concentration in the blood plasma and decrease of the basal level of Hsp70 in rat thymus. The course preventive administration of the extract from *Astragalus membranaceus* prevents the development of the "stress triad" signs surpassing the effect of eleutherococcus extract. The increased resistance to emotional stress against the background of the course administration of the extract from *Astragalus membranaceus* is followed by activation of Hsp70 expression in thymus and leucocytes induced by heat shock in animals of experimental group. It is known that in the cell there is a complex system of proteostasis, the system of interrelated proteins-chaperones; one part of them binds misfolded or partially denaturated proteins and other part provides their ATP-dependent protein folding or controlled proteolysis. Under stress influence, the overload of proteostasis system by misfolded proteins takes place thus resulting in cell death and injury of organs [8]. We have revealed that the course administration of the *Astragalus membranaceus* extract to animals under stress reliably increases the Hsp70 level in thymus and prevents the development of injuries in inner organs. We suppose

Table 1

Influence of DEAM on the manifestation of "Selye's triad" in acute emotional stress (M±m)

Animal groups	n	Relative weight mg/100 g		Number of erosions in stomach mucosa per one animal
		adrenal glands	thymus	
Intact	6	17.3±0.83	242.6±9.18	0.0
Control (stress)	6	22.0±1.42*	173.4±19.53*	3.2±0.52
Experimental 1 (stress+astragalus)	6	17.5±0.11**	231.7±13.41**	1.1±0.43**
Experimental 2 (stress+eletheroccocus)	6	20.3±2.19	234.7±23.77	1.2±0.10**

Note: * - hereinafter differences were significant when p <0.05 as compared to the intact; ** - p <0.05 as compared to the control; n – number of animals.

Table 2

Influence of DEAM on the concentration of NO metabolites in the blood plasma in acute emotional stress (M±m)

Groups	Concentration of NO metabolites (nitrates and nitrites), μmol/l
Intact (n=6)	35.5 ± 3.43
Experimental 0 (astragalus) (n=6)	54.8 ± 1.76
Control (stress) (n=6)	52.1 ± 1.54*
Experimental 1 (stress+astragalus) (n=6)	29.3 ± 1.62**

that the activation of Hsp70 expression in cells is one of the key mechanisms of the stress-protective effect of *Astragalus membranaceus*. Adaptogenic remedy ADAPT-232 derived from *Rhodiola*, *Schisandra*, *Eleutherococcus* reportedly stimulates Hsp70 expression *in vivo* [10]. The course administration of *Schisandra chinensis* prevents apoptosis of hepatocytes induced by TNF-α introduction to D-galactosamine sensibilized mice that is followed by increase in Hsp70 expression in the liver. Shisandrin B isolated from *Schisandra chinensis* has neuro-protective and cardio-protective activity due to activation of Hsp70 synthesis in the models of ischemia-reperfusion of myocardium, intensive physical exertion and emotional stress [11].Though early NO flux due to activity of endothelial nitric oxide synthase is important for vasodilatation sustain, we have obtained data supporting the injuring role of high NO concentrations. The increase in its production seems to cause oxidative injury of cell membranes by peroxynitrite formed during interaction with free radicals in animals undergoing acute stress. One can assume that the given mechanism is due to the presence of flavonoids – calycosin, calycosin-7-O-glycoside, which have the marked antioxidant effect. Antioxidant activity of isoflavonoids involves prevention of the decrease in activity of antioxidant enzymes, chelating of iron and copper ions participating in the production of free radicals and AOK uptake. Besides, polysaccharides of *Astragalus membranaceus* render antioxidant activity both *in vitro* and *in vivo*: they increase the activity of superoxide dismutase, glutathione peroxidase and the level of reduced glutathione and inhibit MDA formation. Polysaccharides of *Astragalus membranaceus* are known to protect mitochondria from oxidative injury and increase the activity of antioxidases in mitochondria of the liver and brain of mice [9, 12]. Thus, the activation of Hsp70 expression and prevention of stress-induced NO generation in tissues is the combined molecular-cell mechanism of *Astragalus membranaceus* stress-protective effect realization due to the presence of flavonoids in its composition: isoflavones – calycosin, calycosin-7-O-glycoside.

Conclusions:

1. Acute emotional stress caused erosive injury of the stomach mucosa, hypertrophy of the adrenal glands and involution of thymus in the control animals followed by 47% increase of nitrate/nitrite concentration in the blood plasma and 15% decrease of the basal level of Hsp70 in thymus (p≤0.05).
2. The course preventive administration of the sum of isoflavones (28-49 mg%) and triterpenes (52-98 mg%) of *Astragalus membranaceus* in experimental-therapeutic dose 50 mg/kg against the background of acute emotional stress has the marked stress-protective effect preventing the development of “Selye’s triad”: involution of thymus (by 34%), erosive injury of the stomach mucosa (by 2.8 times) and hypertrophy of the adrenal glands (by 20%) (p≤0.05).
3. The increased resistance to emotional stress against the background of the course administration of the tested remedy is followed by activation of Hsp70 expression by 2.6 times (p≤0.05), revealed *in vivo* but not *in vitro*.
4. Concentration of NO metabolites in the blood plasma of the control animals against the background of isoflavone sum introduction (28-49 mg%) and triterpenes (52-98mg%) of *Astragalus membranaceus* decreased by 44%. The administration of the tested remedy to the intact animals was followed by the marked activation of NO system (by 54%) (p≤0.05).

Table 3

Influence of DEAM on the basal level of Hsp70 in thymus and synthesis of Hsp70 induced by heat shock in leucocytes in the model of acute emotional shock ODU, (M±SD)

Group	Content of Hsp70	
	in thymus	in leucocytes after heat shock
Intact	5.5±1.85	0.6±0.04
Experimental 0 (Astragalus)	8.1±1.26	0.8±0.11
Control (stress)	4.8±0.68	2.9±0.32*
Experimental 1 (stress+Astragalus)	13.0±2.04**	1.0±0.13**

References

1. Гужова И.В. Механизмы работы шаперона Hsp70 в нормальных клетках и при клеточной патологии: Автореф. дис. … докт. биол.- С.-Петербург.- 2004.- 45 с.[Guzhova I.V. Mechanisms of the Hsp70 chaperone function in normal cells and cell pathology. Author’s abstract of D. Sci. Dissertation. St Petersburg. 2004; 45. (In Russ.)]
2. Манухина Е.Б., Дауни Г.Ф., Маллет Р.Т. и др. Депо оксида азота (NO) и его адаптивная роль в сердечно-сосудистой системе. *Патогенез*.2012; 10 (2):19-27. [Manukhina E.B., Dauni G.F., Malett R.T. et al. NO formation and its adaptive role in the cardio-vascular system. *Patogenez*. 2012; 10 (2):19-27. (In Russ.)]
3. Малышев И.Ю., Манухина Е.Б. Стресс-лимитирующая система оксида азота. *Росс. физиолог. журн. им. И.М. Сеченова*. 2000;10:1283 – 1292. [Malyshev I.Yu., Manuchina E.B. Stress-limiting NO system. *Ross. fiziolog. zhurn. im. I.M. Sechenova*. 2000; 10: 1283-1292. (In Russ.)]
4. Редуктор нитратов [Текст]: пат. 2161303 Рос. Федерация МПК G01N31/22, G01N27/00, G01N21/00, B01F3/00 / заявитель и патентообладатель : Круглов С.В., Амелин В.Г., Ларионов Н.П.; подано 27.02.2000. – опубл. 27.12.2000. – Бюл. № 2000. – 36 с. [Reducer of nitrates [Text] Patent 2161303 Russian Federation G01N31/22, G01N27/00, G01N21/00, B01F3/00, applicant and patentee: Kruglov S.V., Amelin V.G., Larionov N.P., submitted 27.02.2000 – published 27.12.2000. *Bjulleten'* N. 2000:36. (In Russ.)]
5. Способ получения средства, обладающего адаптогенным действием [Текст] : пат. 2582952 Рос. Федерация // Д.Н. Оленников, Г.В. Чехирова, Л.М. Танхаева. И [и др.]; заявитель и патентообладатель ИО и ЭБ СО РАН. – опубл. 27.04.2016. - Бюл. № 12. [Method for obtaining the remedy with adaptogenic effect [Text]: Patent 2582952 Russian Federation// D.N. Olennikov, G.V. Chekhirova, L.M. Tankhaeva et al., applicant and patentee: IGEB SB RAS, published 27.04.2016. *Bjulleten'* N. 12. (In Russ.)]
6. Чичёва М.М. Белок теплового шока HSP70 модулирует нейродегенеративный процесс в трансгенной модели протеинопатии. *Russian Scientist*. 2018: 2(1): 3-12. [Chicheva M.M. Heat shock protein Hsp70 modulates neurodegenerative process in transgenic model of proteopathy. *Russian Scientis*.2018:2(1):3-12. . (In Russ.)]
7. Asea A., Kaur P., Panossian A., Wikman K.G. Evaluation of molecular chaperones Hsp 72 and neuropeptide Y as characteristic markers of adaptogenic activity of plant extracts. *Phytomed*. 2013; 20: 1323-1329.
8. Leak R.K. Heat shock proteins in neurodegenerative disorders and aging. *J. Cell.Commun. Signal*. 2014; 8(4): 293-310.
9. Liu P., Zhao H., Luo Y. Anti-Aging Implications of Astragalus Membranaceus (Huangqi): A Well-Known Chinese Tonic. *Aging Dis*. 2017; 8: 868–886. Doi: 10.14336/AD.2017.0816.
10. Panossian, A., Wikman G. Effects of adaptogens on the central nervous system and the molecular mechanisms associated with their stress-protective activity. *Pharmaceuticals*. 2010; (3): 188-224.
11. Panossian, A. Understanding adaptogenic activity: specificity of the pharmacological action of adaptogens and other phytochemicals. *Annals of the New York Academy of Sciences*. 2017; 1401 (1): 49-64.
12. Zhenzhen G., Yanmei L., Muyan K., Qing L., Zhongqiu L., Jinjun W.A Systematic Review of Phytochemistry, Pharmacology and Pharmacokinetics on Astragali Radix: Implications for Astragali Radix as a Personalized Medicine. *Int. J.Mol. Sci.* 2019; 20(6): 1463.Doi: 10.3390/ijms 20061463

A.V. Gerasimov, D.K. Garmaeva, S.A. Fedorova, V.P. Kostyuchenko, A.V. Potapov, E.Yu. Varacuta, A.A. Denisov, S.V. Logvinov

INFRADIAN RHYTHM OF SECRETORY ACTIVITY AND CALCIFICATION PINEAL GLAND IN EXPERIMENT

DOI 10.25789/YMJ.2020.71.04

УДК 611.814.53:612.4.09

In order to verify the assumption about the lunaphase nature of changes in pineal cells of melatonin-synthesizing mitochondria and pineal calcification in 150 Wistar rats, during the synodic month after decapitation under ether anesthesia, the pineal gland was taken, which was fixed in a mixture of para-formaldehyde and glutaraldehyde, post-fixed OsO4, dehydrated, and enclosed in a mixture of EPON–Araldite. To exclude differences related to overpopulation stress, age, gender, season of the year and time of day, males weighing 180-200 g were kept in cells for 29 days with a constant daily photophase and removed from the experiment daily at the same time of day. Ultra-thin sections of the pineal gland were contrasted with uranyl acetate and lead citrate, examined in a transmission microscope. The spe-cific volume of mitochondria in the cytoplasm of pinealocytes was calculated by applying a test system to electronic micrographs. The Mann-Whitney U–test was used to assess the significance of lunaphase differences at a significance level of $p<0.05$. Changes in the value of the morphometric index in all types of pinealocytes showed an infradian rhythm. In the main hormone-producing pinealocytes with numerous mitochondria, secretory granules and synaptic bands in the cytoplasm, the value of the studied morphometric index reached its maximum at the new moon. In light pinealocytes with calci-fied osmiophilic bodies, the specific volume of mitochondria decreased to a minimum in the first quar-ter of the month. In dark pinealocytes that do not contain secretory granules in the cytoplasm, the spe-cific volume of mitochondria was lowest during the full moon. Calcination of mitochondria in pinea-lo-cytes and the formation of osmophilic bodies with grains of brain sand were more frequent in the first quarter. Calcination of collagen fibers in the pineal gland stroma, degenerative changes in pineal cells, apoptosis and pinealocytophagia - during the full moon and the last quarter. The conclusion is made about mainly fine-dispersed deposition of calcifications in the pineal gland in rats, their rhythmic char-acter associated with lunaphase shifts in the functional state of the pineal gland.

Keywords: pineal gland, rats, ultrastructure, calcification, infradian rhythm.

GERASIMOV Alexander – professor of the department of histology, embryology and cytology of the Siberian state medical university of the Ministry of health of Russia (Tomsk), doctor of medical sci-ences, associate professor, telephone (3822) 901-101-1930, +7 (913) 822-68-26, e-mail: a_gerasimov@sibmail.com, **GARMAEVA Darima** – head of the department of normal and pathological anatomy, operative sur-gery with topographic anatomy and forensic medicine of the Medical institute of the North-Eastern federal university named after M. K. Ammosov, doctor of medical sciences, professor, telephone +7 (914) 234-96-80, e-mail: dari66@mail.ru, **FEDOROVA Sardana** – head of the research laboratory of molecular biology of the Institute of natural sciences, chief researcher of the North-Eastern federal university named after M. K. Ammosov, doctor of biology, telephone +7 (914) 239-31-75, e-mail: sa.fedorova@s-vfu.ru, **KOSTYUCHENKO Vitta** – senior teacher of the department of histology, embryology and cytology of the Siberian state medical university of the Ministry of health of Russia, candidate of medical sci-ences, telephone (3822) 901-101-1930, e-mail: vittacos@yandex.ru, **POTAPOV Alexey** – professor of the department of histology, embryology and cytology of the Siberi-an state medical university of the Ministry of health of Russia, telephone (3822) 901-101-1930, e-mail: potalex@mail.ru, **VARAKUTA Elena** – head of the department of human anatomy with the course of topographic anat-omy and operative surgery of the Siberian state medical university of the Ministry of health of Russia, doctor of medical sciences, telephone (3822) 901-101-1776, e-mail: varelen@rambler.ru, **DENISOV Andrey** – professor of the department of immunology and allergology of the Siberian state medical university of the Ministry of health of Russia (Tomsk), doctor of medical sciences, associate professor, telephone 8 (3822) 901-101-1930, e-mail: denanalex@mail.ru, **LOGVINOV Sergey** – head of of the department of histology, embryology and cytology of the Siberi-an state medical university of the Ministry of health of Russia, doctor of medical sciences, professor, telephone (3822) 901-101-1930, e-mail: s_logvinov@mail.ru.

Introduction. The pineal gland is large in animals in the harsh conditions of survival in the Far North [8]. Due to the secretion of the pineal hormone melatonin, they maintain the normal functioning of the daily clock genes in the pace-mecker neurons of the suprachiasmatic nuclei of the hypothalamus [9]. The hypothalamic-spinal-ganglion-pineal link melatonin "loop" shows activity. The body adapts to the long-term absence of retino-hypothalamic photostimulation, low temperature, and other adverse environmental factors. However, the secretion of melatonin contributes to the calcification of the pine-al gland, which complicates its functioning [7]. The absence of photostimulation during bilateral enu-cleation in the experiment on gerbil was accompanied not only by an increase in melatonin production, but also by a more pronounced calcification of the pineal gland, whereas after ganglioectomy of the upper cervical autonomous nodes, calcification did not appear at all [10]. Under prolonged illumination of rats (45, 90 days), morphological signs of pineal gland hypofunction were observed with a decrease in the specific volume of mitochondria in the cytoplasm of pinealocytes [4]. Mitochondria are the exclusive site of synthesis of the daily chronobiotic melatonin. In pineal cells, melatonin stimulates mitochondrial fusion and suppresses division (mitophagia) when the gland is activated [3]. In addition to daily rhythms in the pineal gland are manifested morphological characteristics lunabase activity [1, 5, 6]. Changes in mitochondria in pineal cells in various phases of the synodic month (29.5 days), and the infradiane rhythm of pineal calcification remain unknown.

The aim of the study was to perform unafanya changes of mitochondria in pinealocytes and the phenomenon of calcification of the pineal gland in rats.

Materials and methods of research. The work was performed on 150 Wistar rats in accord-ance with the rules of laboratory practice (order of the Ministry of health of Russia from 19.06.2003 No. 267). To exclude differences related to overpopulation stress, age, gender, season and time of day, males weighing 180-200 g were kept in cells for 29 days with a constant daily photophase and daily at 16 h, when the activity of the pineal gland reached a minimum, were withdrawn from the experiment [2]. The iron was taken after decapitation under ether anesthesia, fixed in a mixture of 4% paraformal-dehyde and 2.5% glutaraldehyde on a 0.1 M cacodilate buffer (pH 7.4), post–fixed in a 1% solution of osmium tetraoxide, dehydrated in ethanol and acetone, and enclosed in a mixture of EPON-Ar-

aldite resins. The sections were made on an ultratome " Leica EM UC7 "(Austria), contrasted with uranyl acetate and lead citrate, and examined in a transmission microscope" JEM-100 CX II " (JEOL, Japan). The specific volume of mitochondria in the cytoplasm of pinealocytes was calculated using a 368-point test system on 15×22.5 cm electronograms. The data was processed using the "Statistica for Windows" software package, version 7 (StatSoft Inc., USA).

Results and discussion. In rats, hormone-producing cells of the pineal gland can be divided in-to four types. Type IA – the light pinealocytes with a developed rough endoplasmic reticulum (RER), which contain in the cytoplasm not only mitochondria, Golgi complex, secretory granules, synaptic ribbons, polysomes, but also osmophilic bodies with grains of brain sand. Type IB – the largest and numerically predominant light pinealocytes rich in mitochondria, with a Golgi complex, polysomes, single RER cisterns, secretory granules and synaptic ribbons. Type II – the dark small pinealocytes with a more electron-dense cytoplasmic matrix than light cells, many polysomes, few mitochondria, without secretory granules and synaptic ribbons in the cytoplasm (signs of secretory activity, at rest). Type III – the degenerating pinealocytes.

The specific volume of mitochondria in the cytoplasm of IB cells is 1.9–3 times greater than IA cells, and 1.6-1.9 times greater than in type II cells, which makes it possible to consider IB cells as the main pinealocytes that produce melatonin [3]. In type IA cells, in the first quarter of the synodic month, the specific volume of mitochondria decreases by 1.2 times compared to the new moon; in the full moon, compared to the previous phase, it increases by 1.3 times and remains at a high level until the new moon. During the new moon, there are more osmophilic bodies in the body of IA cells than during the full moon, and less in the processes. Intracellular transport is an energy-intensive process, and mitochondria in IA cells are involved not only in the production of melatonin, but also provide en-ergy, apparently, for the transport of osmophilic bodies with grains of brain sand. At full moon, the specific volume of mitochondria in IB cells is 1.1 times less than at new moon, which corresponds to previously published data on lunaphase shifts in pineal gland functional activity in rats and mice [1, 5, 6]. The decrease in the specific volume of mitochondria in pinealocytes IB in the first quarter of the synodic month after the end of the active functioning phase may be associated with the destruction of some of the organelles involved in melatonin synthesis. During the full moon, not only the secretory activity of the pineal gland decreases, but also, obviously, the activity of intracellular processes in pin-eal cells that do not participate in the secretion of melatonin and the transport of osmophilic bodies, since it is during this phase that the specific volume of mitochondria in the cytoplasm of type II cells reaches a minimum (Table).

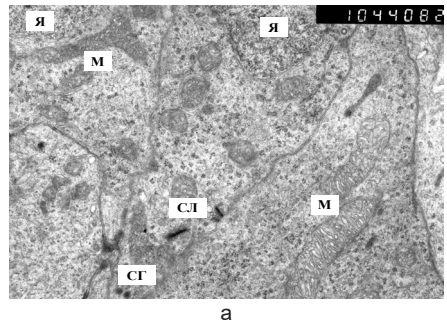
During the new moon in the main hormon-producing cells, mitochondria not only increase in size, but also acquire a spiral and branched T-shape (Fig. 1a, 1b). In the next phase of the synodic month, the mitochondria decrease in size, the mitochondrial matrix is compacted, individual organelles undergo calcification, undergo destructive changes and, with the participation of lysosomes, turn into bodies with an osmophilic matrix and fine inclusions of calcifications (brain sand), characteristic of IA cells (Fig. 1c). During the full moon in part IB cells with mitophagic phenomena, the core shell be-comes folded (Fig. 1d). Morphological signs of programmed pineal cell death appear (Fig. 1e). Apop-totic corpuscles are more often detected in the last quarter of the synodic month, and small grains of calcifications from osmiophilic bodies and cytoplasm fragments at the site of pinealocyte death enter the intercellular space and bind to the collagen fibers of the stroma (Fig. 1f, 1g). Utilization of dead pinealocytes (pinealocytophagy) is carried out, apparently, with the participation of astrocytes also mainly in the phase of the waning moon (Fig. 1i).

Conclusion. Thus, in young sexually mature rats, calcification of the pineal gland is manifested in the form of fine deposits of brain sand in the mitochondria and osmophilic bodies of functioning and degenerating pineal cells, apoptotic bodies at the site of their death, and collagen fibers of the stroma of the organ. Calcification is a consequence of moon-phase changes in mitochondria in pineal cells and secretory activity of the pineal gland. Intracellular calcification of pinealocytes becomes more pronounced in the first quarter of the synodic month after the phase of more active functioning at the new moon. In the suppression phase of secretory activity during the full moon and the last quarter of the lunar cycle, calcifications are released and deposited in the connective tissue against the background of programmed death of some pineal cells.

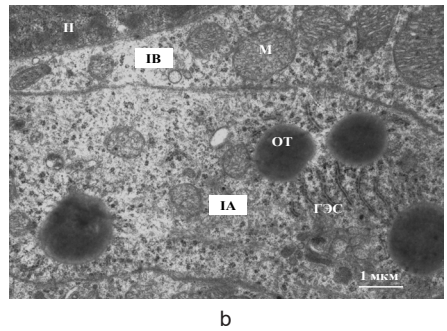
Lunaphase changes in the specific volume of mitochondria in the cytoplasm of various types pinealocytes in rats during the synodic month Me (Q1–Q3)

Phases (day)	Тип IA	Тип IB	Тип II
New moon (1–7)	6.15° (5.57–7.04)	14.41° (13.84–15.098)	8.81 (7.21–9.32)
First quarter (8–15)	5.25*° (4.31–6.08)	15.62° (14.58–16.78)	8.27 (7.12–8.96)
Full moon (16–22)	6.82° (6.11–7.24)	12.96*° (11.46–15.02)	7.53* (5.81–8.35)
Last quarter (23–29)	6.33° (5.56–6.72)	13.64° (12.20–14.56)	8.48 (7.25–9.86)

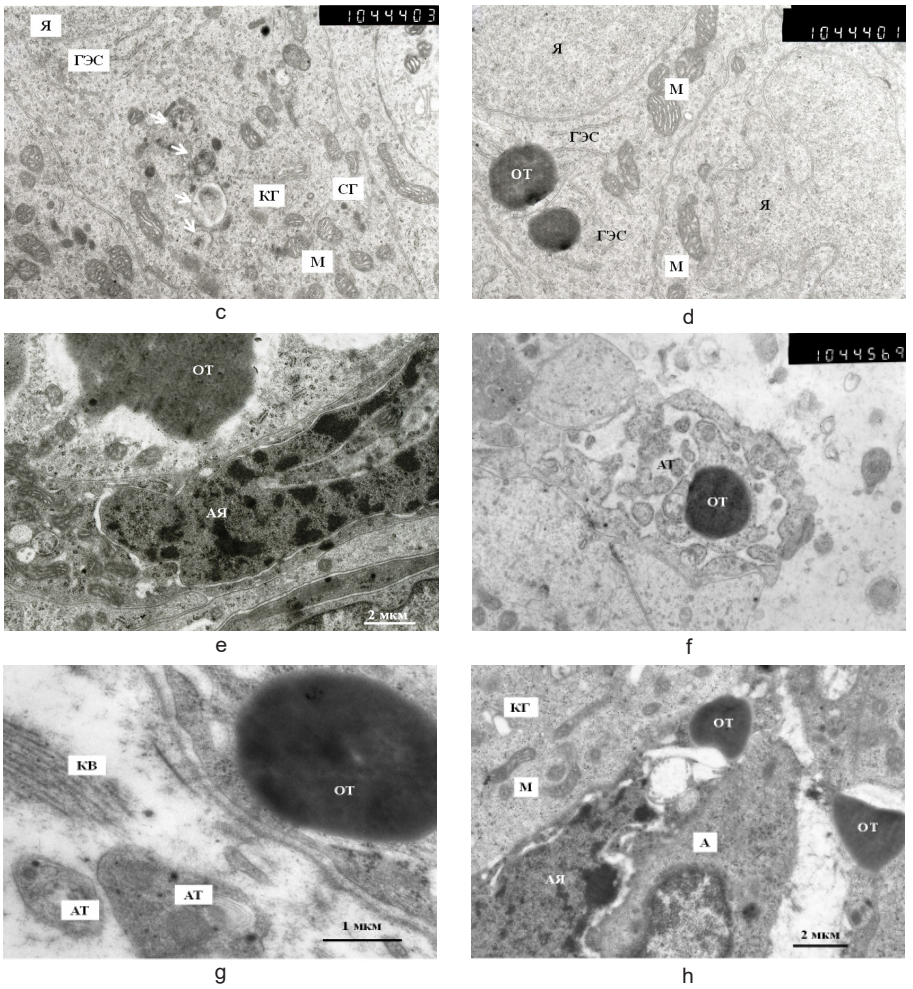
* – significant differences with the new moon, ° – with type II in each phase of the cycle.



a



b



c d e f g h

Fig. 1. Lunaphase changes in the pineal gland ultrastructure in rats: a) and b) – new moon, c) – phase of the growing moon (first quarter), d) and e) – full moon, f), g) and h) – phase of the waning moon (last quarter), A – astrocyte, AN – apoptotic nucleus, AB – apoptotic bodies, RER – rough endoplasmic reticulum, CF – collagen fibers, GA – Golgi apparatus, M – mitochondria, OB – osmiophilic bodies, SR – synaptic ribbons, SG – secretory granules, N – pinealocyte nuclei, IA, IB and II – types of pinealocytes, arrows – forming osmiophilic bodies. Magnification: a) – f), h) – 10000, g) – 30000.

References

1. Gerasimov AV, Kostyuchenko VP, Garmaeva DK, Fedorova SA. Morphological markers for lunaphase changes of organs in rats. *Jakutskij medicinskij zhurnal (Yakut medical journal)*. 2014; 1:13–16. (In Russ.).
2. Gerasimov AV, Kostyuchenko VP, Logvinov SV. Morphological changes in the pineal gland of rats under conditions of long-term exposure to bright light. *Bul. Exp. Biol. Med.* 2010; 150(7):86–88. DOI: org/10.1007/s10517-010-1076-z.
3. Gerasimov AV, Kostyuchenko VP, Potapov AV et al. Infradian rhythm of the content of secretory granules in pinealocyte cytoplasm in mice and rats. *Bul. Exp. Biol. Med.* 2018; 165(2):276–279. DOI: org/10.1007/s10517-018-4147-1.
4. Gerasimov AV, Kostyuchenko VP, Solovieva AS, Olovnikov AM. Pineal gland as an endocrine gravitation lunasensor: manifestation of moon-phase dependent morphological changes in mice. *Biochemistry* (Moscow). 2014;79(10):1064–1074. DOI: org/10.1134/S0006297914100083.
5. Lewczuk B, Nowicki M, Prusik M et al. Diurnal rhythms of pinealocyte ultrastructure, pineal serotonin content and plasma melatonin level in the domestic pig. *Follia Histochem. Cytobiol*. 2004;42(3):155–163.
6. Ralph C. The pineal gland and geographical distribution of animals. *Int. J. Biometeorol*. 1975; 19:289–303. DOI: 10.1007/BF01451040.
7. Suofu Y, Li W, Jean-Alphonse F et al. Dual role of mitochondria in producing melatonin and driving GPCR signaling to block cytochrome c release. *Proc. Natl. Acad. Sci. USA*. 2017; 114:7997–8006. DOI: 10.1073/pnas.1705768114.
8. Tan D, Xu B, Zhou Xi, Reiter R. Pineal calcification, melatonin production, aging, associated health consequences and rejuvenation of the pineal gland. *Molecules.* 2018; 23(2):301. DOI: org/10.3390.
9. Waly N, Hallworth R. Circadian pattern of melatonin MT1 and MT2 receptor localization in the rat suprachiasmatic nucleus. *J. Circadian Rhythms*. 2015; 13:1–7. DOI: 10.5334/jcr.ab.
10. Welsh M. Effects of superior cervical ganglionectomy, constant light and bliding on the gerbil pineal gland: an ultrastructural analysis. *(Abstract) Anat. Rec.* 1977; 187:746.

S.N. Balashova, A.V. Samodova, L.K. Dobrodeeva

THE DEPENDENCE OF THE HEMODYNAMIC ACTIVITY OF HUMAN RESPONSE TO SHORT-TERM GENERAL COOLING OF THE INITIAL CONTENT OF NEUTROPHILS IN THE PERIPHERAL VENOUS BLOOD

DOI 10.25789/YMJ.2020.71.05

УДК 612.13:613.166.9:616.155.34

Aim: to evaluate the hemodynamic activity of human response to general short-term cooling, depending on the initial content of neutrophils in the peripheral venous blood.

Materials and methods. The study of peripheral venous blood from 158 volunteers before and immediately after a short General cooling (5 minutes in cold chamber at -25°C). Plasma and serum was determined by the content of adrenaline and noradrenaline, endothelin-1 by enzyme immunoassay using an automated analyzer "Evolis" (Bio-RAD, France).

Results. Short-term general cooling for 5 minutes causes a decrease in the content of neutrophil granulocytes in venous blood by more than 1.5 times in 35.44% of the surveyed people. The results of the studies surveyed individuals with decrease in concentration of neutrophils were divided into 2 groups according to the level of the initial content of neutrophils (<2.0×109 cells/l and 2.0 to 5.5×109 cells/l). At normal initial level of neutrophils after cooling registers a more pronounced transition cells (neutrophils and lymphocytes) from circulating to marginal pool without the participation of endothelin-1, catecholamines. On the background of neutropenia hemodynamic response to a general cooling is weak. Background neutropenia reduces the activity of the migration of lymphocytes, mainly activated T cells (CD25+, HLA-DRII). Under the influence of the total cooling phagocytic activity of blood neutrophils is almost not reduced in both study groups.

Conclusion. Background neutropenia reduces the hemodynamic response to short-term general cooling, resulting in less pronounced redistribution of neutrophils and lymphocytes from the circulating to the marginal pool. The achievement required in these terms of redistribution requires a more significant vasoconstriction and high concentrations of endothelin-1. Thus, endothelin-1 is involved in the reaction of redistribution of the circulating and marginal pools of cells from peripheral venous blood.

Keywords: hemodynamic reaction, general short-term cooling, neutrophils, lymphocytes, endothelin-1.

BALASHOVA Svetlana Nikolaevna – candidate of Biological Sciences, senior researcher of the laboratory of regulatory mechanisms of immunity of the Institute of Physiology of Natural Adaptations of the N. Laverov Federal Center for Integrated Arctic Research of the Ural Branch of the Russian Academy of Sciences. Address: Russia, 163000, Arkhangelsk, naberezhnaya Severnoy Dviny, d. 23. E-mail: ifpa-svetlana@mail.ru. Office phone: (8182) 21-04-58. ORCID: 0000-0003-4828-6485, **SAMODOVA Anna Vasilyevna** – candidate of Biological Sciences, Head of the laboratory of regulatory mechanisms of immunity of the Institute of Physiology of Natural Adaptations of the N. Laverov Federal Center for Integrated Arctic Research of the Ural Branch of the Russian Academy of Sciences. Address: Russia, 163000, Arkhangelsk, naberezhnaya Severnoy Dviny, d. 23. E-mail: annapoletaeva2008@yandex.ru. Office phone: (8182) 21-04-58. ORCID: 0000-0001-9835-8083, **DOBRODEEVA Liliya Konstantinovna** – doctor of Medical Sciences, Professor, Honored Worker of Science, Chief Researcher of the laboratory of regulatory mechanisms of immunity, Director of the Institute of Physiology of Natural Adaptations of the N. Laverov Federal Center for Integrated Arctic Research of the Ural Branch of the Russian Academy of Sciences. Address: Russia, 163000, Arkhangelsk, naberezhnaya Severnoy Dviny, d. 23. E-mail: dobrodeevalk@mail.ru. Office phone: (8182)652992. ORCID: 0000-0001-5080-6502.

Introduction. The microvasculature is a system of transport blood flow, its functional state varies depending on the state of the tissues provided by the blood in this section of the blood flow [4, 8-10, 19, 22, 29]. Migration and perfusion of cells are provided by a significant slowdown in blood flow in the capillary network of the bloodstream. In this case, it becomes possible for the cell to adhere to the capillary wall with its subsequent exit beyond the vascular bed [17, 20, 32]. Vascular response to the impact of any potential pathogen usually is biphasic. First comes short of vasoconstriction and haemoconcentration in the venous network. The vasoconstrictor effect in the earliest vascular response to the damaging factor is set at the serotonin. Serotonin, depending on the dose and time of influence of damaging factors can cause a spasm, and vasodilation [12, 16, 36]. Ambiguous effects and properties of serotonin more include its ability to stimulate proliferation [14]. The effect of histamine is more clear. During the first 1-1.5 seconds comes almost complete inhibition of electrical activity of smooth muscle of venules and reduce blood vessel at 70-85%. Vasodilation ensures increased blood volume and accelerating its flow. In this phase the activity of aggregation of blood cells are severely reduced, and venous return increases sharply. Simultaneously with the dilation of blood vessels is the divergence of cells with formation of cracks by reducing mainly subplasmalemmal layer. Then, after 20 seconds is reduced and the surface membrane endotheliocyte [1]. Life of histamine-cracks or channels in the endothelium does not exceed 10 min. Restore tone and vascular permeability induced by serotonin, histamine, kinins or with other agents, provide catecholamines. W.G. Spector and D.A. Willoughby in 1968 for the first time had shown that inhibitors of methyltransferase enzymes, and dihydroxyphenylalanine-decarboxylase, dopamine-β-oxidase prevented the inflammation caused by serotonin, histamine, bradykinin and kallikrein [36]. Then in 1976, D.L Marciniak, J.J. Maciejko, and D.E. Dobbins found that noradrenaline prevented lymph flow response to histamine [30].

Neutrophil granulocytes migrate to the site of pathology in the early stages of the disease, however, as in any other pathological process, the neutrophils first appear in the middle of trouble [23, 25, 39]. In determining the direction of migration and to overcome barriers between blood and tissues is dominated by chemotactic signals and specific cell-cell interaction that can be disrupted un-

der the influence of cold. Cell response to cold is mediated by the presence of thermo-receptors on the membrane, and receptors for mediators of cold exposure (catecholamines, cytokines, vasomotor amines, glucocorticoids).

The **aim** of the study was to evaluate the hemodynamic activity of human response to general short-term cooling, depending on the initial content of neutrophils in the peripheral venous blood.

Materials and methods. The study of peripheral venous blood from 158 volunteers before and immediately after being in the cold chamber USHZ-25N (Russia) for 5 minutes at a temperature of -25°C. All the volunteers at the time of the survey had chronic and/or recurrent diseases. The survey was carried out with the written consent of the respondents in compliance with the basic norms of biomedical ethics in accordance with the document "Ethical principles for medical research involving human subjects" (WMA Declaration of Helsinki 1964, amended in 2013). For the study, peripheral venous blood was taken from the ulnar vein in the morning on an empty stomach before and after being in the cold chamber. The number of cells of the leucogram, monocytogram, lymphocytogram, neutrogram was counted in blood smears stained by the Romanovsky-Giemsa method; monocytoram was determined by O.N. Grigorova (1956), lymphocytogram – according to the method of I.A. Kassirsky (1970), neutrogram – according to the method of J. Todorov (1968). The phagocytic activity of neutrophils was studied using the test kit of the chemical company Reacomplex (Chita city). Isolation of mononuclear cells from peripheral blood was performed according to the method of A. Boymn (1976), phenotyping of lymphocytes in an indirect immunoperoxidase reaction using monoclonal antibodies (MedBioSpektr, Sorbent, Moscow) and flow cytometry using an Epics XL apparatus from Beckman Coulter (USA) with Immunotech a Beckman Coulter Company reagents (France). In blood serum by the method of solid-phase, ELISA on an automatic enzyme-linked immunosorbent analyzer "Evolis" company Bio-RAD (Germany) with the appropriate reagents was determined the content of adrenaline and noradrenaline (IBL, Germany), endothelin-1 («Biomedica», Czech). Statistical processing of the obtained data was carried out using the Statistica 10.0 software package (StatSoft, USA). The critical level of significance (p) in the work was taken equal to 0.05.

Results and discussion. Short-term general cooling for 5 minutes causes a decrease in the content of neutrophil granulocytes in venous blood by more than 1.5 times in 56 out of 158 of the surveyed people (35.44%). It is known that the level of background activity of the immune system is a factor contributing to the formation of adaptive reactions. In this regard, the results of the studies surveyed individuals with decrease in concentration of neutrophils were divided into 2 groups according to the level of the initial content of neutrophils The first group included patients with neutropenia, n=22 (the content of neutrophils <2.0×109 cells/l), the second with a normal amount of neutrophils, n=34 (2.0 to 5.5×109 cells/l). Hemodynamic reaction to a general cooling with the transition of neutrophils from the circulating to the marginal pool is more pronounced in the second group of patients. This also applies to the total content of neutrophils and cells in the structure of neutrogram (Table). No change in the level of stab neutrophils and neutrophils with 5 or more nuclear segments in the first group shows that in conditions of neutropenia, the most active and mature cells react to the cold factor. But before the effects of cooling the content of stab neutrophils and cells with 5 nuclear segments on a background of neutropenia was significantly lower (p<0.05-0,01).

Migration is the main responsibility for granulocytes as phagocytic, and secretory functions of them are implemented mainly in the tissues. Under the influence of the total cooling phagocytic activity of blood neutrophils is almost not reduced in both study groups

The composition of peripheral venous blood cells in in persons with background neutropenia and normal neutrophil content before and after short-term cooling (M±m)

Cells, ×109 cells/l	The initial content of neutrophils			
	<2.00×109 cells/l		>2.00×109 cells/l	
	before	after	before	after
Leukocytes	3.40±0.24	2.75±0.18*	4.88±0.34	3.39±0.37**
Neutrophils	1.70±0.11	1.19±0.08*	2.47±0.12	1.65±0.12**
Segmented neutrophils	1.60±0.10	1.11±0.08*	2.26±0.12	1.53±0.11**
Stab neutrophils	0.10±0.02	0.08±0.03	0.21±0.05	0.12±0.03*
Neutrophils, 2 nuclear segments	0.53±0.09	0.31±0.06*	0.76±0.07	0.52±0.05**
Neutrophils, 3 nuclear segments	0.70±0.04	0.51±0.04*	0.94±0.06	0.68±0.06**
Neutrophils, 4 nuclear segments	0.30±0.04	0.23±0.03*	0.45±0.07	0.27±0.03*
Neutrophils, 5 и > nuclear segments	0.06±0.01	0.05±0.01	0.09±0.01	0.05±0.01*
Monocytes (total content)	0.24±0.04	0.18±0.03	0.20±0.03	0.19±0.03
Promonocytes	0.13±0.02	0.13±0.02	0.10±0.02	0.11±0.02
Monocytes mature	0.11±0.01	0.07±0.01*	0.08±0.01	0.06±0.01
Polymorphonuclear monocytes	0.05±0.01	0.02±0.00*	0.04±0.01	0.02±0.01
Eosinophils	0.08±0.02	0.07±0.01	0.10±0.02	0.06±0.01
Natural killer cells CD16+	0.22±0.02	0.17±0.02	0.30±0.04	0.16±0.03*
Lymphocytes	1.38±0.19	1.30±0.13	2.10±0.14	1.49±0.18*
Lymphocytes small	0.71±0.14	0.80±0.14	1.04±0.12	0.56±0.09*
Lymphocytes medium	0.35±0.08	0.23±0.04*	0.34±0.06	0.17±0.03**
Lymphocytes large	0.17±0.04	0.11±0.02*	0.14±0.02	0.07±0.02*
CD3+	0.34±0.03	0.20±0.04*	0.34±0.06	0.13±0.03**
CD10+	0.27±0.04	0.19±0.02*	0.29±0.04	0.18±0.03*
CD71+	0.30±0.05	0.19±0.05*	0.36±0.06	0.18±0.03*
CD25+	0.22±0.03	0.19±0.03	0.28±0.05	0.17±0.02*
HLADR	0.25±0.03	0.22±0.04	0.33±0.06	0.16±0.02*
CD8+	0.31±0.03	0.22±0.03*	0.26±0.05	0.13±0.02*
CD4+	0.26±0.03	0.21±0.04	0.32±0.05	0.13±0.03*
CD95+	0.20±0.02	0.15±0.02*	0.25±0.03	0.15±0.02*

Note: * - p<0.05, ** - p<0.01.

(57.50±5.45 и 52.63±4.78%; 64.55±4.77 и 56.36±3.32%). Neutrophils perform 2 major functions – phagocytosis and secretion of biologically active substances; cells with increased phagocytic function less actively secrete biologically active substances [5, 6]. In this situation the manifestations of migration activity of neutrophils relate to cells, initiating the preventive approach of protection to ensure the regulatory response of the tissues through humoral mediators.

In this study, there were no changes in the total concentration of monocytes in both groups, but in neutropenia after short-term cooling, the structure of the monocytogram shows a decrease in the content of mature and polymorphonuclear monocytes. The tissue pool of monocytes is significantly 3.5 times higher than the content of tissue neutrophils [31], so the migration processes of monocytes are less intense and less pronounced.

In humans, which are characterized by higher background levels of neutrophils, after a short-term cooling is recorded a more significant decrease in the concentration of lymphocytes than patients with neutropenia. This pattern is identified and the structure of lymphocytogram, and the content of mature CD3+, activated CD25+, CD71+, HLADRII, CD10+, differentiated T-cells and natural killer cells CD16+, and lymphocytes labeled to apoptosis CD95+. The decrease in the content of lymphocytes in neutropenia is due to the medium and large lymphocytes, the content of small cells does not change. This fact is most interesting because the population of small lymphocytes has a high nuclear-cytoplasmic ratio, a high penetrating ability and the ability of recycling [27, 37]. Cells capable of recirculation through weak ties lymphocytes with stromal cells of lymphoid organs and relatively high degree of mobility. Lymphocytes (especially T cells) actively recycle back to lymphoid organs through the mechanism of "homing" based on the affinity of adhesion molecules on lymphocytes and endothelial cells of lymphoid organs. T cells recirculate more rapidly than B cells, for the implementation of cycle recirculation time consuming 4 times less, the most actively recycle T-helper cells [13, 15].

It is established that hemodynamic response to a general cooling of neutropenia and normal level of neutrophils is ensured without the participation of the catecholamines. On the background of neutropenia noradrenaline is 229.80(200.00-361.72) and 190.66(56.60-234.04) pg/ml, adrenaline – 28.95(20.76-43.84) and 30.00(23.08-34.64) pg/ml. In individuals with a normal amount of neutrophils in the blood, statistically significant changes in the concentration of noradrenaline and adrenaline also not installed (112.55(24.80-344.68) and 70.62(45.37-276.60) pg/ml, 39.01(16.16-66.08) and 34.17(25.40-68.62) pg/ml). In neutropenia, an increase in the concentration of endothelin-1 was found from 0.36(0.24-0.48) to 0.77(0.25-1.19) fmol/ml, p=0.028, frequency response of endothelin-1 on the background of neutropenia made 62.50±9.83%; under normal levels of neutrophils the concentration of endothelin-1 was recorded 20.00±4.45% the average values of the peptide 0.99(0.56-1.68) and 0.67(0.40-1.06) fmol/ml. Thus, when the background of neutropenia hemodynamic response to a general cooling is manifested by secretion of endothelin-1. Endothelins increase vascular permeability, activate neutrophils and mast cells, activate T-lymphocytes in the thymus, enhancing the immune response [7]. It should be noted the role of endothelin-1 as an inhibitor of apoptosis [35]. Endothelin-1 enhances the expression of adhesion molecules on endothelial cells of blood vessels and stimulates, thus, aggregation of neutrophils, which in turn may contribute to endothelial dysfunction [40].

Thus, the hemodynamic reaction to total short-term cooling, with the transition of neutrophils from the circulating to the marginal pool, revealed in 35,44% of the surveyed people. At normal initial level of neutrophils after cooling registers a more pronounced transition cells (neutrophils and lymphocytes) from circulating to marginal pool without the participation of endothelin-1, catecholamines. On the background of neutropenia hemodynamic response to a general cooling is weak: the contents of circulating neutrophils are reduced to 0.51±0.08×109 cells/l, while the normal background levels of neutrophils, this difference was 0.82±0.07×109 cells/l. this ratio, but more pronounced set and relative content of lymphocytes (respectively 0.08±0.02×109 cells/l and 0.61±0.09×109 cells/l). Background neutropenia reduces the activity of the migration of lymphocytes, mainly activated T cells (CD25+, HLA-DRII). Neutrophils have the ability to intraphagosomal and extracellular degranulation, so they can regulate the functional activity of many cells, including lymphocytes, at the autocrine and paracrine levels [5, 26, 33, 41]. In addition, the expression of CD40, CD80, CD86, and HLA-DR determines the ability of neutrophils to act as antigen-presenting cells for T-lymphocytes [24 34]. Neutrophil granulocytes are one of the main participants in the formation of the cytokine network, secreting almost all known regulatory, pro-inflammatory (IL-1a, IL-1β, CSF, IFNα, IFNγ) and anti-inflammatory (IL-4, IL-10, IL1Ra) cytokines [11, 21]. Neutropenia is accompanied by an increase in the blood content of IL-17F and IFN-γ [2, 3]. IL-17 stimulates neutrophil infiltration, primarily activating migration ability [38]. We consider the IL-17 reaction as a compensatory one that activates the population of these cells in case of their insufficiency. IFN-γ increases the effectiveness of antibody-dependent cytotoxicity of neutrophils and eosinophils through the expression of high-affinity genes FcγR (CD64) [28].

Conclusion. Background neutropenia reduces the hemodynamic response to short-term general cooling, resulting in less pronounced redistribution of neutrophils and lymphocytes from the circulating to the marginal pool. The achievement required in these terms of redistribution requires a more significant vasoconstriction and high concentrations of endothelin-1. Thus, endothelin-1 is involved in the reaction of redistribution of the circulating and marginal pools of cells from peripheral venous blood. The influence of a general cooling in a climatic chamber at -25°C for 5 minutes does not cause reactions of adrenaline and noradrenaline.

The study was performed within the framework of fundamental studies on the topic "Role of extracellular pool of adhesive molecules and short-chained peptides in the formation and outcome of adaptive reactions on the change of light pattern" (№ AAAA-A17-117033010123-0).

References

1. Алексеев О.В., Чернух А.М. Эндотелиальное сокращение: оперативная структурализация миофибриллярного аппарата при образовании «меченых сосудов» и ее триггерный механизм. В кн.: О проблемах микроциркуляции (функция и структура). Москва: Медицина; 1977; 132-4. [Alekseev O.V., Chernukh A.M. Endothelial contraction: operative structuralization of the myofibrillar apparatus during the formation of "labeled vessels" and its trigger mechanism. In: On the problems of microcirculation (function and structure). Moscow: Medicina; 1977; 132-4. (In Russ.).].

2. Балашова С.Н. Механизм формирования нейтропении у жителей Арктического региона. Арктические исследования: от экстенсивного освоения к комплексному развитию: материалы I международной молодежной научно-практической конференции. Архангельск: САФУ; 2018; 149-52. [Balashova S.N. The mechanism of neutropenia formation in the inhabitants of the Arctic region. In: Arctic research: from extensive exploration to integrated development: materials

of the I international youth scientific-practical conference. Arkhangelsk: NArFU; 2018; 149-52. (In Russ.).].

3. Балашова С.Н., Добродеева Л.К. Формирование нейтропении как следствие активации миграции нейтрофилов у практически здоровых людей, проживающих на Севере. *Экология человека.* 2018; 8:41-5. [Balashova S.N., Dobrodeeva L.K. Neutropenia's Formation as a Consequence of Neutrophil Migration Activation in Almost Healthy People in the North. *Ekologiya cheloveka [Human Ecology].* 2018; 8:41-5. (In Russ.).].

4. Бархатов И.В. Применение лазерной допплеровской флоуметрии для оценки нарушений системы микроциркуляции крови человека. *Казанский медицинский журнал.* 2014; 95(1):63-9. [Barkhatov I.V. The use of laser Doppler flowmetry to assess violations of the human blood microcirculation system. *Kazanskij medicinskij zhurnal.* 2014; 95(1):63-9. (In Russ.).].

5. Нестерова И.В., Ковалева С.В., Чудилова Г.А. и др. Двойственная роль нейтрофильных гранулоцитов в реализации противоопухолевой защиты. *Иммунология.* 2012; 5:281-7. [Nesterova I.V., Kovaleva S.V., Chudilova G.A. et al. The dual role of neutrophils in the antitumor protection. *Immunologiya.* 2012; 5:281-7. (In Russ.).].

6. Долгушин И.И., Бухарин О.В. Нейтрофилы и гомеостаз. Екатеринбург: УрО РАН; 2001. [Dolgushin I.I., Bukharin O.V. Neutrophils and homeostasis. Ekaterinburg: UrO RAN; 2001. (In Russ.).].

7. Дремина Н.Н., Шурыгин М.Г., Шурыгина И.А. Эндотелины в норме и патологии. *Международный журнал прикладных и фундаментальных исследований.* 2016; 10-2:210-4. [Dremina N.N., Shurygin M.G., Shurygina I.A. Endothelins in health and disease. *Mezhdunarodnyj zhurnal prikladnyh i fundamental'nyh issledovanij.* 2016; 10-2:210-4. (In Russ.).].

8. Козлов В.И., Соколов В.Г. Исследование колебаний кровотока в системе микроциркуляции. Материалы Второго Всероссийского симпозиума «Применение лазерной допплеровской флоуметрии в медицинской практике». Москва; 1998; 8-13. [Kozlov V.I., Sokolov V.G. Study of blood flow fluctuations in the microcirculation system. In: Materials of the Second All-Russian Symposium "The use of laser Doppler flowmetry in medical practice". Moscow; 1998; 8-13. (In Russ.).].

9. Кречина Е.К., Козлов В.И., Маслова В.В. Микроциркуляция в тканях десны пародонта. Москва: ГЭОТАР-Медиа; 2007. [Krechina E.K., Kozlov V.I., Maslova V.V. Microcirculation in periodontal gum tissues. Moscow: GEOTAR-Media; 2007. (In Russ.).].

10. Крупаткин А.И. Новые возможности оценки иннервации микрососудов кожи с помощью спектрального анализа колебаний микрогемодинамики. *Регионарное кровообращение и микроциркуляция*. 2004; 4:52-9. [Krupatkin A.I. New possibilities for assessing the innervation of microvasculature of the skin using spectral analysis of oscillations of microhemodynamics. *Regionarnoe krovoobrashchenie i mikrocirkulyaciya.* 2004; 4:52-9. (In Russ.).].

11. Нехаев С.Г., Григорьев Ю.И. Полиморфноядерные лейкоциты как система антиэндотоксикационной защиты организма. *Иммунология.* 2010; 31(3):116-8. [Nekhaev S.G., Grigor'ev YU.I. Polymorphonuclear leukocytes as a system of antiendotoxic defense of the body. *Immunologiya.* 2010; 31(3):116-8. (In Russ.).].

12. Сперанская Т.В., Александров П.И. Прижизненное изучение влияния антагонистов гистамина и серотонина на процесс воспаления. *Бюллетень экспериментальной биологии и медицины.* 1975; 79(3):26-9. [Speranskaya T.V., Aleksandrov P.I. Intravital study of the effects of histamine and serotonin antagonists on inflammation. *Byulleten' eksperimental'noj biologii i mediciny.* 1975; 79(3):26-9. (In Russ.).].

13. Супрун Е.Н. Рециркуляция лимфоцитов. *Аллергология и иммунология в педиатрии.* 2014; 1(36):32-6. [Suprun E.N. Lymphocyte recirculation. *Allergologiya i immunologiya v pediatrii.* 2014; 1(36):32-6. (In Russ.).].

14. Чернух А.М. Воспаление. Москва: Медицина; 1979. [Chernukh A.M. Inflammation. Moscow: Medicina; 1979. (In Russ.).].

15. Ярилин А.А. Основы иммунологии. Москва: Медицина; 1999. Yarilin A.A. [The basics of immunology. Moscow: Medicina; 1999. (In Russ.).].

16. Akers R.P., Zweifach B.W. Effect of vasoactive materials on the peripheral blood vessels in the hamster. *The American journal of physiology.* 1955; 183(3):529-34. DOI: 10.1152/ajplegacy.1955.183.3.529

17. Ambrus C.M., Ambrus J.M. Regulation of the leukocyte level. *Ann. N. Y. Acad. Sci.* 1959; 77:445-86. DOI: 10.1111/j.1749-6632.1959.tb36920.x

18. Bhatnagar S., Shinagawa K., Castellano F.J., Schorey J.S. Exosomes released from macrophages infected with intracellular pathogens stimulate a proinflammatory response in vitro and in vivo. *Blood.* 2007; 110(9):3234-44. DOI: 10.1182/blood-2007-03-079152

19. Bergstrand S., Lindberg L.G., Ek A-C. et al. Blood flow measurement at different depths using photoplethysmography and laser Doppler techniques. *Skin. Res. Technol.* 2009; 15:139-47. DOI: 10.1111/j.1600-0846.2008.00337.x

20. Carper H.A., Hoffman P.L. The intravascular survival of transfused canine Pelger-Huet neutrophils and eosinophils. *Blood.* 1966; 27:739-43. DOI: 10.1182/blood.V27.5.739.739

21. Cascao R., Rosario H.S., Fonseca J.E. Neutrophils: warriors and commanders in immune mediated inflammatory diseases. *Acta Reumatol.* Port. 2009; 34(2B):313-26.

22. Choi C.M., Bennett R.G. Laser Dopplers to determine cutaneous blood flow. *Dermatol. Surg.* 2003; 29:272-80.

23. Coussens L.M., Werb Z. Inflammatory cells and cancer: think different! *J. Exp. Med.* 2001; 193:23-6. DOI: 10.1084/jem.193.6.F23

24. Sandilands G.P., Ahmed Z., Perry N. et al. Cross-linking of neutrophil CD11b results in rapid cell surface expression of molecules required for antigen presentation and T-cell activation. *Immunology.* 2005; 114:354-68. DOI: 10.1111/j.1365-2567.2004.02114.x

25. Di Carlo E., Forni G., Musiani P. Neutrophils is the antitumoral immune response. *Chem. Immunol. Allergy*. 2001; 83:182-203. DOI: 10.1159/000071561

26. Hess C., Sadallah S., Hefti A. et al. Ectosomes released by human neutrophils are specialized functional units. *J. Immunol.* 1999; 163(8):4564-73.

27. Ford W.L., Gowans J.L. The traffic of lymphocytes. *Seminars in hematology.* 1969; 6:67-83.

28. Huizinga T.W., Van der Schoot C.E., Roos D., Weening R.S. Induction of neutrophil FC-gamma receptor I expression can be used as a marker for biologic activity of recombinant interferon-gamma in vivo. *Blood.* 1991; 77:2088-90.

29. Humeau A., Steenbergen W., Nilsson H., Stromberg T. Laser Doppler perfusion monitoring and imaging: novel approaches. *Med. Biol. Eng. Comput.* 2007; 45:421-35. DOI: 10.1007/s11517-007-0170-5

30. Marciniak D.L., Maciejko J.J., Dobbins D.E. The failure of locally administered histamine to increase lymph flow and protein efflux in the presence of norepinephrine in forelimbs of anesthetized dogs. *Fed. Proc.* 1976; 35:851-863.

31. Meuret G., Hoffman G. Monocyte kinetic studies in normal and disease states. *Brit. J. Haematol.* 1973; 24:275-85. DOI: 10.1111/j.1365-2141.1973.tb01652.x

32. Meuret G., Fliedner T.M. Neutrophil and monocyte kinetics in a case of cyclic neutropenia. *Blood.* 1974; 43:565-71. DOI: 10.1182/blood.V43.4.565.565

33. Nathan C. Neutrophils and immunity: challenges and opportunities. *Nature Reviews Immunology.* 2006; 6(3):173-82. DOI: 10.1038/nri1785

34. Megiovanni A.M., Sanchez F., Robledo-Sarmiento M. et al. Polymorphonuclear neutrophils deliver activation signals and antigenic molecules to dendritic cells: a new link between leukocytes upstream of T lymphocytes. *J. Leukoc. Biol.* 2006; 79:977-88. DOI: 10.1189/jlb.0905526

35. Shichiri M., Sedivy J.M., Marumo F., Hirata Y. Endothelin-1 is a potent survival factor for c-Myc-dependent apoptosis. *Mol Endocrinol.* 1998; 12:172-80. DOI: 10.1210/mend.12.2.0064

36. Spector W.G., Willoughby D.A. The pharmacology of inflammation. London: English universities Press Ltd.; 1968.

37. Springer T.A. Traffic signals for lymphocytes recirculation and leukocyte emigration: the multistep paradigm. *Cell.* 1994; 76(2):301-14. DOI: 10.1016/0092-8674(94)90337-9

38. Fogli L.K., Sundrud M.S., Goel S. et al. T cell-derived IL-17 mediates epithelial changes in the airway and drives pulmonary neutrophilia. *J. Immunol.* 2013; 191(6):3100-11. DOI: 10.4049/jimmunol.1301360

39. Prestwich R.J., Errington F., Hatfield P. et al. The immune system – is it relevant to cancer development, progression and treatment? *Clin. Oncol.* 2008; 20:101-12. DOI: 10.1016/j.clon.2007.10.011

40. Kowalczyk A., Kleniewska P., Kolodziejczyk M. et al. The role of endothelin-1 and endothelin receptor antagonists in inflammatory response and sepsis. *Arch. Immunol. Ther. Exp.* 2015; 63:41-52. DOI: 10.1007/s00005-014-0310-1

41. Yang D., Chen Q., Chertov O., Oppenheim J.J. Human neutrophil defensins selectively chemoattract naive T and immature dendritic cells. *J. Leukoc. Biol.* 2000; 68(1):9-14.

N.V. Zaitseva, T.S. Ulanova, O.V. Dolgikh, T.V. Nurislamova

ASSOCIATION ANALYSIS-BASED STUDY RESULTS IN EVALUATING THE AROMATIC HYDROCARBONS CONTAMINATION LEVEL OF BIO MEDIA AND IMMUNOTROPIC EFFECTS OF OIL AND GAS ENTERPRISES EMPLOYEES FROM VARIOUS WORK EXPERIENCE GROUPS

DOI 10.25789/YMJ.2020.71.06

УДК 54.06:575:576:616

A study of the working area air and the bio-media both of the workers, differing in the duration of contact with harmful chemical factors at oil and gas production facilities, and the employees of the administrative apparatus, have been conducted. The aim of this work was an association analysis of the study results of the biological media contamination level by aromatic hydrocarbons and immunotropic effects in workers of various employment groups working in oil and gas production enterprises.

Materials and methods. A study of the working area air and blood of working employees at oil and gas production facilities (observation group, n = 298) and employees of the administrative management apparatus (comparison group, n = 80), which differ in the duration of contact with harmful chemical factors has been performed. Studies of the biological media for the content of benzene and its homologues were executed by gas chromatographic method. Markers of cell differentiation, proteins that control apoptosis, expression of the tumor necrosis factor receptor were determined by flow cytometry. The content of immunoglobulin G (IgG) specific to benzene was determined by the allergosorbent method.

Results. Analysis of air samples from the working area showed the presence of benzene, its homologues in 100% of samples at workplaces. It was found that the level of contamination with aromatic hydrocarbons in the body increases depending on the length of service. A comparative assessment of the associative relationships of the immuno-endocrine status indicators of workers with length of service of more than 20 years corresponds to a high level of specific sensitivity to the control group according to the criterion of immunoglobulin G to benzene. This work experience period is characterized by inhibition of CD95 + expression, an imbalance of membrane and intracellular factors, with a simultaneous negative genetic background, which indicates a decrease in stress resistance processes, controlling cell death associated with polymorphic changes in candidate genes and the formation of negative effects from the immune, cardiovascular systems. Conclusion. An analysis of the research results allowed us to identify chains of stable associative relationships: the content of aromatic hydrocarbons in the working area air - the level of blood contamination of workers with benzene and its homologues - work experience - immunosuppression.

Keywords: biological media contamination with hydrocarbons, stage employees, CD markers, gene polymorphism.

ZAITSEVA Nina Vladimirovna – Doctor of Medical Sciences, Professor, Academician of the Russian Academy of Sciences, Scientific Director of the Federal Budgetary Scientific Institution “Federal Scientific Center for Medical and Preventive Health Risk Management Technologies”, 614045, Perm, Russian Federation, Monastyrskaya str., 82; e-mail: znv@fcrisk.ru; tel. +7(342) 237-25-34, ORCID: 0000-0003-2356-1145, **ULANOVA Tatyana Sergeevna** – Doctor of Medical Sciences, Head of the Department of Chemical and Analytical Research Methods of the Federal Budgetary Scientific Institution “Federal Scientific Center for Medical and Preventive Health Risk Management Technologies”, 614045 Perm, Russian Federation, Monastyrskaya str., 82; e-mail: ulanova@fcrisk.ru; tel. +7(342) 236-39-30, ORCID: 0000-0002-9238-5598, **DOLGIKH Oleg Vladimirovich** – Doctor of Medical Sciences, Head of the Department of Immunobiological Diagnostic Methods, Federal Budgetary Scientific Institution “Federal Scientific Center for Medical and Preventive Health Risk Management Technologies", 614045 Perm, Russian Federation, Monastyrskaya str., 82; e-mail: oleg@fcrisk.ru; tel. +7(342) 236-39-30, ORCID: 0000-0003-4860-3145, **NURISLAMOVA Tatyana Valentinovna** –Doctor of Medical Sciences, Head of the Laboratory of Gas Chromatography Methods, Federal Budgetary Scientific Institution “Federal Scientific Center for Medical and Preventive Health Risk Management Technologies”, 614045 Perm, Russian Federation, Monastyrskaya str., 82; e-mail: nurtat@fcrisk.ru; tel. +7(342) 236-39-30, ORCID: 0000-0002-2344-3037.

Introduction. Among the sectors of the economy that determine the level of scientific and technological progress of the country and its economic development, one of the leading places belongs to the oil and gas industry. This industry is the basic segment of Russian industry and is quite dangerous for the health of its workers [6]. Disorders of health associated with work at an oil and gas producing enterprise are caused by a set of adverse factors in the working environment [2, 4]. The main and constant chemical production factor is the sum of various hydrocarbons (benzene, its homologues) [5], the main route of entry into the body is inhalation. Under exposure to benzene vapours and its homologues through the respiratory organs as a result of diffusion through the alveolar capillaries, contaminants penetrate directly into the bloodstream [1], adversely affecting a wide range of functional-metabolic systems at the molecular, cellular and systemic levels [3]. Studies on the genotoxic effect of aromatic hydrocarbons show that the occupational contact with these products is a source of oxidative stress and DNA damage [2]. Preserving the occupational health, professional longevity with the help of modern technologies, reducing the level of morbidity and injuries is one of the main tasks of society, the most important function of the Government and the basis of its social policy.

The aim of this work became the associative analysis-based study results of the level of biological media contamination by aromatic hydrocarbons and immunotropic effects in workers of various work experience groups at oil and gas production facilities.

Materials and methods. The object of the study was the working area air, the biological environment (blood) of employees working at oil and gas production facilities (observation group, n = 298) and employees of the administrative control apparatus (comparison group, n = 80), including those with pathology of the cardiovascular system. Air sampling of the working zone for the content of aromatic hydrocarbons: benzene, toluene, ethylbenzene, o-, m-p-xylene was carried out

on sorption tubes, followed by thermal desorption and analysis on a Crystal 5000 gas chromatograph. Blood tests were performed by analysis of the equilibrium vapour phase on a gas chromatograph, on an HP-FFAP capillary column with a flame ionization detector. As criteria for assessing the content of aromatic hydrocarbons in the blood of people working at oil and gas production facilities, background regional levels were used.

Determination of lymphocyte differentiation markers ($CD95^+$, $CD127^-$), expression level to tumor necrosis factor-type 1 TNFR, bcl-2, bax proteins, intracellular p53 protein apoptosis marker was determined by cytofluorimetric method based on the interaction of the corresponding monoclonal membrane antibodies receptors. Data collection was performed on a FACSCalibur flow cytometer. The content of IgG specific for benzene was determined by the allergosorbent method.

The study of the occurrence of gene polymorphism was based on the real-time Polymerase Chain Reaction (PCR) method on a BioRAD CFX96 instrument. Genetic diagnostic study of candidate genotypes in the development of diseases of the cardiovascular system was performed: adducin gene ADD1 rs 4961 Gly460Trp, cytochrome CYP1A1 rs 1048943 C/T, dopamine receptor DRD2 rs 1800497 C / T, scurfin gene FOXP3 rs 37615 47 T(-3499) C, major histocompatibility complex HLA-DRA rs 3135388 C/T, integrin receptor gene ITGA2 rs 1126643 C807T, LDL oxidative receptor gene OLR1 rs 1050283 C1073T, TERT rs 10054203 C309G telomerase reverse transcriptase gene, tumor necrosis factor gene TNF rs 1800629 G4682A, tumor suppressor gene TP53 rs 1042522 Pro72Arg. Mathematical models describing the analyzed dependencies were evaluated by the Fisher criterion (F> 3.86). The analysis of immunological studies and estimation of model parameters were performed using the Statistica 10.0 application software package, and the Student and U-Mann-Whitney parametric t-tests. The differencies were considered significant at $p \leq 0.05$. The frequencies of gene polymorphisms for compliance were estimated according to Hardy-Weinberg equilibrium. The differences were considered significant with the level of $p<0.05$. For pairwise comparisons, the Bonferroni correction was also used, establishing a significance level of $p<0.008$.

Results and its discussion. During the observation period (April-September 2019), the concentrations of benzene and its homologues in the working zone air varied for benzene in the range from 0.0003 to 0.0008 mg / m^3, for toluene from 0.0003 to 0.0012 mg / m3, for xylenes from 0.0003 to 0.0025 mg / m^3. The simultaneous content in the working zone air of a combination of highly hazardous chemicals of benzene and its homologues of hazard class II with prolonged exposure has a combined effect and enhances the effect of each other. In the process of research it was found that the average concentrations of benzene, toluene, ethylbenzene and o-xylene in all the studied blood samples of the observation group workers were determined at concentrations higher than in the blood of comparison group workers at 1.5, 1.2, 1.2 and 1.6 times, respectively. The content of p, m-xylene in the blood of the observation and comparison groups has not been established (Table 1).

Workers who were in contact with harmful production factors of 1 year and more than 20 years were examined. Based on the results of a chemical blood test (Table 2), it was found that the average group content of benzene, toluene and o-xylene in the blood of workers significantly increased with duration of work experience: up to 10 years from 0.00041 mg / dm^3, 0.00043 mg / dm^3 and 0.00024 mg / dm^3, respectively, up to 0.00084 mg / dm^3, 0.00078 mg / dm^3 and 0.00064 mg / dm^3 with work experience exceeding 20 years. The content of benzene, toluene and o-xylene in the blood of employees of the administrative apparatus also increased depending on the experience: up to 10 years from 0.00029 mg / dm^3, 0.00036 mg / dm^3 and 0.00016 mg / dm^3 with an experience of more than 20 years 0.00054 mg / dm^3, 0.00051 and 0.00036 mg / dm^3. The average concentrations of benzene, toluene and o-xylene in the blood of the examined workers were significantly 1.8 times higher ($p<0.05$) than in the blood of the comparison group. A study of the content of benzene and its homologues in the blood of workers depending on the length of service in the concentration range for benzene 0.00041-0.00084 mg / dm^3

Table 1

Comparative assessment of the content of contaminants in the blood of observation groups working at oil and gas production facilities, comparison groups and background concentrations

Indicators	Background levels mg / ml	Comparison Group (M ± m) n = 80	Observation group	P_1	P_2
Benzene	0	(M±m) n=298	0.00057±0.00005	0.00	0.00
o-xylene	0	0,0003±0,00011	0.00037±0.00008	0.00	0.00
p, m-xylene	0	0±0	0±0	0.01	0.00
Toluene	0	0,00045±0,00007	0.00054±0.00004	0.00	0.00
Ethylbenzene	0	0,00006±0,00004	0.00009±0.00004	0.06	0.00

P1 - difference with background concentrations by average values;
P2 - intergroup difference in average values.
P1 - difference with background concentrations according to average values;
P2 - intergroup difference in average values.

Table 2

The biomonitoring results of workers at oil and gas production facilities from 1 year and more than 20 years (observation group) and the administrative apparatus group (comparison group) (concentration, mg / dm3)

Ingredient	Comparison group (M±m)	Observation group (M±m)	Intergroup difference in means (p)
Experience from 1 to 10 years (n = 185)			
Benzene	0.00029±0.00012	0.00041±0.00006	0.00
O-xylene	0.00016±0.00012	0.00024±0.00004	0.00
Toluene	0.00036±0.00009	0.00043±0.00006	0.00
Experience from 11 to 20 years (n = 100)			
Benzene	0.00039±0.00013	0.00055±0.00010	0.00
O-xylene	0.00032±0.00021	0.00041±0.00018	0.00
Toluene	0.00045±0.00013	0.00052±0.00008	0.00
Experience over 20 years (n = 93)			
Benzene	0.00054±0.00012	0.00084±0.00006	0.00
O-xylene	0.00036±0.00026	0.00064±0.00017	0.00
Toluene	0.00051±0.00015	0.00078±0.00004	0.00

and o-xylene 0.00024-0.00064 mg / dm^3 made it possible to establish a significant directly proportional dependence. In the process of modeling, statistically significant linear dependences of the concentration of benzene and o-xylene found in the blood of employees of the oil and gas industry, on the length of service, are obtained and described by the equation of the form: y=0.00037+0.00001 x and y=0.00013+0.00001 x. Based on the analysis of the obtained dependencies, it can be concluded that the rate of benzene and o-xylene contamination in the blood of employees of the observation group is proportional to the work experience and has a linear relationship. The established determination coefficient for benzene was R^2 = 0.68, for o-xylene, the determination coefficient tends to 1 (R^2 = 0.81), which characterizes the obtained models as statistically significant linear relationships.

Significant deviations of the CD immunogram values are observed in comparison with the reference level: the content of the transcription factor p53 is significantly increased in 100.0% of workers of primary professions, the level of histocompatibility receptor TNFR (75.0% of workers), bcl2 (68.8% of workers), hyperproduction of T-reg lymphocytes (56.2% of workers), proapoptotic factors are activated - Annexin V negative cells (56.2% of workers), Annexin V positive cells (87.5% of workers), bcl2 (68.8% of workers), and the cell death receptor CD95 + (87.5% of workers) controlling the aggression of the immune response is also inhibited (p <0.5). In the workers of the observation group the significant inhibition of the content of the activation cell marker CD95 + was revealed, which is responsible for the implementation of cell death, in relation to the control group by 1.4 times (in 87.5% of the workers of the main observation group, in 75.0% in the control). There is also a 1.2-fold increase with respect to the control of Annexin V level of negative cells, indicating a predominant cell death along the apoptosis pathway (no significant differences were found). The level of specific sensitization of IgG to benzene was reliably increased in 51.2% of workers in basic professions in relation to the norm. The content of specific IgG to benzene was increased (by 9.6%) in relation to the control group without significance of differences, p> 0.05. For workers in basic professions, the data obtained indicate excessive activation of the speed of the life cycle of immune cells and at the same time inhibition of the receptor activity of immunocompetent cells. A comparative assessment of the immune status indicators of workers with the length of service of more than 20 years, with workers with the length of service of less than 20 years, indicates a consistently high level of specific sensitivity with respect to the control group since the “length of service period” of 10 years, observed only in this period, reliable inhibition of the activation marker $CD95^+$ in relation to the control group, which in general may indicate a decrease in cell death control processes, which, along with existing polymorphic changes in candidate genes, can lead to the formation of negative effects on the part of the immune (autoimmune processes), nervous (astheno-neurotic syndrome, Alzheimer's syndrome), cardiovascular (hypertension, atherosclerosis) systems. The level of specific sensitivity to benzene according to the IgG criterion for benzene increases from 0.173 ± 0.02 for work experience of up to 10 years, reaching a maximum of 0.194 ± 0.033 for length of services of > 20 years. However, at the same time, the reliability of the difference with the same age group of comparison is levelled, which is probably due to the migration of workers who worked in contact with harmful working conditions and were transferred to administrative positions due to contraindications. A similar trend is observed in the experience groups with respect to the tumor suppressor transcription factor p53.

Relative to the control group there are increased levels of the frequency of the variant allele of the following genes: genes regulating processes of the nervous system – the dopamine receptor gene DRD2 rs1800497, immunoassociated proteins genes HLA-DRA rs3135388 gene of his TNF co-receptor rs1800629, rs10054203 telomerase TERT gene, the p53 rs1042522 gene; genes associated with metabolic processes, pathology of the cardiovascular system-the receptor - 1 gene of oxidized low-density lipoprotein *OLR1* rs1050283 (due to variant homozygosis), the integrin 2 itga2 rs1126643 – α–integrin gene, is associated with the risk of developing coronary atherosclerosis. The control group is characterized by an excess frequency of polymorphism of the FOXP3 rs3761547 suppressor lymphocyte protein gene, the glucose transporter gene 7 transcription factor TCF7L2-1 rs7903146, the first phase detoxification gene cytochrome CYP1A11048943, the α-adductin *ADD1* rs4961 gene (transport of sodium and potassium ions) - a tendency to hypertension of the renal type.

Conclusion. An analysis of the research results allowed us to identify chains of stable associative relationships between the following indicators: the content of aromatic hydrocarbons in the working area air - the level of blood contamination of workers with benzene and its homologues – work experience - immunosuppression. Analysis of air samples from the working demonstrated the presence of benzene and its homologues in 100% of samples at workplaces and in the office building. According to the results of the study of associative relations between April and September 2019, it was found that a change in the concentration of benzene and o-xylene in the blood is directly dependent on: the work experience at oil and gas production facilities for more than 20 years forms the excess of blood contamination with benzene and its homologues in the studied groups more than 2 times in relation to the level of contamination of workers whose experience does not exceed 10 years. The use of modern immunological and genetic technologies has revealed that in workers contaminated with aromatic hydrocarbons, deviations in immunological parameters and polymorphic variants of genes involved in immunoregulation are closely associated with the pathology of the cardiovascular system with the formation of coronary atherosclerosis.

References

1. Бандман А.Л., Войтенко Г.А., Волкова Н.В. и др. Вредные химические вещества. Галоген и кислородсодержащие органические соединения: справочное издание под ред. В.А.Филова и др. СПб.: Химия, 1994:688. [Bandman AL, Vojtenko GA, Volkova NV i dr. Harmful chemicals. Halogen and oxygen-containing organic compounds: reference edition, ed. VA Filov et al. SPb.:*Himija*, 1994:688. (In Russ.).]

2. Бойко В.И. Гигиена производственной и окружающей среды в нефтяной промышленности. *Гигиена труда*. 1976;6:37-39. [Bojko VI, Occupational and environmental hygiene in the oil industry. *Gigiena truda*. 1976;6:37-39. (In Russ.).]

3. Зайцева Н.В., Землянова М.А., Тарантин А.В. Нарушения белкового состава крови человека в условиях воздействия ароматических углеводородов. *Экология человека*. 2013;15-25. [Zajceva NV, Zemljanova MA, Tarantin AV. Violations of the protein composition of human blood under the influence of aromatic hydrocarbons. *Ekologiya cheloveka*. 2013; 15-25. (In Russ.).]

4. Макаров Г.В., Васин А.Я., Маринина Л.К. и др. Охрана труда в химической промышленности. М.:Химия, 1989:496. [Makarov GV, Vasin AJa, Marinina LK i dr. Labor protection in the chemical industry. M.:Himija, 1989:496 (In Russ.).]

5. Российская энциклопедия по медицине труда под ред. Н.Ф. Измерова. М.:Медицина, 2005;653. [Russian Encyclopedia of Occupa-

tional Medicine ed. NF Izmerov. M.:Medicina, 2005:653. (In Russ.).]

6. Шамсиахметова Г.И. Профессиональные заболевания на предприятиях нефтяной промышленности. *Молодой ученый*. 2016;16:460-463. [Shamsiahmetova GI, Occupational diseases at the enterprises of the oil industry. *Molodoy uchenyy.* 2016;16: 460-463. (In Russ.).]

DIAGNOSTIC AND TREATMENT METHODS

I.V. Averyanova

INFORMATIVE VALUE OF CARDIOHEMODYNAMIC AND GAS ANALYSIS INDICATORS WHEN EVALUATING RESPONSES TO RETURN BREATHING IN YOUNG MALE RESIDENTS OF THE FAR EASTERN REGION

DOI 10.25789/YMJ.2020.71.07

УДК 612.176+612.014.43+612.899

Objective: studying the effect of breathing in a confined space (return breathing) on the indicators of heart rate variability (HRV), blood pressure (BPS, BPD) and gas composition of exhaled air in order to identify the degree of reactivity of the systems as markers of the tension in the body functional state adaptation to the extremes of the North-East of Russia, in particular, when living in the continental or coastal climatic zones.

Research methods: The study involved 316 young men aged 17-21, of them 214 were young male residents of the city of Magadan, Magadan Region, 45 young men from the city of Anadyr, Chukotka Autonomous District, a coastal natural-climatic zone, and 57 young men residing in the settlement of Susuman, Magadan Region (continental climatic zone). The studied indicators were main characteristics of the cardiovascular system, heart rate variability, and gas exchange at rest and after a test with return breathing (re-respiration).

The results obtained testify to changes in heart rate, hemodynamics, and gas analysis that occur in response to re-breathing depending on the region of residence. Young residents of the continental zone demonstrated a pronounced reactivity of the hemodynamic system and gas analysis while no response to the test was observed in the heart rate variability. Of note that, young men of the coastal zones (the cities of Magadan and Anadyr), on the contrary, showed pronounced shifts in the indicators of the heart rate variability with less reactivity observed in the cardiovascular system and gas analysis.

Conclusions: It has been established that the severity of rearrangements of the parasympathetic component of autonomic nervous system is interconnected with hemodynamic and gas patterns in response to hypoxic-hypercapnic effects and together they are highly informative indicators for assessing the degree of tension of the functional state when living in different regions of the Far North.

Keywords: young males, re-respiration, cardiovascular system, gas analysis, heart rate variability, natural and climatic zones.

Introductio. Compensation mechanisms for moderate forms of hypoxia, hypercapnia, or their combination in a healthy body have a certain adaptive value in the development of adaptive reactions aimed at increasing the body's resistance to a range of extreme factors [1]. Changing heart rate in response to a disturbing factor is a universal operative reaction of the whole organism in response to any environmental impact and characterizes the balance between sympathetic and parasympathetic tone [8]. It is known that the analysis of HRV at rest allows to quantitatively assess the current functional state of the body, and to determine its adaptive reserves during functional tests [6].

AVERYANOVA Inessa Vladislavovna – Biological Candidate, Leading Researcher, Laboratory for Physiology of Extreme States, Scientific Research Center "Arktika", Far Eastern Branch of the Russian Academy of Sciences (SRC "Arktika" FEB RAS), 24 Karl Marx street, 24, Magadan, 685000. e-mail: Inessa1382@mail.ru, tel.: 7(924)-69-111-46. http://orcid.org/0000-0002-4511-6782; eLibrary SPIN: 9402-0363.

Therefore, one of the objectives of our research was to study the rearrangements in gas analysis, cardiovascular system, and heart rate during hypoxic-hypercapnic exposure in order to identify the degree of reactivity of the body systems as markers of the tension at adaptation to the extreme conditions of Northeast Russia, in particular, when living in the continental or the coastal climate zones. The study suggested that the degree of responses and lability of those systems based on a quantitative assessment of the influence of each link of the ANS on heart rate, as well as responses of hemodynamic and gas analysis indicators would be objective criteria for the body functional state as a criterion for the stress degree being one of quantitative indicators of adaptability to conditions of the North which if taken into account would be useful for assessing the adaptive reserves of the organism as a whole.

Materials and methods. In total, 214 young Caucasian men from the city of Magadan, 57 young men from Susuman and 45 young Caucasians from Anadyr underwent a re-breathing test. Due to the small number of sympathotonic subjects in the sample, the functional indicators of young men of this type were not analyzed in this series of studies. The sample for statistical analysis included individuals with vagotonic and normotonic types of autonomic regulation.

A test with return breathing (re-respiration) without CO2 absorption was used as a functional test. Prior to the test, the subjects were examined to determine the content of CO2 (%) and O2 (%) in the expired air using a portable gas analyzer manufactured by the "Karbonik" LLC. To perform the test, the subject had to make 3 deep exhalations into a plastic sealed bag (the Douglas type) from where the process of inhalation and exhalation with a total duration of 3 minutes was further done; the nose was closed with a clip [4]. After completion of the test with return breathing, the gas mixture remaining in the sealed bag was analyzed for the level of CO2 and O2 in% using the same device.

The VARICARD-KARDi software and the methodical recommendation by Russian experts [2] were used to register cardiorhythmogram at rest before

the test as well as during its performing. After that, the heart rate and the original type of autonomic regulation were analyzed, which is presented in details in the work [4].

Using a Nessei DS-1862 automatic blood pressure monitor (Japan), we analyzed the systolic and diastolic blood pressure indicators at rest prior to the test and at the peak of the test (the end of the 3rd minute) with simultaneous, at the same points of the test, recording of the oxyhemoglobin values (HbO2,%) using the "NPB-40" pulse oximeter (USA). All studies were carried out in the morning in a room with a comfortable temperature of 19-21° C.

The criterion for inclusion in the study was at least 3200 mL VC of the lungs as well as the subjects' reporting of having no chronic diseases in the acute stage or health complaints. The study was performed in accordance with the principles of the Helsinki Declaration. The study protocol was approved by the Bioethics Commission of the Federal State Institution of Science, IBPN FEB RAS (No. 001/019 of March 29, 2019). All volunteers gave their written informed consent prior to inclusion in the study.

The results were statistically processed using the Statistica 7.0 application software package. The distribution of the measured variables was checked out for normality based on the Shapiro-Wilk test. The results of nonparametric processing methods have been presented as the median (Me) and interquartile range of 25 and 75 percentiles, and parametric values as the average value and its error (M ± m). When comparing related samples, the statistical significance of differences was determined using the Student's t-test for dependent samples with a normal distribution and the nonparametric Wilconson criterion for samples with other than normal distribution. When comparing unrelated samples, the statistical significance of the differences was determined using the Student's t-test for independent samples with a parametric distribution, and the nonparametric Mann-Whitney criterion was applied for samples with non-normal distribution. The critical significance level (p) in the study was taken to be equal to or less than 0.05 [3].

Research results. Table 1 presents the main indicators of the cardiovascular system, gas exchange, and arterial blood saturation in young residents of different regions of the Far East (Magadan Region with the settlement of Susuman and Magadan city, and Chukotka Autonomous District with the city of Anadyr). From the data it can be seen that, the young men of Magadan at rest were significantly lower in systolic blood pressure as compared to those from Anadyr and Susuman. As for the young men from Susuman, their baseline rates of diastolic blood pressure and heart rate were significantly higher.

The analysis of the data has revealed pronounced intergroup region-related differences in gas analysis at rest: lower carbon dioxide values and higher oxygen values in exhaled air were observed among the Anadyr subjects while the highest CO2 concentrations and lowest O2 levels were recorded in group of Susuman subjects. No intergroup differences in the saturation of arterial blood were detected.

The analysis of the cardiovascular system indicators has revealed more pronounced responses to the hypoxic-hypercapnic test demonstrated by young male subjects from Susuman, as compared with the examinees from other regions of residence, which could be seen in a significant growth in BPS, BPD, and HR. Of note that, young male residents from the city of Magadan showed rather low BPS and HR values at the peak of the test while the subjects from Anadyr proved to be the lowest in BPD.

In response to the re-breathing test, a significant increase in carbon dioxide concentration and a decrease in oxygen concentration could be seen in all the examinees; such reaction was found more pronounced among the Susuman subjects. During the test, as well as at rest, the Susuman group demonstrated the highest concentration of carbon dioxide with low oxygen levels while the Anadyr group were, on the contrary, the lowest in CO2 and had high O2 values. The indicators of gas analysis, both at rest and at the peak of the test, in the young men of Magadan were characterized by average indicators in comparison with those from Anadyr and Susuman. At the peak of the re-breathing test, a significant decrease in arterial blood saturation was

Table 1

Indicators of the cardiovascular system, gas exchange, and arterial blood saturation at rest and when performing the re-breathing test in young male residents of different regions of Northeast Russia

Examined Cohorts				Difference Significance		
Indicators	Magadan Caucasians (1)	Susuman Caucasians (2)	Chukotka Caucasians (3)	1-2	2-3	1-3
Baseline						
BPS, mmHg	124.0±0.7 *	127.9±1.3 *	127.2±1.4 *	p<0.01	p=0.72	p<0.05
BPD, mmHg	76.3±0.8 *	77.1±1.2 *	73.3±1.2 *	p<0.05	p<0.05	p=0.50
HR, beat per min	70.9±0.8 *	74.1±1.0 *	71.3±1.0 *	p<0.001	p<0.05	p=0.22
CO2 concentration in the exhaled air, %	3.8±0.1 *	4.2±0.1 *	3.2±0.1 *	p<0.001	p<0.001	p<0.001
O2 concentration in the exhaled air, %	16.3±0.1 *	15.8±0.1 *	17.1±0.1 *	p<0.001	p<0.001	p<0.001
HbO2, %	98.5±0.1 *	98.5±0.1 *	98.6±0.1 *	p=0.74	p=0.51	p=0.76
Return Breathing						
BPS, mmHg	133.0±1.1	136.2±1.2	138.1±2.2	p<0.05	p=0.49	p<0.05
BPD, mmHg	89.5±1.1	86.9±1.1	77.6±1.7	p<0.05	p<0.001	p<0.01
HR, beat per min	75.1±0.9	85.3±1.6	81.3±1.4	p<0.001	p<0.05	p<0.001
CO2 concentration in the exhaled air, %	7.1±0.1	8.4±0.2	6.6±0.2	p<0.001	p<0.001	p<0.05
O2 concentration in the exhaled air, %	12.5±0.1	10.7±0.2	13.1±0.2	p<0.001	p<0.001	p<0.05
HbO2, %	96.2±0.2	95.2±0.1	96.6±0.1	p<0.001	p<0.001	p<0.05

Note: * is for the significant differences between baseline vs. test.

observed through all the examined groups, but the subjects from the continental zone of residence (Susuman) showed a more pronounced dynamics of this indicator in response to the hypoxic-hypercapnic exposure.

Table 2 presents the heart rate variability values at rest and during the re-breathing test by young male residents of Northeast Russia. The data testify to a statistically significant dynamics observed during the test in the studied heart rate indices at the background of the baseline. Of note that, the pNN50 and HF values increased while the Mo, VLF, LF / HF and IC decreased in all the examinees. The groups of Magadan and Anadyr had running up RMSSD and TP and lowered AMo; the tendency was not typical of the residents from Susuman. At the same time only the Magadan young males tended to have a significant decrease in SI at the peak of the test. In addition, the groups of Magadan and Susuman subjects were found to have positive dynamics in MxDMn.

On the whole, of all the 13 studied heart rate indicators, the test with return breathing has shown the following statistically significant differences that could be seen, compared to the baseline, in as many as 12 in the group of Magadan, in 10 indicators with the Anadyr group, and in 6 indicators with those of Susuman.

The discussion of the results. The analysis of the cardiovascular indicators presented in table. 1 has revealed region-related profiles suggested by the return breathing test with regard to BPS, BPD, HR values. The subjects from Magadan were lower in BPS at both baseline and the peak of re-breathing. At the same time, the BPD values in the test statistically significantly grew higher in all the studied groups, with the least pronounced dynamics in the Anadyr subjects. Based on the different patterns of hemodynamic responses we calculated the degree of reactivity without taking into account the sign of dynamics (increase, decrease) as a reflection of the lability of the body system in response to a disturbing factor. The increase observed in heart rate and blood pressure in response to a disturbing factor is undoubtedly typical adaptive reactions of the body that depend on the region of residence of the subjects. The examinees from Magadan, in response to the re-breathing test, experienced the following degree of the growth in their cardiovascular system reactivity: BPS by 7%, BPD - 17%, and HR - 6% in total amounted to 30%. In the group of Anadyr examinees a less pronounced growth was observed: BPD by 6%, BPS by 9% and HR by 14% in total amounted to 29%. The Susuman subjects showed the highest increase: BPS by 6%, BPD - 13% and HR - 15%, which amounted to 34%.

The analysis of the gas exchange results indicated that in the three analyzed groups during the return breathing there could be seen a statistically significant increase in carbon dioxide concentration and a decrease in oxygen with a simultaneous decrease in arterial blood saturation. Those changes were more

Table 2

Heart rate indicators at rest and when performing the re-breathing test in residents of different regions of Northeast Russia

Indicators	Baseline									Difference Significance		
	Magadan Caucasians			Susuman Caucasians			Anadyr Caucasians					
	Me	25	75	Me	25	75	Me	25	75	Magadan-Susuman	Susuman-Anadyr	Magadan-Anadyr
MxDMn, мс	368.2 *	290.0	459.6	404.5	322.3	539.5	377.0	285.0	418.1	p<0.01	p<0.01	p=0.44
RMSSD, мс	46.2 *	36.5	65.4	61.7	50.4	75.0	48.2 *	32.1	66.3	p<0.001	p<0.001	p=0.96
pNN50, %	21.4 *	13.6	35.1	34.1 *	23.2	44.1	23.9 *	11.2	47.5	p<0.001	p=0.14	p=0.81
SDNN, мс	68.0 *	54.0	84.7	73.9	62.3	93.5	66.3 *	47.5	84.2	p=0.11	p<0.05	p=0.47
Mo, мс	827.6 *	774.0	928.0	779.0 *	711.4	875.6	822.4 *	728.0	879.0	p<0.001	p=0.14	p=0.25
AMo50, мс	30.5 *	25.0	38.5	29.0	25.8	34.6	31.7*	24.3	39.9	p=0.49	p=0.38	p=0.59
SI, arb. unit	48.6 *	31.1	79.7	44.8	30.8	58.2	49.0	36.5	94.1	p=0.37	p=0.15	p=0.27
TP, мс^2	3751.9 *	2387.2	5871.0	4375.0	2577.3	6429.2	3765.9*	1906.5	4940.4	p<0.05	p=0.22	p=0.36
HF, мс^2	873.5 *	501.4	1543.1	1579.4*	922.7	2599.7	912.4*	374.8	1938.9	p<0.001	p<0.01	p=0.98
LF, мс^2	1351.6	928.6	2118.8	1306.7	799.6	2034.7	1310.6	838.2	1577.4	p=0.69	p=0.35	p=0.17
VLF, мс^2	592.9 *	363.7	968.8	531.7*	328.0	866.7	572.0*	373.8	1000.0	p=0.26	p=0.31	p=0.95
LF/HF, arb. unit	1.6 *	1.0	2.7	0.8 *	0.6	1.2	1.2 *	0.6	2.3	p<0.001	p<0.05	p=0.13
IC, arb. unit	2.5 *	1.6	3.9	1.2 *	0.9	1.9	2.1 *	1.0	3.5	p<0.001	p<0.01	p=0.28
Return Breathing												
MxDMn, мс	395.5	318.3	498.5	431.0	367.0	561.4	363.0	294.3	443.3	p<0.001	p<0.01	p=0.24
RMSSD, мс	61.7	46.5	82.7	70.0	55.4	88.7	58.8	41.2	85.7	p=0.12	p=0.12	p=0.48
pNN50, %	38.3	25.6	53.4	41.4	27.4	53.3	35.4	20.3	48.3	p=0.75	p=0.22	p=0.21
SDNN, мс	78.3	62.1	105.5	78.9	64.5	99.8	76.3	57.5	89.4	p=0.70	p=0.37	p=0.22
Mo, мс	774.3	676.8	875.7	678.7	621.1	776.1	725.3	676.4	777.4	p<0.001	p<0.05	p<0.05
AMo50, мс	26.5	21.5	31.4	29.0	22.7	32.1	28.6	22.9	35.8	p=0.10	p=0.53	p<0.01
SI, arb. unit	43.3	27.5	65.5	45.2	32.3	73.0	52.2	39.5	79.7	p=0.77	p=0.18	p<0.05
TP, мс^2	4948.7	2974.9	7236.5	4105.8	3141.9	7761.3	4556.7	2126.0	7308.3	p=0.27	p=0.93	p=0.46
HF, мс^2	2002.3	1107.4	3488.0	2139.7	1180.0	3277.4	1803.6	739.9	4170.6	p=0.88	p=0.79	p=0.54
LF, мс^2	1292.0	750.7	2359.3	1107.8	722.6	2197.2	1066.3	605.3	2169.7	p=0.26	p=0.84	p=0.43
VLF, мс^2	498.1	284.3	824.4	382.5	261.8	700.0	413.5	240.1	997.9	p=0.12	p=0.54	p=0.67
LF/HF, arb. unit	0.6	0.3	1.2	0.5	0.4	0.8	0.7	0.4	1.1	p=0.59	p=0.44	p=0.77
IC, arb. unit	0.9	0.5	1.7	0.8	0.5	1.2	0.9	0.6	1.7	p=0.50	p=0.33	p=0.63

Note: * is for the significant differences between baseline vs. test

pronounced in the group from the continental zone of residence. Of note that, the Susuman subjects decreased in the O2 content in the bag by 10.7%, and increased in the CO2 content by 8.4%, while those from Magadan and Anadyr demonstrated the oxygen decrease by 12.5% and 13.1%, and their carbon dioxide did not exceed 7.1% and 6.6%, respectively. As no significant differences in the saturation of arterial blood were found at rest during hypoxic-hypercapnic exposure, that indicator was down to 96.2% in the subjects from Magadan, to 96.6% in the Anadyr people, and to 95.2% in the group of Susuman examinees.

According to the results of the study, the group of young men of the continental zone of residence had more pronounced oxygen consumption in response to the test with return breathing, which was the highest (∆O2, 5.1%) in comparison with 4.0% and 3.8% in those from Anadyr and Magadan, respectively. The similar trend was observed with respect to the carbon dioxide concentration: the group of Susuman had the highest value of this parameter (∆CO2, 4.2%) as compared to the examinees from the cities of Magadan (∆CO2, 3.3%) and Anadyr (∆CO2, 3.4 %). It is necessary to point out that the highest values of the baseline vs. test difference on the arterial blood saturation could be also seen in the group of Susuman (3.3%). On the whole, having analyzed the overall percentage dynamics as well as taking into account the degree of decrease or increase in gas analysis indicators in response to the test through the examined groups from the three regions of residence, we found that, the young male subjects from both Magadan and Anadyr demonstrated this parameter (∆ of gas analysis) equal to 9.4% while in Susuman it was 12.6%.

The analysis of changes in heart rate variability in response to the re-breathing test indicates that activity of the autonomous regulatory contour is running up due to an increase in respiratory rate, which always occurs under significant hypoxic and hypercapnic effects. Our studies showed that short-term hypoxic-hypercapnic exposure leads to changes in the heart rate indicators which have a number of differences depending on the region of residence. These changes tend to be accompanied by the growth in the spectrum power in HF and pNN50 against the background of the decrease in Mo, VLF, LF / HF, and IC. In the group of Magadan and Anadyr, in addition to the above changes, the examined subjects were higher in RMSSD and SDNN, and lower in AMo, which reflects the activation of the parasympathetic link of the ANS, and was not recorded in the group of Susuman.

It should be noted that the indicators of the high-frequency component of the spectrum of heart rate variability (HF) which were significantly lower at baseline among the examinees from Magadan and Anadyr, did not statistically differ at the peak of the return breathing test. The growth in TP that was only noted in the group of Magadan and Anadyr was due to a pronounced increase in the high-frequency component of the spectrum of the heart rhythm in response to re-breathing in those examined groups. Such dynamics was caused by running up HF in the young male subjects of the coastal zone of residence (more than 129% growth with the Magadan group and 97% among Anadyr people), which undoubtedly reflects the high degree of activation of the parasympathetic link and ascertains the structure of the heart rate during re-breathing whereas the Susuman examinees did not exceed the 35% growth in the HF value. Nowadays the HF indicator which is a marker of vagal activity [5], is associated with sinus arrhythmia that ensures the optimal concentration of the blood gas and optimizes gas exchange during breathing by making perfusion comparable with heart rate [9] and with the rate of oxygen consumption [7].

Based on this, we can assume that such a pronounced growth in the high-frequency component of the spectrum of heart rate variability in young men in the coastal zone of residence (Magadan, Anadyr) is aimed at increasing the gas exchange rate in the alveoli, which indicates an increase in the efficiency of oxygen consumption and carbon dioxide excretion and is comparable with the ∆ of gas analysis that proved to be lower in the group of Magadan and Anadyr people (9.4%). It should be noted that such changes in HRV indices in young men of the three regions of Northeast Russia occurred against the background of the significant decrease in the VLF component of the heart rate spectrum.

We have analyzed the heart rate reactivity indicators to the return breathing test by summing the percentage of the degree of the responses without taking into account whether it runs up or down, we have found the following degree of the HRV reactivity: 463% for the Magadan group, 348% for the Anadyr group, and 171% in the group of Susuman.

Conclusion. Thus, the analysis of the changes occurred in the heart rate, hemodynamics, and gas analysis indicates the region-related profiles maintaining the functional state of the body in response to hypoxic-hypercapnic exposure. Our data indicate that, qualitatively identical shifts in HRV indicators can be observed which characterize the activation of the parasympathetic component of the autonomic nervous system, but their severity depends on the region of residence. As for the examinees from the coastal zone of residence, a pronounced growth in the activity of the parasympathetic component of the ANS, in particular, the heart rate high-frequency component (HF) was typical for them, which optimizes gas exchange at the re-breathing test and is confirmed by lower values of Δ CO2, ∆O2, and ∆HbO2 against the background of a less pronounced reaction from the BPS, BPD, and HR which amounted 30%, 29%, and 34% in the studied groups from Magadan, Anadyr, and Susuman, respectively.

Summarizing the above, it should be noted that, the observed more pronounced degree of the gas analysis responses is comparable with the high reactivity of cardiovascular system to the test, which was observed against the background of the inactivity of the heart rate variability indicators in the group of young men from Susuman.

Our data obtained from the analysis of the baseline vs. test differences, have explicitly demonstrate that, the main characteristics of cardiovascular system, heart rate, arterial blood saturation, and, to a greater extent, the gas exchange can serve an indicator of the tension degree in response to the influence of factors of different extremeness. Of note that, the most specific parameters that reflect differences in the studied systems changing in response to the return breathing test in the subjects of the three examined groups, are ΔCO2, ΔO2, and ΔHbO2, as well as the degree of reactivity of the cardiovascular system and heart rate.

An essential aspect established in the research is the young men of the coastal zone of residence (the cities of Magadan and Anadyr) were who demonstrated the most optimal indicators of hemodynamics, gas exchange, and heart rate, as well as the degree of their reactivity in response to the re-breathing test, which testifies to a good level of adaptation by those subjects to the surrounding climatic conditions and their best functional body reserves as compared to the young examinees of the continental zone of residence (Susuman).

References

1. Агаджанян Н.А., Чижов А.Я. Гипоксические, гипокапнические и гиперкапнические состояния. М. Медицина. 2003; 93. [Agadzhanjan NA, Chizhov AJa. Hypoxic, hypocapnic and hypercapnic States. M: Medicina, 2003; 93 (in Russ.)]. https://search.rsl.ru/ru/record/01002386001

2. Баевский Р.М., Иванов Г.Г., Чирейкин Л.В. и др. Анализ вариабельности сердечного ритма при использовании различных электрокардиографических систем (методические рекомендации). Вестник аритмологии. 2001; 24:65-83. [Baevskij RM, Ivanov GG, Chirejkin LV i dr. Analysis of Heart Rate Variability When Using Different Electrocardiographic Systems (Methodical Recommendations). Bulletin of Arrhythmology. 2001; 24:65-83 (In Russ.)]. http://www.vestar.ru/article.jsp?id=1267

3. Боровиков В. Statistica. Искусство анализа данных на компьютере: Для профессионалов. (2-е издание). СПб.: Питер, 2003; 688. [Borovikov V. Statistica. Statistica. The Art of Analyzing Data on a Computer: For Professionals]. VSpb.:Piter, 2003; 688. (In Russ.)]. http://www.statosphere.ru/books-arch/statistica-books/borkat.html

4. Максимов А.Л., Аверьянова И.В. Перестройки вариабельности кардиоритма у лиц с различными исходными типами вегетативной регуляции в процессе переспирации. Российский физиологический журнал им. И.М. Сеченова. 2016; 102(5): 606-617. [Maksimov AL, Averyanova IV. Heart rate variability changes demonstrated by individuals with different vegetative regulation types in origin under rerespiration session. Ross. Fiziol. Zh. im. I.M. Sechenova. 2016; 102(5): 606-617. (In Russ.)] https://elibrary.ru/item.asp?id=25950101

5. Grossman P, Wilhelm FH, Spoerle M. Respiratory sinus arrhythmia, cardiac vagal control, and daily activity. American Journal of PhysiologyHeart and Circulatory Physiology. 2004; 287(2): 728-734. https://doi.org/10.1152/ajpheart.00825.2003 https://search.crossref.org/?q=Grossman%2C+P.%2C+Wilhelm%2C+F.H.%2C+Spoerle%2C+M.%2C+2004.+Respiratory+sinus+arrhythmia%2C+cardiac+vagal+control%2C+and+daily+activity.+American+Journal+of+PhysiologyHeart+and+Circulatory+Physiology+287+%282%29%2C+29.

6. Malliani A, Pagani M, Lombardi F. Importance of appropriate spectral methodology to assess heart rate variability in the frequency domain. Hypertension. 1994;24:140–142. https://doi.org/10.1161/01.hyp.24.1.140 https://search.crossref.org/?q=Malliani+A%2C+Pagani+M%2C+Lombardi+F.+Importance+of+appropriate+spectral+methodology+to+assess+heart+rate+variability+in+the+frequency+domain.+Hypertension+1994%3B24%3A140%E2%80%931

7. Perini R, Orizio C, Baselli G et al. The influence of exercise intensity on the power spectrum of heart rate variability. European Journal of Applied Physiology. 1990;61:143–148. https://doi.org/10.1007/bf00236709

8. Sassi R, Cerutti S, Lombardi F, Malik M, Huikuri HV, et al. Advances in heart rate variability signal analysis: joint position statement by the e-Cardiology ESC Working Group and the European Heart Rhythm Association co-endorsed by the Asia Pacific Heart Rhythm Society. Ep Europace. 2015;17:1341-1353. https://doi.org/10.1093/europace/euv015 https://search.crossref.org/?q=Sassi+R%2C+Cerutti+S%2C+Lombardi+F%2C+Malik+M%2C+Huikuri+HV%2C+et+al.+%282015%29+Advances+in+heart+rate+variability+signal+analysis%3A+joint+position+statement+by+the+e-Cardiology+ESC+Working+Group+and+the+European+Heart+Rhythm+Association+co-endorsed+by+the+Asia+Pacific+Heart+Rhythm+Society.+Ep+Europace+17%3A+1341-1353.

9. Shamailov BT, Paton J. Evaluating the physiological significance of respiratory sinus arrhythmia: looking beyond ventilation-perfusion efficiency. J. Physiol. 2012;590(8):1989-2008. https://doi.org/10.1113/jphysiol.2011.222422 https://search.crossref.org/?q=Shamailov+B.+T-.%2C+Paton+J.+Evaluating+the+physiological+significance+ofrespiratory+sinus+arrhythmia%3A+looking+beyond+ventilation-perfusionefficiency.+J.+Physiol.+2012%2C+590+%288%29%2C+pp.+1989-2008

10. Yasuma F, Hayano J. Respiratory sinus arrhythmia: why does the heartbeat synchronize with respiratory rhythm? Chest J. 2004; 125(2): 683-690. https://doi.org/10.1378/chest.125.2.683 https://search.crossref.org/?q=Yasuma+F.%2C+Hayano+J.+Respiratory+sinus+arrhythmia%3A+why+does+the+heartbeatsynchronize+with+respiratory+rhythm%3F+Chest+J.+2004%2C+125+%-282%29%2C+pp.+683-690.

E.N. Suprun, G.P. Evseeva, S.V. Suprun, O.A. Lebedko, T.V. Pivkina

TRACE ELEMENT STATUS IN CHILDREN WITH BRONCHIAL ASTHMA

DOI 10.25789/YMJ.2020.71.08

УДК 616.248:577.118:612.015.31]-053

SUPRUN Evgeniy Nikolaevich – PhD, Senior Researcher of Groups of health and environmental problems of mother and child health; associate Professor of the Department of hospital and faculty Pediatrics with a course of propaedeutics of children's diseases Far Eastern State Medical University, orcid.org/0000-0002-1089-8884, evg-suprun@yandex.ru +7(914)772-76-85, **EVCEEVA Galina Petrovna** – Doctoral Degree in Medicine,, Main staff scientist of Groups of health and environmental problems of mother and child health, orcid.org/0000-0002-7528-7232; **SUPRUN Stefaniya Viktorovna** – Doctoral Degree in Medicine,, Main staff scientist of Groups of health and environmental problems of mother and child health, orcid.org/0000-0001-6724-3654; **LEBED'KO Olga Antonovna** – Doctoral Degree in Medicine, Director Research Institute of Maternity and Childhood Protection, Senior Researcher Laboratory of Integral Methods of Bronchopulmonary and Perinatal Pathology Research, orcid.org/0000-0002-8855-7422; **PIVKINA Tatyana Vladimirovna** – Researcher of Groups of health and environmental problems of mother and child health.

A series of studies conducted in this country has shown that BA prevalence may vary almost threefold among the regions; BA severity structure also varies from region to region. One factor underlying the environmental diversity is specific geochemical features of the region. Specifically, Khabarovsk Krai is known for high concentrations of manganese, copper, chrome, cobalt, zinc, molybdenum and shortage of selenium as well as technogeniclead pollution with Pb. It is of great interest to study body assimilation of trace elements and involvement thereof in metabolic processes in bronchial asthma.

At the first stage, we studied the content of some trace elements (Ni, Co, Cu, Li, Mn, Se, Pb, Zn, Fe) in blood serum and corpuscles of 314 children residing in Khabarovsk Krai. The main group consisted of 135 patients with BA, comparison group – of 189 non-asthmatic individuals. To assess the impact of trace element concentration changes on the body state at a cell-molecule level in BA children, 40 patients were examined, assessment was made of RBC membrane transformation as a model of cell membrane status in general. Medical condition of children with BA was assessed in accordance with GINA-2015 standards, performance status of bronchopulmonary system – by means of respiratory function test.

The study showed a significant decrease in nickel (0.15 vs 0 μmol/l) and zinc (39.2 vs 34.0 μmol/l) levelsinblood serum and copper (10.7 vs 6.4 μmol/l) and selenium levels (1.96 vs 1.27 μmol/l) in blood corpuscles; an elevated concentration of cobalt (0.96 vs 1.2 μmol/l) in corpuscles and of lead (0 and 1.28 vs 0.0051 and 1.8 μmol/l) in serum and corpuscles in BA patients versus the comparison group children. Patients with severe BA were found to have lower levels of copper in blood corpuscles (4.9 mmol/l)than the patients with mild (6.6 μmol/l) and moderately severe (6.8 μmol/l) BA. Selenium content in both serum and corpuscles was found to decrease with an increase in the BA severity (severe BA 0.70 and 0.83 μmol/l, moderately severe – 1.05 and 1.22 μmol/l, mild - 1.57 and 1.55 μmol/l). Children with severe BA were found to have elevated levels of lithium in corpuscles (1.81 μmol/l) as compared to the patients with mild (1.4 μmol/l) and moderately (1.46 μmol/l) severe BA. Elevated levels of manganese in corpuscles of patients with severe BA (2.02 μmol/l) as compared to the patients with mild BA (1.32 μmol/l). In the study of the effect on the RBC membrane transformation produced by the model trace elements chosen, the exposure to lead salts was found to result in a significant difference in the element levels in comparison between the patients with BA of different degrees of severity and the comparison

group (discocytes %: comparison group – 83, mild BA - 69, moderate BA – 64, severe BA – 58; transitional%: comparison group – 24, mild BA - 27, moderate BA – 30, severe BA – 36; destructive%: comparison group – 1.5, mild BA – 3.5, moderate BA – 4.9, severe BA – 5.5). A series of significant and pathogenically relevant correlation relationships was found: strong reverse causality (R=-0.74) between the degree of severity and a lead exposure-induced change in the number of discocytes, strong direct causality (R=0.73) between the degree of severity and a lead exposure-induced change in the number of transitional forms of red blood cells, strong direct causality (R=0.92) between the degree of severity and a lead exposure-induced change in the number ofspheroids,on the one part, and strong reverse causality (R=-0.68) between the degree of severity and a lead exposure-induced change in the number of echynocytes, on the other part. A series of significant correlation relationships with RBC membrane transformation in BA children was also foundfor selenium: strong direct causality (R=0.75) between the number of spheroids produced by the action of selenium and the degree of BA severity and a strong reverse causality (R=-0.81) between the degenerative forms of RBC produced by the action of selenium in generalandspherocytes(R=-0.79) in particular and FEV1 values.

The data obtained open the way for individualized prediction, diagnosis and correction of trace element imbalance in BA patients.

Keywords: trace elements, bronchial asthma, children, RBC membrane transformation.

Introduction. BA prevalence has amounted to 15% of the total pediatric population in the world [9]. According to official statistics, about 10% of children in Russia suffer from BA [2], but the actual figures are notably higher – 15-20% [3]. BA prevalence rate in the current century has been increasing all over the world. From 2005 to 2015, this rate in teenagers and children under 14 years of age of Khabarovsk Krai increased twofold (from 12‰ to 14‰, 11‰ and 18‰) [8]. The increase is due to the change in the total genetic makeup of developed countries and, as such, it wouldn't respond to correction; therefore, the efforts of medical community are focused on the improvement of BA control efficiency. However, apparent inherited predisposition as a main factor of BA development is modified by the environmental exposure [4, 6]. A series of studies conducted in this country has shown that BA prevalence may vary almost threefold among the regions; BA severity structure also varies from region to region [1, 10].These differences pose a challenge to identify factors responsible for the specific BA course in any particular region. One factor underlying the environmental diversity is specific geochemical features of the region. Specifically, Khabarovsk Kray is known for high concentrations of manganese, copper, chrome, cobalt, zinc, molybdenum and shortage of selenium as well as technogeniclead pollution with Pb concentrations significantly higher than the background value [5, 7, 11, 12]. However, environmental factors act equally upon all members of the local population, but the disorders associated therewith take place in certain cases only; therefore, it is of great interest to study body assimilation of trace elements and involvement thereof in metabolic processes in bronchial asthma.

Materials and Methods. At the first stage, we studied the content of some trace elements (Ni, Co, Cu, Li, Mn, Se, Pb, Zn, Fe) in blood serum and corpuscles of 314 children residing in Khabarovsk Krai. The main group consisted of 135 patients with BA, comparison group – of 189 non-asthmatic individuals; the groups were randomized by sex and age. Trace element contents were measured by means of atomic absorption spectrometry (Hitachi-Z3000, Japan). To assess the impact of trace element concentration changes on the body state at a cell-molecule level in BA children, 40 patients were examined, assessment was made of RBC membrane transformation as a model of cell membrane status in general by means of light microscopy of unstained whole blood smear and exposure to lead and selenium salts in vitro. Lead was chosen as a typical toxicant, while selenium – as an essential element reported as most deficient in this region, and the first stage of study revealed a significant difference in the levels thereof in the comparison group and in BA patients. Medical condition of children with BA was assessed in accordance with GINA-2015 standards, performance status of bronchopulmonary system – by means of respiratory function test. The study was conducted under the principles of the current revision of the Declaration Helsinki (64th WMA General Assembly, Fortaleza, Brazil, October, 2013) and approved by the Ethics Committee, Research Institute of Mother and Child Health Care. Statistical analysis of the results was made using Statistica10.0 software package.

Results and Discussion. The study showed a significant decrease in nickel and zinc levelsinblood serum and copper and selenium levels in blood corpuscles; an elevated concentration of cobalt in corpuscles and of lead in serum and corpuscles (Table 1) in BA patientsversus the comparison group children.

What comes under notice is the relative decrease of, apart from selenium, the trace elementswidespread in our geochemical region (copper, zinc) in BA patients, which may be indicative of abnormal assimilation rather than poor supply thereof. Relatively low selenium and high toxicant (lead and nickel) content is predictable in view of the pathogenic model of the disease.

Upon comparison of trace element levels in patients with BA of different degrees of severity, significant differences were detected as follows: Patients with severe BA were found to have lower levels of copper in blood corpuscles than the patients with mild and moderately severe BA. Selenium content in both serum and corpuscles was found to decrease with an increase in the BA severity. Children with severe BA were found to have elevated levels of lithium in corpuscles as compared to the patients with mild and moderately severe BA. Elevated levels of manganese in corpuscles and of lead in serum of patients with severe BA as compared to the patients with mild BA (Table 2).

It follows from the data obtained that with the course of the disease turning more severe a change in the content and trends takes place in the same trace elements that displayed significant difference upon comparison between BA patients and apparently healthy children. The results speak for the important role thereof in the pathogenesis of the disease.

In the study of the effect on the RBC membrane transformation produced by the model trace elements chosen, the exposure to lead salts was found to result in a significant difference in the element levels in comparison between the patients with BA of different degrees of severity and thecomparison group (Table 3).

A series of significant and pathogenically relevant correlation relationships was found: strong reverse causality (R=-0.74) between the degree of severity and a lead exposure-induced change in the number of discocytes, strong direct causality (R=0.73) between the degree of severity and a lead exposure-induced change in the number of transitional forms of red blood cells, strong direct causality (R=0.92) between the degree of severity and a lead exposure-induced change in the number ofspheroids,on the one part, and strong reverse causality (R=-0.68) between the degree of severity and a lead exposure-induced change in the number of echynocytes, on the other part. That is, lead affects cell membranes to a greater degree in patients with a

Table 1

Trace Element Levels in BA Children (μmol/L)

Trace element	Comparison group (n=189)	BA patients (n=135)	P value
Ni_c	0 (0 – 0.1509)	0 (0 – 0)	<0.001
$Co_{эл}$	0.9601±0.086	1.2038±0.0638	<0.05
$Cu_{эл}$	10.6514±0.658	6.424±0.2699	<0.001
$Se_{эл}$	1.9608±0.186	1.2675±0.1321	<0.001
Pb_c	0 (0 – 0.1737)	0.0051 (0 – 0.0167)	<0.01
$Pb_{эл}$	1.2778±0.1786	1.8066±0.0654	<0.05
Zn_c	39.273±0.6937	34.0539±1.961	<0.05

Table 2

Trace Element Levels versus BA Severity (μmol/L)

Trace element	Mild BA (n=32)	Moderately severe BA (n=88)	Severe BA (n=15)
$Cu_{эл}$	6.5685±0.6116	6.7743±0.3798	4.9589±0.4878*/°
$Li_{эл}$	1.4049±0.0994	1.4664±0.078	1.8168±0.156*/°
$Mn_{эл}$	1.3209±0.1288	1.6323±0.1295	2.0246±0.2635*
Se_c	1.5676±0.264	1.048±0.0672	0.6963±0.1127**/°
$Se_{эл}$	1.5508±0.357	1.2213±0.1373	0.8371±0.074*/°
Pb_c	0.0087 (0–0.0191)	0 (0 – 0.013)	0.0058 (0 – 0.013) *

Note. - Statistical significance of difference in valuesin mild and severe BA groups: *-p≤0,05; **-p≤0,01; - Statistical significance of difference in valuesin moderate and severe BA groups: °-p≤0,05;

Table 3

Red Blood Cell Membrane Transformation under Exposure to Lead *in vitro* (%)

RBC variation	Comparison group	Mild BA	Moderate BA	Severe BA
Discocytes	83.00	68.64	63.64	58.25
Transitional	23.93	27.18	30.15	36.20
Destructive	1.58	3.53	4.89	5.48

Note. Statistical significance of difference in values between the patients with BA of different degrees of severity and the comparison group and one another: - p≤0,05

more severe BA course, which results in the greater decrease in the number of normally shaped red blood cells (discocytes) and, accordingly, greater increase in the number of transitional and destructive forms thereof. As for the particular transformation pathway, the strong inverse correlation displayed by the number of echynocytesprompts suggestions thatlead causes transformation to follow a stomatocytic pathway. A series of significant correlation relationships with RBC membrane transformation in BA children was also foundfor selenium: strong direct causality (R=0.75) between the number of spheroids produced by the action of selenium and the degree of BA severity and a strong reverse causality (R=-0.81) between the degenerative forms of RBC produced by the action of selenium in generalandspherocytes(R=-0.79) in particular and FEV1 values.That is, the more severe asthma the given patient has, the greater beneficial effect of selenium is.

Conclusion. In the course of study it was found that in spite of the common geochemical region of residence there is a trace element imbalance in BA patients as compared to the comparison group – statistically significant decrease in copper, selenium and zinc content and elevated levels of lead, nickel and cobalt. Relationship between the changes in the trace element status and degree of severity was identified. As BA turns more severe, selenium and copper decrease and lead, manganese and lithium increase are observed. According to the data on RBC transformation in BA patients, an impact of metal (lead, selenium) salts on the functional state of cell membranes is significantly dependent on the degree of severity which is also manifested in the correlation relationships. The data obtained open the way for individualized prediction, diagnosis and correction of trace element imbalance in BA patients.

References

1. Батожаргалова Б.Ц. Мизерницкий Ю.Л., Подольная М.А. Метаанализ распространенности астмоподобных симптомов и бронхиальной астмы в России (по результатам программы ISAAC). *Российский вестник перинатологии и педиатрии*. 2016; 61(4):59-69. [Batozhargalova B.Ts. Mizernitsky Yu.L., Podolnaya M.A. Metaanalysis of asthma-like symptoms and bronchial asthma prevalence in Russia (according to ISAAC data). *Rossiyskiy Vestnik Perinatologii i Pediatrii*. 2016; 61(4):59-69. (In Russ.).] DOI: 10.21508/1027-4065-2016-61-4-59-69.

2. Клинические рекомендации. Бронхиальная астма. Москва: Министерство здравоохранения Российской федерации; 2016. [Clinical Recommendations. Bronchial Asthma. Moscow: Russian Federation Ministry of Health; 2016. (In Russ.).]

3. Национальная программа «Бронхиальная астма у детей. Стратегия лечения и профилактика»: 5-е изд., перераб. и доп. Москва, Оригинал-макет; 2017. [National program "Bronchial asthma in children. Treatment strategy and prevention»: 5-th edition revised and expanded. Moscow: Original-Maket; 2017. (In Russ.).].

4. Оконенко Т.И., Гатилов Д.В., Макаров С.В. Состояние загрязнения атмосферного воздуха и влияние аэрополлютантов на обращаемость больных бронхиальной астмой. *Вестник Новгородского государственного университета им. Ярослава Мудрого*. 2013; 71(1): 27-30. [Okonenko T.I., Gatilov D.V., Makarov S.V. Atmospheric pollution state and effect of air pollutants on incidence of asthmatic outpatient visits. *Annals of Yaroslav-the-Wise Novgorod State University*. 2013; 71(1):27-30. (In Russ.).].

5. Супрун С.В., Козлов В.К., Евсеева Г.П. и др. Особенности микроэлементного статуса крови у детей Приамурья. *Дальневосточный медицинский журнал. Приложение*. 1997; 2:71-73. [Suprun S.V., Kozlov V.K., Evseeva G.P. et. al. Specifics of blood trace element status in children of Amur River Region. *Far East Med J. Addendum*. 1997; 2:71-73. (In Russ.).].

6. Панасенкова Е.В., Косенкова Т.В., Легонькова Т.И. Влияние антропогенной нагрузки на течение и формирование бронхиальной астмы у детей. *Профилактическая и клиническая медицина*. 2013; 4:13-19. [Panasenkova E.V., Kosenkova T.V., Legonkova T.I. Effect of anthropogenic stress on bronchial asthma onset and course in children. *Preventive and Clinical Medicine*. 2013; 4:13-19. (In Russ.).].

7. Супрун С.В., Козлов В.К. Анемические состояния у беременных женщин (клинико-патогенетические аспекты, исходы). – Хабаровск; 2013. [Suprun S.V., Kozlov V.K. Anaemic conditions in pregnant women (clinical and pathogenetic aspects, outcomes). Khabarovsk: 2013. (In Russ.).].

8. Евсеева Г.П., Холодок Г.Н., Морозова Н.В. и др. Эпидемиология бронхолегочных заболеваний детей и подростков Хабаровского края. *Бюллетень физиологии и патологии дыхания*. 2016; 61:31-35. [Evseeva G.P., Kholodok G.N., Morozova N.V. et. al. Epidemiology of bronchopulmonary diseases in children and teenagers of Khabarovsk Kray. *Bûlleten' fiziologii i patologii dyhaniâ = Bulletin Physiology and Pathology of Respiration*. 2016; 61:31-35. (In Russ.).].

9. Global Initiative for Asthma. Global strategy for asthma management and prevention. (Updated 2017); 2017. http://ginasthma.org/2017-gina-report-global-strategy-for-asthma-management-and-prevention

10. Kamaltynova Ye.M. Deyev I.A., Belonogova Ye.G. Comparative epidemiological characteristics of bronchial asthma according to data of International Study of Asthma and Allergy in Childhood (ISAAC). *Bulletin of Siberian medicine*. 2009; 4;92-98.

11. Evseeva G.P., Suprun S.V., Ivakhnishina N.M. et. al. The contents of trace elements in lung tissue in fetuses with developmental abnormality. *Journal of Trace Elements in Medicine and Biology.* 2017; 41(1): 3. DOI: 10.1016/j.jtemb.2017.03.339

12. Suprun S.V., Evseeva G.P., Larina T.N. et. al. Trace elements and cell membranes at pregnancy. *Journal of Trace Elements in Medicine and Biology*. 2017; 41(1):21. DOI: 10.1016/j.jtemb.2017.03.093

DOI 10.25789/YMJ.2020.71.09

УДК 616.8-009.17-057.874(571.513)

L.S. Evert, S.A. Bakhshieva, N.Yu. Grishkevich, N.S. Shibanova, Yu.R. Akhmeldinova

CHARACTERISTIC AND COMORBIDE ASSOCIATIONS OF ASTHENIC SYNDROME IN KHAKASIA SCHOOLCHILDREN

The aim of the study is to assess the frequency of occurrence, age-sex characteristics and comorbid associations of asthenic syndrome (AS) in schoolchildren of Khakassia. Materials and methods: 1351 schoolchildren of 12-17 years old of both sexes were examined, 132 of them were teenagers with AS and 1219 - without AS. The presence of AS, recurrent pain (headache, abdominal), and back pain (dorsalgia) was assessed using a screening questionnaire. The data were processed in the program "Statistica 12". Results: AS is registered in 9.8% of schoolchildren of Abakan, with a higher frequency in girls, more often in the non-indigenous population than in the indigenous population, the prevalence of AS increases with age. Discussion: The presence of asthenic syndrome in schoolchildren of Abakan is more associated with frequent episodes of headaches, abdominal pains and back pains. Conclusions: A distinctive feature of the AS comorbidity in schoolchildren of Abakan is its more pronounced association with cephalgias (both frequent and infrequent episodic), frequent pains in the abdomen and back.

Keywords: schoolchildren, asthenic syndrome, headaches, abdominal pain, back pain.

EVERT Lidiya Semyonovna – Doctor of Medical Sciences, Chief Researcher of the Clinical Department of Somatic and Mental Health of Children, Scientific Research Institute of Medical Problems of the North, Federal Research Center "Krasnoyarsk Scientific Center of the Siberian Branch of the Russian Academy of Sciences"; Professor of the Department of General Professional Disciplines, Federal State Budgetary Educational Institution of Higher Education “Khakass State University named after N.F. Katanova"; tel. 8-950-436-6532, e-mail: lidiya_evert@mail.ru https://orcid.org/0000-0003-0665-7428, **BAKHSHIEVA Svetlana Alekseevna** – pediatrician, assistant of the Department of Nursing and Clinical Care, Federal State Budgetary Educational Institution of Higher Education “Krasnoyarsk State Medical University named after Professor V.F. Voino-Yasenetsky" Ministry of Health of Russia, tel. 8-963-260-4102, e-mail: s.bahshieva@mail.ru ; https://orcid.org/0000-0002-2709-8373, **GRISHKEVICH Natalya Yurievna** – Candidate of Medical Sciences, Associate Professor of the Department of Children's Diseases with a course of postgraduate education Federal State Budgetary Educational Institution of Higher Education “Krasnoyarsk State Medical University named after Professor V.F. Voino-Yasenetsky" Ministry of Health of Russia, tel. 8-913-514-9543, e-mail: grishkevitch@mail.ru, https://orcid.org/0000-0002-8643-201X, **SHIBANOVA Nina Semyonovna** – Candidate of Medical Sciences, Associate Professor of the Department of Hygiene Federal State Budgetary Educational Institution of Higher Education “Krasnoyarsk State Medical University named after Professor V.F. Voino-Yasenetsky" Ministry of Health of Russia, tel. 8-923-360-1471, e-mail: shibanova1958@mail.ru, **AKHMELDINOVA Yulia Rinatovna** – 6th year student of the pediatric faculty Federal State Budgetary Educational Institution of Higher Education “Krasnoyarsk State Medical University named after Professor V.F. Voino-Yasenetsky" Ministry of Health of Russia, tel.8-913-196-1527, e-mail: axmeldinova@mail.ru; https://orcid.org/0000-0001-6233-6472

Introduction. The most important area of modern pediatrics is the conduct of screening epidemiological studies of functional somatic disorders (functional somatic disorders - FSD) in pediatric teenage populations with mandatory consideration of ethnosocial factors and age-gender differences. Such medically unexplained disorders account for up to 50% of visits to specialists of various profiles, they are associated with significant physical and psychological disorders and can have a negative impact on the functional state and well-being of children and young people in the long term [16].

Functional somatic disorders are common in children and adolescents and include physical symptoms that are either disproportionate or incompatible with history, physical examination, laboratory and other findings [13]. FSD includes, inter alia, disorders such as asthenic syndrome, recurrent head and abdominal pain, and back pain. Several recent studies have shown that FSD is associated with functional differences in hypothalamic-pituitary-adrenal function, imbalance in vagal-sympathetic tone, increased regulation of immune-inflammatory function, and primary cognitive-emotional responses that provide and enhance responsiveness to threatening stimuli. thereby contributing to the subjective experience of somatic symptoms [11].

Asthenic syndrome is one of the clinical options for FSD in children and adolescents. This is a psychopathological syndrome, the characteristic manifestations of which are a state of general weakness, excessive exhaustion, irritability, impaired attention and memory. Translated from Greek, asthenia means weakness, impotence, and this is the most characteristic symptom of this disease. The child constantly feels tiredness, weakness, weakness, weakness, often apathy develops against this background.

Recurrent headache (RH) is the most common functional somatic disorder found in children and adolescents. Pain can be caused by mechanical, chemical or thermal effects on sensitive receptors found in the soft tissues of the head - skin, muscles, in the walls of the superficial arteries of the head, the dura mater, in the vessels of the base of the brain. One of the typical mechanisms for the development of frequent headaches is associated with impaired vascular regulation. The presence of episodes of headache (especially frequent) negatively affects school and social functions, and the quality of life.

According to foreign population studies, recurrent headaches are one of the most common complaints in adolescence. Among schoolchildren of 7-15 years old, from 26 to 82% suffer from headaches. At the same time, prima-

ry headaches are detected in pediatric practice in 18.6–27.9% of children and adolescents, mainly in the form of migraines and tension headaches (HDN) [9]. According to studies by various authors, the incidence of headache increases from 3–8% among preschoolers to 57–82% in adolescents [7]. The prevalence of headaches in children varies widely depending on the region of residence, methodology, genetic differences, and diagnostic criteria used. Thus, the prevalence of headaches in the child population of Italy is 41.0% [9], Germany - 53.2% [5], Norway - 29.8% (Zwart J. et al., 2004), Sweden - 24.4 % [12], Turkey - 46.2% [17], China - 9.7% [8], Jordan - 24.0% [10], India - 25.5% [14], Korea - 29.1 % [15] and Kuwait - 28.8% [6].

According to Russian studies, the prevalence of cephalalgia in the pediatric population varies over a wide range - from 6.8 to 82%, apparently, this is due to differences in the choice of both study groups and diagnostic criteria for interpreting headache in children [1]. N. A. Schneider (2015) also believes that the heterogeneity of the data obtained is related to the difference in the methodology of epidemiological studies [3]. The true prevalence of HD in the child population may be higher, as many adolescents with mild to moderate hypertension do not seek medical attention.

Another disorder related to FSD is *Recurrent Abdominal Pain* Syndrome (RAP). In the vast majority of cases, it is not possible to identify any organic cause of abdominal pain. Often they are caused by experiences, stresses or other psychological factors. Quite often, abdominal pain is a sign of inorganic (psychogenic, functional) disorders of the gastrointestinal tract. More often, such pains are rare, short-term, weak or medium intensity, passing on their own or after a simple change in diet, symptomatic use of antispasmodics, enzyme and antisecretory drugs.

Recurrent abdominal pain occurs in 7–25% of children. S.Yu. Tereshchenko et al. (2014) indicate that RAP in adolescents is comorbid with a wide range of other symptoms (recurrent headache, back pain, dizziness, and asthenic syndrome). Considering that psychosomatic pathogenic components are at the heart of the development of these conditions, the authors believe that the diagnosis and treatment of RAP in adolescents should require an assessment and correction of their mental health [2].

Dorsalgia is another representative of psychosomatic pain syndromes. Dorsalgia is back pain, regardless of the origin, nature, accompanying symptoms and the exact location of the pain syndrome. Depending on the location, it is customary to distinguish three main types of back pain - cervicalgia, thoracalgia and lumbodynia. In some cases, combined types of pain are distinguished, for example, cervico-thoracalgia or cervic-dorsalgia. All types of back pain can be divided into two large categories - pain of vertebral origin (the cause of pain is pathology of the spine) and pain, the true cause of which lies outside the spinal column, or non-vertebral dorsalgia (psychogenic, myofascial syndrome, myositis). The intensity of the pain can range from moderate to intense.

At present, the problem of comorbid associations of these disorders (including asthenic syndrome) with the obligatory consideration of ethnogeographic, age and gender differences is also extremely urgent, requiring an urgent solution [4,17]. In connection with the above, the *purpose of our study* was to assess the frequency of occurrence, age-sex characteristics and comorbid associations of asthenic syndrome in schoolchildren of Khakassia.

Materials and research methods. The object of the study was students of 4 general educational institutions of the city of Abakan - the administrative center of the Republic of Khakassia. 1351 students of 12-17 years old were examined, of which 666 (49.3%) were boys and 685 (50.7%) girls, the average age of the examined was 15.3 ± 1.2 years. The main group consisted of 132 adolescents with asthenic syndrome, the control group included 1219 schoolchildren without asthenic syndrome. The study was approved by the Ethics Committee of the Research Institute of the Ministry of Railways of the Federal Research Center of the Kola Scientific Center of the SB RAS (protocol dated 05.06.2020). The survey was carried out after obtaining written informed consent to participate in the study of parents of adolescents under 15 years of age or adolescents themselves over the age of 15 years.

In our screening questionnaire developed by prof. S.Yu. Tereshchenko et al. (2014), questions about the presence and frequency of pain (headaches, abdominal and back pain - dorsalgia) are included. Pain in the abdomen and back was considered frequent if they were noted more than 2 times a month, rare - 1-2 times a month, no pain - if there is no pain at all or they occur less than 1 time per month. The criteria for recurrent headache (Recurrent Headaches - RH) were the presence and frequency of headache in the last 3 months: if the head was sick no more than 1 time per month (or did not get sick at all), they concluded that there was no RH, with a headache frequency of 1 to 15 days in month - RH was considered rare, with a headache frequency of more than 15 days per month - RH was regarded as frequent. The presence of asthenic syndrome (AS) was evaluated according to one of the sections of the screening questionnaire. The criterion for the presence of speakers was the sum of the points assigned to the questions in this section, ≥ 10 points, with a score of less than 10 - a conclusion was made about the absence of speakers. *Statistical processing* of the obtained data was carried out by non-parametric statistics methods in the program "Statistica 12". The results of the analysis are presented as % share and boundaries of the confidence interval estimated by the Wilson method. The achieved level of significance of differences (p) for binary signs was evaluated by the Pearson $\chi 2$ criterion. Differences between groups were considered statistically significant at a achieved level of $p \leq 0.05$.

Results and discussion. The analysis of the frequency of occurrence of AS in adolescents of various age and gender groups was carried out. It was shown that asthenic syndrome was recorded much more often in girls –7.5% (101/1351, CI = 6.2–9.0%) compared with boys - 2.3% (31/1351, CI = 1.6 -3.2%; p <0.0001). AS was noted more often in the older age group - 6.0% (81/1351, CI = 4.9-7.4%) than the younger - 3.8% (51/1351, CI = 2.9-4.9 %), although the revealed differences did not reach the level of statistical significance (p1-2 = 0.4244). According to the results of a comparative analysis of the incidence of AS in schoolchildren of the indigenous (Khakass) and non-indigenous (Caucasoid) adolescent population, it was found that schoolchildren of the non-indigenous population were distinguished by a higher incidence of AS - 5.8% (78/1351, CI = 4.7-7.1%) in comparison with the root - 4.0% (54/1351, CI = 3.1-5.2%; p1-2 = 0.0298).

One of the objectives of our study was to study the incidence rate of relapsing headaches (RH) in adolescents as manifestations of functional somatic disorders. It was found that in general, in the entire population of adolescents included in the examination, episodic headache with a frequency of 1 to 15 days per month was found in 32.6% (440/1351), CI = 30.1-35.1%; frequent headache was recorded in 6.2% (84/1351), CI = 5.0-7.6%, and there were no episodes of cephalgia (or they were less than 1 time per month) in

a larger number of examined patients - in 61.2% (827/1351), CI = 58.6-63.8%. In total, rare and frequent cephalgias were recorded in 524 adolescents out of 1351 examined, which amounted to 38.8%.

We carried out a comparative analysis of the incidence and severity of RH in adolescents with asthenic syndrome, the results of the analysis are illustrated in Figure 1a.

The highest was % of adolescents without recurring cephalgia in the group without AS - 64.5% (786/1219, CI = 61.8-67.1%) compared with the group with AS - 31.1% (41/132, CI = 23.8-39.4%. The differences between the compared groups were statistically significant: p1-2 <0.0001, df = 1, χ2 = 56.02. Rare cephalgias were significantly more often recorded in schoolchildren with AS - 53.8% (71/132, CI = 45.3-62.1%) versus 30.3% (369/1219, CI = 27.8-32.9%), and the revealed differences had statistical significance: p1-2 <0.0001, df = 1, χ2 = 29.99. Frequent cephalgia was also more characteristic of schoolchildren with AS - 15.1% (20/132, CI = 10.0-22.2%; p1-2 < 0.0001, df = 1, χ2 = 20.02).

Recurrent abdominal pain is included in the structure of functional somatic disorders. We found that in the entire sample of the examined, rare abdominal pains (1-2 times a month) were found in 39.3% (531/1351), CI = 36.7-41.9%; frequent abdominal pains were recorded in 9.9% (134/1351), CI = 8.4-11.6%, and there were no episodes of RAP (or they were less than 1 time per month) in a larger number of examined patients - in 50.8 % (686/1351), CI = 48.1-53.4%.

The results of a comparative analysis of the incidence of RAP in adolescents with asthenic syndrome are illustrated in Figure 1b.

The highest was the number of adolescents who were not bothered by episodes of abdominal pain, in the group without AS - 53.1% (647/1219, CI = 50.3-55.9%) and the proportion of such people among the examined with AS was less - 29 5% (39/132, CI = 22.4-37.8%; p1-2 <0.0001, df = 1, χ2 = 26.39. The frequency of occurrence of rare episodes of abdominal pain was comparable in groups with AS and without AS (Figure 1b). Frequent abdominal pain was significantly more common among schoolchildren with AS - 25.8% (34/132, CI = 19.1-33.8%; p1-2 <0.0001, df = 1, χ2 = 41.08).

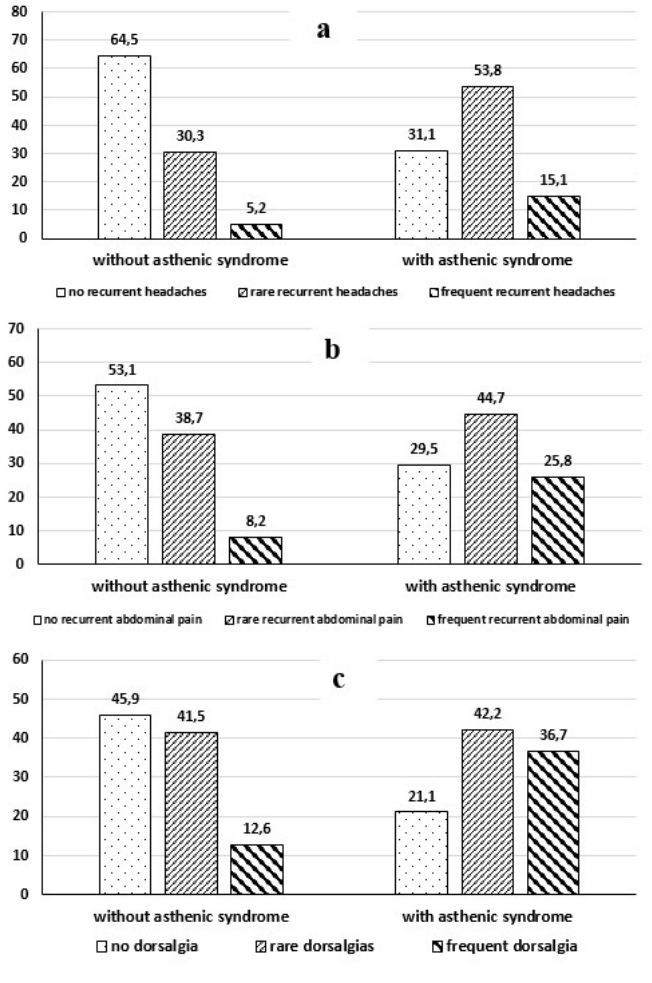
Periodically occurring pains in various parts of the back (in the cervical, thoracic or lumbar) with a frequency of 1-2 times a month were observed in 41.6% (542/1303, CI = 38.9-44.3%) of the examined students. Frequent episodes of dorsalgia were characteristic of 15.0% of the examined (195/1303, CI = 13.1-17.0%). 43.4% of adolescents were not bothered by back pain (566/1303, CI = 40.8-46.1%; p1-2 <0.0001, df = 1, χ2 = 28.84).

The results of a comparative analysis of the frequency of dorsalgia in adolescents with asthenic syndrome are illustrated in Figure 1c.

The number of adolescents with frequent dorsalgia was significantly higher in the group with AS compared with those examined without AS - 36.7% (47/128, CI = 28.9-45.3%) and 12.6% (148/1175, respectively). CI = 10.8-14.6%), p1-2 <0.0001, df = 1, χ2 = 52.78. The ratio of adolescents with rare back pain was comparable in the compared groups and did not depend on the presence of AS (42.2 and 41.5%, respectively, p1-2 = 0.8864). At the same time, the number of schoolchildren without an episode of dorsalgia in history was significantly higher in the group without AS - 45.9% (539/1175, CI = 43.0-48.7%) versus 21.1% (27/128, CI = 14.9-29.0%) among those examined with AS (p1-2 <0.0001, df = 1, χ2 = 28.84).

Conclusion: We have carried out a screening examination of 1351 schoolchildren of 12-17 years old in 4 general educational institutions in Abakan. According to the results of the study, it was found that the frequency of occurrence of AS was 9.8% of the entire sample of the surveyed, asthenic syndrome is more typical for girls (7.5%) than boys (2.3%). There was a tendency towards a higher prevalence of AS in the older age group (6.0%) in comparison with the younger (3.8%). Schoolchildren of the non-indigenous population (5.8%) than the indigenous (4.0%) have a higher prevalence of AS.

In the entire sample surveyed, the prevalence of recurrent cephalgias was 38.8%. In the structure of RSL, rare episodes of cephalgia accounted for 32.6%, frequent episodes - 6.2%, absence of cephalgia - 61.2%. Of the 1351 examined, the proportion of adolescents with rare abdominal pain was 39.3%, with fre-



Structure of recurrent headaches (a), of recurrent abdominal pain (b), dorsalgia structure (c) in adolescents without AS and with AS, %. Note: Statistical significance of differences according to Pearson's χ2 criterion (p) for all indicators - p1-2 <0.0001, except: for the indicator "rare RAP" p1-2 = 0.1817, "rare dorsalgias" - p1-2 = 0.8864.

quent pains - 9.9%, and with the absence of BC - 50.8%. The total number of adolescents with dorsalgias was revealed, both rare episodes (41.6%) and frequent (15.0%).

In adolescents with asthenic syndrome, the frequency and structure of functional somatic disorders associated with this syndrome - recurrent pain syndromes (headaches, abdominal and back pain) - were analyzed. It has been shown that asthenic syndrome has statistically significant comorbid associations with recurrent cephalgias, both rare (53.8% versus 30.3% in the group without AS) and frequent (15.1% versus 5.2% in the group without AS) ... Frequent abdominal pains (25.8% versus 8.2% in individuals without AS) and frequent dorsalgia (36.7% and 12.6%, respectively) are associated with the presence of AS.

Considering the fact that many studies in the field of psychosomatic pathology emphasize the fact that the presence of concomitant disorders in children with functional somatic symptoms complicates their treatment, and in the case of late diagnosis and lack of treatment, can lead to the formation of chronic psychosomatic pathology, all this indicates the feasibility and necessity of an individually oriented approach to adolescents with comorbid functional somatic disorders, and their timely correction.

Findings:

1. The frequency of occurrence of asthenic syndrome in the total sample of examined schoolchildren in Abakan was 9.8%. AS was more often recorded in adolescents of non-indigenous populations (Caucasians), than indigenous (Khakasses), more often in girls than boys, the frequency of the syndrome increased with age.

2. A characteristic feature of comorbid associations of asthenic syndrome in schoolchildren of Abakan was a more pronounced conjugation of AS with headaches (both frequent and infrequent episodic), frequent abdominal pain and frequent dorsalgia.

3. To improve the health and quality of life of schoolchildren with asthenic syndrome, a comprehensive approach is needed, including timely diagnosis and correction of concomitant functional somatic disorders in the form of recurrent pain syndromes.

References

1. Алиджанова Д.А., Маджидова Ё.Н., Нурмухамедова М.А. Патогенетические механизмы развития головной боли напряжения у детей и принципы ее профилактики. *Сибирское медицинское обозрение*. 2017;1:5-10. [Alidzhanova DA, Madzhidova JoN, Nurmuhamedova MA. Pathogenetic mechanisms of tension headache development in children and principles of its prevention. *Sibirskoe medicinskoe obozrenie*. 2017;1:5-10. (In Russ).] DOI: 10.20333/2500136-2017-1-5-10

2. Терещенко С.Ю. Шубина М.В., Горбачева Н.Н. [и др.]. Рецидивирующие боли в животе у подростков: сопутствующие заболевания и их взаимосвязь с психоэмоциональными факторами, маркерами дефицита железа и воспалительного процесса. *Забайкальский мед. вестник*. 2014;2:55–59. [Tereshhenko SJu, Shubina MV, Gorbacheva NN [i dr.]. Recurrent abdominal pain in adolescents: comorbidities and their relationship with psychoemotional factors, markers of iron deficiency and inflammation. *Zabajkal'skij med. vestnik*. 2014;2:55–59. (In Russ).]

3. Шнайдер Н.А., Кондратьев А.В., Шульмин А.В. Эпидемиология головных болей. *Современные проблемы науки и образования*. 2015;6:23. URL: http://science-education.ru/ru/article/view?id=22811 (дата обращения: 07.05.2020). [Shnajder N.A., Kondrat'ev A.V., Shul'min A.V. Epidemiology of headaches. *Sovremennye problemy nauki i obrazovanija*. 2015; 6:23. (In Russ).] URL: http://science-education.ru/ru/article/view?id=22811 (date of the application: 07.05.2020).

4. Almirall P, Núñez FA, Bello J [et al.]. Abdominal pain and asthenia as common clinical features in hospitalized children for giardiasis. *Acta Trop*. 2013;127(3):212-5. DOI: 10.1016/j.actatropica.2013.05.005.

5. Albers L, Andreas M. Straube, Mirjam N. Landgraf [et al.]. Migraine and tension type headache in adolescents at grammar school in Germany – burden of disease and health care utilization. *The Journal of Headache and Pain*. 2015 (First Publication: 4 June 2015).

6. Al-Hashel Jasem Yousef, Samar Farouk Ahmed, Raed Alroughani. Prevalence and Burden of Primary Headache Disorders in Kuwaiti Children and Adolescents: A Community Based Study. *Front Neurol*. 2019;10:793. Published online 2019 Jul 30. DOI: 10.3389/fneur.2019.00793

7. Blume HK. Headache of childhood: an overview. *Pediatrician Ann*. 2017;46:155–65. 10.3928 / 19382359-20170321-02

8. Jin Z, Shi L, Wang JJ [et al.]. Headache prevalence among children and adolescents in Shanghai, China. *J Clin Neurosci*. 2013;20:117-21. 10.1016 / j.jocn.2012.02.02.020

9. Cinzia Cavestro, Francesca Montrucchio, Paola Benci [et al.]. Headache prevalence and related symptoms, family history and treatment habits in a representative population of children in Alba, Italy. *Pediatrician Neurol.* 2014;51:348-53. 10.1016 / j.pediatrneurol.2014.05.022

10. Hussein FA, Hussein AB. The prevalence of headaches and migraines among schoolchildren in Jordan. *Sudan J. Public Health*. 2006;1:307–8.

11. Kozlowska K. Functional somatic symptoms in childhood and adolescence. *Curr Opin Psychiatry.* 2013;26(5):485-92. DOI: 10.1097/YCO.0b013e3283642ca0.

12. Lorell K, Larsson B, Aig-Olofsson O. The prevalence of headache in Swedish schoolchildren with an emphasis on intense type of headache. *Cephalgia*. 2004;24:380–8. 10.1111 / j.1468-2982.2004.00681.x

13. Malas N, Ortiz-Aguayo R, Giles L [et al.]. Pediatric Somatic Symptom Disorders. *Curr Psychiatry Rep*. 2017;19(2):11. DOI: 10.1007/s11920-017-0760-3. PMID: 28188588 Review.

14. Mehta S. Study of various social and demographic variables associated with primary headache disorders in 500 schoolchildren of central India. *J Pediatr Neurosci*. 2015;10:13–7. 10.4103 / 1817-1745.154319

15. Jung JD, Lee YT, Lee IG [et al.]. Primary headaches in children and adolescents - experience at the only headache center in Korea. *BMC Neurol*. 2018;18:70. 10.1186 / s12883-018-1073-9

16. Bonvani IYa, Kalleso KKh, Janssens KAM [et al.]. Psychological interventions for children with functional somatic symptoms: a systematic review and meta-analysis. *J pediatrician*. 2017;187:272–281.

17. Işik U, Topuzoğlu A, Ay P [et al.]. The prevalence of headache and its association with socioeconomic status among schoolchildren in Istanbul, Turkey. *Headache*. 2009;49(5): 697-703. DOI: 10.1111/j.1526-4610.2009.01339.x.

Y. Akhverdyan, B. Zavodovsky, Y. Polyakova, L. Seewordova, E. Papichev

DIAGNOSTIC VALUE OF DETERMINATION OF CONCENTRATIONS OF BONE REMODELING MARKERS IN BLOOD SERUM OF PATIENTS WITH RHEUMATOID ARTHRITIS

DOI 10.25789/YMJ.2020.71.10

УДК616.72-002-031.13

The aim: to study the diagnostic value of determining the concentrations of bone remodeling markers in the blood serum of patients with rheumatoid arthritis (RA) by constructing ROC curves to predict the development of osteoporosis (OP).

Materials and methods. We observed 88 patients (all women) aged 21 to 81 years (mean age 47.51+9.63). The average disease duration was 7.92+5.21 months. The diagnosis of RA was established in accordance with the ACR/EULAR 2010 criteria. In all patients, the serum 25-OH level of vitamin D, the specific sequence of type I collagen, the products of type I collagen degradation by ELISA using commercial kits were determined.

Results. In the study of the diagnostic value of determining type I collagen C-telopeptide by the ROC-curve method, it was found that the determination of this marker in the diagnosis of OD in RA is of good quality (the area under the curve is 0.595), the diagnostic decision point is 0.988 ng/ml. A sensitivity of 36.36% and a specificity of 90.91% corresponds to this value. A study of the diagnostic value of P1NP showed that it is a valuable marker in the diagnosis of OP in RA (area under the curve of 0.785), the point of diagnostic decision for P1NP is 50.225 ng/ml. When using this cut-off point, the specificity of the method was 71.21%, sensitivity 81.82%. In the study of the diagnostic value of 25-OH vitamin D, it was found that the determination of this marker in the diagnosis of OD in RA is of good quality (the area under the curve is 0.696), the diagnostic decision point is 58.741 ng/ml. A sensitivity of 95.45% and a specificity of 37.88% correspond to this value.

Findings. Thus, these bone remodeling markers are of good quality and can be used to predict the development of OP in patients with RA.

Keywords: rheumatoid arthritis, osteoporosis, markers of bone remodeling.

Introduction. The pathogenesis of osteoporosis (OP) in rheumatoid arthritis (RA) is based on the imbalance between the processes of bone tissue creation and destruction [3]. Bone is a metabolically active structure that undergoes constant remodeling throughout life. Bone formation occurs with the help of osteoblasts. The newly formed bone contains an organic matrix, which consists mainly of collagen of the first type, which has undergone a mineralization process. The process of bone resorption is controlled by osteoclasts - large multinucleated cells that initially isolate one of the segments of the bone surface, forming a gap. Further, under the conditions of an acidified environment, the mineral component dissolves, and under the action of acidic proteases, the degradation of organic components, including collagen, occurs. Under optimal physiological conditions, bone resorption occurs after about 10 days, and bone formation takes about 3 months. Up to 20% of the skeleton, it can be replaced by remodeling every year [8].

Various biomolecules released into the blood circulation during resorption and bone formation are called markers of bone metabolism [2].

Pro-inflammatory cytokines (IL-1, 3, 6, 11, TNF-α, GM-CSF) contribute to the stimulation of bone resorption and are involved in the regulation of local and systemic inflammatory reactions. Chronic inflammation in RA is associated with overproduction of IL-1, TNF-α, IL-6 and a lack of synthesis of anti-inflammatory cytokines: IL-4, IL-10. A group of cytokines and their receptors, RANKL, RANK, and osteoprotegerin (OPG), are also involved in bone remodeling [1].

Important role in the development of OP has violation synthesis and metabolism of vitamin D. Vitamin D deficiency 25-OH process causes increased bone resorption due to bone formation disorders and negative calcium balance. Inhibition of T-lymphocyte activation and cell proliferation also occurs [7].

Markers of bone formation and markers of bone resorption are distinguished among markers of bone remodeling.

Type I N-terminal pro-peptide collagen (PINP) are peptides resulting from post-translational cleavage of type I collagen molecules with proteases at the N-terminus. PINPs come predominantly from proliferating osteoblasts and fibroblasts with little contribution from the skin, tendons, dentin, and cartilage. PINP is preferred for clinical use as a marker for bone formation. Initially, PINP is present in the form of trimeropeptides of 3 protein chains in type 1 collagen and is subsequently converted into monomeric forms in the bloodstream. Accordingly, analyzes can measure both monomeric and trimeric PINP (common PINP) or only trimeric PINP (intact PINP) [5, 6].

Collagen 1 C-terminal telopeptide is a degradation product of bone type 1 collagen generated by cathepsin K enzyme activity. The native type 1 collagen C-terminal telopeptide exists in two forms: α- and β-isomerized forms. These isomerized forms undergo further isomerization to form D and L forms. Spontaneous β-isomerization of α-isoforms occurs with

AKHVERDYAN Yuri Rubenovich – PhD in Medicine, Senior Researcher at the Laboratory of Methods for the Treatment and Prevention of Joint Diseases of the FSBI "RICER named after A.B. Zborovsky", address: 400138, Volgograd, Zemlyachki St., 76; phone (work): (8442) 789098, e-mail: doctor_2001@mail.ru. **ZAVODOVSKY Boris Valerievich** – Doctor of Medical Sciences, Deputy Director for Research, Federal State Budget Scientific Institution Scientific Research Institute of the FSBI "RICER named after A.B. Zborovsky", the head. laboratory of methods of treatment and prevention of joint diseases, address: 400138, Volgograd, Zemlyachki St., 76; tel. work: (8442) 78-90-93, e-mail: pebma@pebma.ru. **POLYAKOVA Julia Vasilievna** – PhD in Medicine, Researcher of the FSBI "RICER named after A.B. Zborovsky"; address: 400138, Volgograd, Zemlyachki St. 76, tel. slave: (8442) 78-90-93. **SEEWORDOVA Larisa Evgenievna** – PhD in Medicine, leading researcher at the laboratory of methods for the treatment and prevention of joint diseases of the of the FSBI "RICER named after A.B. Zborovsky"; address: 400138, Volgograd, 76 Zemlyachki St., tel. office: (8442) 78-90-93 address: 400138, Volgograd, 76 Zemlyachki St., tel. slave: (8442) 78-90-93. **PAPICHEV Evgeny Vasilievich** – Junior Researcher at the Laboratory for Methods of Treatment and Prevention of Joint Diseases of the FSBI "RICER named after A.B. Zborovsky"

protein aging. Therefore, the altered ratio of α- and β-isomerized forms occurs with the formation of a new bone, as in physiological conditions, such as growing children, and pathological conditions, such as rheumatic diseases.

Objective: to study the diagnostic value of determining the concentrations of bone remodeling markers in the blood serum of patients with rheumatoid arthritis by constructing ROC curves to predict the development of osteoporosis.

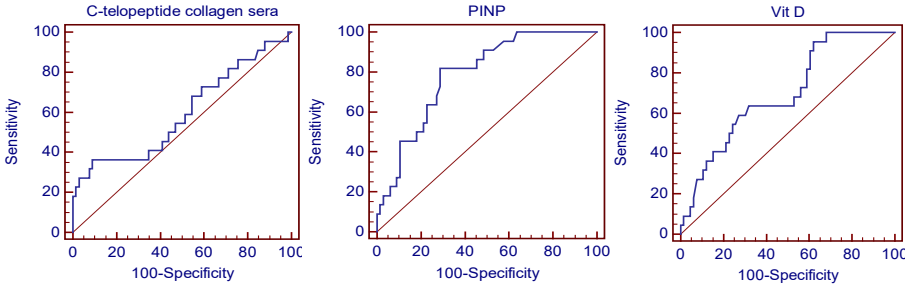
Materials and methods. We observed 88 patients (all women) aged 21 to 81 years (mean age 47.51+9.63). The average disease duration was 7.92+5.21 months. The diagnosis of RA was established in accordance with the criteria of ACR/EULAR 2010. After osteodensitometry, the diagnosis of OP was made to 22 patients, 66 patients did not show signs of OP.

Serum levels of 25-OH vitamin D in blood serum were determined in all patients by ELISA using commercial Vit D ELISA kits 25 (OH). The specific sequence of type I collagen in blood serum was determined by ELISA ELISA kit Procollagen I N-terminal Propeptide (PINP) SEA957Hu (CLOUD-CLONE CORP., USA). To determine CrossLaps in blood serum, a set of reagents was used to quantify the products of type I collagen degradation by ELISA using commercial Cross Laps ELISAKit kits (IDS, UK).

For statistical processing, the software package "STATISTICA 10.0 for Windows" was used. Assessment of the normality of the distribution was carried out by the Kolmogorov-Smirnov criterion. The significance of differences between the groups was compared using variation statistics (ANOVA) methods. The results were considered statistically significant at $p<0.05$.

Results. To analyze the diagnostic accuracy of laboratory tests, characteristic (ROC) curves were constructed with sensitivity (Se) and specificity (Sp) for each cut-off threshold from 0 to 1 in steps of 0.01. ROC-curves were evaluated by calculating the area under the curve (AUC – Area Under Curve), which varied from 0.5 (lack of diagnostic test effectiveness) to 1.0 (maximum test efficiency). This calculation allows us to conclude about the prognostic significance of the laboratory test [4].

Curves are shown for significantly significant laboratory tests in Figures 1-3. Data on the area under the curves, the points of the diagnostic decision (cut-off points) are presented in the tables below (tables 1-4).



ROC-curve characterizing the diagnostic value of determining the C-telopeptide of type I collagen (a), P1NP (b), 25-OH vitamin D (c) in the development of OP at RA

From table 1 it is seen that the area under the ROC curve (0.595) characterizes the good quality of the laboratory test of type I collagen C-telopeptide for the diagnosis of OP in RA. The optimal cut-off point of type I collagen C-telopeptide is 0.988 ng/ml. Based on the data in table 2, it is clear that for a given cut-off value, the specificity of the method is 90.91%, and the sensitivity is 36.36%.

By evaluating similar ROC curves, the diagnostic value of determining the serum N-terminal type I procollagen collagen (P1NP) and 25-OH vitamin D was also studied.

Table 3 shows that the area under the ROC curve (0.785) characterizes the good quality of the laboratory test P1NP for the diagnosis of OP in RA. The optimum cut-off point P1NP is 50.225 ng / ml. We determined that at a given cut-off value, the specificity of the method is 71.21%, and the sensitivity is 81.82%.

The area under the ROC curve (0.696) characterizes the good quality of the laboratory test of 25-OH vitamin D for the diagnosis of OP in RA (Table 4). By analogy with the above examples, it was calculated that the optimal value of the cut-off point of 25-OH vitamin D is 58.741 ng/ml, with this value of the cut-off point, the specificity of the method is 37.88%, and the sensitivity is 95.45%.

Findings. According to modern concepts, the determination of markers of bone remodeling is recommended before starting therapy of OP, as well as in dynamics to monitor the effectiveness of treatment. Also, the study of these markers is indicated when deciding whether it is necessary to prescribe repeated courses of therapy. A significant advantage is the non-invasiveness of this technique. In combination with the study of bone mineral density by the DXA method, the determination of markers of bone remodeling significantly increases the level of initial diagnosis and allows for earlier identification of patients with a higher risk of developing OP.

One of the generally accepted methods for assessing the diagnostic accuracy of laboratory tests is the construction of the ROC curve and the calculation of the area under the ROC curve (AUC). This model allows calculating the values of the sensitivity Se and the specificity of the Sp test.

Conclusions. When investigating the diagnostic value of type I collagen C-telopeptide by the method of constructing ROC curves, it was found that the determination of this marker in the diagnosis of OP at RA is of good quality (the area under the curve is 0.595). When analyzing ROC curves, the point of making a diagnostic decision was determined for the type I collagen C-telopeptide (0.988 ng/ml). A sensitivity of 36.36% and a specificity of 90.91% corresponds to this value.

A study of the diagnostic value of P1NP by constructing ROC curves showed that it is a valuable marker in the diagnosis of OD in RA (the area under the curve is 0.785). Based on the analysis of ROC

Key descriptive characteristics of the ROC curve

Index	Collagen type I C-telopeptide	P1NP	25-OH vitamin D
Area under the ROC curve ROC (AUC)	0.595	0.785	0.696
Standard error [a]	0.0765	0.0509	0.0624
95% confidence interval [b]	0.485 to 0.698	0.685 to 0.866	0.589 to 0.790
Z statistics	1.238	5.608	3.146
Significance Level P (Area = 0.5)	0.2159	<0.0001	0.0017

[a] DeLongetal., 1988, [b] Binomial accuracy

curves, a new diagnostic decision point was proposed for P1NP (50.225 ng/ml). Using this cut-off point, the specificity of the method was 71.21%, sensitivity 81.82%.

When examining the diagnostic value of 25-OH vitamin D by constructing ROC curves, it was found that the determination of this marker in the diagnosis of OD in RA is of good quality (the area under the curve is 0.696). When analyzing ROC curves, the point of making a diagnostic decision was determined for 25-OH vitamin D (58.741 ng / ml). A sensitivity of 95.45% and a specificity of 37.88% correspond to this value.

References

1. Дыдыкина И.С., Кожевникова П.О. Изучение локальной и генерализованной потери костной ткани у больных ревматоидным артритом. *Науч.-практич. ревматология.* [Dydykina IS, Kozhevnikova PO. The study of local and generalized bone loss in patients with rheumatoid arthritis. *Nauchno-prakticheskaya revmatologiya.* 2019; 57(3):328-332 (In Russ.).]
2. Carey JJ, Licata AA, Delaney MF. Biochemical markers of bone turnover. *Clin Rev Bone Miner Metab.* 2006; 4:197–212.
3. Clarke B. Normal bone anatomy and physiology. *Clin J Am Soc Nephrol.* 2008; 3:131–139.
4. Davis J, Goadrich M. The Relationship between Precision-Recall and ROC Curves. Proc. Of 23 International Conference on Machine Learning, Pittsburgh, PA, 2006
5. Koivula M.-K, Ruotsalainen V, Björkman M, Nurmenniemi S, Ikäheimo R., Savolainen K. et al. Difference between total and intact assays for N-terminal propeptide of type I procollagen reflects degradation of pN-collagen rather than denaturation of intact propeptide. *Ann Clin Biochem.* 2010; 47:67–71.
6. Garnero P, Vergnaud P, Hoyle N. Evaluation of a fully automated serum assay for total N-terminal propeptide of type I collagen in postmenopausal osteoporosis. *Clin Chem.* 2008; 54:188–96.
7. Li Y, Zhu J, Gao C, Peng B. Vitamin D receptor (VDR) genetic polymorphisms associated with intervertebral disc degeneration. *J Genet Genome.* 2015; 42(4):135–140.
8. Zaidi M. Skeletal remodelling in health and disease. *Nature Med.* 2007; 13:791–801.

O.S. Olifirova, A.A. Kozka, E.A. Volosenkova, L.K. Reshetnikova

MODERN COMPREHENSIVE APPROACH TO THE TREATMENT OF THE II DEGREE FROSTBITES

DOI 10.25789/YMJ.2020.71.11

УДК 617-089:616-08-031.81

Research objective. Purpose of the research is to study the results of the use of antioxidant therapy and hyperbaric oxygenation (HBO) in the complex treatment of patients with the II-degree frostbite.

Materials and methods. The main group (MG) consisted of 22 patients, the clinical comparison group (CCG) included 21 patients. Inclusion criteria: age - from 18 to 58 years; localization of frostbite: upper and lower extremities; duration of the wound process: from 10 days or more; terms of admission to the hospital from the moment of cold injury: from 6 to 24 hours. Exclusion criteria: frostbite of the III-IV degree. Patients of the main group received antioxidant therapy with the bioflavonoid dihydroquercetin and arabinogalactan (1:3) in the form of a biologically active supplement (BAS) orally and dihydroquercetin powder – topically on the wound surface. Simultaneously with antioxidant therapy, 10 HBO sessions were performed. CCG patients received conventional treatment.

Results. The wound process in MG patients with the II degree frostbites was more favorable than in CCG. The use of antioxidants and HBO allowed to reduce the intensity of lipid peroxidation processes and activate the antioxidant defense system, reduce the hyperproduction of pro-inflammatory interleukins (IL-1β, IL-6, Il-8, TNF-α) in patients of the main group. Due to the elimination of persistent inflammation and activation of reparative processes, the duration and intensity of pain in patients of the main group decreased, as well as the time for wound healing in comparison with CCG.

Conclusion. The use of antioxidant therapy and HBO is pathogenetically justified and has a positive effect on the course of the wound process in the II degree frostbites.

Keywords: frostbite, dihydroquercetin, hyperbaric oxygenation, wound process.

OLIFIROVA Olga Stepanovna – MD, associate Professor, head of the Department of surgical diseases of the Faculty of postgraduate additional education of the FSBEI HE of the Amur SMA of the Ministry of Health of Russia, olif.oc@mail.ru, tel: 89145544652. **KOZKA Alexandra Aleksandrovna** – Candidate of medical Sciences, assistant of the Department of surgical diseases of the Faculty of postgraduate additional education of the FSBEI HE of the Amur SMA of the Ministry of Health of Russia, kozka.a.89@mail.ru, tel: 89638488407. **VOLOSENKOVA Elena Alekseevna** – senior teacher of the Department of philosophy, history of the Fatherland and foreign languages of the FSBEI HE of the Amur SMA of the Ministry of Health of Russia, tel: 89246778541, **RESHETNIKOVA Larisa Konstantinovna** – Candidate of medical Sciences, assistant of the Department of faculty and outpatient therapy of the FSBEI HE of the Amur SMA of the Ministry of Health of Russia.

Introduction. Frostbite is one of the most common and severe types of thermal injury, since almost all people who come into contact with low temperatures are at risk. The specific weight of cold injury among all surgical diseases reaches 10% [7]. According to the Amur regional clinical hospital, those affected by the cold in 2018 averaged 9.8% of the total number of hospitalized patients. The problem of frostbite is the subject of various studies that reflect the issues of diagnosis and treatment at different stages of the course of cold injury [4,5,11].

In the wound process with frostbite, there are several periods: pre-reactive, early and late reactive; granulation, epithelization, scarring; long-term consequences. In addition, there are several degrees of damage depth (from the I to the IV degree) [8]. If timely assistance in the pre-reactive period gives a good therapeutic effect, then in the late reactive period, doctors have to deal with the formed necroses and their spread to the surrounding tissues, the addition of microbial infection, sepsis and multiple organ failure [4, 10]. In the early reactive period, treatment problems are mainly caused by the depth of penetration of the cold agent. With frostbites of the II degree, there is a necrosis of the corneal and granular layers of the epidermis, an increase in edema with the appearance of blisters filled with transparent exudate. Wounds are characterized by significant pain when touched, there is stiffness in the interphalangeal joints, increased skin sensitivity [8]. The main task in the treatment of the II degree frostbites is to improve microcirculation, prevent blood clots in the area of damaged tissues and prevent their necrosis, as well as activate regenerative and reparative processes.

Natural epithelization of wounds must occur as a result of adequate treatment of this group of victims.

One of the aspects of studying the molecular basis of the wound process in thermal lesions is the study of the role of free radical reactions of lipid peroxidation (LPO). It was found that the effect of low temperatures on the body of experimental animals is accompanied by activation of free-radical processes leading to protein denaturation and inactivation, disruption of cell division and growth [9]. In this regard, the search for modern methods of treatment of frostbite that pathogenetically contribute to the correction of LPO is relevant.

To achieve this goal, antioxidants can be used as inhibitors of LPO processes and activators of reparative regeneration processes [6]. They include a natural antioxidant, the bioflavonoid - dihydroquercetin (Taxifolin), obtained from the wood of the Dahurian larch (Gmelin larch) [1,3].

In addition, one of the methods that activates regenerative-reparative processes is hyperbaric oxygenation (HBO). The therapeutic effect of HBO is associated with the activation of cascades of biochemical reactions that lead to the formation of growth factors, cytokines and other substances involved in wound healing. It was found that HBO promotes angiogenesis and collagen synthesis in the wound, and increases the effectiveness of antibacterial therapy [2].

Taking into account the above, it seems appropriate to use a modern approach with the inclusion of antioxidant therapy and HBO in the complex treatment of the II degree frostbites.

Research objective. Purpose of the research is to study the results of the use of antioxidant therapy and hyperbaric oxygenation (HBO) in the complex treatment of patients with the II degree frostbites.

Materials and methods. The analysis of treatment results of 43 patients aged 18 to 58 years with the II degree frostbites who were treated in the burn Department of the Amur regional clinical hospital was carried out. There were 32 (74.4%) men and 11 (25.6%) women. Localization of frostbites: upper extremities - in 13 patients (30.2%), lower extremities – in 30patients (69.8%). The time of admission to the hospital from the moment of cold injury ranged from 6 to 24 hours.

For a comparative analysis of treatment results, two groups were identified from the total number of patients. The main group (MG) included 22 patients who received antioxidant therapy and HBO. Patients (21) of the clinical comparison group (CCG) received conventional treatment. The complex of therapeutic measures in both groups included warming, vessels' dilating, antibacterial and symptomatic therapy. Inclusion criteria: the II degree frostbites, age - from 18 to 58 years. Exclusion criteria: the III-IV degree frostbites. The MG and CCG patients are comparable by gender, age, and degree of lesion.

Complex treatment of the MG patients was as follows: after a thorough cleaning of the wound with the removal of the detached epidermis and treatment with an antiseptic (0.5% solution of chlorhexidine bigluconate), bandages with dihydroquercetin powder were applied every other day until the wound was completely healed. It was applied to the wound surface with a microbial contamination of no more than 103-4 of m. b. per 1 cm2 of 1-2 mm layer. Atraumatic coating "parapran" was placed over the wound. At the same time, HBO of 1.5-1.8 atmospheres lasting 40 minutes daily for 10 days was performed and oral intake of biologically active supplements (BAS) containing dihydroquercetin and arabinogalactan (1:3), 1 capsule 2 times a day with meals daily for 10 days was prescribed. The results were analyzed according to indicators of the course of the wound process, clinical data, the level of pro-inflammatory interleukins (IL-1β, IL-6, IL-8, TNF-α), indicators of LPO and antioxidant protection (AOP) on the first and the 10th day of treatment.

The activity of LPO processes was assessed by the content of diene conjugates (DC) and malondialdehyde (MDA) in the blood. The content of DC and MDA was determined in lipid extracts from blood serum spectrophotometrically on the basis of their ability to absorb light in the ultraviolet part of the spectrum.

The state of AOP was studied by the quantitative content of vitamin "E" and ceruloplasmin in the blood serum. The content of ceruloplasmin in blood serum was measured photoelectrocalorimetrically. The content of vitamin "E" was determined in lipid extracts from blood serum by color reaction with dipyridyl and FeCl3.

Interleukins: IL-1β, IL-6, IL-8, and TNF-α were studied by IEA using standard sets of reagents from "Vector-best" (Novosibirsk, Russia). As normal values of interleukins, the control parameters attached to the set of reagents from "Vector-best": IL-1β – 0-11 pg/ml, IL-6 – 0-10 pg/ml, IL-8 – 0-10 pg/ml, TNF-α - 0 – 5pg/ml were used.

Statistical analysis was performed using descriptive statistics. The findings were checked for the normality of the distribution visually using the histogram and the Kolmogorov-Smirnov test. The quantitative parameters with normal distribution are given as an arithmetic mean (M) and average error (m); in cases where the distribution differed from the normal – in the form of median (Me) and inter-quartile range (25th lower quartile and 75th lower quartile). The T-Student criterion was used to analyze differences in normal distribution and data. If the distribution was significantly different from normal, nonparametric methods were used: for comparing independent samples- the Whitney – Mann test, for analyzing repeated changes - the Wilcoxon test, and the sign test. The significance of differences in quality indicators was determined using the $\chi 2$ criterion and the two-sided Fisher exact method for a four-field table. The degree of difference was considered significant at $p < 0.05$.

Results and discussion. Analyzing the results of the study, a smoother course of the wound process was noted in patients of the MG compared to CCG. The duration of the pain syndrome on the VAS scale in the MG was 5.7±0.4 cm, and the duration of the temperature reaction was 3.4±0.3 days, which is less than in the CCG - 8.1±0.5 cm and 6.5±0.3 days, respectively ($p < 0.05$).

In patients of the MG who received antioxidant therapy and HBO, earlier periods of epithelization (2.3±1.3 days) and complete wound healing by natural epithelization (11.8±1.7 days) were observed than in CCG (4.3±1.6 days and 18.9±2.1 days, respectively; $p < 0.05$). The dynamics of the wound process in the MG patients is shown in figures 1 and 2: before treatment (Fig. 1) and after treatment (Fig.2).

Indicators of LPO (DC, MDA) and AOP (vitamin "E", ceruloplasmin) at the beginning of treatment in the MG and CCG did not have significant differences.

However, by the 10th day of treatment there was a significant decrease in the content of LPO products in the MG patients in comparison with those of the CCG. DC values in blood serum decreased by 7.7 % (from 43.3 ± 0.7 nmol/ml to 40 ± 0.6 nmol/ml; $p < 0.01$), MD – by 25.7 % (from 6.2 ± 0.2 nmol/ml to 4.6 ± 0.1 nmol/ml; $p < 0.01$), while in the CCG DC – by 2.2 % (from 43.3 ± 0.5 nmol/ml to 42.4 ± 0.5 nmol/ml; $p = 0.27$), MD – by 4.9% (from 6.0 ± 0.1 nmol/ml to 5.7 ± 0.1 nmol/ml; $p = 0.17$).

In the same period of time, the content of the AOP components increased significantly more in the MG than in the

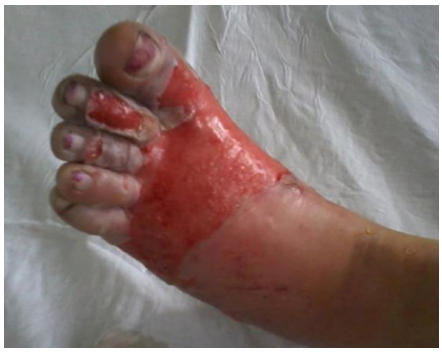


Fig. 1. Frostbite of the left foot of the II degree 1 day after injury.

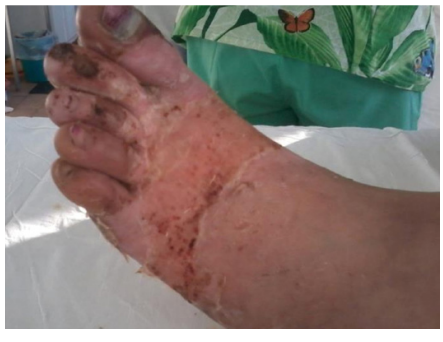


Fig. 2. Frostbite of the left foot of the II degree on the 11th day after the injury during treatment with antioxidants and HBO.

CCG. In the MG patients, the content of vitamin "E" in the serum increased by 17.3% (from 19.7 ± 0.3 mg/100 ml to 23.1 ± 0.3 mg/100 ml; p <0.01) and ceruloplasmin – by 14.8% (from 19.7 ± 0.3 mg/100 ml to 23.1 ± 0.5 mg/100 ml; p<0.01), and in the CCG – only by 4.2 % (from 41.9 ± 0.8 mg/ml to 43.7 ± 0.3 mg/ml; p = 0.37) and 1.1% (from 20.2 ± 0.3 mg/100 ml to 20 ± 0.2 mg/100 ml; p = 0.68), respectively.

The obtained data indicate the activation of pro-inflammatory interleukins in the blood of patients with frostbites of the II degree in both groups at the time of admission to the hospital compared with normal indicators. High levels of IL-1β were found in the MG - 25.8 times to 130.7 [13.9; 216.7] pg/ml at the norm <5 pg/ml; IL-6 – 28.7 times to 122.4 [89.9; 213.7] pg/ml at the norm <4.1 pg/ml; IL-8 – 3.4 times to 202.9 [67.7; 267] pg/ml at the norm <62 pg/ml and TNF-α – 4.5 times to 35.4 [13.7; 67] pg/ml at the norm <8.2 pg/ml.

In the CCG patients with frostbites of the II degree the following indices were noted: the increase in the level of IL-1β - 23.8 times to 124 [17; 292] pg/ml with normal <5 pg/ml; IL-6 – 27.2 times to 115.6 [35; 216,7] pg/ml at norm <4,1 pg/ml; IL-8 – 3.8 times to 230.4 [60,2; 325,6] pg/ml at norm <62 pg/ml and TNF-α – 4.9 times to 41.9 [14; 79,6] pg/ml when normal is <8,2 pg/ml.

In the course of complex treatment with the use of antioxidants and HBO, a significant decrease in the levels of IL-1β, IL-6, Il-8, and TNF-α was observed in patients of the MG by the 10th day of treatment. In the MG, the serum level of IL-1β decreased by 41.7% (from 130.7 [13.9; 216.7] pg/ml to 77.4 [8.0; 115.8] pg/ml; p <0.01); IL-6 – by 44.5 % (from 122.4 [89.9; 213.7] pg/ml to 66.8 [12.9; 100] pg/ml; p <0.01); IL-8 – by 34.6% (from 202.9 [67.7; 267] pg/ml to 130.7 [54.6; 228.6] pg/ml; p <0.01) and TNF-α – by 54.7 % (from 35.4 [13.7; 67] pg/ml to 16.2 [6.7; 48.9] pg/ml; p <0.01). In contrast to the significant dynamics of interleukin indicators in the MG, the level of pro-inflammatory interleukins (IL-1β, IL - 6, IL-8, TNF-α) in the CCG patients did not change significantly on the 10th day of treatment.

Due to the more active course of reparative processes, the average duration of the inpatient stage of treatment in the MG was 12.0 ± 0.4 days compared to the CCG-20.7±0.3 days.

The conducted research has shown the effectiveness of using antioxidant therapy and HBO in the complex treatment of patients with the II degree frostbites in comparison with conventional treatment. Excessive and prolonged hyperproduction of pro-inflammatory interleukins (IL-1β, IL-6, IL-8, TNF-α) maintains a state of persistent inflammation, which slows down reparation in the wound. This is also facilitated by the intensity of LPO processes. The use of the proposed method allowed to reduce the level of pro-inflammatory interleukins and LPO products, as well as to increase the activity of AOP, contributing to the activation of reparative processes. Thus, the use of antioxidant therapy (dihydroquercetin powder and dietary supplements containing dihydroquercetin and arabinogalactan) with HBO in the complex treatment of the II degree frostbites is pathogenetically justified and is accompanied by a significant anti-inflammatory effect. As a result of treatment, there was a reduction in the time of wound healing and the length of the MG patients' stay in the hospital by 1.7 times.

Conclusions

1. The use of antioxidant therapy with dihydroquercetin and HBO in the complex treatment of the II degree frostbites is pathogenetically justified and has a favorable effect on the course of the wound process.
2. Due to the activation of reparative processes, the time of the hospital treatment of patients with the II degree frostbites was reduced by 1.7 times.

References

Доровских В.А., Целуйко С.С., Доровских Ю.В. и др. *Антиоксиданты в профилактике холодового стресса и перегревания.* Благовещенск: ГБОУ ВПО Амурская ГМА; 2014:124. [Dorovskikh V.A., Tseluiko S.S., Dorovskikh Yu.V. et al. *Antioksidanty v profilaktike kholodovogo stressa i peregrevaniya.* Blagoveshchensk: FSBEI HPE, the Amur SMA;2014:124. (In Russ.).]

2. Багаев В.Г., Сергеева В.В., Боброва А.А. и др. Гипербарическая оксигенация в комплексной терапии ран у детей. *Раны и раневые инфекции.* 2014;1(2):31-37. [Bagaev V.G., Sergeeva V.V., Bobrova A.A. et al. Hyperbaric oxygenation in complex therapy of wounds in children. *Rany i ranevyye infektsii.* 2014;1(2):31-37. (In Russ.).]

3. *Лавитол (дигидрокверцетин)* [Электронный ресурс]. Режим доступа: https://www.ametis.ru/production/lavitol-dihydroquercetin, свободный. Загл. с экрана. Яз. рус. [*Lavitol (Digidrokvertsetin*) [electronic resource]. Access mode: https://www.ametis.ru/production/lavitol-dihydroquercetin, free. Title from the screen. (In Russ.).]

4. Гостищев В.К., Липатов К.В., Бородин А.В. и др. Лечебная тактика при отморожениях. *Хирургия. Журнал им. Н.И. Пирогова.* 2010;(6):10-15. [Gostishchev V.K., Lipatov K.V., Borodin A.V. et al. Therapeutic tactics in frostbites. *Khirurgiya. Zhurnal im. N.I. Pirogova.* 2010;(6):10-15. (In Russ.).]

5. Алексеев Р.З., Томский М.И., Гольдерова А.С. и др. Предупреждение развития некроза при отморожениях с оледенением тканей. *Международный журнал прикладных и фундаментальных исследований.* 2015;8(1):35-41. [Alekseev R.Z., Tomskiy M.I., Golderova A.S. et al. *Mezhdunarodnyy zhurnal prikladnykh i fundamental'nykh issledovaniy.* 2015;8(1):35-41. (In Russ.).]

6. Мельникова Н.Б., Сидорова М.В., Малыгина Д.С. и др. Разработка и исследование гидрофильных гелей с фитиновой кислотой и ксимедоном для лечения ожоговых ран в эксперименте на крысах. *Медицинский альманах.* 2014;3(33):165-169. [Melnikova N.B., Sidorova M.V., Malygina D.S. et al. Development and research of hydrophilic gels with phytic acid and xymedon for the treatment of burn wounds in an experiment on rats. *Meditsinskiy al'manakh.* 2014;3(33):165-169. (In Russ.).]

7. Шакиров Б.М. Тагаев К.Р. Наш опыт лечения местной холодовой травмы конечностей. *Вестник экстренной медицины.* 2017;X(1):29-32. [Shakirov B.M. Tagaev K.R. Our experience in the treatment of local cold injury of extremities. *Vestnik ekstrennoy meditsiny.* 2017;X(1):29-32. (In Russ.).]

8. Шаповалов К.Г. Отморожения в практике врача анестезиолога-реаниматолога. *Вестник анестезиологии и реаниматологии.* 2019;16(1):63-68. [Shapovalov K.G. Frostbites in the practice of an anesthesiologist-resuscitator. *Vestnik anesteziologii i reanimatologii.* 2019;16(1):63-68. (In Russ.).]

9. Bhattacharya S. Reactive oxygen species and cellular defense system. Free radicals in human health and disease. 2015;17-29.

10. Hassi J., Lehmuskallio E., Juhani J. et al. Frostbite and other problems of skin expose to cold. Duodecin. 2005;121(4):454-461.

11. McIntosh S.E., Opacic M., Freer L. et al. Wilderness medical society practice guidelines for the prevention and treatment of frostbite: 2014 update. Wilderness Environ Med. 2014;25(4):43-54.

B.M. Gasanova, M.L. Polina, N.I. Douglas, F.I. Chotchayeva

AN AUDIT OF METABOLISM FEATURES AMONG PREGNANT WOMEN WITH IRON DEFICIENCY AND ANEMIA OF CHRONIC DISEASES

DOI 10.25789/YMJ.2020.71.12

УДК 61.618.2

The article presents the results of studies in a sample of pregnant women with anemia of various genesis. Differences in the development of iron deficiency – true and functional – have been established, the sequestration of which in macrophages is associated with a chronic inflammatory or autoimmune process in the body of a pregnant woman.

Anemia of chronic diseases (ACD) was characterized by the activity of acute phase inflammation reactions. Placental dysfunction resulted in large perinatal morbidity in the group with ACD compared to IDA due to significant metabolic changes. The low activity of adaptive mechanisms should be considered as a consequence of chronic hypoxia on the background of metabolic features in "infect anemia" - chronic immunostimulation determined by the cytokine production vector according to the Th-1 variant.

Keywords: iron deficiency anemia, anemia of chronic diseases, perinatal outcomes, oxidative stress.

Introduction. Anemia is a global health problem affecting nearly 529 million women of reproductive age, including 38% of all pregnant women [34].

Iron deficiency (ID) is one of the most significant points at issue in the spectrum of micronutrient deficiency and the leading cause of anemia in the world [13].

The statement that programming of long-term fetal health is determined by the nutritional status of the mother confirms the significant role of iron and other essential micronutrients in ensuring the processes of growth, development and metabolism of the placenta [20].

Iron is an important mineral for maintaining cellular homeostasis and a cofactor of biological reactions. It is responsible for electron transfer with the formation of divalent (Fe 2+) and trivalent (Fe 3+) forms, oxygen transport, deoxyribonucleic acid (DNA) synthesis, and ATP formation [11].

In the human body, iron exists in complex forms connected to a protein (hemoprotein) in the form of heme compounds (hemoglobin or myoglobin), heme enzymes, or non-heme complexes [4].

The needs of pregnant women for iron increase sharply: up to a gram is required for the development and functioning of the placenta and fetal growth [14]. The perinatal ID (iron deficiency) consequences in the periconceptual period are associated with a violation of the regulatory mechanisms of transport – embryonic and placental, and understanding of the genesis of anemia [30].

The effectiveness of anemia treatment deals with an understanding of ID genesis –true in IDA or functional, in which changes in homeostasis are connected to infectious, inflammatory or autoimmune diseases [7, 16].

Anemia of chronic diseases (ACD) is associated with impaired proliferation of the erythroid sprout on the background of acute phase inflammation reactions, increased C-reactive protein (CRP), excessive production of pro-inflammatory cytokines [26].

ID in urogenital and inflammatory diseases of the genitourinary system corresponds to the term "infect anemia" [1].

New data on complex iron homeostasis indicate the need to clarify the features of not only ferrokinetics, but also metabolism associated with the presence of chronic and infectious inflammatory diseases.

The ferritin-controlled regulation of iron release acts as a molecular-cellular defense mechanism in case of deficiency and excess [8,21]. The content of ferritin, a globular complex of 24 protein subunits in the plasma of less than 15 mcg/ l shows the depletion of iron reserves [36,37].

Ferritin is considered to be an endogenous antioxidant due to its ability to sequester potentially toxic labile iron [9].

The need to provide oxygen to the placenta and the growing fetus results in the development of oxidative stress (OS) [27].

The loss of physiological balance between the generation of oxidative types (malondialdehyde) and the antioxidant potential of blood serum (catalase, glutathione peroxidase, thiol compounds) is accompanied by an "oxidative explosion" [6]. An excess of free radicals is associated with defective enzymes of the antioxidant system and damage to the elimination mechanisms of toxic metabolites in chronic inflammatory diseases, obstetric complications [31,33].

ID causes the risk of cell-membrane disorganization, contributes to the oxidative damage to red blood cells and a feto-placental unit, the development of infectious and inflammatory processes [15].

The intrauterine development of the fetus in ID is programmed by the structural and functional failure of the placenta, the villous tree and the peculiarities of vascularization [17].

High perinatal morbidity is caused by the decrease in iron bioavailability in the bloodstream of mothers with anemia and the violation of its transfer through the placenta to the fetus [25].

Anemia is associated with intrauterine growth retardation, preterm birth (PB), low birth weight, the risk of maternal and prenatal mortality, decreased cognitive abilities and impaired psychomotor development of newborns [23].

The probability of adverse outcomes for the fetus with low maternal hemoglobin (<110 g / l) is shown in a review that included 272 studies, a meta-analysis of 95 studies, as well as for the mother (postpartum hemorrhage, pre-

GASANOVA Bakhtykei Musalavovna – PhD in Medical Sciences, Doctoral Candidate of the Department of Obstetrics and Gynaecology with a course in Perinatology of the Medical Institute of the Federal State Autonomous State Educational Institution of Higher Education «People's Friendship University of Russia», Moscow, Russia, e-mail: gsbhms69@gmail.com, **POLINA Miroslava Leonidovna** – PhD in Medical Sciences, Gynecologist at the Women's Health Medical Center, Moscow, Russia, e-mail: polina.ml@mail.ru, **DOUGLAS Natalya Ivanovna** – Grand PhD in Medical Sciences, Head of the Department of Obstetrics and Gynecology, Medical Institute of North-Eastern Federal University, Yakutsk, Russia, e-mail: nduglas@yandex.ru, **CHOTCHAYEVA Fatima Ismailovna** – Head of the Republican State Budget Medical Treatment and Prevention Institution "Republican Perinatal Center", Cherkessk, Russia, e-mail: asiyat1967@mail.ru

eclampsia and blood transfusion) [24].

Chronic hypoxia of pregnant women with anemia is connected to the development of a stress reaction leading to excessive production of corticotropin-releasing hormone, which acts as a trigger for PB. Prematurity and low birth weight were more often observed with pregnant hemoglobin levels of less than 7 g/dl [29].

The effect of the severity of anemia and the duration of ID manifestation on pregnancy outcomes is debated [5]. The development of anemia in the third trimester is associated with a lower frequency of prematurity, low weight, including for the gestational period [18].

Data on the influence of features of iron homeostasis and an oxidative blood profile are promising from the point of predicting perinatal risks.

Objective of the study: to assess the nature of iron metabolism disorders and the oxidative blood profile of pregnant women with anemia, their effect on perinatal outcomes.

Materials and methods. 310 pregnant women with anemia (hemoglobin less than 110 g / l, serum iron less than that) were examined: group I – with IDA (n = 108), II – with ACD (“infect anemia”) (n = 202). The control group included 34 women with physiological pregnancy.

Inclusion criteria: singleton progressive pregnancy, the presence of anemia before pregnancy.

The women’s informed consent to use biological material for scientific purposes was obtained.

At 14-16 weeks of gestation, a total blood count, transferrin, ferritin (ELISA-ferritin test system (St. Petersburg)), total blood protein, serum iron (Iron (Fe) rx-series, “RANDOX”test system), were evaluated in a sample of women with anemia.

The distribution of anemia types was carried out on the basis of the level of acute phase proteins – C-reactive protein (CRP), ferritin.

The oxidant blood level was evaluated using test systems (malondialdehyde (MDA), erythrocyte and serum catalase, ceruloplasmin, sulfhydryl groups (SH-groups)) .

The cytokine profile of pregnant women with anemia was evaluated based on the ratio of pro-inflammatory interferon-gamma (IFN-γ) to interleukin-4 (IL-4).

Statistical processing of the results was carried out using the program Statistica 10.0 (manufacturer StatSoft Inc., USA). The sample was checked for compliance with the normal distribution using the Shapiro-Wilk test. Methods of descriptive statistics included: estimation of the arithmetic mean (M), average error of the mean (M) —for features that have a continuous distribution; frequency of features with discrete values. Student’s t-test was used to identify differences in features having a continuous distribution. An analysis of intergroup differences in terms of quality was carried out using the χ2 criterion; less than five – the accurate two-sided Fisher test. The significance level (p) when testing statistical hypotheses was taken p≤0,05.

Results and discussion. The average age of pregnant women with ACD was 27.4 ± 3.4 years, IDA – 26.8 ± 3.7 years, without significant intergroup differences. Moderate anaemia found in 228 pregnant women (73.5%), average – 82 (26.5%).

The anamnesis of pregnant women with ACD was characterized by a predominance of sinusitis and upper respiratory tract diseases (47.0% versus 12.9%, p = 0.0005), lower bronchopulmonary system diseases (27.2% and 6.5%, p = 0.0006), kidney diseases (33.2% and 12.9%, p = 0.0008). Inflammatory diseases of the uterus and appendages were detected among almost half of pregnant women with ACD and a quarter with true iron deficiency (p = 0.0009).

Belonging of pregnant women to the category with a high infectious risk was determined by the exacerbation of chronic extragenital diseases (chronic pyelonephritis, bronchopulmonary diseases) – 3.5 times (p = 0.0005), gestational pyelonephritis – 3 times (p = 0.005). Sonographic markers of intrauterine infection of the fetus and premature maturation of the placenta among pregnant women with ACD were noted almost three times more often than with IDA (16.3% versus 6.0%, p <0.05). Episodes of acute respiratory viral infections (ARVI) (with a temperature rise), reactivations of chronic ones (TORCH) were observed among 30.2% of pregnant women with ACD, three times less often (9.2%) – in IDA (p = 0.0006). Urogenital infections (ureaplasma, chlamydia, herpes) were found in a quarter of pregnant women with ACD and 12.0% IDA (p = 0.007).

A biochemical blood test showed compliance ferritin with reference values among 17.8% of pregnant women with ACD, an increased rate among 82.2%.

The level of serum iron in the group with true iron deficiency was 8.2 ± 2.6 μmol/l, ACD – 11.8 ± 2.8 μmol/l.

Markers of acute phase inflammation reactions were an increase in CRP, changes in the leukocyte formula (lymphocytosis and / or monocytosis, an increase in ESR) among 67.8% of pregnant women with “infect anemia”.

The ratio of INF-γ/IL-4 was increased (0.73±0.03) among 44.6% of women with ACD (1.55±0.16/2.11±0.2), corresponding to the upper limit of the norm – 55.4% (0.68±0.04) (1.48±0.11/2.18±0.2). Among pregnant women with IDA, the INF-γ/IL-4 index (1.48±0.2/2.46±0.2) made up 0.6±0.04.

Pregnant women with IDA were distinguished by a low ferritin level (less than 30 mcg / l) and an increased transferrin level (2.4±0.6 g/l), in contrast to the other groups (ACD (1.5±0.3 g/l)(p<0.05) and pregnant women with FP (2.7±0.3) (p<0.05). The content of total protein in anemia was reduced, to a greater extent in the group with high CRP (70.8±3.6 g/l и 67.4±2.6 g/l, p<0.05).

A study of the pro- and antioxidant activity of pregnant women with anemia, the results of which are presented in Table 1, showed an increased production of free radicals in the group with ACD compared to IDA.

Inhibition of erythropoiesis in ACD was accompanied by excessive activity of lipid peroxidation (LPO) – MDA (p<0.05) on the background of a decrease in the antioxidant potential (plasma catalase and red blood cells, sulfhydryl groups) (p<0.05).

Pregnant women with IDA were characterized by an increased level of MDA with a moderate increase in antioxidant defense markers (plasma and red blood cells catalase, sulfhydryl groups). The content of ceruloplasmin, capable of duality in peroxidation reactions, did not differ significantly in groups.

Caesarean section (CS) delivery was performed among 13.0% of women with anemia. The PB frequency among pregnant women with ACD was one and a half times higher than with IDA. Childbirth at 26-28 weeks and 29-33 weeks was 4.2% and 4.6%, respectively. Delivery at 34-37 weeks was performed among 15.8% of pregnant women with ACD, 6.5% with true iron deficiency (p = 0.03).

PB was more common in the group with ACD than IDA in the period of 29-33 weeks. The relationship between adverse perinatal outcomes and anemia was significant taking into account the manifestation of the disease from an early stage / pre-existing pregnancy.

The predominance of PB at a hemoglobin level of pregnant women less than 110 g / l is 2.75 times higher compared to the normal indicator, which was also noted by other authors [22].

Perinatal dysfunction was determined by the worst anthropometric parameters of newborns from mothers with ACD in

comparison with IDA (2970 ± 280 g / l and 2850 ± 140 g / l), but without significant differences. The relationship of low birth weight in anemia of mothers from the first trimester was marked by other authors too [10]. The decrease in body weight and length of newborns from anemic mothers is associated with a change in the metabolism of iron-containing enzymes, which is consistent with observations of a decrease in the brain of mice fetuses with ID, their length and weight [12, 17].

Perinatal outcomes in moderate anemia of pregnant women (Hb <100 g / l) differed in a higher frequency of undermaturity (2 times, 17.1% versus 8.8%, p = 0.04) and small gestational age (2 times, 36.6% versus 17.9%, p = 0.001), compared with pregnant women with mild anemia, similar to other authors [32].

20.8% of women with ACD and 14.8% with IDA had low Apgar scores for newborns ("6 points") in the first minute after birth, but without significant differences. A "7-point" mark was found among one third of all newborns and 18.9% - in physiological pregnancy, "8-point" – one and a half times more often with ACD (27.7% versus 16.7%, p = 0.04).

Perinatal consequences of chronic hypoxia among pregnant women with anemia (100% placental insufficiency in ACD and 64.8% IDA) were reflected by a 7-point mark among 18.9% of newborns, an 8-point mark was given among 53.1% on average. Reduction in Apgar scores for maternal anemia was also reported by other authors [19].

The number of newborns with 9-10 points remained unchanged: in the first minute – twice as many mothers with IDA compared to ACD (37.9% vs 19.3%, p = 0.001), the fifth – without significant intergroup differences (31.5% and 22.3%, p> 0.05).

In newborns from mothers with ACD, there is an increase in indicators of infectious and inflammatory morbidity (omphalitis, dacryocystitis, vesiculopustulosis, conjunctivitis) in comparison with IDA - 2.7 times (14.8 and 5.5%, respectively, p = 0.02), morphofunctional immaturity - 2 times (32.7 and 15.7%, respectively, p = 0.003).

Cerebral damage in the outcome of chronic fetal hypoxia was observed in almost a third in the group with ACD and half as much – in IDA group (29.7% и 16.7%, p = 0.02). Hypotrophy was noted among 21.8% of newborns, 8.9% were premature.

High perinatal morbidity of women with anemia should be considered as a consequence of uterine angiopathy followed by pregnancy, which determined placental ischemia from early pregnancy [2, 3, 35]. Hypoxic damage to the brain of newborns from mothers with ID is probably due to an imbalance in the processes of absorption, redistribution and consumption of trace elements.

Iron deposition with the development of functional deficiency acts as a protective measure for a pregnant woman with "infect anemia" in a competition for a micronutrient resource with pathogens. The peculiarities of iron metabolism among pregnant women with ACD were revealed in violation of the proliferation of erythroid progenitors on the background of index vectorization according to the Th-1 variant (INF-γ / IL-4 ratio) and increased CRP values.

The detection of acute phase inflammation reactions with iron sequestration in macrophages corresponded to chronic immunostimulation in case of persistent infection or autoimmune process in the pelvic organs of pregnant women with ACD.

Hypoxia caused by "infect anemia" contributed to the development of a systemic inflammatory reaction with excessive production of free radicals on the background of iron-containing antioxidant enzymes failure.

The degree of placental dysfunction in pregnant anemia reflects the degree of adaptive capabilities.

Metabolic reactions of the placenta and fetal tissues to hypoxia and OS are caused by different activity of compensatory mechanisms aimed at maintaining optimal mitochondrial respiration.

The decrease in the oxygenation of the placenta of pregnant women with anemia is determined by varying degrees of OS and trophic disorders.

Low compensatory-adaptive reactions among pregnant women with ACD were connected to the presence of chronic immunostimulation due to high infectious potential, high endointoxication and limited antioxidant reserves.

Lower perinatal morbidity in IDA is due to the early implementation of diagnostic and treatment measures, compensation for dysmetabolic disorders and restoration of iron homeostasis.

The degree of placental dysfunction in pregnant anemia reflects the degree of adaptive capabilities. Metabolic reactions of the placenta and fetal tissues to hypoxia and OS are caused by different activity of compensatory mechanisms aimed at maintaining optimal mitochondrial respiration.

The data obtained suggest that there are causal relationships between anemia of pregnant women and adverse outcomes of pregnancy, the greater frequency of which during ACD is due to significant dysmetabolic shifts.

Conclusion. Maladaptive shifts and the degree of placental dysfunction among women with anemia go along with the activity of markers of persistent inflammatory process with varying degrees of oxidative damage.

The decrease in compensatory and adaptive resources, adversely affecting ante- and perinatal well-being, was more vivid among pregnant women with ACD.

Perinatal consequences among pregnant women with anemia reflect the necessity to expand the scope of examination in groups with high infectious risk (cytokine and oxidant profiles, proteins of acute phase inflammation reactions) and the effectiveness of compensation for hypoxic disorders of the fetoplacental complex.

Pro- and antioxidant factors in samples of pregnant women with anemia of various genesis

Parameters of an oxidatic profile	Iron deficiency anemia (n=108)	Anemia of chronic diseases (n=202)	Healthy pregnant women	p
Malonic dialdehyde, μmol/l	1.43 ±0.1	1.78 ±0.1	1.2 ± 0.4	p_{1-3}=0.003 p_{2-3}=0.02
Erythrocyte catalase, mkat/l	72.6 ± 1.5	88.2 ±3.8	74.7 ± 0.9	p_{1-3}=0.005 p_{2-3}=0.02
Serum catalase, mkat/l	16.4 ± 1.6	28.6 ± 2.7	15.7 ± 0.5	p_{1-3}=0.003 p_{2-3}=0.005
Ceruloplasmin, mg/l	376.3±11.2	393.5±13.6	366.9± 13.5	p>0.05
SH-groups, mmol/l	12.6±0.5	18.1±1.6	12.4±0.4	p_{1-3}=0.002 p_{2-3}=0.04

References

1. Рукавицын О.А. Актуальные вопросы диагностики и лечения анемии при хронических заболеваниях. *Клиническая онкогематология.* 2012; 5(4): 296–304. [Rukavitsyn OA. Topical issues of diagnosis and treatment of anemia in chronic diseases. *Klinicheskaya onk-*

ogematologiya. 2012; 4 (5): 296–304. (In Russ.)].

2. Радзинский ВЕ, Оразмурадов АА. Беременность ранних сроков. От прегравидарной подготовки к здоровой гестации. 3-е изд., испр. и доп. М., Status Praesens. 2018; 800 с. [Radzinskij VE, Orazmuradov AA. Pregnancy of early terms. From pregravid preparation to healthy gestation, 3rd ed., M., Status Praesens. 2018; 800. (In Russ.).]

3. Апресян С.В. Беременность и роды при экстрагенитальных заболеваниях. под ред. В. Е. Радзинского. 2-е изд., перераб. и доп. М.: ГЭОТАР-Медиа. 2015; 536 с. [Apresyan SV. Pregnancy and childbirth in extragenital diseases. under ed. VE. Radzinskij. The 2nd prod. M.: GEOTAR-Media. 2015; 536. (In Russ.).]

4. Abbaspour N, Hurrell R, Kelishadi R. Review on iron and its importance for human health. *J Res Med Sci.* 2014; 19: 164–174.

5. Abdel-Raoufabdel-Aziz AR, Ali DK, Talkhan HM. Pregnancy outcome and the effect of maternal nutritional status. *J Egypt Soc Parasitol.* 2013; 43(1): 125-132. DOI: 10.12816/0006372.

6. Al-Gubory KH, Fowler PA, Garrel C. The roles of cellular reactive oxygen species, oxidative stress and antioxidants in pregnancy outcomes. *Int J Biochem Cell Biol.* 2010; 42: 1634–1650. DOI: 10.1016/j.biocel.2010.06.001.

7. Abioye I, Park S, Ripp K. et al. Anemia of Inflammation during Human Pregnancy Does Not Affect Newborn Iron Endowment. *J Nutr.* 2018; 148(3): 427–436. DOI: 10.1093/jn/nxx052.

8. Kohgo Y, Ikuta K, Ohtake T. et al. Body iron metabolism and pathophysiology of iron overload. *Int J Hematol.* 2008; 88: 7–15. DOI: 10.1007/s12185-008-0120-5.

9. Bresgen N, Eckl PM. Oxidative stress and the homeodynamics of iron metabolism. *Biomolecules.* 2015; 5: 808–847. DOI: 10.3390/biom5020808.

10. Broek van den NR, Jean-Baptiste R, Neilson JP. Factors associated with preterm, early preterm and late preterm birth in Malawi. *PLoS One.* 2014; 9(3): e90128. DOI: 10.1371/journal.pone.0090128.

11. Duck KA, Connor JR. Iron uptake and transport across physiological barriers. *Biometals.* 2016; 29(4): 573-91. DOI: 10.1007/s10534-016-9952-2.

12. Hubbard AC, Bandyopadhyay S, Wojczyk BS. et al. Effect of dietary iron on fetal growth in pregnant mice. *Comp Med.* 2013; 63(2): 127-135.

13. Fanzo J, Hawkes C, Udomkesmalee E. et al. 2018 Global Nutrition Report. 2019; 161.

14. Fisher AL, Nemeth E. Iron homeostasis during pregnancy. *Am J Clin Nutr.* 2017;106(Suppl 6):1567S–1574S. DOI: 10.3945/ajcn.117.155812

15. Valko M, Leibfritz D, Moncol J. et al. Free radicals and antioxidants in normal physiological functions and human disease. *Int J Biochem Cell Biol.* 2007; 39: 44–84. DOI: 10.1016/j.biocel.2006.07.001

16. Raiten DJ, Sakr Ashour FA, Ross AC. et al. Inflammation and Nutritional Science for Programs/Policies and Interpretation of Research Evidence (INSPIRE). *J Nutr.* 2015;145:1039S–108S. DOI: 10.3945/jn.114.194571

17. Lelic M, Bogdanovic G, Ramic S. et al. Influence of maternal anemia during pregnancy on placenta and newborns. *Med Arch.* 2014;68(3):184-7. DOI: 10.5455/medarh.2014.68.184-187

18. Yuan X, Hu H, Zhang M. et al. Iron deficiency in late pregnancy and its associations with birth outcomes in Chinese pregnant women: a retrospective cohort study. *Nutr Metab (Lond).* 2019;16:30. DOI: 10.1186/s12986-019-0360-9. eCollection 2019

19. Laflamme EM. Maternal hemoglobin concentration and pregnancy outcome: a study of the effects of elevation in El Alto, Bolivia. *McGill J Med.* 2012; 11(1): 47.

20. Lewandowska M, Lubiński J. Serum Microelements in Early Pregnancy and their Risk of Large-for-Gestational Age Birth Weight. *Nutrients.* 2020; 12(3): 866. DOI: 10.3390/nu12030866

21. Zhang Y, Mikhael M, Xu D. et al. Lysosomal proteolysis is the primary degradation pathway for cytosolic ferritin and cytosolic ferritin degradation is necessary for iron exit. *Antioxid Redox Signal.* 2010; 13: 999–1009. DOI: 10.1089/ars.2010.3129.

22. Zhang Q, Ananth CV, Li Z. et al. Maternal anaemia and preterm birth: a prospective cohort study. *Int J Epidemiol.* 2009; 38: 1380–1389. DOI: 10.1093/ije/dyp243.

23. Rahmati S, Delpishe A, Azami M. et al. Maternal anemia during pregnancy and infant low birth weight: a systematic review and meta-analysis *Int J Reprod Biomed (Yazd.)*. 2017; 15: 125–134.

24. Young MF, Oaks BM, Tandon S. et al. Maternal hemoglobin concentrations across pregnancy and maternal and child health: a systematic review and meta-analysis. *Ann N Y Acad Sci.* 2019;1450(1):47-68. DOI: 10.1111/nyas.14093

25. Milman N. Iron and pregnancy–a delicate balance. *Ann Hematol.* 2006; 85: 559–565. DOI: 10.1007/s00277-006-0108-2

26. Northrop-Clewes CA. Interpreting indicators of iron status during an acute phase response–lessons from malaria and human immunodeficiency virus. *Ann Clin Biochem.* 2008;45:18–32. DOI: 10.1258/acb.2007.007167

27. De Lucca L, Fabiane R, Letícia BJ. et al. Oxidative Profile and δ-Aminolevulinate Dehydratase Activity in Healthy Pregnant Women with Iron Supplementation. *Int J Environ Res Public Health.* 2016; 13(5): 463. DOI: 10.3390/ijerph13050463

28. Sangkhae V, Nemeth E. Placental iron transport: The mechanism and regulatory circuits. *Free Radic Biol Med.* 2019;133:254-261. DOI: 10.1016/j.freeradbiomed.2018.07.001.

29. Zhao GG Xu, Min Z, Yaping J. et al. Prenatal Iron Supplementation Reduces Maternal Anemia, Iron Deficiency, and Iron Deficiency Anemia in a Randomized Clinical Trial in Rural China, but Iron Deficiency Remains Widespread in Mothers and Neonates. *J Nutr.* 2015; 145(8): 1916–1923. DOI: 10.3945/jn.114.208678

30. Kidanto HL, Mogren I, Lindmark G. et al. Risk for preterm delivery and low birthweight are independently increased by severity of maternal anemia. *S Afr Med J.* 2009;99:98–102.

31. Schmidt PJ. Regulation of iron metabolism by hepcidin under conditions of inflammation. *J Biol Chem.* 2015; 290: 18975–18983. DOI: 10.1074/jbc.R115.650150.

32. Scholl TO. Maternal iron status: Relation to fetal growth, length of gestation, and iron endowment of the neonate. *Nutrition Reviews.* 2011;69(1):S23–S29. DOI: 10.1111/j.1753-4887.2011.00429.x.

33. Agarwal A, Aponte-Mellado A, Premkumar BJ. et al. The effects of oxidative stress on female reproduction: A review. *Reprod Biol Endocrinol.* 2012; 10: 49-80. DOI: 10.1186/1477-7827-10-49

34. The global prevalence of anaemia in 2011. Geneva: World Health Organization. Accessed August 28, 2017.

35. Yildiz Y, Ozgü E, Unlu SB. et al. The relationship between third trimester maternal hemoglobin and birth weight/length; results from the tertiary center in Turkey. *J Matern Fetal Neonatal Med.* 2014;27(7):729-32. DOI:10.3109/14767058.2013.837445

36. Theil EC, Elizabeth C. Ferritin protein nanocages-The story. Nanotechnol. *Percept.* 2012; 8: 7–16. DOI: 10.4024/n03th12a.ntp.08.01.

37. Pavord S, Myers B, Robinson S. et al. UK guidelines on the management of iron deficiency in pregnancy. *Br J Haematol.* 2012;156:588–600. DOI: 10.1111/j.1365-2141.2011.09012.x.

S.G. Afanasyev, V.V. Faltin, S.V. Avdeev, A.V. Avgustinovich, A.Yu. Dobrodeev, M.Yu. Volkov, L.V. Gerdt, U.B. Urmonov

PERIOPERATIVE CHANGES IN HEMOSTASIS DURING COMBINED ANESTHESIA WITH XENON AND DEXMEDETOMIDINEINRADICALSURGERY FOR GASTRIC CANCER

DOI 10.25789/YMJ.2020.71.13

УДК 616.89-008

AFANASYEV Sergey G. – MD, DSc, Professor, Head of the Department of Abdominal Oncology, Cancer Research Institute, Tomsk National Research Medical Center, Russian Academy of Sciences. E-mail: Afanasievsg@oncology.tomsk.ru. SPIN-код: 9206-3037. ORCID: 0000-0002-4701-037. AuthorID: 264236. Researcher ID (WOS): D-2084-2012. Author ID (SCOPUS): 21333316900. Тел. 89039132574. **FALTIN Vladimir V.** – MD, Anesthesiologist, Department of Anesthesiology and Critical Care, Cancer Research Institute, Tomsk National Research Medical Center, Russian Academy of Sciences. E-mail: faltin.vladimir@yandex.ru. SPIN-код: 7209-2620. ORCID: 0000-0002-9989-1979. AuthorID: 809913. Researcher ID (WOS): S-5474-2017. **AVDEEV Sergey V.** – MD, Dsc, Anesthesiologist, Department of Anesthesiology and Critical Care, Cancer Research Institute, Tomsk National Research Medical Center, Russian Academy of Sciences; e-mail: svanaesth@oncology.tomsk.ru. SPIN-код:8433-4153. ORCID: 0000-0002-4609-9187. AuthorID: 254000. Researcher ID (WOS): S-6135-2017. **AVGUSTINOVICH Aleksandra V.** – MD, PhD, Senior Researcher, Department of Abdominal Oncology, Cancer Research Institute, Tomsk National Research Medical Center, Russian Academy of Sciences. E-mail: aov862@yandex.ru. SPIN-код: 2952-6119, Researcher ID (WOS): D-6062-2012. AuthorID: 558788. ORCID: 0000-0001-7301-7581, **DOBRODEEV Alexey Yu.** – MD, DSc, Leading Researcher, Department of Abdominal Oncology, Cancer Research Institute, Tomsk National Research Medical Center, Russian Academy of Sciences. E-mail: dobrodeev@oncology.tomsk.ru. SPIN-код (РИНЦ): 5510-4043. ORCID: 0000-0002-2748-0644. Researcher ID: B-5644-2017. Author ID (SCOPUS): 24832974200. **VOLKOV Maxim Yu.** – MD, PhD, Surgeon, Department of abdominal oncology, Cancer Research Institute, Tomsk National Research Medical Center, Russian Academy of Sciences. E-mail: dok75@sibmail.com. SPIN-код: 8052-9941. ORCID: 0000-0001-6776-4898, **GERDT Lyubov V** – MD, PhD, Laboratory Assistant, Laboratory of Molecular Oncology and Immunology, Cancer Research Institute, Tomsk National Research Medical Center, Russian Academy of Sciences. E-mail: immune@oncology.tomsk.ru, **URMONOV Umidjon B.** – MD, Postgraduate, Department of Abdominal Oncology, Cancer Research Institute, Tomsk National Research Medical Center, Russian Academy of Sciences. E-mail: UrmonovUB@oncology.tomsk.ru. SPIN-код (РИНЦ): 7150-7291. ORCID: 0000-0003-2804-4227. Author ID: 967755.

The effect of anesthesia with xenon and dexmedetomidine combined with epidural analgesia on blood hemostasis was assessed using routine coagulogram and rotational thromboelastometry (Rotem). The prospective randomized study included 63 patients with operable stage II–III gastric cancer, who underwent total gastrectomy (n=33) and distal subtotal gastrectomy (n=30). Patients of the study group (n=32) received anesthesia with xenon and dexmedetomidine in combination with epidural analgesia. Patients of the control group (n=31) received anesthesia with sevoflurane in combination with epidural analgesia.

Anesthesia with xenon and dexmedetomidine in combination with epidural analgesia was shown to reduce the perioperative physiologic stress response to surgery and thereby decrease thrombotic and hemorrhagic postoperative complications.

Keywords: xenon; dexmedetomidine; ROTEM; thromboelastometry; surgical stress; gastric cancer.

Introduction. The current understanding of the patient's response to stress is firmly rooted in Walter B. Cannon's concept of homeostasis [9, 14]. At the beginning of the last century, Dr. George Creel put forward the idea of reducing surgical stress, consisting of a combination of nitrous oxide inhalation and tissue infiltration with procaine, a local anesthetic drug. Later, he proposed a combination of sedation, local and regional anesthesia and called it "stress-free anesthesia", which formed the basis of the modern concept of "proactive anesthesia" [9]. These recommendations constitute the principles of modern anesthesiology and are aimed at adequate protection of the body from surgical stress, which is one of the main reasons for the development of postoperative complications [8].

Surgical stress is known to cause a wide and varied response at several levels depending on the type and duration of surgery, anesthetic technique, age and gender of the patient, and previous state of health [8, 9]. Stress response is induced by a skin incision, which causes afferent excitation of nerve cells, activation of the hypothalamic-pituitary-adrenal system, endothelial dysfunction, inflammation and coagulopathy. Complement activation is an integral part of the surgical stress and intersects as well as amplifies the coagulation activation pathways, thus leading to coagulopathies and hyperfibrinolysis [9, 11]. It should be noted that uncontrolled expression of tissue factor (TF) upon activation of pro-inflammatory cytokines can also lead to coagulopathy [16]. Perioperative coagulopathic hemorrhage and hyperfibrinolysis are serious complications in abdominal surgery. In the prospective multicenter cohort study of 1134 patients, postoperative non-surgical hemorrhagic complications were observed in 9.5% of cases, with a mortality rate of 12% [5, 9].

A previous study of the anesthetic technique including xenon (NMDA receptor antagonist) and dexmedetomidine (alpha2-agonist) in combination with epidural analgesia showed a high anti-stress protection during radical surgery for gastric cancer [3, 4]. In abdominal oncology, anesthesia with xenon and dexmedetomidine in combination with epidural analgesia should guarantee the maximum degree of anti-stress protection; therefore further studies are required to assess the effect of this combined anesthesia on the hemostatic system.

The purpose of the study was to analyze perioperative changes in the hemostatic system of patients, who received anesthesia with xenon and dexmedetomidine in combination with epidural analgesia in radical surgery for gastric cancer.

Materials and methods. The study included 63 patients with operable stomach cancer (SC), who were treated at the Department of Abdominal Oncology, Research Institute of Oncology, Tomsk National Research Medical Center from 2012 to 2019. Depending on the anesthetic technique, patients were divided into 2 groups (Table 1). In the study group (n=32), anesthesia with xenon and dexmedetomidine was combined with

epidural analgesia. In the control group (n=31), anesthesia with sevorflurane was combined with epidural analgesia. Gastrectomy was performed in 33 (52.4%) patients and subtotal distal gastrectomy in 30 (47.6%). There were no statistically significant differences between the treatment groups (p>0.05).

The hemostatic system was assessed using standard parameters: platelet count, prothrombin time (PTT), activated partial thromboplastin time (APTT), thrombin time (TT), dissolved fibrin-monomer complex (DFMC), fibrinogen. Antithrombin was determined using the Tech-Antithrombin-Test Technology Standard reagent kit. Plasminogen was determined using the ChromoTech-Plasminogen Technology Standard reagent kit. The D-dimer levels were measured using the semi-quantitative method of the HELENA test system (I – 0-250; II – 250-500; III – 500-1000). In addition, the rotational thromboelastometry parameters were determined using ROTEM device (Germany): CT – the condensation time required to reach an amplitude of 2 mm, CFT – the clot formation time (time interval between the periods of reaching 2 and 20 mm amplitudes), □-angle, characterizing the kinetics of clot formation, A (10) is the clot firmness after 10 minutes, MCF – the maximum clot firmness (a measure of the clot firmness, which is the maximum amplitude reached by the time the fibrinolysis process starts to decrease), MCE – the maximum clot elasticity reflecting its mechanical properties. To determine the coagulogram indices, venous blood samples were taken the day before surgery, during surgery, after extubation of patients, and on the 1-st day after surgery.

To perform anesthesia, the AXEOMA machine (Finland) designed for low-flow anesthesia with xenon and sevoflurane was used. Standard premedication was performed in the operating room by administering atropine at a dose of 0.5 mg and fentanyl at a dose of 0.1 mg. A catheter was inserted into the epidural space of the patients at the Th7-Th9 level. A continuous epidural infusion of 0.2% solution of napropine (94 mg) + 0.005% solution of fentanyl (0.1 mg) + 0.1% solution of adrenaline (0.01 mg) was injected through the catheter at a rate of 5-15 ml/h. Anesthesia was induced with diprivan 2 mg/kg, and tracheal intubation with muscle relaxants was then performed.

After epidural catheter insertion, intravenous dexmedetomidine at a dose of 0.6-0.8 μg/kg/h was administered to the patients of the study group. After tracheal intubation, denitrogenation was performed by mechanical ventilation controlled by volume in standard ventilation mode with 100% oxygen, while maintaining EtCO2 at 35 ± 2 mm Hg for 10-15 minutes. During denitrogenation, only dexmedetomidine was used for sedation. Xenon was then delivered into the closed circuit anesthetic machine to fill the breathing bag, and ventilation was continued with fresh Xe flow of 250-400 ml/min under FiO2 control until the oxygen concentration in the respiratory circuit was 40% and the concentration of Xe was 60%. When achieving a stable equilibrium in the ratio Upon reaching a stable equilibrium in the Xe:O2 ratio of 60:40, low-flow anesthesia was performed: the Xe flow was reduced to 0-160 ml / min, and oxygen was supplied to the circuit at a dose of 4 ml/kg. Xenon was the main hypnotic agent, but dexmedetomidine infusion was continued during surgery with the necessary dose correction to ensure the necessary depth of sedation when assessed by clinical scoring tools and by BIS. Dexmedetomidine infusion was stopped 20 minutes before the end of surgery. The average Xe consumption during surgery was 16 (15-17.5) liters.

After intubation, patients in the control group underwent mechanical ventilation with volume control in the normal ventilation mode of FiO2 0.4 and maintenance of EtCO2 at the level of 35 ± 2 mm Hg. They received low-flow anesthesia with sevoflurane in the required concentration. Dexmedetomidine was not administered.

Fentanyl was given to patients of both treatment groups at the discretion of the anesthetist. Considering the absence of blood loss, intraoperative infusion therapy in both treatment groups was minimal and was given according to the same scheme.

Before surgery, patients did not receive anticoagulant therapy. In the postoperative period, elastic compression stockings for the lower extremities were used to prevent thrombosis, and enoxaparin (low-molecular-weight heparin) at a dose of 0.4 mg was administered 2 times a day.

The statistical analysis of the data was carried out using the STATISTICA 8 software package. P-value less than 0.05 was considered as statistically significant.

Results and discussion. The effect of anesthesia technique on the hemostatic system was assessed using routine coagulogram and rotational thromboelastometry (ROTEM). Before surgery, the values of these parameters in the treatment groups were the same (Tables 2 and 3).

The platelet count decreased in both groups equally during surgery and returned to the initial levels on day 1 after surgery. It is most likely associated with intraoperative hemodilution. The levels of TT, APTT, fibrinogen and FMC were equally increased in both groups after administering low molecular weight heparins to prevent thrombosis. Moreover, in both treatment groups, the levels of antithrombin III and plasminogen C were significantly lower after extubation of patients and on day 1 after surgery than before surgery (p<0.05). Statistically significant differences in the traditional coagulogram parameters between the treatment groups were the decreased level of plasminogen C on day 1 after surgery with sevoran anesthesia and decreased level of APTT after extubation with combined xenon – dexmedetomidine anesthesia compared to sevoran anesthesia (Table 2).

Table 1

Patient characteristics between treatment groups

Characteristics		Xenon (n=32)	Sevoran (n=31)
Age	<40 40-49 50-59 60<	1 (3.2) 9 (28.1) 17 (53.1) 5 (15.6)	2 (6.3) 8 (25.8) 16 (51.6) 5 (16.1)
Male	Female Male	14 (43.8) 18 (56.2)	15 (48.4) 16 (51.6)
ASA	I II III	4 (12.6) 14 (43.7) 14 (43.7)	5 (16.2) 13 (41.9) 13 (41.9)
Stage	II III	13 (40.7) 19 (59.3)	14 (45.2) 17 (54.8)
Tumor localization	The cardia The body of the stomach The pyloric antrum Subtotal lesion	6 (18.8) 11 (34.3) 12 (37.5) 3 (9.4)	5 (16.1) 9 (29.1) 13 (41.9) 4 (12.9)

Significant changes were revealed in rotationa thromboelastometry (ROTEM) parameters. Two ROTEM tests: EXTEM – external moderate activation of hemostasis and INTEM – internal moderate activation of hemostasis were used to assess the hemostatic system at a particular time [19]. It should be noted that thromboelastometry parameters were within acceptable limits at all control points; they were the same before surgery (Table 3). However, during surgery and extubation, the levels of most parameters of ROTEM tests were significantly lower in the study group than in the control group. The inverse relationship was observed in the CFT level: an increase in the CT and CFT levels and decrease in □-angle and MCE levels was observed on day 1 after surgery in the study group patients ($p<0.05$).

The laboratory data correlated with clinical results. Combined xenon and dexmedetomidine anesthesia resulted in more favorable postoperative period than sevoran anesthesia (postoperative complications in 5 (15.6%) cases versus 12 (38.7%) cases ($p<0.05$).

The use of xenon, dexmedetomidine, epidural analgesia, as well as xenon in combination with epidural analgesia is reported to result in a decrease in the severity of the humoral and inflammatory response to surgical stress [6, 7]. The combined use of a xenon, an NMDA receptor antagonist and dexmedetomidine, alpha2-adrenoreceptor agonist, not only enhances the effectiveness of individual compounds via synergism, but also reduces the likelihood of undesirable side effects that can occur when each of these agents is used individually [10]. Our anesthesia technique provides stable hemodynamic parameters due to xenon, which has a positive inotropic effect, increases cardiac output, and has the properties of a vasoconstrictor with increased vascular resistance, both in the small and large circles of blood circulation [1, 3, 13].

Blood coagulation is initiated by the impact of platelets on subendothelial components, the activation of TF and plasma factors through the extrinsic pathway [17]. The secondary coagulation pathway, known as the intrinsic pathway, is also very important in the formation of a clot [15]. The relationship between the extrinsic and intrinsic pathways during surgery has been little studied [16], there are suggestions about a decrease in TF production during xenon anesthesia [12], but in the presence of a pronounced inflammatory and immune response, hemostasis is unbalanced with the occurrence of coagulopathies, thus leading to the development of postoperative complications associated with microthrombosis in the vascular bed of the gastrointestinal tract, such as postoperative pancreatitis and anastomotic leakage [2].

Many authors report that the significance of standard laboratory tests for controlling hemostasis in the surgical unit is limited [18]. The use of thromboelastometry made it possible to evaluate global hemostatic function at a specific point in time, which enabled us to conduct perioperative monitoring of hemostasis. Thromboelastometry showed that in the study group, hemostatic function tended to hypercoagulation via both the extrinsic and intrinsic pathways during surgery and after extubation of patients in comparison with hemostatic parameters observed in the control group. In patients, who received sevoflurane anesthesia, a tendency towards hypocoagulation occurring through the extrinsic pathway and in both groups through the intrinsic pathway was observed after extubation of patients. Based on the results obtained, it can be concluded that there is less risk of intraoperative and early postoperative bleeding when using xenon and dexmedetomidine. The increase in the maximum clot's flexibility observed in the control group with sevoflurane anesthesia indicates super-proportional clot strength parameters, which in turn can increase the risk of thromboembolic complications.

Thus, we can state that our anesthesia technique has a less pronounced effect on hemostasis during surgery, while maintaining its reserve capacity, lower risk of coagulopathy and bleeding during surgery, and thromboembolism in the postoperative period. Anesthesia with xenon and dexmedetomidine in combination with epidural analgesia led to a decrease in postoperative complications during surgery and in the early postoperative period. Our anesthesia technique including xenon and dexmedetomidine in combination with epidural analgesia corresponds to the concept of "proactive anesthesia".

Conclusion. The approach to the

Table 2

Changes in coagulogram parameters in the treatment groups

Parameter	Before surgery		During surgery		After extubation		Day 1 after surgery	
	Xe	Sevoran	Xenon	Sevoran	Xenon	Sevoran	Xenon	Sevoran
Platelet.103/mm3	263.0 (218.0-313.0)	262.0 (226.0-331.5)	199.0** (189.0-279.0)	207.0** (178.0-284.0)	243.0 (210.0-284.0)	227.09 (207.0-294.0)	253.0 (189.0-315.0)	256.5 (194.0-310.5)
PT. sec	13.0 (13.0-14.0)	14.0 (13.0-14.0)	14.0 (14.0-15.0)	15.0 (14.0-16.0)	14.0 (14.0-15.0)	15.0 (14.0-16.0)	16.0** (15.0-16.0)	16.0** (15.0-17.0)
APTT. sec	31.0 (28.0-34.0)	30.5 (27.0-34.0)	31.0 (30.0-35.0)	31.0 (30.0-37.5)	29.0 (28.0-31.0)	35.0* (32.5-38.0)	48.0** (44.0-52.0)	48.0** (43.5-55.0)
TT. sec	16.0 (15.0-16.0)	16.0 (14.5-16.0)	16.0 (16.0-17.0)	16.5 (16.0-18.0)	16.0 (16.0-17.0)	16.0 (15.0-17.0)	16.0 (15.0-17.0)	15.0 (14.0-15.5)
DFMC. mg/ml	4.0 (3.5-6.8)	3.75 (3.5-6.4)	3.5 (3.5-4.9)	3.95 (3.5-4.7)	4.8 (3.8-6.0)	4.35 (3.5-5.0)	6.5** (4.8-8.0)	7.0** (4.9-8.6)
Fibrinogen. g/l	4.0 (3.5-5.3)	4.0 (3.4-5.2)	3.0 (2.8-5.1)	3.4 (2.9-5.0)	4.2 (3.4-4.7)	4.0 (3.3-4.7)	6.0** (5.6-6.5)	6.1** (5.5-7.0)
Antitrombin III. %	96.5 (89.0-102.0)	95.5 (94.0-105.0)	91.5 (84.0-95.0)	93.0 (85.0-95.0)	87.5 ** (80.0-91.0)	87.0** (81.0-89.0)	83.0** (78.0-84.0)	82.0** (79.0-86.0)
D-dimer. ng/ml	<250	<250	<250	<250	<250	250-500	500-1000	500-1000
Plasminogen C.%	105.0 (100.0-110.0)	103.5 (94.0-108.0)	96.0 ** (92.0-99.0)	98.0 (94.0-102.0)	94.0** (92.0-97.0)	95.5 (94.0-100.0)	84.0* ** (84.0-90.0)	82.0** (79.0-84.0)

Note: * – differences are statistically significant compared to the control group ($p<0.05$); ** – differences are statistically significant compared to preoperative parameters ($p<0.05$).

Table 3

Changes in the parameters of rotational thromboelastometry (ROTEM) in the treatment groups

Parameter	Before surgery		During surgery		After extubation		Day 1 after surgery	
	Xe	Sevoran	Xenon	Sevoran	Xenon	Sevoran	Xenon	Sevoran
EXTEM								
CT. sec	50.0 (45.0-58.0)	52.0 (51.0-57.0)	48.0* (43.0-54.0)	54.0 (49.0-63.0)	54.0* (50.0-64.0)	62.0** (59.0-70.0)	65.5* ** (59.5-73.5)	59.0** (56.0-62.0)
A(10). mm	58.5 (52.0-63.0)	57.0 (54.0-65.0)	54.0* (50.0-56.0)	58.0 (54.0-59.0)	56.0 (54.0-59.0)	54.0 (52.0-56.0)	59.0 (55.0-63.0)	59.0 (56.0-62.0)
CFT. sec	65.0 (60.0-78.0)	74.0 (49.0-84.0)	73.0* (68.0-97.0)	98.0** (79.0-101.0)	77.0* (59.0-103.0)	96.0** (87.0-103.0)	89.5* ** (85.0-98.0)	80.0** (75.0-93.0)
α	76.0 (74.0-78.0)	76.0 (74.0-79.0)	76.0 (75.0-78.0)	72.0 (70.0-76.0)	72.0 (70.0-75.0)	73.0 (70.0-76.0)	70.5* ** (68.0-72.5)	75.5 (74.0-77.0)
MCF. mm	64.5 (60.0-69.0)	67.0 (63.0-71.0)	67.0 (65.0-69.0)	63.0 (61.0-65.0)	64.0 (62.0-68.0)	62.0 (60.0-63.3)	62.0 (57.5-63.5)	65.0 (64.0-67.0)
MCE. mm	179.5 (152.0-224.0)	203.0 (175.0-247.0)	187.0 (160.0-213.0)	180.0 (169.0-201.0)	157.0* (140.0-177.0)	187.0 (172.0-211.0)	203.0* (189.0-228.5)	230.0 (189.5-249.0)
INTEM								
CT. sec	120.5 (111.0-131.0)	124.0 (109.0-142.0)	136.0* ** (127.0-145.0)	160.0 (140.5-175.0)	137.0* ** (124.0-146.0)	155.0** (145.0-167.0)	155.0* ** (144.5-167.5)	142.5** (130.5-152.0)
A(10). mm	54.5 (53.0-59.0)	56.0 (54.0-63.0)	54.0* (50.0-59.0)	56.0 (53.5-61.0)	56.0 (55.0-59.0)	54.0 (53.0-57.0)	58.5* ** (55.0-65.0)	62.0** (60.0-66.0)
CFT. sec	57.5 (52.0-67.0)	58.0 (49.0-68.0)	82.0* ** (62.0-90.0)	59.0 (46.5-75.5)	66.0* ** (58.0-78.0)	78.0** (73.0-89.0)	87.5* ** (79.0-98.0)	76.0 (67.5-81.0)
α	77.0 (74.0-79.0)	78.0 (75.0-80.0)	75.0 (70.0-77.0)	77.0 (75.5-80.0)	77.0 (74.0-78.0)	75.0 (72.0-76.0)	77.5* (74.5-79.0)	78.0 (77.0-79.5)
MCF. mm	61.5 (59.0-67.0)	64.0 (62.0-69.0)	62.0** (58.0-66.0)	63.0 ** (61.0-67.0)	64.0* ** (60.0-66.0)	58.0** (56.0-62.0)	61.0 (57.5-62.5)	64.0 (60.5-67.0)
MCE. mm	177.0 (154.0-233.0)	194.0 (181.0-234.0)	163.0* ** (139.0-191.0)	172.0** (153.5-206.0)	178.0* ** (164.0-189.0)	179.0** (167.0-207.0)	180.0* ** (161.5-227.5)	187.5 (175.0-231.0)

choice of anesthetic technique based on perioperative protection allows us to solve the problem of surgical stress by affecting on different levels of homeostasis imbalance. Xenon and dexmedetomidine anesthesia in combination with epidural analgesia in radical surgery for gastric cancer can reduce surgical stress, which is reflected in physiological hemostasis, and provides a favorable course of surgery, thereby decreasing thrombohemorrhagic complications.

References

1. Куликов А.Ю., Кулешов О.В., Лебединский К.М. Влияние анестезии ксеноном на гемодинамику: что нам известно к 2015 г. *Анестезиология и реаниматология.* 2015; 60(6): 71-74. [Kulikov AYu, Kuleshov OV, Lebedinskii KM. Effects of xenon anesthesia on hemodynamics: what do we know until 2015. *Anesteziologiya i reanimatologiya.* 2015; 60(6): 71-74. (in Russ.).]

2. Самсонова Н.Н., Климович Л.Г., Рогальская Е.А. Патогенез послеоперационных коагулопатий. *Клиническая физиология кровообращения.* 2014; 4: С. 17-24. [Samsonova NN, Klimovich LG, Rogalskaya EA. The pathogenesis of post-operative coagulopathies. *Clinical Physiology of Circulation.* 2014; 4: 17-24. (in Russ.).]

3. Фальтин В.В., Авдеев С.В., Афанасьев С.Г. [и др.]. Стресс-ответ при комбинированной анестезии ксеноном и дексмедетомидином при радикальных операциях по поводу рака желудка. *Вестник интенсивной терапии имени А.И. Салтанова.* 2018; 2: 40-45. [Faltin VV, Avdeev SV, Afanasyev SG [et al.]. Stress response during combined anaesthesia xenon and dexmedetomidine in radical surgery for gastric cancer. *Alexander Saltanov Intensive Care Herald.* 2018; 2: 40-45. (in Russ.).] doi: 10.21320/1818-474X-2018-2-40-45

4. Авдеев С.В., Афанасьев С.Г., Фальтин В.В. [и др.] Стресс-ответ при радикальных операциях по поводу рака желудка. *Сибирский онкологический журнал.* 2017; 16(6): 25-30. [Avdeev S.V., Afanasyev S.G., Faltin V.V. [et al.]. Stress-response in radical surgery for gastric cancer. *Siberian Journal of Oncology.* 2017; 16(6): 25-30. (in Russ.).] doi: 10.21294/1814-4861-2017-16-6-25-30

5. Albaladejo P., Charbonneau H., Samama C.M. [et al]. Bleeding complications in patients with coronary stents during non-cardiac surgery. *Thromb Res.* 2014; 15(2): 268–272. doi: 10.1016/j. thromres.2014.05.015

6. Bugada D., Ghisi D., Mariano E.R. Continuous regional anesthesia: a review of perioperative outcome benefits. *Minerva Anestesiol.* 2017; 83: 1089-1100.doi:10.23736/S0375-9393.17.12077-8

7. Soliz J.M., Ifeanyi I.C., Katz M.H. [et al.]. Comparing postoperative complications and inflammatory markers using total intravenous anesthesia versus volatile gas anesthesia for pancreatic cancer surgery. *Anesth Pain Med.* 2017; 7(4): e13879. doi: 10.5812/aapm.13879

8. Desborough J.P. The stress response to trauma and surgery. *Br J Anaest.* 2000; 85(1): 109-117. doi: 10.1093/bja/85.1.109

9. Dobson G.P. Addressing the global burden of trauma in major surgery. *Front Surg.* 2015; 2: 43-69. doi: 10.3389/fsurg.2015.00043

10. Franks N.P. Molecular targets underlying general anesthesia. *Br J Anaest.* 2006; 147(1): 72-81. doi: 10.1038/sj.bjp.0706441

11. Gutierrez T., Hornigold R., Pearce A. The systemic response to surgery. *Surgery (Oxford).* 2011; 29(2): 93-96. –DOI: 10.1016/j.mpsur.2010.11.010

12. Amara U., Rittirsch D., Flierl M. [et al.] Interaction between the coagulation and complement system. *Adv Exp Med Biol.* 2008; 632: 71-79.

13. Law L.S., Lo E.A., Gan T.J. Xenon Anesthesia: a systematic review and meta-analysis of randomized controlled trials. *Anesth Analg.* 2016; 122(3): 678-97. doi: 10.1213/ANE.0000000000000914

14. Moore F.D. The growth of surgical biology. *Ann Surg.* 1953; 138: P. 807-822. doi:10.1097/00000658-195311000-00023

15. Pan X., Lu J., Cheng W. [et al]. Pulmonary static inflation with 50% xenon attenuates decline in tissue factor in patients undergoing stanford type A acute aortic dissection repair. *J Thorac Dis.* 2018; 10(7): 4368-4376. doi: 10.21037/jtd.2018.06.95

16. Rao L.V., Pendurthi U.R. Regulation of tissue factor coagulant activity on cell surfaces. *J Thromb Haemost.* 2012; 10(11): 2242-53. doi: 10.1111/jth.12003

17. Rao L.V., Pendurthi U.R. Tissue factor-factor VIIa signaling. *Arterioscler Thromb Vasc Biol.* 2005; 25(1): P. 47-56.

18. Durila M., Jonas J., Durilova M. [et

al.]. Thromboelastometry as an alternative method for coagulation assessment in pediatric patients undergoing invasive procedures: a pilot study. Eur J Pediatr Surg. 2019; 29(3): 298-301. doi: 10.1055/s-0038-1667111

19. Vymazal T., Astraverkhava M., Durila M. Rotational thromboelastometry helps to reduce blood product consumption in critically ill patients during small surgical procedures at the intensive care unit - a retrospective clinical analysis and literature search. *Transfus Med Hemother.* 2018; 45(6): 385-387. doi: 10.1159/000486453

A.A. Chakhov, I.D. Ushnitsky, O.M. Kolosova, A.V. Yurkevich, M.M. Vinokurov, D.V. Mikhalchenko, A.D. Semenov

CLINICAL CHARACTERISTIC OF PSYCHOPHYSIOLOGICAL INDICES DYNAMICS AT DENTAL CONSULTATION STAGES USING MODIFIED MANDIBULAR ANESTHESIA METHOD

DOI 10.25789/YMJ.2020.71.14

УДК616.31-085:612.821.1

The **research aim** was a comparative analysis of clinical-physiological parameters dynamics of examined patients by a modified method of mandibular anesthesia at dental consultation stages.

The **materials and methods** were related to the assessment of psychophysiological parameters of patients at the stages of dental consultation and psychoemotional patients' anxiety at the stages of dental care, the determination of the level of patients' anxiety and the study of the functional state of regulatory systems of the body, as well as emotional-pain stress and the study of analgesic effect of mandibular at consultation.

Results. We obtained psychophysiological parameters that indicated the presence of an anxious condition in the examined patients of the main and experimental groups at the dentist's. At the same time, the level of patients' anxiety in both examined groups before consultation and during anesthesia did not have reliable differences. There was a decrease in anxiety level after consultation in both groups. The group difference at the treatment stage was when patients of the main group had pronounced analgesic effects, which lead to a decrease of the level of patients' anxiety. The results of our study of situational and personal anxiety showed that there were no people with low levels of anxiety among patients who had come to the dentist. According to the heart rate variability of patients, vegetative balance was determined at different stages of medical consultation, which was characterised by voltage index of regulatory systems that determined the activity of sympathetic regulation mechanisms and the state of the central regulation circuit. **Discussion.** The main group showed a moderate anxiety by the end of consultation, the experimental group – a condition between moderate and high situational anxiety. Low level of anxiety in patients of the main group at consultation was carried out with Ushnitsky-Chakhov's method of mandibular anesthesia.

Conclusion. The obtained data characterize the possibility of use of mandibular anesthesia method in practical dentistry, as it does not cause any additional psychoemotional stress.

Keywords: mandibular, local anesthesia, inferior dental nerve, psychoemotional stress.

M.K. Ammosov MI NEFU: **CHAKHOV Alexander Alexandrovich** – Candidate of Medical Sciences, Associate Professor, alex-alex41169@mail.ru, orcid.org/0000-0002-6371-0734, **USHNITSKY Innokenty Dmitrievich** – Doctor of Medical Sciences, Prof., Head of Department, incadim@mail.ru, orcid.org/0000-0002-4044-3004, **VINOKUROV Mikhail Mikhailovich** – Doctor of Medical Sciences, Prof., Head of department, mmv_mi@rambler.ru; **SEMENOV Alexander Dmitrievich** – Candidate of Medical Sciences, Associate Professor, semenovs777@list.ru; **KOLOSOVA Olga Nikolaevna** – Doctor of Biological Sciences, Chief Researcher Institute of biological permafrost problems SB RAS, kololgonik@gmail.com; **YURKEVICH Alexander Vladimirovich** – Doctor of Medical Sciences, Dean of the Faculty, Head of Department of FESMU, Khabarovsk, dokdent@mail.ru; **MIKHALCHENKO Dmitry Valerievich** – Doctor of Medical Sciences, Associate Professor, Head of Department of Volgograd State Medical University, Ministry of Health of the Russian Federation, Vologograd, karta007@rambler.ru.

Introduction. Medical practice shows that a large proportion of dental patients have a certain psychoemotional setting for pain that they feel without even seeing tools [7, 9, 33]. Meanwhile, patients feel anxiety and fear thinking of forthcoming medical manipulation and possible pain [2, 3, 4, 24]. Though reduction of pain sensitivity at dental consultation is carried out by local anesthesia [32]. The quality of pain relief depends as on the individual and anatomical-topographic features of the patient's maxillofacial structure as on his psychophysiological condition, and also on methods of treatment [1, 21, 22, 29, 30, 31]. Therefore, it is important to develop modified anaesthesia methods that will improve the effectiveness and safety of anaesthesia [10, 11, 12, 13, 14, 15, 17, 18, 19, 20, 25, 27, 28]. At the same time, painless medical and preventive measures will have positive effect on the psychoemotional condition of patients [2, 6, 23, 31]. Thus, researches aimed at solving these problems are relevant, which are of important scientific, theoretical and practical importance.

The **research aim** was to carry out a comparative analysis of clinical and physiological parameters dynamics of examined patients during mandibular anesthesia using a modified method of mandibular anesthesia at dental consultation.

Material and methods of the research. The research of dynamics of psychoemotional condition of patients at stages of dental visit with the use of classical palpatory method and Ushnitsky-Chakhov's modified method of mandibular anesthesia (application No. 2019140398 09.12.2019) included 148 people, 107 were from the main group (MG) and 41 from experimental group (EG), aged from 18 up to 50 years old. The width of the mandible branch was first measured with Ushnitsky-Chakhov device to carry out the modified mandibular anaesthesia method. We determined the needle depth using this obtained index (Patent No. №196101 17.02.2020).

Further, the obtained index was set by means of a movable needle immersion depth limiter on Ushnitsky-Chakhov's device for mandibular anesthesia (Patent No. 184398). The device was then placed in the oral cavity of the patients in the area of deep concavity of the anterior

Table 1

Ushnitsky-Chakhov's table to determine the depth of needle immersion taking into account the width of the mandible branch, mm

Index of the smallest width of the mandible branch according to the device data for determining the width of the mandible branch	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45
Depth of needle immersion	15.3	15.9	16.5	17.1	17.7	18.3	18.9	19.5	20.1	20.6	21.2	21.8	22.4	23.0	23.6	24.2	24.8	25.4	26.0	26.5

edge of the mandible branch until a stop was reached. The handle of the device was placed vestibularly from the dental rows, moving the angle of the mouth and cheek outwards at the level of interocclusion height with the open mouth. Then, a carpular syringe with anaesthetic and needle was installed parallelly to the device at the level of premolars on the opposite side. The needle was placed in the plane formed between the limiter plates and brought to the sulcus of the tip end and further advanced into the tissue until bone was reached, where 0.3 ml of anesthetic was administered to turn off the lingual nerve, then the syringe was moved to the frontal teeth and the needle was advanced back, without losing contact with bone until stop against pre-installed limiter, wherein the needle reached the target point and an aspiration sample was carried out with further administration of 1.5 ml of anesthetic. After anaesthesia, the carpulous syringe was removed from the device and then the device itself from the oral cavity. Anesthesia occurred 7 minutes later, pain relief zone follow the standard technique. The following methods were used to assess the psychoemotional condition: C. Spielberger's test (1989) to detect the level of situational (SA) and reactive anxiety (RA) in patients at the stages of dental care; determination of emotional pain stress at the stages of medical and preventive care was carried out by G.G. Grishanin's method (1998); determination of patient's anxiety level at treatment stages using visual-analogue scale by S.A. Rabinovich's method, Moskovets O.N., (2001); diagnostics of the dynamics of the functional state of the patient 's regulatory systems at various stages was carried out by a comprehensive assessment of heart rate variability for 5 minutes with Neurosoft hardware and software complex "VNS-Micro" (Ivanovo). The study was conducted on the clinical bases of the dental polyclinic of M.K. Ammov North-Eastern Federal University, dental clinic "Eurostat" (Yakutsk). M.K.Ammosov North-Eastern Federal University Medical Institute Local Ethical Committee permission was received for the research (protocol No. 43, 15.10.2018).

Statistical processing of the research data was carried out using standard methods of variation statistics with calculation of mean value, standard error using packages of application programs "MicrosoftExcel" 2009 (MicrosoftCorporation, 2000-2016). The results were grouped by a combination of identical features. Correlation analysis of clinical material with Pearson's coefficient (r) determination was performed using the average program package "SPSS," version 22.

Results and discussion. The assessment results of the anxiety level of patients using the visual-analogue scale according to Rabinovich S.A., Moskovets O.N., (2001) show that the level of anxiety (LA) of patients in both examined groups before consultation and during anesthesia does not have reliable differences (Figure 1). During the therapeutic measures, LA increases in experimental group patients by 0.41 points ($p < 0.05$). There is LA decrease after consultation in both groups: in patients of main group by 4.51 point, and in patients of experimental group - 4.53 points ($p > 0.05$). The difference in group values at the treatment stage may be due to the fact that the patients of the main group had a pronounced analgesic effect, which probably leads to a LA decrease in patients.

Since anxiety refers to the level of individual personality properties and is understood as the expectation of a dysfunctional outcome in relatively neutral, non-real-threat situations (V.R. Kislovskaya, 1971), a study of personal (PA) and situational anxiety (SA) has been conducted. PA characterizes stable excitation focus in the cortex cerebri (CC) and is a stable characteristic of a person who tends to perceive a sufficiently large range of situations negatively and respond to them by increasing the level of anxiety. The SA reflects the human condition at the moment and reflects the excitation processes in the CC in response to a particular situation of increased excitability. A high level of SA can cause attention violations, accuracy of complex tasks, manifestation of negative emotions, etc.

The obtained SA and PA results show that there are no people with low levels of anxiety among patients who came to dental consultation (Figure 2). The average level of anxiety, both personal and situational, found in 68.4% and 78.9% of patients, respectively, is the most optimal, aimed at mobilizing compensation-adaptation systems of the body to activate metabolic processes of the organism in response to stress factors. More than a third of patients (31.6%) have high PA levels and every fifth patient has high SA levels (21.1%).

The level of PA in scores in patients of both groups was slightly increased at the end of consultation, but no reliable differences between the groups were found (Figure 3). At the same time, SA levels in main group patients decrease significantly more ($p < 0.05$) than in experimental group patients. The obtained data of SA level dynamics during consiltation show that tension, anxiety and nerve during in patients of main group decreased significantly more by - 1.03 than in experimental group ($p < 0.05$). This is due to the fact that the blockade is carried out taking into account the individual anatomical and topographic features of the lower and upper jaws in the developed conductor methods of pain relief, which, accordingly, increase the analgesic effect.

Emotional stress increases the energy consumption of the body and, as a result, changes in metabolic processes. These processes are expressed more significantly in the case of negative emotions. The higher the emotional tension, the more the corresponding psychophysiological mechanisms are connected, which we have studied with the method of heart rate variability analysis.

When assessing statistical indicators of heart rate variability of patients at different stages of dental consultation, it is necessary to note decrease of SDNN value in patients of both groups. The decrease in SDNN value in the course of treatment, which is a total indicator of the variability of RR interval values over the whole period under consideration, indicates the activation of the effects of the sympathetic nervous system on the body by the end of treatment. The activity

index of the parasympathetic vegetative regulation link (RMSSD) reflects the activity of the autonomous regulation loop. The higher RMSSD value, the more active the parasympathetic regulation link is. Normal values of this indicator are in the range of 20-50 ms. Patients of main and experimental groups showed a decrease in parasympathetic regulation by the end of treatment, because RMSSD decreased in experimental group patients from 40.03 ms to 37.58 ms, in main group patients from 40.16 ms to 37.69 ms.

According to researches, MO increase > 900 ms is typical for an increase in the activity of the parasympathetic part of the vegetative nervous system (vagotonia), and AMO increase > 50%, IT > 100. IVE > 145 r.u. - for sympathetic (Smirnov V.M., 2002). Our results characterize the vegetative balance during consultation as follows (Table 2).

The voltage index of regulatory systems characterizes the activity of sympathetic regulation mechanisms, the state of the central regulation loop. Patients in both groups showed a significant increase in the Range ($p < 0.05$) by the end of the treatment, indicating the activation of sympathetic regulation as a result of external stress. Since the patients show a decrease in the spread of cardiointervals duration and an increase in the number of similar intervals (AMO growth from 40.73% to 42.96%) by the end of treatment, it can be said that the performed therapeutic manipulations make a psychoemotional load in both groups of patients, which is the reason for the increase of sympathetic regulation.

The heart rate variability (HRV) analysis of spectral parameters showed a decrease in total power of TP spector, ms2 at patients of both groups that correlates (r=0.73) with decrease in the SA level during dental consultation. A more detailed analysis of the spectral parameters of HRV of main group patients indicates that the proportion of low frequency component of the spectrum of all waves (VLF) is more than half of the spectrum of all waves - 53.35%. It should be noted that the high level of anxiety in patients who have come for dental care leads to the activation of extremely expensive duplicate central, neurogumoral link of regulation by all metabolic processes, in the organism which can cause reduction of adaptation resources, disadaptation and desynchronization of functions. Therefore, effective anesthesia and painless treatment for such patients also leads to normalization of body functions. By the end of medical manipulations, the percentage of influences of the sympathetic nervous system (LF) and central neuro-humoral regulation (VLF) changes towards energetically better intersystem sympathetic regulation, which should have a positive impact on the recovery of body functions after medical manipulations.

The correlation analysis has established a relationship between the anxiety level and the average duration of the R-R intervals (RRNN, ms) - Pearson linear correlation coefficient was 0.45. There is also a relationship between the anxiety level and the frequency of consecutive R-R intervals with a difference of more than 50 ms (PNN50,%) (r = 0.43). We interpreted the results as the average level of relationship between the parameters being compared. Correlation analysis data suggest changes in the functional activities of the autonomic nervous system at different stages of dental consultation, manifested by an anxiety level reduction when we use block anesthesia methods for the maxillofacial area.

Table 2

Geometric indicators of heart rate variability of patients of the main group and experimental group during dentist's consulatation (M ± SD)

Показатель	Groups	Before consultation	After Anesthesia	After consultation
MO, ms	1	666.67±42.13	648.26±43.17	663.46±48.01
	2	667.34±43.07	647.79±42.05	662.86±41.07
AMO, %	1	41.12 ± 3.16	42.19 ± 3.51	43.39 ± 4.99
	2	40.73 ± 2.13	41.67 ± 2.56	42.96 ± 4.45
VAR, s.	1	202.11±19.13	198.10±19.12	194.35±21.11
	2	200.14±18.11	193.10±19.09	192.25±20.12
IVE, r.u.	1	242.14±19.21	258.21±19.98	265.48±18.65
	2	240.16±17.24	257.23±19.21	264.47±18.28
IAPR, r.u.	1	63.13± 6.45	66.89±6.12	67.02± 8.92
	2	62.56± 6.16	66.19±5.17	66.19± 7.11
IT, r.u.	1	187.35±27.11	205.13±21.98	205.99±23.12
	2	186.82±26.13	206.29±21.24	206.21±18.23

Note. Mo (mode) - the most frequent value of the cardiointerval in this dynamic series; AMo (mode amplitude) is the number of cardio intervals corresponding to the mode value, in% to the sample size; VAR - variation range; IVE - vegetative equilibrium index; IAPR is an indicator of the adequacy of regulation processes, IT – tension index.

Conclusion. The obtained psychophysiological parameters indicate the presence of an anxiety condition in the examined patients of the main and experimental groups at the dental consultation. At the same time, moderate anxiety is revealed in the main group and a condition between moderate and high situational anxiety in the experimental group by the end of dentist's visit. Low level of anxiety in main group patients at dental consultation, which was carried out with Ushnitsky-Chakhov's method of mandibular anesthesia, determined its possible application in practical dentistry, as it did not cause additional psychoemotional stress.

References

1. Чахов А.А., Ушницкий И.Д., Пинелис И.С. Анатомо-топографическая характеристика вариабельности показателей угла ветви нижней челюсти. *ЭНИ Забайкальский медицинский вестник.* 2018;3:87-92. [Chakhov AA, Ushnitsky ID, Pinelis IS. Anatomical-topographic characteristic of variability of lower jaw branch angle indicators. *ENI Zabaikalskii medicinskii vestnik*. 2018; 3:87-92. (In Russ.).]

2. Рабинович С.А., Зорян Е.В., Бобкова А.Х. Выбор анксиолитических препаратов на амбулаторном стоматологическом приеме. *Образование, наука и практика в стоматологии по объединенной тематике «Обезболивание в стоматологии»: сб. тр. 6-й Всерос. науч.-практ. конф.*/под ред. О.О. Янушевича, И.Ю. Лебеденко, С.А. Рабиновича. – СПб.: Человек, 2009:73-76. [Rabinovich SA, Zoryan EV, Bobkova AKh. Selection of anxiolytic drugs at outpatient dental consultation. *Obrazovanie, nauka i praktika v stomatologii po obedinennoi tematike «Obezbolivanie v stomatologii»: sb. tr. 6-j Vseros. nauch.-prakt. konf.* Edited by O.O. Yanushevich, I.U. Lebedenko, S.A. Rabinovich. – Saint-Petersburg: Chelovek, 2009:73-76. (In Russ.).]

3. Анисимова Е.Н., Орехова И.В., Сохов С.Т. Выбор местного обезболивания у пациентов с артериальной гипертензией в амбулаторной стоматологической практике. *Стоматология.* 2016; 95(1):31-34. [Anishimova EN, Orekhova IV, Sokhov ST. Choice of local pain relief in patients with arterial hypertension in outpatient dental practice. *Stomatologiya*. 2016; 95(1):31-34. (In Russ.).] DOI:10.17116/stomat201695131-34

4. Гажва С.И., Степанян Т.Б., Горячева Т.П. Психоэмоциональный статус пациентов до и после стоматологического вмешательства. *Современные проблемы науки и образования*. 2014; 4:296. [Gazhva SI, Stepanyan TB, Goryacheva TP. Psichoemotional status of patients before and after dental intervention. *Sovremennye problemy nauki i obrazovaniya*. 2014; 4:296. (In Russ.).]

5. Кисловская В.Р. Зависимость между социометрическим статусом и симптомом тревожности ожиданий в социальном общении в возрастном плане: *дис. … канд. мед. наук*: 21.9.67. Кисловская Валентина Романовна.

M., 1971:194. [Kislovskaya VR. The relationship between sociometric status and symptom of anxiety expectations in social communication in age terms: *dis. … kand. med. nauk*.: 21.9.67. Kislovskaya Valentina Romanovna. M., 1971: 94. (In Russ.).]

6. Ушницкий И.Д., Чахов А.А., Винокуров М.М. Клиническая характеристика способов блокады нижнего луночкового нерва. *Якутский медицинский журнал*. 2019; 68(4):103-105. [Ushnitsky ID, Chakhov AA, Vinokurov MM. Clinical methods characteristic of the inferior dental nerve blockade. *Yakutskii medicinskii zhurnal*. 2019; 68(4):103-105. (In (Russ.).] DOI 10.25789/YMJ.2019.68.29

7. Рабинович С.А., Стош В.И., Ремарк Е.Р. Применение безыгольного шприца нового поколения в стоматологической практике. М.: АНМИ, 2002:23. [Rabinovich SA, Stosh VI, Remark ER. Use of a new generation needle-free syringe in dental practice. M.: ANMI, 2002: 23. (In Russ.).]

8. Смирнов В.М. Физиология человека : учебник. М.: «Медицина», 2002; 608. [Smirnov VM. Human physiology: textbook. M.: «Medicine», 2002: 608 (In Russ.).]

9. Сокольская О.Ю. Изменение психоэмоционального состояния детей в процессе диспансерного наблюдения у детского стоматолога. *Стоматология детского возраста и профилактика*. 2014; 49(2):27-31. [Sokolskaya OYu. Change of psychoemotional state of children in the course of dispensary observation at children 's dentist. *Stomatologiya detskogo vozrasta i profilaktika*. 2014; 49(2):27-31. (In Russ.).]

10. Ушницкий И.Д., Чахов А.А. Способ мандибулярной анестезии Ушницкого-Чахова: патент №2019140398, 22.07.2020. [Ushnitsky ID, Chakhov AA. Ushnitsky-Chakhov's method of mandibular anesthesia: Patent № 2019140398, 22.07.2020. (In Russ.).]

11. Ушницкий И.Д., Чахов А.А. Способ мандибулярной анестезии Ушницкого-Чахова по методу Гоу-Гейтса: патент №2695895, 29.07.2019. [Ushnitsky ID, Chakhov AA. Ushnitsky-Chakhov's method of mandibular anesthesia by Gow-Gates's method: Patent № 2695895, 29.07.2019. (In Russ.).]

12. Ушницкий И.Д., Чахов А.А. Способ мандибулярной анестезии Ушницкого-Чахова, учитывающий угол ветви нижней челюсти: патент №2695896, 29.07.2019. [Ushnitsky ID, Chakhov AA. Ushnitsky-Chakhov's method of mandibular anesthesia, taking into account the angle of the branch of the lower jaw: Patent № 2695896, 29.07.2019. (In Russ.).]

13. Ушницкий И.Д., Чахов А.А. Способ ментальной анестезии для блокады подбородочного и резцовой ветви нижнего луночкового нерва: патент №2699736, 09.09.2019. [Ushnitsky ID, Chakhov AA. Methods of mental anesthesia for blockade of mental region and incisal branch of the inferior dental nerve: Patent № 2699736, 09.09.2019. (In Russ.).]

14. Ушницкий И.Д., Чахов А.А. Способ определения целевого пункта при мандибулярной анестезии: патент № 2682457, 19.03.2019. [Ushnitsky ID, Chakhov AA. Method of determination of target point in mandibular anesthesia: Patent № 2682457, 19.03.2019. (In Russ.).]

15. Ушницкий И.Д., Чахов А.А. Способ определения ширины ветви нижней челюсти: патент № 2727579, 22.07.2020. [Ushnitsky ID, Chakhov AA. Method of determination of the lower jaw branch width: Patent № 2727579, 22.07.2020. (In Russ.).]

16. Стош В.И., Рабинович С.А., Зорян Е.В. Руководство по анестезиологии и оказанию неотложной помощи в стоматологии. М.: МЕДпресс-информ, 2002;288. [Stosch VI, Rabinovich SA, Zoryan EV. Manual on anesthesiology and emergency care in dentistry. M. : MEDpress-inform, 2002: 288. (In Russ.).]

17. Чахов А.А., Ушницкий И.Д. Устройство Ушницкого-Чахова для измерения ширины ветви нижней челюсти: патент №196101, 17.02.2020. [Chakhov AA, Ushnitsky ID. Ushnitsky-Chakhov's device for measuring the width of the lower jaw branch: Patent № 196101, 17.02.2020. (In Russ.).]

18. Чахов А.А., Ушницкий И.Д. Устройство Ушницкого-Чахова для проведения мандибулярной анестезии: патент №184398, 24.10.2018. [Chakhov AA, Ushnitsky ID. Ushnitsky-Chakhov's device for mandibular anesthesia: Patent № 184398, 24.10.2018. (In Russ.).]

19. Чахов А.А., Ушницкий И.Д. Устройство Ушницкого-Чахова для проведения мандибулярной анестезии по методу Гоу-Гейтса: патент №189949, 29.12.2018. [Chakhov AA, Ushnitsky ID. Ushnitsky-Chakhov's device for mandibular anesthesia according to Gow-Gates method: patent № 189949, 29.12.2018. (In Russ.).]

20. Чахов А.А., Ушницкий И.Д. Устройство Ушницкого-Чахова для проведения ментальной анестезии по методу С. Маламед: патент № 189950, 26.02.2019. [Chakhov AA, Ushnitsky ID. Ushnitsky-Chakhov's device for mental anesthesia according to S. Malamed's method: patent № 189950, 26.02.2019. (In Russ.).]

21. Ушницкий И.Д., Чахов А.А. Дистресс пациентов на этапах оказания стоматологической амбулаторно-поликлинической помощи. *Актуальные проблемы и перспективы развития стоматологии в условиях Севера: сб. науч. ст. межрег. науч.-практ. конф., посв. 10-летию Ассоциации стоматологов г. Якутска Республики Саха (Якутия) и 50-летию высшего медицинского образования в Республике Саха (Якутия)*. Якутск, 2007:54-57. [Ushnitsky ID, Chakhov AA. Distress of patients at the stages of dental outpatient and polyclinic care. *Aktual'nye problemy i perspektivy razvitiya stomatologii v usloviyah Severa : sb. nauch. st. mezhreg. nauch.-prakt. konf., posv. 10-letiyu Associacii stomatologov g. Yakutska Respubliki Saha (Yakutiya) i 50-letiyu vysshego medicinskogo obrazovaniya v Respublike Saha (Yakutiya)*. Yakutsk, 2007:54-57. (In Russ.).]

22. Ушницкий И.Д., Чахов А.А. Проводниковая анестезия по Гоу-Гейтсу с применением вспомогательного устройства. *Актуальные проблемы ортопедической стоматологии и ортодонтии: материалы I региональной научно-практической конференции врачей стоматологов Федерального Сибирского округа*. Под ред. проф. Писаревского Ю.Л., доц., Бородулиной И.И. Чита, 2011:144-146. [Ushnitsky ID, Chakhov AA. Gow-Gates Conduction anesthesia with the auxiliary device. *Aktual'nye problemy ortopedicheskoj stomatologii i ortodontii: materialy I regional'noj nauchno-prakticheskoj konferencii vrachej stomatologov Federal'nogo Sibirskogo okruga*. Edited by professor Pisarevsky YuL, Borodulina II. Chita, 2011:144-146. (In Russ.).]

23. Ушницкий И.Д., Чахов А.А., Винокуров М.М. Современная концепция патофизиологических механизмов болевого синдрома, психоэмоционального напряжения и их профилактика на стоматологическом приеме. *Стоматология*. 2018; 97(6):67-71. [Ushnitsky ID, Chakhov AA, Vinokurov MM. Modern concept of pathophysiological mechanisms of pain syndrome, psychoemotional tension and their prevention at dentist's. *Stomatologiya*. 2018; 97(6):67-71. (In Russ.).] doi.org/10.17116/stomat20189706167

24. Чахов А.А., Ушницкий И.Д., Колосова О.Н. Эмоциональный статус и эффективность проводниковой анестезии на нижней челюсти у пациентов на амбулаторно-поликлиническом стоматологическом приеме. *Сиб. мед. журн.* Приложение к №2. 2007; 22:100-102. [Chakhov AA, Ushnitsky ID, Kolosova ON. Emotional status and effectiveness of conductor anesthesia on the lower jaw in patients at outpatient and polyclinic dental consultation. *Sib. med. zhurn*. *Prilozhenie k №2*. 2007; 22:100-102. (In Russ.).]

25. Чахов А.А., Ушницкий И.Д. Клинико-физиологическое обоснование эффективности обезболивания нижнелуночкового нерва по Гоу-Гейтсу с использованием вспомогательного устройства. *Якутский мед. журн.* Якутск, 2009; 27(3):17-19. [Chakhov AA, Ushnitsky ID. Clinical-physiological justification of the effectiveness of Gow Gates's anesthesia of the inferior dental nerve using an auxiliary device. *Yakutskij med. zhurn.* Yakutsk, 2009; 27(3): 17-19. (In Russ.).]

26. Чахов А.А., Ушницкий И.Д. Модифицированный способ блокады нижнего луночкового нерва. *Якутский мед. журн.* Якутск, 2009; 28(4):114-116. [Chakhov AA, Ushnitksy ID. Modified method of inferior dental nerve blockade. *Yakutskij med. zhurn*. Yakutsk, 2009; V.28(4):114-116. (In Russ.).]

27. Чахов А.А., Ушницкий И.Д. Роль и значение анатомо-топографических особенностей нижней челюсти при проведении мандибулярной анестезии. *Якутский мед. журн*. 2017; 59(3):116-118. [Chakhov AA, Ushnitsky ID. Role and significance of anatomical and topographic lower jaw features during mandibular anesthesia. *Yakutskij med. zhurn*. 2017; 59(3):116-118. (In Russ.).]

28. Чахов А.А., Ушницкий И.Д. Клиническая характеристика факторов и средств, влияющих на эффективность и безопасности местной анестезии в стоматологии. *Стоматология*. 2018; 4:77-81. [Chakhov AA, Ushnitsky ID. Clinical characterization of factors and agents affecting the effectiveness and safety of local anaesthesia in dentistry. *Stomatologiya*. 2018. 4: 77-81. (In Russ.).].

29. Чахов А.А., Ушницкий И.Д., Исаков Л.О. Модифицированный способ ментальной анестезии по методу Маламед. *Эндодонтия. Today*. 2019; 17(4):12-15. [Chakhov AA, Ushnitsky ID, Isakov LO. Modified method of mental anesthesia by Malamed's method. *Endodontiya. Today*. 2019; 17(4):12-15. (In Russ.).] doi.org/10.36377/1683-2981-2019-17-4-12-15

30. Сидоров Р.Б., Куприянов Г.Я., Чахов А.А. Характеристика клинической значимости угла ветви нижней челюсти в блокаде нижнего луночкового нерва. *Бюллетень СГМУ*. 2018; 40(1):53-54. [Sidorov RB, Kupriyanov GJ, Chakhov AA. Characteristic of clinical significance of the branch angle of the lower jaw in the inferior dental nerve blockade. *Byulleten' SGMU*. 2018; 40(1):53-54. (In Russ.).]

31. Brignardello-Petersen R. Uncertainty about the relative effects of upright versus supine positions for inferior alveolar nerve block. *J. Am. Dent. Assoc*. 2018; 149(5):83-84. DOI: 10.1016/j.adaj.2017.12.027

32. Nogueira da Costa J.K., Wambier L.M., Maran B.M. Different anesthetics on the efficacy of inferior alveolar nerve block in patients with irreversible pulpitis. *J. Am. Dent. Assoc*. 2020; 151(2):87-97. DOI: 10.1016/j.adaj.2019.09.002

33. Lapidus D., Goldberg J., Edward H. Effect of premedication to provide analgesia as a supplement to inferior alveolar nerve block in patients with irreversible pulpitis. *J. Am. Dent. Assoc*. 2016; 147(6):427-437. DOI: 10.1016/j.adaj.2016.01.006

HEALTHCARE, MEDICAL SCIENCE AND EDUCATION ORGANIZATION

S.A. Evseeva, N.M. Gogolev, V.A. Sotnikov, T.E. Burtseva, E.A. Borisova, M.P. Slobodchikova

ROLE OF THE SANITARY AVIATION IN CASES OF EMERGENT MEDICAL ASSISTANCE AND MEDICAL EVACUATION OF THE POPULATION IN THE REPUBLIC OF SAKHA (YAKUTIA)

DOI 10.25789/YMJ.2020.71.15

УДК 614.2

The article represents the analysis of emergency service by means of sanitary aviation (specialized sanitary air medical service) and medical evacuation of the population according to the data of the Republican Center for disaster medicine under the Ministry of health in the Republic of Sakha (Yakutia) for the period of 2017-2019. The analysis took into account the district division in the Republic of Sakha (Yakutia). According to the results of analysis it is obvious that the Arctic regions show high demand in specialized medical assistance and medical evacuation of the pregnant women and injured patients, South-Western parts and Zarechniy regions require evacuation of the patients with vascular disorders.

Keywords: sanitary aviation, air medical service, Yakutia, the Far North, the Arctic.

Introduction: The research is carried out in the Republic of Sakha (Yakutia), one of the nine regions of the Far East federal districts of the Russian Federation. Yakutia, occupying the biggest territory of the Russian Federation, is referred to the Far North territories with a very low density of population. The Republic is located in the North-Eastern part of Eurasia, the total area of the continental and insular parts (including the Lyakhovsky, the Anzhu and the De Long islands composing the New Siberian islands proper in the Arctic ocean) composes 3.1 mln. sq.km. More than 40% of the territory of Yakutia is located above the Polar circle.

The territory of Yakutia covers three time zones, varying from GMT +9, +10, +11. Until now Yakutia is still the most isolated and the most difficult for access regions in the world: the year-round transport infrastructure is unavailable for 90% of its territory. The territories are accessible only by air, road and water transports (depending on the season).

The Republic is characterized by climatic and geographic peculiarities, associated with the periods of freezing up and ice drifting, frequent change of air mass originated from the Arctic, extremely unstable weather, moreover absence of the developed road communications, high number of water barriers, remote healthcare services in rural areas and islands are most common. As of 01.01.2019 only 22.6% parts of the road communication for the common use of the Republic of Sakha (Yakutia), 47.0% roads of the Far North Federal district, and 43.1% roads in the Russian Federation could be defined within the required norms. A number of areas are accessible only by air and seasonal water transports. Thus the Republican Center for disaster medicine under the Ministry health in the Republic of Sakha (Yakutia) becomes one of the key emergency organizations specializing in sanitary medical assistance and medical evacuation.

Objectives: The research of frequency and cause of the sanitary aviation in emergent cases of medical assistance and medical evacuation of the population of the Republic of Sakha (Yakutia) are investigated.

Materials and methods: The article represents the analysis of the emergent medical assistance by means of sanitary aviation and medical evacuation of the population of the Republic of Sakha (Yakutia) according to the district division of the of the Republican Center for disaster medicine under the Ministry health in the Republic of Sakha (Yakutia) for the period of 2017-2019.

Results: Emergent medical consultation and medical evacuation of the populations of the Republic of Sakha (Yakutia) are carried out by the central station of the medical aviation (city Yakutsk), local branches of the sanitary aviation and the bases of the aircrafts in the regions. The local branches of the sanitary aviation are located and referred to the Central district hospitals in Srednekolymsk town, Srednekolymsky region, Batagai village, Verkhoyansky region, Nyurba town, Nyurbinsky region, Mirniy town, Mirninsky region, Neryungry town, Neryungrinsky region and Tiksi village, Bulunsky region.

5 Mil Mi-8 helicopters, equipped with modern medical module, were obtained in accordance with the regional program "On the development of emergent specialized medical assistance to the population living in remote areas of the Republic of Sakha (Yakutia) by means of aircrafts 2017-2019", approved by the Government of the Republic of Sakha (Yakutia) in 14.02.2017. These helicopters are based on the branches of the Republican Center for disaster medicine under the Ministry of health in the Republic of Sakha (Yakutia) including Yakutsk, Srednekolymsk, Batagai, Nyurba, and Magan. Thus, they provide emergent medical assistance to those living in remote areas in due time. Mil Mi-8 helicopters are provided with all necessary medical equipment necessary for medical assistance during the flight, such as resuscitation module including ventilator, defibrillator, monitor, infusion pumps, oxygen therapy, console for medical gases and electricity, ECG apparatus.

The highest number of flights for the period of 2017-2019 is registered from the Central station of the sanitary aviation in Yakutsk (2017 – 516 flights, 2018 – 602 flights, and in 2019 – 675), Nyurbinsky branch (2017 – 137 flights, 2018 – 122 flights, and in 2019 – 150 flights), Srednekolymsky branch (2017 – 75 flights, 2018 – 109 flights, and in 2019 – 100 flights), Tiksi branch (2017 – 89 flights, 2018 – 86 flights, and in 2019 – 71 flights), as it is represented in the table 1.

ЕВСЕЕВА Сардана Анатольевна – к.м.н., с.н.с. ЯНЦ КМП, sarda79@mail.ru; **ГОГОЛЕВ Николай Михайлович** – к.м.н., директор МИ СВФУ им. М.К. Аммосова, gogrcemp@mail.ru; **СОТНИКОВ Владимир Андреевич** – гл. врач Республ. центра медицины катастроф МЗ РС(Я); **БУРЦЕВА Татьяна Егоровна** – д.м.н., проф. МИ СВФУ им. М.К. Аммосова, зав. лаб. ЯНЦ КМП, bourtsevat@yandex.ru; **БОРИСОВА Елена Афраимовна** – к.м.н., доцент МИ СВФУ им. М.К. Аммосова, bolenaff@yandex.ru; **СЛОБОДЧИКОВА Майя Павловна** – преподаватель СПбГПМУ.

Table 1

Key performance indicators (KPI) for the branches of sanitary aviation of the Republican Center for disaster medicine under the Ministry health in the Republic of Sakha (Yakutia)

Sanitary aviation	2017		2018		2019	
	Number of flights	Number of patients	Number of flights	Number of patients	Number of flights	Number of patients
Central station	516	933	602	1084	675	1469
Филиалы						
Nyurba	137	126	122	175	150	215
Batagai	115	144	71	163	84	131
Srednekolymsk	75	212	109	176	100	167
Mirniy	86	176	78	104	25	32
Chulman	36	48	13	15	17	17
Tiksi	89	110	86	109	71	111
Total number of flights from the basic stations	1054		1081		1122	
Flights from other places of bases	121		42		12	

To analyze the KPI of the emergent specialized medical assistance and medical evacuation of the patients of the Republic of Sakha (Yakutia) by means of sanitary aviation we have divided the Republic into the following groups of regions: Arctic, Northern, South-Western, Zarechny, Viluysky and Central. As it can be seen in the table 8 most of the calls are from the Arctic regions. Half of them are evacuated within the region and about 30% are evacuated to Yakutsk (Table 2). It can be explained by the absence of necessary emergent medical assistance in the regions and transport infrastructure peculiarities. Historically all the specialized medical assistance was accessible only in Yakutsk that is why the patients are still transported there. About 20% of all the calls (651) are pediatric (132) the calls for the patients of the first year of life compose 15% (23).

Most of the Northern regions evacuate the patients to Yakutsk in 2019 – 85.2% (2016 – 73.9%, 2017 – 79.8%, 2018 – 85.2%) due to the absence of intraregional primary departments for the patients with initial vascular disorders and traumatological centers of the second level, or by objective reasons, e.g. absence of the qualified narrow medical specialists.

The patients of the South-Western, Zarechny and Viluysky regions are also evacuated to Yakutsk, in 2019 – 69.2% of all the flights in these regions were evacuated to Yakutsk. According to the analysis for the period of 2016-2018 the mean number of evacuated patients was 69.8%, they required high-tech specialized medical assistance under special conditions of the hospitals of the 3rd level of medical service. It can be explained by the high density of population in these regions and presence of the hospitals of the 2nd level of medical service in their regions.

These data once again confirm high demand and reasons for developing the Republican Center for disaster medicine under the Ministry of health in the Republic of Sakha (Yakutia) for specialized emergent medical assistance and evacuation of the patients by means of sanitary aviation.

Structurally the calls are classified the following way: firstly, the disorders of the circulatory system, secondly, traumas and poisonings, thirdly, evacuation of the pregnant women [3,5].

Table 3 shows the highest number of the evacuated patients with cardiovascular disorders in 2019, being hospitalized to the medical institutions of the 3rd level of medical service from Viluisky, South-Western, and Arctic group of regions.

Totally in 2019, 378 patients with acute coronary syndrome were medically assisted, this number is 30.1% higher than in 2018. 195 patients out of 378 were able-bodied, 165 amongst them were males. There is a noticeable increase of the patients in all the regions, most of them are in South-Western group 193 (51%), Viluysky group 64 (17%) and the Arctic group – 42 (11%).

In 2015, 355 patients with acute stroke were medically assisted, this number is 5.3% higher than in 2018. Out of 355 acute strokes 145 cases were in able bodied patients, 100 amongst them were males. Most of the cases 101 (28%) were registered in Zarechny group, 93 (26%) in Viluysky group, and 66 (18.5%) in the Arctic group (table 4).

As for the trauma patients most of them are from the Arctic group of regions 145 (2017 – 12, 2018 – 109), then South-Western 93 (2017 – 101, 2018 – 61) and Viluysky 67 (2017 – 100, 2018 – 82) (table5).

Conclusions: Dynamical increase of the sanitary aviation calls as well as huge territory, underdeveloped road infrastructure, geographical and climatic conditions provide evidence of necessity and validity to develop regional centers for disaster medicine and sanitary avia-

Table 2

Key performance indices of the branches of the Republican Center for disaster medicine under the Ministry of health in the Republic of Sakha (Yakutia)

Group of regions	Total calls number	Examined calls	Specialist calls	Evacuation within the region	Evacuation within the regions	Evacuation to Yakutsk
Arctic	478	453	8	262	31	152
Northern	150	135	0	19	1	115
South-Western	410	396	11	82	14	289
Zarechny	284	271	13	16	37	205
Viluysky	272	269	8	50	57	154
Central	46	41	7	11	0	23
Total	1640	1566	47	440	140	938

Table 3

Dynamics of the patients number evacuated with the disorders of the circulatory system, 2017-2019

Groups of regions	2017	2018	2019
Artic	145	127	152
Northern	46	71	85
South-Western	147	136	331
Zarechny	133	227	166
Viluysky	122	120	204
Central	19	16	12
Total	611	697	950

Table 4

Dynamics of the number of patients evacuated with acute coronary syndrome and cerebral circulation disorders, 2018-2019

Group of regions	Number of the patients with acute coronary syndrome		Number of the patients with cerebral circulation disorders	
	2018	2019	2018	2019
Arctic	26	42	74	66
Northern	21	33	32	32
South-Western	137	193	64	59
Zarechny	30	39	76	101
Viluysky	48	64	78	93
Central	2	7	12	4
Total	264	378	336	355

Table 5

Dynamics of the number of the patients evacuated with traumas, 2017-2019

Group of regions	2017	2018	2019
The Arctic group	126	109	145
The Northern group	46	34	39
The Viluysky group	100	82	67
The South-Western group	101	61	93
The Zarechny group	57	70	58
The Central group	20	14	14
Total:	450	370	416

Table 6

Dynamics of the number of the pregnant women, evacuated with the pathology, 2017-2019

Group of regions	2017	2018	2019
Arctic group	170	132	132
Northern group	42	43	38
Viluysky group	49	50	50
South-Western group	42	54	81
Zarechny group	55	59	61
Central group	4	3	0
Total	362	341	362

tion for emergent specialized medical assistance and evacuation of the patients. The analysis determined the structure of the calls similar to the other regions of the Russian Federation, having remote areas [1,3,5]. For the discussing period a remarkable increase of the calls associated with cardio-vascular pathology was noticed, the number of the calls for trauma patients does not decrease, as well as the calls for medical assistance to the pregnant women. High demand of medical assistance associated with trauma patients and pregnant women evacuation is common for the Arctic regions, whereas South-Western and Zarechny groups require medical assistance associated with vascular disorders. Availability of due time emergent specialized medical assistance in the areas with low density of population can be achieved only with the help of sanitary aviation and the service of the Center for disaster medicine [2,4,5].

Funding. *It is a part of research of the Medical science centre of complex medical problems "Children health condition monitoring in the Republic of Sakha (Yakutia)" (state registration number: 0120-128-07-98); the project of the Ministry of science and education of the Russian Federation (FSRG-2020-0016); and with a financial support from RFBR according to the research project #18-05-60035_ Arctica.*

References

1. Басавина Т.Д., Фалевич Я.Ю. Служба санитарной авиации Архангельской области - флагман современной медицины. *Бюллетень Северного государственного медицинского университета,* 2019.1(42):234-235. [Basavina TD, Falevich YaYu. Service of the sanitary aviation of Arkhangelsky region is the leader of modern medicine. *Bulleten Severnogo gosudarstvennogo meditsinskogo universiteta*, 2019.1:234-235. (In Russ.).]

2. Зосимова О.С. Основные проблемы безопасной эвакуации из учреждений охраны материнства и детства. *Естественные и технические науки*, 2018.8:204-207. [Zosimova OS. The main problems of safe evacuation from maternity and child healthcare institutions. *Estestvenniye i tekhnicheskiye nauki*, 2018.8:204-207. (In Russ.).]

3. Курнявка П.А., Суханов А.В., Катик А.А. Роль санитарной авиации в оказании экстренной медицинской помощи и проведении медицинской эвакуации в Хабаровском крае. *Медицина катастроф*, 2017.2(98): 58-62. [Kurnyavka PA, Sukhanov AV, Katik AA. The role of air ambulance in emergency medical care and medical evacuation in the Khabarovsky territory. *Medicina katastrof*, 2017.2 (98): 58-62. (In Russ.).]

4. Адамян Л.В., Артымук Н.В., Белокриницкая Т.Е. и др. Организация медицинской эвакуации беременных женщин, рожениц и родильниц при неотложных состояниях. *Проблемы репродукции*, 2018.6(24):268-276. [Adamyan LV, Artymuk NV, Belokrinitskaya TE. et al. Organization of medical evacuation of pregnant women, parturient and puerperant women in emergency conditions. *Problemy reproduktsii*, 2018.6(24):268-276. (In Russ.).]

5. Гулин А.Н., Гончаров С.Ф., Гармаш О.А. и др. Пути развития системы экстренной консультативной медицинской помощи и медицинской эвакуации (санитарной авиации) в Российской Федерации. *Медицина катастроф*, 2012.3(79):41-44. [Gulin AN, Goncharov SF, Garmash OA. et al. Ways to develop the system of emergency consutative medical care and medical evacuation (air ambulance) in the Russian Federation. *Medicina katastrof.* 2012.3(79):41-44. (In Russ.).]

6. Evseeva Sardana Anatolievna – Ph.D., senior staff scientist of the laboratory of pediatric health level monitoring, Yakut science centre of complex medical problems, sarda79@mail.ru

7. Gogolev Nikolai Mikhailovich – PhD, a head of the Medical institute of the North-Eastern federal university, +7-924-168-7966, gogrcemp@mail.ru

8. Sotnikov Vladimir Andreyevich – a head doctor of the Republican center for disaster medicine under the Health Ministry of the Republic of Sakha (Yakutia)

9. Burtseva Tatiana Egorovna – MD, professor of the department of pediatrics and pediatric surgery, Medical institute of the North-Eastern federal university, a head of the laboratory of the Yakut science centre of complex medical problems, +7-914-294-3244, bourtsevat@yandex.ru

10. Borisova Elena Afraimovna – PhD, associate professor of department of public health and healthcare, hygiene and bioethics, Medical institute of the North-Eastern federal university, +7-914-273-6232, bolenaff@yandex.ru

11. Slobodchikova Maya Pavlovna – a senior lecturer of the department of foreign languages with the courses of Russian and Latin languages, St. Petersburg state pediatric medical university, +7-911-908-7772, limelight@mail.ru

B.O. Shcheglov, I.V. Galkina, A.O. Lembikov, E.M. Shchelkanov, I.A. Baranchugov, S.N. Shcheglova, M.Yu. Shchelkanov

RHINOLARYNGOLOGICAL SIMULATORS BASED ON 3D PRINTING OPEN UP NEW OPPORTUNITIES FOR PROFESSIONAL TRAINING

DOI 10.25789/YMJ.2020.71.16

УДК 004:614.23:611.2

This article shows the advantages and disadvantages of using the existing various rhinolaryngological simulators for training specialists. The positive technological and educational-methodical aspects of creating personalized digital manikins based on medical tomographic images processed using the Slicer 3D software are indicated. The authors, based on the proposed methodology, created digital and physical models using 3D printing, which are proposed to be introduced as an educational tool.

The use of 3D printing of otorhinolaryngological simulators (ORLS) based on digital data of computed or magnetic resonance imaging allows not only to dramatically reduce the cost of their production, practically transferring ORLS from the category of "equipment" to "consumables", but also to bring the educational process to a qualitatively new level. It is possible to create personalized 3D ORLS to prepare for complex surgical procedures as well as to take into account the age features and biological diversity of the human population. A convenient method is to use a sequence of 3D ORLS of gradually decreasing scale (from highly enlarged models to real size ones). The use of transparent plastics for 3D printing allows for additional detail and increased visibility of the manipulations performed. Additional application of dyes to various parts of the nasal cavity and oropharynx allows practicing the skills of the collecting deep swabs without contamination of the separated mucous surfaces of neighboring departments, which is necessary for the development of microbiological research methods.

Keywords: medical training, simulation equipment, practical skill, 3D modeling, otorhinolaryngology.

SHCHEGLOV Bogdan Olegovich – Head of the Student Association «3D Modelling for Biomedicine» in the Centre of Project Activities (CPA) of Far Eastern Federal University (FEFU) (Vladivostok); e-mail: b.shcheglov@mail.ru; phone.: 89147189825. **GALKINA Irina Vyacheslavovna** – Ph.D. in Medicine, Leader Researcher of the International Scientific and Educational Center for Biological Security (ISECBS) in FEFU (Vladivostok); e mail: galkina333@mail.ru; phone: 89031705864.**LEMBIKOV Alexei Olegovich** – member of the Student Association «3D Modelling for Biomedicine» in CPA of FEFU (Vladivostok); e-mail: letsa1970@mail.ru; phone: 89996161281. **SHCHELKANOV Egor Mikhailovich** – student of Phystech-School of Biological and Medical Physics of Moscow Institute of Physics and Technology (Dolgoprudny); e mail: egorshchelkanov@mail.ru; phone: 89243215162. **BARANCHUGOV Ilya Alexandrovich** – post-graduate of the School of Engineering of FEFU (Vladivostok); e mail: baranchugov_ia@dvfu.ru; phone: 89146611718. **SHCHEGLOVA Svetlana Nikolaevna** – Ph.D. in Pedagogics, Chief of the Department of Higher Mathematics in Northern-East State University (Magadan); e-mail: schegloff@mail.ru; phone: 89148624028. **SHCHELKANOV Mikhail Yurievich** – Doctor of Sciences in Biology, Chief of ISECBS in FEFU (Vladivostok); Chief of the Laboratory of Virology in the Federal Scientific Center of the East Asia Terrestrial Biodiversity, Far Eastern Branch of Russian Academy of Sciences (FEBRAS) (Vladivostok); Leader Researcher of the Laboratory of Marine Mammals in the National Scientific Center of Marine Biology, FEBRAS (Vladivostok); e mail: adorob@mail.ru; phone: 89032689098.

The nasopharynx performs an important barrier function preventing the penetration of foreign objects, particles of dust and aerosols with microorganisms in the respiratory and digestive tract. A large number of options for trauma of the nose and trachea including the penetration of foreign bodies as well as the further development of methods for collecting of deep nasopharyngeal and tracheal swabs for the needs of infectology forces rhinopharyngologists to maintain a high level of qualification for implementing penetrating techniques with minimal possible damage to the mucous surfaces. These same techniques cause serious difficulties for students. In this regard silicone otorhinolaryngological simulators (ORLS) of various designs occupied a worthy place in the corresponding educational technologies both in Russia [3, 4, 6, 7] and other countries [13-15]. The relevance of the widespread introduction of ORLS was clearly illustrated in the process of deploying a large-scale system of molecular genetic diagnostics of SARS-CoV-2, which caused the COVID-19 pandemic in 2020 [1, 12], since it was repeatedly emphasized that the efficiency of taking oropharyngeal swabs significantly depends on analysis [8, 9].

At the same time, silicone ORLS as a part of special mannequins, which have proven themselves in simulation centers for passing qualification exams, have a number of limitations in their use for the routine training: commercial silicone ORLS that reproduce not only topological and anatomical, but also tactile properties of human tissues remain quite expensive educational equipment, manipulation with which is to be carried out under the supervision of a qualified teacher; commercial ORLS are purchased ready-made and their production in order to develop skills in manipulating patients of a specific age or with specific pathologies although theoretically possible but practically unattainable for economic reasons; placing of the real foreign objects in the body cavities of mannequins quickly renders them unusable.

This drawback of ORLS is deprived by 3D printing of topological models based on digital data of computed or magnetic resonance imaging. Acrylonitrile butadiene styrene is suitable as a printing material, which allows obtaining flexible samples at an affordable price (several hundred rubles depending on size). All parts can be printed in a single cascade or, if possible, assembled / disassembled. To obtain 3D models the freely distributed free Slicer 3D program was used (Fig. 1) [5, 11].

3D-ORLS are so low that they are real “consumables” and could be widely utilized even for independent educational process by students without teacher supervision. Real hard-to-remove foreign bodies can be placed in the cavities of such ORLS, and students have the opportunity to make incisions in order to visualize manipulations. As experience shows, the very possibility to repeat 3D printing at any time and therefore not pay attention to the safety of ORLS significantly increases the effectiveness of training.

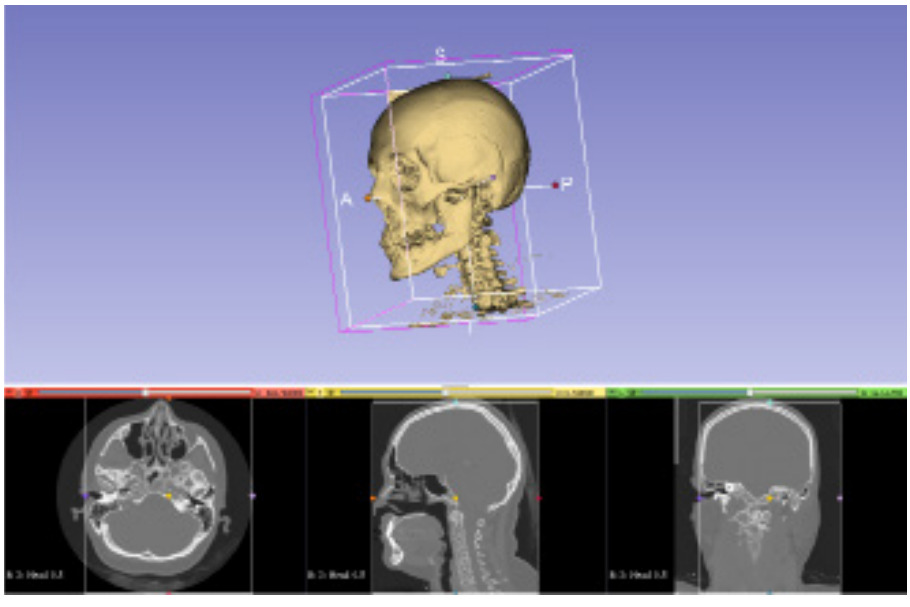


Fig. 1. A fragment of the work on creating a 3D model of the skull with the upper respiratory tract in the Slicer 3D program based on computer tomography data of the patient's head in different projections.

3D-printed ORLS also open up fundamentally new prospects for increasing the efficiency of training of medical specialists.

Using 3D printing, it is possible to quickly get ORLS that reproduce the upper respiratory tract of people of different ages, up to individual characteristics. This helps students not to get used to the "average mannequin" and be prepared to constantly face the age-related features and anatomical diversity of the human population as it happens in practice. In addition, there is the possibility of personalized 3D ORLS to prepare for complex surgical procedures.

The digital model allows to print at any scale (Fig. 2). Of particular value for the educational process are 3D ORLS, which are increased in comparison with real objects –this makes it possible to increase the visibility of the details of the studied objects and the manipulations carried out. Using a sequence of 3D ORLS of gradually decreasing scale is a convenient method for eliminating unnecessary movements that increase the risk of mucosal damage.

One can use transparent materials (for example, VisiJet, PET GT-Glass, ABS-plastic, etc.) for 3D printing. The ability to look inside otorhinolaryngological cavities brings the level of training upto a qualitatively new level.

The production of relatively cheap 3D replicas of ORLS allows adding to them elements of improving the educational process, which are almost impossible for much more expensive silicone mannequins. For example, one can apply a thick wet dye (we used a mixture of glycerol and gouache) of different colors to different parts of the nasal cavity and oropharynx to practice the skills of collecting deep swabs without contamination of the separated mucous membranes by neighboring departments [4]. This technique is necessary for reliable diagnosis and study of receptor specificity of etiological agents of acute respiratory diseases [2, 10].

Introduction of the described digital technologies in the educational process will allow not only to integrate educational modules of various disciplines into a single complex, but also to form students ' commitment to modern concepts of digitalization of Russian medicine.

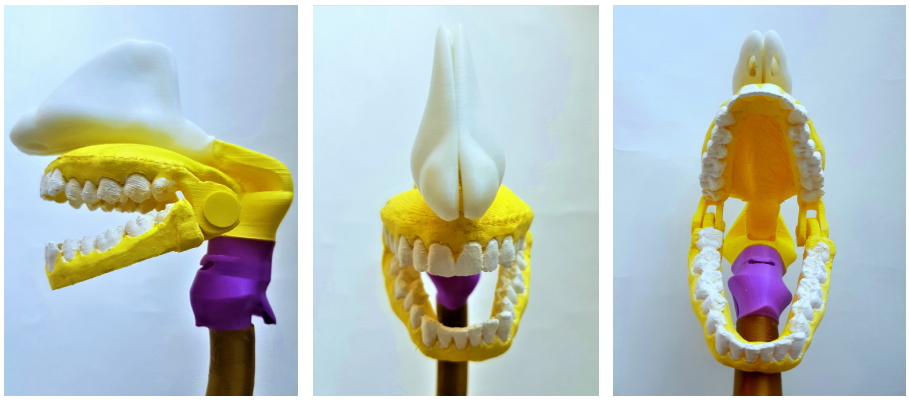


Fig. 2. Photo of a 3D otorhinolaryngological simulator: in profile (A), full face (B), full face with open mouth (C).

References

1. М.Ю. Щелканов, А.Ю. Попова, В.Г. Дедков [и др.]. История изучения и современная классификация коронавирусов (Nidovirales: Coronaviridae). Инфекция и иммунитет. 2020; 10 (2): 221–246. DOI: 10.15789/2220-7619-HOI-1412 [M.Yu. Shchelkanov, A.Yu. Popova, V.G. Dedkov [et al.]. History of investigation and current classification of coronaviruses (Nidovirales: Coronaviridae). *Russian Journal of Infection and Immunity*. 2020; 10 (2): 221–246. (In Russ.) DOI: 10.15789/2220-7619-HOI-1412]

2. Кудряшов С. Е., Козлов В. С. Ринологический симулятор: модернизация и оценка эффективности применения. *Вестник оториноларингологии*. 2018; 3: 50-55. DOI: 10.17116/otorino201883350 [Kudryashov SE, Kozlov VS. The rhinological simulator: the upgrade and validation study with the evaluation of the effectiveness of its application. *Bulletin of Otorhinolaryngology*. 2018; 3: 50-55. (In Russ.) DOI: 10.17116/otorino201883350]

3. Лембиков А. О. Использование ринологического симулятора для совершенствования процедуры взятия мазков со слизистой различных отделов респираторного тракта при комплексной диагностике гриппа А. Владивосток: изд-во Дальневосточного федерального университета. В сб.: *Инновации и технологии в биомедицине. Сборник материалов научно-практической конференции (10-13 июня 2019 г.)*, 2019: 287-288. Режим доступа: https://www.dvfu.ru/science/publishing-activities/catalogue-of-books-fefu/ [Lembikov AO. The use of a rhinological simulator to improve the procedure for taking swabs from the mucosa of various parts of the respiratory tract in the complex diagnosis of influenza A. Vladivostok: Far Eastern Federal University. *Innovations and Technologies in Biomedicine. Collection of materials of the scientific and practical conference* (June 10-13, 2019). 2019: 287-288. (In Russ.) Access mode: https://www.dvfu.ru/science/publishing-activities/catalogue-of-books-fefu/]

4. Львов Д. К., Щелканов М. Ю., Бовин Н. В. [и др.] Корреляция между рецепторной специфичностью штаммов пандемического вируса гриппа А (H1N1) pdm09, изолированных в 2009-2011 гг., структурой рецептор-связывающего сайта и вероятностью развития летальной первичной вирусной пневмонии. *Вопросы вирусологии*. 2012; 1(57): 14-20. [Lvov DK, Shchelkanov MYu, Bovin NV [et al.] Correlation between the receptor specificities of pandemic influenza A (H1N1) pdm09 virus strains isolated in 2009–2011 and the structure of the receptor-binding site and the probabilities of fatal primary virus pneumonia. *Voprosy Virusologii*. 2012; 1 (57): 14-20. (In Russ.)]

5. Львов Д. К., Яшкулов К. Б., Прилипов А. Г. и др. Обнаружение аминокислотных замен аспарагиновой кислоты на глицин и глутаминовую кислоту в рецептор-связывающем сай-

те гемагглютинина в штамме пандемического вируса гриппа H1N1 от больных с летальным исходом и со средне-тяжелой формой заболевания. *Вопросы вирусологии*. 2010; 3 (55): 15–18. [Lvov DK, Yashkulov KB, Prilipov AG [et al.] Detection of amino acid substitutions of asparaginic acid for glycine and asparagine at the receptor-binding site of hemagglutinin in the variants of pandemic influenza A/H1N1 virus from patients with fatal outcome and moderate form of the disease. *Voprosy Virusologii*. 2010; 3(55): 15–18. (In Russ.)]

6. Матвеева И. В., Колосова М. М. Обработка медицинских данных в программе 3D-Slicer: 3D-моделирование челюстей и их фрагментов, полученных из результатов конусно-лучевой компьютерной томографии. *Бюллетень Северного государственного медицинского университета*. 2018; 1: 23-25. [Matveeva IV, Kolosova MM. Processing of medical data in the 3D-Slicer program: 3D modeling of jaws and their fragments obtained from the results of cone beam computed tomography. *Bulletin of the Northern State Medical University*. 2018; 1: 23-25. (In Russ.)]

7. Тимофеев М. Е., Шаповальянц С. Г., Полушкин В. Г. [и др.] Медицинские симуляторы: история развития, классификация, результаты применения, организация симуляционного образования. *Вестник Новгородского государственного университета им. Ярослава Мудрого*. 2015; 2: 63-59. [Timofeev ME, Shapoval'iants SG, Polushkin VG [et al.] Medical simulators: the history, classification, application, and simulation training organization. *Bulletin of Novgorod State University of Yaroslav the Wise*. 2015; 2: 63-59. (In Russ.)]

8. Новая коронавирусная инфекция (COVID-19): этиология, эпидемиология, клиника, диагностика, лечение и профилактика. Учебно-методическое пособие / В.В. Никифоров, Л.В. Колобухина, С.В. Сметанина [и др.] // М.: Департамент здравоохранения города Москвы, 2020. – 71 с. [Novel coronavirus infection (COVID-19): etiology, epidemiology, clinics, diagnostics, treatment, and prophylaxis. Educational and methodological guide / V.V. Nikiforov, L.V. Kolobukhina, S.V. Smetanina [et al.] // Moscow: Department of Public Health of Moscow city, 2020. – 71 p. (In Russ.)]

9. В.В. Грибова, Д.Б. Окунь, Е.А. Шалфеева [и др.]. Облачный сервис для дифференциальной клинической диагностики острых респираторных вирусных заболеваний (в том числе – связанных с особо опасными коронавирусами) методами искусственного интеллекта. *Якутский медицинский журнал*. 2020; 2: 44–47. DOI: 10.25789/YMJ.2020.70.13 [V.V. Gribova, D.B. Okun, E.A. Shalfeeva [et al.]. Cloud service for the differential clinical diagnostics of acute respiratory viral diseases (including those associated with highly contagious coronaviruses) with an application of methods of artificial intelligence. *Yakut Medical Journal*. 2020; 2: 44–47. DOI: 10.25789/YMJ.2020.70.13]

10. Чернышева О. Е., Герасименко В. В., Климовицкий Ф. В. [и др.] Медицинские симуляторы и манекены как элемент обучения студентов практическим навыкам в педиатрии. *Травма*. 2017; 1(18): 103-105. DOI: 10.22141/1608-1706.1.18.2017.95598 [Chernyshova OYe, Gerasymenko VV, Klymovytsky FV [et al.] Medical simulators and mannequins as an element of teaching practical skills in pediatrics. *Trauma*. 2017; 1 (18): 103-105. (In Russ.) DOI: 10.22141/1608-1706.1.18.2017.95598]

11. Щеглов Б. О., Галкина И. В., Щеглова С. Н. [и др.] Эффективность внедрения лаборатории 3D-моделирования в лечебно-диагностических учреждениях. *Якутский медицинский журнал*. 2019; 3: 109-111. DOI: 10.25789/YMJ.2019.67.30 [Shcheglov BO, Galkina IV, Shcheglova SN [et al.] Effectiveness of 3D modelling laboratory implementation into therapeutic and diagnostic medical institutions. *Yakut medical journal*. 2019; 3: 102-104. DOI: 10.25789/YMJ.2019.67.30]

12. М.Ю. Щелканов, Л.В. Колобухина, О.А. Бургасова [и др]. COVID-19: этиология, клиника, лечение. *Инфекция и иммунитет*. 2020; 10 (3): 421-445. DOI: 10.15789/2220-7619-CEC-1473 [M.Yu. Shchelkanov, L.V. Kolobukhina, O.A. Burgasova [et al.]. COVID-19: etiology, clinic, treatment. *Russian Journal of Infection and Immunity*. 2020; 10 (3): 421-445. DOI: 10.15789/2220-7619-CEC-1473]

13. Chang D. R., Lin R. P., Bowe S. [et al.] Fabrication and validation of a low-cost, medium-fidelity silicone injection molded endoscopic sinus surgery simulation model. *Laryngoscope*. 2017; 4(127): 781-786. DOI: 10.1002/lary.26370

14. Lee A. Y., Fried M. P., Gibber M. Improving rhinology skills with simulation. *Clin. North Am.: Otolaryngol.* 2017; 5(50): 893-901.

15. Musbahi O., Aydin A., Al Omran Y. [et al.] Current status of simulation in otolaryngology: a systematic review. *J. Surg. Educ*. 2017; 2(74): 203-215.

HYGIENE, SANITATION, EPIDEMIOLOGY AND MEDICAL ECOLOGY

T.E. Popova, O.G. Tikhonova, A.N. Romanova, A.A. Tappakhov, Andreev M.E.

DOI 10.25789/YMJ.2020.71.17

УДК 616.92:616:93:578.834.1

ANALYSIS OF THE EPIDEMIOLOGICAL SITUATION ON COVID-19

The epidemiological situation of the new coronavirus infection COVID-19 in a number of countries and the Republic of Sakha (Yakutia) has been studied. There were analyzed the prevalence, morbidity, mortality and lethality, dependence of the detection of new cases on the level of average air temperature. Three zones of the spread of the new coronavirus infection were conditionally identified. The "red" zone includes countries with a prevalence of more than 100 cases per 100000 of the population and a high rate of spread of the disease (USA and Brazil). The "yellow" zone includes those countries in which the prevalence varies within 30-100 cases per 100000 of the population: Spain, Russia, Great Britain, Italy and France. The "green" zone includes countries with a prevalence of the disease of less than 30 cases per 100000 the population and the most favorable rates of spread of the new coronavirus infection: China, Norway and Finland.

Keywords: new coronavirus infection, COVID-19, epidemiology, prevalence, incidence.

Introduction. 17 years after the SARS (Severe Acute Respiratory Syndrome) pandemic caused by the SARS coronavirus and originated in the Chinese province of Guangzhou, China has once again presented the world with a new coronavirus infection. On the 31th of December, 2019, the Chinese authorities informed the World Health Organization (WHO) of an outbreak of unknown pneumonia. The pandemic of the new coronavirus infection began with the detection of a group of idiopathic pneumonia cases in hospitals in Wuhan (Hubei Province, China) on the 31th of December, 2019 [7]. Although the location of the Wuhan seafood market where the infection is likely to have originated has been clearly identified, it is still unclear whether this is the source of the infection. From the first 41 patients, only 27 (66%) had an epidemiological link to the seafood market. First cases, which began to be registered on the 1st of December, had no relation to the market [11].

Koczkodaj WW et al. based on heuristic reports on the situation, WHO predicted that by the 30th of March, 2020, 1,000,000 cases of COVID-19 will be registered outside China [26]. So as of April 22, 2020, 1,188,324 confirmed cases and 106,374 deaths were registered in the world. The total cumulative morbidity is 127.7 cases per 100000 of the population, the total cumulative mortality is 114.3 per 100000 of the population [30].

The identified pathogen was named SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2) [1, 6, 21]. On the 11th of February, 2020, the disease was named the new coronavirus disease COVID-2019 (the English abbreviation for COV - coronavirus and ID - infectious disease) [28].

Chinese researchers found a close relationship between SARS-CoV-2 with coronavirus, which was isolated from patients with severe acute respiratory syndrome in 2018 in Zhoushan, bat SARS-like coronaviruses (88%), SARS-CoV (79%) and less - with the MERS virus (50%) [16].

COVID-19 is a single positive strand RNA virus enclosed in a lipid bilayer [4, 16]. The lipid bilayer fuses with the host cell membrane, releasing RNA into the cytoplasm and causing the translation of various viral proteins. The replicated RNA genome and synthesized viral proteins are reassembled into new viruses that break out of the cell [10, 20].

The virus enters cells through the binding of two proteins. The viral analogue is the spike protein (S-protein), a glycoprotein expressed as a homotrimer on the viral envelope [18]. Each S-protein consists of two subunits. The S1 subunit includes a receptor-binding domain that targets receptors in host cells, and S2 regulates membrane fusion. This viral S-protein binds to the human protein receptor ACE2 [9]. ACE2 is widely distributed in the lungs, heart, kidneys, and adipose tissue [2, 25]. Binding of the S-protein to ACE2 facilitates membrane fusion and the introduction of COVID-19 RNA into the cell. Compared to SARS, COVID-19 uses the same mechanism to enter host cells, but at a slower rate. However, COVID-19 is accumulating in the system more than SARS. This explains why COVID-19 has a longer incubation period and is more infectious, while SARS has more symptoms and disease severity [22]. Varga Z. et al. demonstrated that SARS-CoV-2 infection promotes the induction of endotheliitis in multiple organs as a direct consequence of involvement. COVID-19 endotheliitis may explain the systemic impairment of microcirculatory function in various vascular beds and their clinical consequences in patients with COVID-19 [15]. This hypothesis provides a therapeutic rationale for the use of anti-inflammatory anticytokine drugs, ACE inhibitors, and statins to stabilize the endothelium in the fight against viral replication [5, 8, 13, 23, 24].

Mehta P et al. showed that a subset of patients with severe COVID-19 have cytokine storm syndrome [12]. The authors recommend identifying and treating hyperinflammation using existing, validated therapies with a proven safety profile to reduce the increase in mortality. Secondary hemophagocytic lymphohistiocytosis (sHLH) is an underestimated,

POPOVA Tatyana E. – Grand PhD in Medical Sciences, deputy Director for Science of Scientific Center for Complex Medical Problems (Yakutsk). Add: 4, Sergellahskoe shosse, str, Yakutsk, 677019, Republic of Sakha (Yakutia). 8(4112)321981; e-mail: tata2504@yandex.ru, **ROMANOVA Anna N.** – Grand PhD in Medical Sciences, Director for Science of Scientific Center for Complex Medical Problems (Yakutsk). Add: 4, Sergellahskoe shosse, str, Yakutsk, 677019, Republic of Sakha (Yakutia). 8(4112)321981; e-mail: ranik@mail.ru, **TIKHONOVA Olga G.** – Scientific Secretary of Scientific Center for Complex Medical Problems (Yakutsk). Add: 4, Sergellahskoe shosse, str, Yakutsk, 677019, Republic of Sakha (Yakutia). 8(4112)321981; e-mail: tichonova_olga@mail.ru, **TAPPAKHOV Alexey A.** – Candidate of Sciences in Medicine, Associate Professor at the Department of Neurology and Psychiatry of the Medical Institute (North-Eastern Federal University), Head Researcher of the Laboratory of Neuropsychophysiological Researches (Medical Clinic of NEFU); Senior Researcher, Center for Neurodegenerative Diseases, Scientific Center for Complex Medical Problems (Yakutsk). Add: 36, Kulakovsky str., Yakutsk, 677000, Republic of Sakha (Yakutia). 8(4112)49-67-00, e-mail: dralex89@ mail.ru, **ANDREEV Michil E.** – Researcher of the Laboratory of Neuropsychophysiological Researches (Medical Clinic of NEFU). Add: 36, Kulakovsky str., Yakutsk, 677000, Republic of Sakha (Yakutia). 8(4112)49-67-00, e-mail: michil90@mail.ru.

hyperinflammatory syndrome characterized by fulminant and fatal hypercytokinemia with multiple organ failure. In adults, sHLH is most often caused by a viral infection [3] and occurs in 3.7–4.3% of cases of sepsis. The cardinal features of sHLH include persistent fever, cytopenia, hyperferrinemia; lung damage (including ARDS) develops in about 50% of patients. Cytokine profile resembling sHLH associated with COVID-19 disease severity characterized by increased interleukins IL-2, IL-7, stimulating factor granulocyte, IFN-γ inducible protein 10, monocyte chemoattractant protein 1, inflammatory macrophage 1-α protein and TNF-α [11].

One of the factors in the spread of infection is presumably meteorological factors. Thus, local weather conditions with low temperatures, moderate daily temperature ranges and low humidity are likely to facilitate transmission of COVID-19 [17].

Wu Y. et al. analyzed the impact of temperature and humidity on daily new cases and new deaths from COVID-19 in 166 countries. As of March 27, 2020, there were 509,164 cumulatively confirmed cases and 23,335 deaths worldwide. Italy, the United States, and Spain were the three countries with the most COVID-19 cases outside of China. Furthermore, Italy, Spain and Iran have the highest number of COVID-19 deaths outside of China. Average temperatures ranged from -5.28 to + 34.30°C, while average relative humidity ranged from 11.39% to 88.42%. A 1°C increase in temperature was associated with a 3.08% decrease (95% CI: 1.53%, 4.63%) in new daily cases and a 1.19% decrease (95% CI: 0.44%, 1.95%) new daily deaths. A 1% increase in RH was associated with a 0.85% decrease (95% CI: 0.51%, 1.19%) in new daily cases and 0.51% (95% CI: 0.34%, 0.67%) in daily new deaths [14].

A particular problem is the tactics of managing patients with severe coronavirus infection. However, to date, there is no specific antiviral treatment for coronavirus infection. In different countries of the world, vaccines against COVID-19 are being developed, clinical trials have begun, but the question of the persistence of the formation of an immune response both after the infection and after vaccination remains open.

In connection with the current unfavorable epidemiological situation in the world, we made an attempt to analyze the epidemiological situation of COVID-19 in a number of countries with different climatic characteristics and the Republic of Sakha (Yakutia).

Purpose of the work: to assess the dynamics of the spread of COVID-19 in the Republic of Sakha (Yakutia) in comparison with other regions of the Russian Federation and a number of foreign countries

Tasks:

1. Conduct a comparative analysis of the spread of COVID-19 in different regions of the world
2. Calculate the growth rate of the spread of COVID-19 in the Republic of Sakha (Yakutia) and Russia
3. Analyze the dependence of the intensity and rate of spread of COVID-19 on the average air temperature in different regions of the world

Materials and methods. Epidemiological data for SARS-CoV-2 were obtained using an online platform that collects data from government agencies from January 1 to July 31, 2020, the coverage was 29 weeks of observation (in different regions, due to different dates of the onset of the pandemic, the observation period varied from 20 to 29 weeks) [27, 29, 31]. The study included the following countries: China, USA, Spain, Italy, France, Germany, Great Britain, Russia, Brazil, Norway, Finland, Thailand. For the Russian Federation, a comparison was made of data in Moscow, St. Petersburg and the Republic of Sakha (Yakutia). Since the onset of disease growth varied from country to country, we conducted a weekly analysis over a period of 29 weeks, starting from the official onset date in each country, to search for common patterns of infection. We analyzed the following indicators: the number of confirmed cases, new cases of COVID-19 in 29 weeks, mortality, mortality in% during the observation period as of July 31, 2020, the prevalence rate of coronavirus infection according to the formula (Rt = number of patients registered in the last week, divided by the number of patients registered in the previous week). We also made an attempt to analyze the dependence of the growth in the spread of COVID-19 depending on the temperature regime in the analyzed regions, in this regard, regions with different temperature regimes were included in the analysis.

Research results. The official dates for the spread of the novel coronavirus infection COVID-19 in the countries included in the study are: China - 12/31/2019, Thailand - 01/13/2020, USA - 01/21/2020, France - 01/24/2020, Germany - 01/27/2020, Finland - 01/29 .2020. In four countries (Spain, Italy, Great Britain, Russia), the COVID-19 epidemic began on January 31, 2020. In Brazil, Norway, the first cases of COVID-19 were registered on 02/26/2020. In large cities of the Russian Federation, such as Moscow and St. Petersburg, the dates of registration of the first cases fall on 03/02/2020 and 03/07/2020, respectively. In the Republic of Sakha (Yakutia), the largest region of the Russian Federation, the first patients with COVID-19 were registered on 03/18/2020. Analyzing the spread of COVID-19 across countries, we can conclude that the proximity of borders, for example, China and Thailand, plays a role in the first place. In Europe, the spread began with France, which has common borders with Germany, Spain, Italy, Great Britain, which determined the patterns of the spread of the new coronavirus infection in this part of the world. Therefore, most likely, the closure of borders between neighboring countries was one of the leading measures to prevent the spread of COVID-19.

Next, we analyzed the growth in the total number of patients in the compared countries in dynamics in terms of per 100000 of the population (prevalence) for 29 weeks of follow-up (end date 07/31/2020). The highest prevalence of a new coronavirus infection was registered in the USA - 1433.8, in Brazil - 1227.7, in Spain - 712.3, followed by Russia with an indicator of 572.4, and the UK in fifth place - 452.6 cases per 100000 of the population (Figure 1). The lowest prevalence of COVID-19 was recorded in China, with 6.1 cases per 100,000 of the population.

We also calculated new cases of coronavirus infection per 100000 of the population weekly (Figure 2). Regularities common for all analyzed regions in identifying new cases were not noted, however, we can distinguish three groups of countries in which we identified different scenarios of the spread of infection: we designated countries with an unfavorable scenario as a "red" zone, countries with an average spreading rate - as a "yellow" zone and countries with a favorable scenario of spread - as a "green" zone. The "red zone" includes the United States and Brazil, where there is a steady increase in the number of cases and the highest prevalence rates, no trend towards a decrease in incidence has been noted. In this zone, the incidence rates exceed 100 cases per 100000 of the population. In the "yellow" zone, weekly incidence rates range from 40 to 90 cases per 100000 of the population, this zone includes Spain, Russia, Great Britain, Italy, France. In the "yellow" zone, a general trend is noted: an increase in incidence occurs from 3-5 weeks of the development of a pandem-

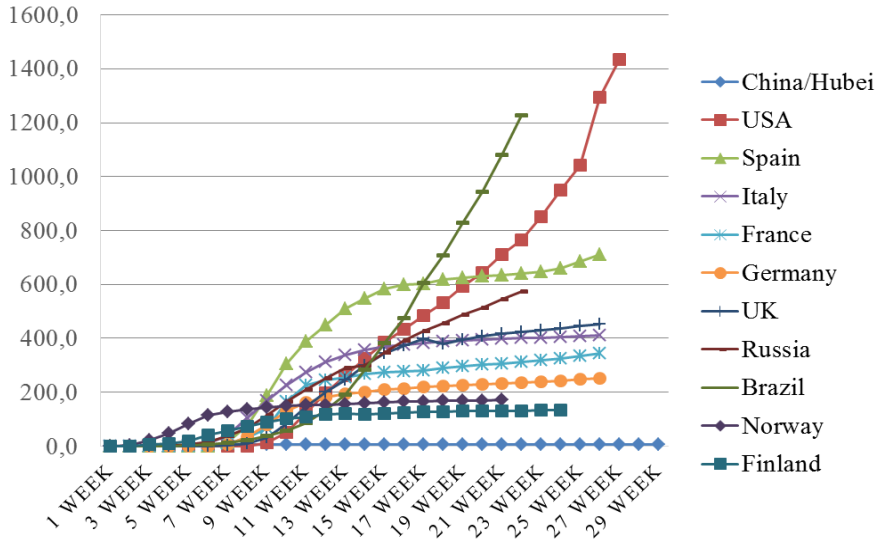


Fig. 1. The number of confirmed cases of COVID-19 (per 100000 of the population) in the compared countries.

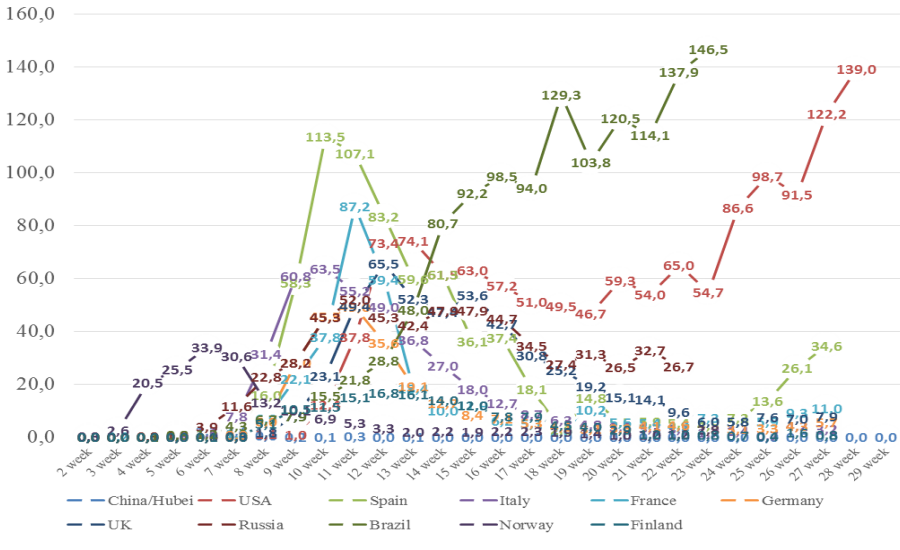


Fig. 2. New cases of COVID-19 (per 100000 of the population) over 13 weeks (since the beginning of the epidemic) in the compared countries

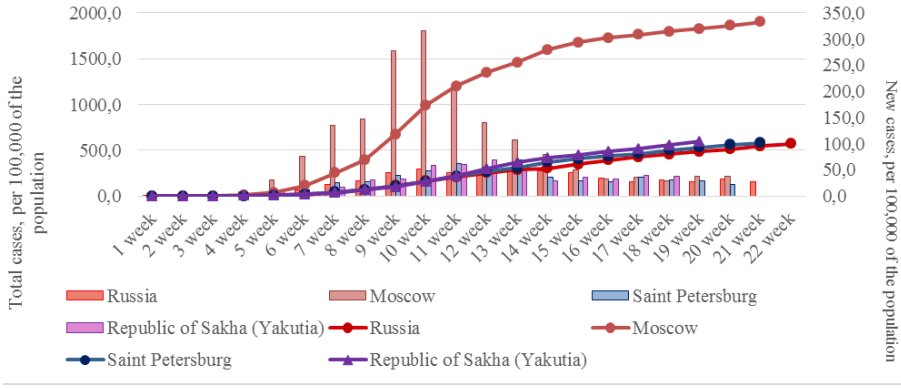


Fig. 3. The number of confirmed and new cases of COVID-19 in the Republic of Sakha (Yakutia) and Russia (per 100000 of the population), on 05/30/2020.

ic, a peak is reached by 9-10 weeks, followed by a decrease by 13 weeks. In the "green" zone were China, Norway, Finland, where the weekly incidence did not exceed 30 cases per 100000 of the population. As can be seen from the current picture of the disease, the level of health care does not affect the rate of spread of the new coronavirus infection.

In the Republic of Sakha (Yakutia), the first case of a new coronavirus infection was registered on March 18, 2020. By July 31, in the Republic of Sakha (Yakutia) reached 20th week, and in Russia – 22nd week of the COVID-19 epidemic. During the analyzed period in the Republic of Sakha (Yakutia), the prevalence of COVID-19 reached 595.4 per 100000 of the population, the prevalence rate of a new coronavirus infection was at 1.1 on 20th week. Indicators of both prevalence and morbidity are comparable with the epidemiological situation in Russia. In the Russian Federation, an increase in the number of cases was observed from 5 weeks with a maximum at 11 weeks, followed by a plateau for 5 weeks and a further decrease in the number of new cases. In Russia, Moscow is in first place in terms of prevalence and incidence of COVID-19. The maximum of newly detected cases in Moscow reached at 11th week of the pandemic, amounting to 315.39 per 100000 of the population, with a subsequent decrease in new cases of COVID-19 (Figure 3).

We calculated the mortality and lethality rates in the compared regions, taking into account the total number of deaths in 29 weeks of observation on July 31, 2020 (Table 1). The indicator reflects the risk of dying from a given disease for the population, and mortality - the probability of dying from a given disease in the event of a disease. The highest death rate in Spain is 63.5 (per 100,000 people), followed by the UK with 58.2, then the United States with 51.7. The lowest death rate in China, at 0.33. Mortality in the countries included in the study ranged from 1.5 to 12.9%. The lowest mortality rate in Russia is 1.5%. France and Great Britain (12.9%) are in first place in mortality, Spain is in second (8.8%), and Italy is in third (6.7%). In China, the case fatality rate has reached 5.5%. In Sakha (Yakutia) on July 31, 2020, the mortality rate reached 1, which is 2 times lower than in Moscow.

We have carried out a correlation between the average level of air temperature and the newly detected cases also weekly (Table 2). As a result of the analysis, it was found that there is no correlation between the average temperature

Table 1

Mortality and mortality rates for 29 weeks of observation (as of July 31, 2020)

Country	Population	Number of deaths as of July 31, 2020	Mortality rate	Mortality, %
China/Hubei	1 393 000 000	4643	0.33	5.5
USA	328 200 000	169795	51.7	3.6
Spain	46 528 966	29534	63.5	8.8
Italy	60 288 522	16606	27.5	6.7
France	65 273 512	29141	44.6	12.9
Germany	83 149 300	7445	8.9	3.5
UK	66 993 318	38995	58.2	12.9
Brazil	217 175 869	80431	37	3
Norway	5 367 580	248	4.6	2.7
Finland	5 543 233	338	6.1	4.5
Russia	146 745 098	13011	8.9	1.5
Moscow	12 692 466	4792	37.8	2
Saint Petersburg	5 392 992	1787	33,1	5,7
Republic of Sakha (Yakutia)	971 996	58	6	1

Table 2

Correlation between temperature and new cases of COVID-19

Country/City	Spearman's rank correlation coefficient	p-value
China	–0.497	0.006
Italy	0.274	0.15
UK	0.52	0.003
Saint Petersburg	0.86	< 0.001
Finland	0.39	0.037
USA	0.88	< 0.001
France	0.47	0.01
Russia	0.806	< 0.001
Brazil	0.47	0.01
Thailand	–0.14	0.46
Spain	0.12	0.53
Germany	0.36	0.05
Moscow	0.71	< 0.001
Norway	0.325	0.08
Republic of Sakha (Yakutia)	0.87	< 0.001
Total	0.433	< 0.001

regime and the incidence of COVID-19. A positive correlation was obtained only in Russia, but this does not prove the effect of the average air temperature on the rate of spread of the new coronavirus infection, since a temporary coincidence with the beginning of the warm season and the beginning of the decline in COVID-19 cases is more likely.

Discussion. In general, the analysis of the epidemiological situation for COVID-19 showed that three zones of the spread of the new coronavirus infection can be conditionally distinguished, depending on the rate of spread. The United States and Brazil are in the "red" zone with an unfavorable scenario and a steady increase in COVID-19, the same countries showed the highest prevalence rates of the SARS-CoV2 virus: 1433.8 and 1227.7 per 100000 of the population, respectively. The "yellow" zone includes Spain, Russia, Great Britain, Italy, France, where the increase in the detection of new cases starts from 3-5 weeks, reaching a maximum at 9-10 weeks, with a subsequent decline by 13 weeks. Norway, Finland, China are in the "green" zone, where both the prevalence and incidence rates of COVID-19 remained at a low level throughout the observation period.

The spread of a new coronavirus infection in the Republic of Sakha (Yakutia) obeys general patterns, the indicators are comparable to the average indicators for the Russian Federation. At the time of this writing, the 20th week of the spread of COVID-19 has been reached in the Republic of Sakha (Yakutia).

COVID-19 leads to severe complications in immunocompromised patients, leading to deaths, mostly in people over 65 years of age. The highest death rate in the UK is 58.2, the lowest death rate is in China, at 0.33. Mortality ranged from 1.5 to 12.9%. The lowest mortality rate in Russia is 1.5%. France and Great Britain are in first place in mortality (12.9%). In the Republic of Sakha (Yakutia), as of July 31, 2020, the mortality rate reached 1%.

A wide variety of factors affect the spread of the new coronavirus infection in the world. The closure of international borders has played a huge role in preventing the spread of COVID-19. At the beginning of the pandemic, the spread of infection began in countries with common borders, which turned out to be most indicative for European countries. In general, such a rapid spread of the new coronavirus infection is due to the processes of globalization in the world.

The level of development of healthcare, as our observation shows, does not affect the growth rate of infection; in most developed countries, the incidence of COVID-19 was at a high level. We found that there is no correlation between the average temperature regime and the rate of spread of COVID-19, which is consistent with the literature data.

What threats can we expect from the new coronavirus infection?

1) In general, unlike influenza, the new coronavirus infection does not spread seasonally, which puts the risk of a "second" and subsequent waves of the disease.

2) Few published data on the absence of persistent humoral immunity casts doubt on the effectiveness of the alleged vaccination [19].

3) It cannot be ruled out that the newly emerged mutations of the SARS-CoV2 virus may lead to the appearance of another outbreak, possibly more dangerous for humanity, which in turn will reduce the effectiveness of vaccination.

4) The features of the pathogenesis of the new coronavirus infection indicate the expected increase in the number of patients with systemic diseases and chronic obstructive pulmonary diseases and, possibly, neurodegenerative diseases, in particular Parkinson's disease.

When to expect a new outbreak is difficult to predict, it may be in 18-20 years, as in the case of the SARS (severe acute respiratory syndrome) pandemic caused by the SARS coronavirus. But the current situation with COVID-19 must be perceived by the scientific, medical community, as well as by the whole of humanity, as a lesson in order to counter them even more effectively with future epidemiological threats.

References

1. Пшеничная Н.Ю., ьВеселова Е.И., Семенова Д.А. [и др.] COVID-19 – новая глобальная угроза человечеству. *Эпидемиология и инфекционные болезни: актуальные вопросы*. 2020;1:6-13. [Pshenichnaya NYu., Veselova EI, Semenova DA. [et al.]. COVID-19 - a new global threat to humanity. *Epidemiologiya i infekcionnye bolezni: aktual'nye voprosy.* 2020;1:6-13 (in Russ).]

2. Gupte M, Boustany-Kari CM, Bharadwaj K. [et al.]. ACE2 is expressed in mouse adipocytes and regulated by a high-fat diet. *Am. J. Physiol.*

Regul. Integr. Comp. Physiol. 2008; 295 (3): 781-788. DOI: 10.1152/ajpregu.00183.2008.

3. Ramos-Casals M, Brito-Zeron P, Lopez-Guillermo A. [et al.] Adult haemophagocytic syndrome. *Lancet.* 2014; 383: 1503-1516. DOI: 10.1016/S0140-6736(13)61048-X.

4. Wu F, Zhao S, Yu B. [et al.] A new coronavirus associated with human respiratory disease in China. *Nature*. 2020; 579 (7798): 265-269. DOI: 10.1038/s41586-020-2008-3.

5. Flammer AJ, Sudano I, Hermann F. [et al.] Angiotensin-converting enzyme inhibition improves vascular function in rheumatoid arthritis. *Circulation*. 2008; 117 (17): 2262-2269. DOI: http://dx.doi.org/10.1161/CIRCULATIONAHA.107.734384

6. Zhu N, Zhang D, Wang W. [et al.]. A novel coronavirus from patients with pneumonia in China, 2019. *N. Engl. J. Med*. 2020;382:727-733. DOI: 10.1056/NEJMoa2001017.

7. Wang C, Horby PW, Hayden FG, Gao GF. A novel coronavirus outbreak of global health concern. *Lancet.* 2020; 395 (10223): 470-473. DOI: https://doi.org/10.1016/S0140-6736(20)30185-9.

8. Hurlimann D, Forster A, Noll G. [et al.]. Anti-tumor necrosis factor-alpha treatment improves endothelial function in patients with rheumatoid arthritis. *Circulation*. 2002; 106: 2184-2187. DOI: 10.1161/01.cir.0000037521.71373.44

9. Zhou P, Yang XL, Wang XG. [et al.]. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature*. 2020; 579 (7798): 270-273. DOI: 10.1038/s41586-020-2012-2017.

10. Qin P, Du EZ, Luo WT. [et al.] Characteristics of the life cycle of porcine deltacoronavirus (PDCoV) in vitro: replication kinetics, cellular ultrastructure and virion morphology, and evidence of inducing autophagy. *Viruses.* 2019; 11 (5): 455. DOI: https://doi.org/10.3390/v11050455.

11. Huang C, Wang Y, Li X. [et al.] Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *The Lancet.* 2020; 395 (10223): 497–506. DOI: https://doi.org/10.1016/S0140-6736(20) 30183-5.

12. Mehta P, McAuley DF, Brown M. [et al.] COVID-19: consider cytokine storm syndromes and immunosuppression. *Lancet.* 2020; 395 (10229): 1033-1034. DOI: 10.1016/S0140-6736(20)30628-0.

13. Taddei S, Virdis A, Ghiadoni L. [et al.] Effects of angiotensin converting enzyme inhibition on endothelium-dependent vasodilatation in essential hypertensive patients. *A. J. Hypertens.* 1998; 16: 447-456. DOI:10.1097/00004872-199816040-00006

14. Wu Y, Jing W, Liu J. [et al.] Effects of temperature and humidity on the daily new cases and new deaths of COVID-19 in 166 countries. Sci Total Environ. 2020; 729: 139051. DOI: 10.1016/j.scitotenv.2020.139051

15. Varga Z, Flammer AJ, Steiger P. [et al.] Endothelial cell infection and endotheliitis in COVID-19. *The Lancet*. 2020; 395 (10234): 1417-1418. DOI:https://doi.org/10.1016/S0140-6736(20)30937-5.

16. Lu R, Zhao X, Li J. [et al.] Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. *The Lancet*. 2020; 395: 565–74. DOI: https://doi.org/10.1016/ S0140-6736(20)30251-8.

17. Liu J, Zhou J, Yao J. [et al.] Impact of meteorological factors on the COVID-19 transmission: A multi-city study in China. *Sci Total Environ*. 2020; 726: 138513. DOI: 10.1016/j.scitotenv.2020.138513.

18. Li F. Structure, function, and evolution of coronavirus spike proteins. *Ann. Rev. Virol.* 2016; 3 (1): 237-261. DOI: 10.1146/annurev-virology-110615-042301.

19. Wu F, Wang A, Liu M. [et al.] Neutralizing antibody responses to SARS-CoV-2 in a COVID-19 recovered patient cohort and their implications. *medRxiv*. 2020. DOI: 10.1101/2020.03.30.20047365.

20. Qinfen Z. The life cycle of SARS coronavirus in Vero E6 cells. *J. Med. Virol.* 2004; 73 (3): 332-337. DOI: 10.1002/jmv.20095

21. Fang J, Deng L, Zhang L. [et al.] Review of the clinical characteristics of coronavirus disease 2019 (COVID-19). *J. Gen. Intern. Med*. 2020; 35 (10223): 1545-1549. DOI: 10.1007/s11606-020-05762-w.

22. Su Z, Wu Y.A multiscale and comparative model for receptor binding of 2019 novel coronavirus and the implications of its life cycle in host cells. *BioRxiv*. 2020. DOI: https://doi.org/10.1101/2020.02.20.958272

23. Anderson TJ, Meredith IT, Yeung AC. [et al.] The effect of cholesterol-lowering and antioxidant therapy on endothelium-dependent coronary vasomotion. *Engl J Med*. 1995; 332: 488-493. DOI: 10.1056/NEJM199502233320802.

24. Feldmann M, Maini RN, Woody JN. [et al.] Trials of anti-tumour necrosis factor therapy for COVID-19 are urgently needed. *Lancet.* 2020; 395 (10234): 1407-1409. https://doi.org/10.1016/S0140-6736(20)30858-8.

25. Turner AJ, Hiscox JA, Hooper NM. ACE2: from vasopeptidase to SARS virus receptor. *Trends Pharmacol. Sci*. 2004; 25 (6): 291-294. DOI: 10.1016/j.tips.2004.04.001.

26. Koczkodaj WW, Mansournia MA, Pedrycz W. [et al.] 1,000,000 cases of COVID-19 outside of China: The date predicted by a simple heuristic. *Glob Epidemio*l. 2020; 23: 100023. DOI: 10.1016/j.gloepi.2020.100023

27. https://covid19.who.int/region

28. https://www.who.int/ru/news-room/detail/08-04-2020-who-timeline---covid-19

29. https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports

30. https://who.maps.arcgis.com/apps/opsdashboard/index.html#/a19d5d1f86ee4d99b013eed5f637232d

31. https://www.worldometers.info/coronavirus/country/italy/

S.I. Semenov, A.V. Fadeev, M.M. Pisareva, A.B. Komissarov, A.A. Egorova, K.M. Stepanov, T.D. Rumyantseva

DOI 10.25789/YMJ.2020.71.18

УДК 616.36-002 (571.56)

MIXT-CHRONIC VIRAL HEPATITIS IN THE REPUBLIC OF SAKHA (YAKUTIA)

Studies of serological and molecular biological markers of viral hepatitis in different regions of the Republic of Sakha (Yakutia) were carried out to study the wide distribution of serological, molecular biological markers of viral hepatitis and assess the epidemiological situation of viral hepatitis in the Republic of Sakha (Yakutia). It was shown that in the structure of mixed viral hepatitis, viral hepatitis C with virus G (21.42%) is more common than mixed viral hepatitis B + C (14.28%). A high proportion of mixed viral hepatitis C + E was also determined (42.6%), and the share of mixed viral hepatitis B + E was only 9.1%. Patients with triple mixed viral hepatitis E + B + C and quadruple mixed viral hepatitis E + B + C + D were identified in equal shares in 9.1% of cases.

Double, triple mixed viral hepatitis should always be considered as a potentially serious disease.

Keywords: mixed chronic viral hepatitis, HBsAg, HBV DNA, a-HCV, HCV RNA, a-HDV, a-HEV.

Introduction. Mixed viral hepatitis with the greatest frequency occurs in persons with a rich parenteral history (drug addicts using intravenous drug use, patients who require blood and blood products in treatment). According to S.S. Sleptsova, parenteral interventions such as operations and blood transfusions (in 64% of cases), the introduction of narcotic drugs in 1.3%, and tattooing in 1.3% made up most of the overall structure of the probable routes of transmission of the hepatitis C virus. Moreover, in the anamnesis of patients infected with genotype 3a of virus C, 38.5% of cases (genotype 2a in 25%, genotype 1b in 22%) indicated frequent visits to the dentist, 7.7% - to injecting drugs. Also, this author indicates that in acute viral hepatitis B, the transmission of the B virus in 43.7% of cases was realized through the blood. At the same time, in 3.3% of cases, the occurrence of AVH B was assumed after blood transfusion, in 10.0% - after surgical treatment, 6.0% indicated various gynecological interventions, including medical abortion, 12.0% - dental manipulations [7]. Similar routes of transmission, characteristic of parenteral hepatitis, lead in some patients to infection with several hepatotropic viruses and the development of mixed infection. First of all, this concerns the combination of viral hepatitis B (VH B), D (VH D) and C (VH C). For the most part, this can be a layering of the second virus on the previous chronic viral hepatitis - superinfection or simultaneous infection with several viruses - co-infection. Where mixed viral hepatitis B + C is present, markers of hepatitis D virus (a-HDV or HDV RNA) - "triple" mixed viral hepatitis, can also be detected in some patients. Double, triple mixed viral hepatitis should always be considered as a potentially serious disease [12]. Antibodies to the hepatitis E virus class G (a-HEV IgG) can also be detected, indicating the transferred hepatitis E, transmitted by the fecal-oral route [17]. In this regard, the detection of any hepatitis virus requires mandatory testing of markers of other viruses, not only parenteral hepatitis, but also enteral.

The purpose of this work is to study the breadth of distribution of serological, molecular biological markers of viral hepatitis and to assess the epidemiological situation of viral hepatitis in the Republic of Sakha (Yakutia).

Materials and research methods. The data of the republican register of viral hepatitis were used [9]. As of January 2020, the republican register includes 14,723 patients with viral hepatitis of various etiologies (B, C and D).

Studies were carried out for the presence of serological and molecular biological markers of viral hepatitis (VH) in 148 people. from different regions of the republic - 25 people. from Tattinsky district, 35 - from Ust-Aldansky, 11 - from Srednekolymsky, 18 - from the infectious diseases department of the YAGKB and 59 - employees of the republican boarding school for the elderly and disabled (DIPI) in Yakutsk. The subjects were divided into 2 groups. Group I - conditionally healthy people (71 people) who have not previously been tested for viral hepatitis. Group II consisted of 77 patients with various parenteral viral hepatitis who underwent ELISA and PCR to clarify the diagnosis.

Study of the breadth of distribution of molecular biological markers of parenteral viral hepatitis in 2014-2015. carried out by PCR studies for the presence of HBV DNA, HCV RNA, HDV RNA in 651 people, 365 of them tested for HGV RNA. The collection of material was carried out during expeditionary work in the following areas: in the Zhigansky region (n-270), Namsky (n-82), Tattinsky (n-50), Srednekolymsky (n-49), Ust-Aldansky (n-41), Khangalassky (n-24), Vilyuisky (n-50), Yakutsk (n-22). PCR studies were carried out in the laboratory of biology of viruses of the Federal State Budgetary Institution "Research Institute of Influenza" of the Ministry of Health of the Russian Federation (Ph.D. M.M. Pisareva) in 2017-2019. and in the laboratory of molecular diagnostics of the FBSI Central Research Institute of Epidemiology of Rospotrebnadzor of the Russian Federation in 2014-2015. (Doctor of Medical Sciences, Professor V.P. Chulanov).

ELISA studies were carried out for the presence of HBsAg, a-HBsAg (mU / ml),

SEMENOV Sergey Innokentievich – Doctor of Medical Sciences, Leading Researcher, Scientific Research Center Medical Institute, FGAOU NEFU named after M.K. Ammosova, 677000, Yakutsk, Belinsky, 56, E-mail: insemenov@mail.ru, **PISAREVA Maria Mikhailovna** – Candidate of Biological Sciences, Leading Researcher, Federal State Budgetary Institution "Research Institute of Influenza" of the Ministry of Health of Russia, 197376, Russia, St. Petersburg, st. prof. Popova 15/17, E-mail: pisarevamm@gmail.com, **KOMISSAROV Andrey Borisovich** – Researcher of the Federal State Budgetary Institution "Research Institute of Influenza" of the Ministry of Health of Russia, 197376, Russia, St. Petersburg, st. prof. Popova 15/17, E-mail: a.b.komissarov@gmail.com, **FADEEV Artem Viktorovich** – Researcher, Research Institute of Influenza, Ministry of Health of Russia, 197376, Russia, St. Petersburg, st. prof. Popova 15/17, E-mail: afadeew@gmail.com, **EGOROVA Anna Andreevna** – laboratory assistant of the Federal State Budgetary Institution "Research Institute of Influenza" of the Ministry of Health of Russia, 197376, Russia, St. Petersburg, st. prof. Popova 15/17, E-mail: anna.egorova@influenza.spb.ru, **STEPANOV Konstantin Maksimovich** – Doctor of Agricultural Sciences, Professor of the Department of food technology and catering Arctic State Agrotechnological University, 677007, Yakutsk, sh. Sergelyakhskoe, 3 km, house 3. E-mail: Stenko07@mail.ru, **RUMYANTSEVA Tatiana Dmitrievna** – Leading Researcher, Arctic State Agrotechnological University, 677007, Yakutsk, sh. Sergelyakhskoye, 3 km, house 3. E-mail: tanya_rum@mail.ru.

a-HBc-total, a-HBc IgM, a-HBeAg, a-HBe IgG, a-HDV total, a-HCV, a-HEV-IgG ... For the detection of markers of viral hepatitis, the test systems "Hepascan HBsAg" and "Hepaskrin" produced by ZAO Bioservice, Moscow, and Vektohep D - antibodies-strip, manufactured by ZAO Vector-Best, Novosibirsk were used. The quantitative determination of anti-HBs was carried out using the anti-HBs Quant EIA II Roche enzyme immunoassay on an automatic analyzer Cobas Core II, manufactured by Hoffmann La Roche (Switzerland).

To isolate nucleic acids, reagent kits "RIBO-prep", DNA HBV, RNA HCV and RNA HDV were used - reagent kits "AmpliSens® HBV-FL", "AmpliSens® HCV-FL" and "AmpliSens® HDV-FL", reverse transcription - kits of reagents "REVER-TA-L". All reagents are produced by FBUN "Central Research Institute of Epidemiology" of Rospotrebnadzor.

Statistical processing of the material was carried out on a personal computer using the IBM SPSS Statistics version 21 software package. To assess the relationship between the studied indicators, parametric (Pearson) correlation analysis methods were used.

Results and discussion. By order of Rospotrebnadzor dated 17.03.2008 No. 88 "On measures to improve monitoring of pathogens of infectious and parasitic diseases", a Reference Center for monitoring viral hepatitis was created on the basis of the Central Research Institute of Epidemiology of Rospotrebnadzor. The reference center has developed an automated computer program for collecting and analyzing information on viral hepatitis - a monitoring system for viral hepatitis patients "Register of patients with viral hepatitis" [5, 6].

According to the republican register, as of January 2020, 14729 patients with viral hepatitis of various etiologies are registered with an infectious disease doctor. Of these, the largest number (14714 people, 99.8%) are patients with chronic viral hepatitis (CVH), and only 0.11% (15 people) of cases are acute viral hepatitis (AVH). The most common cause of chronic forms of the disease were hepatitis B and C viruses. Chronic viral hepatitis B and C account for 49.53 and 45.52% of patients, respectively. Among 6194 patients with chronic hepatitis B, the register includes 4 children under 14 years old and 9 adolescents 15-17 years old, among 6698 patients with chronic hepatitis C - 13 children under 14 years old and 14 adolescents 15-17 years old.

According to the register, 559 patients with combined forms of hepatitis B and C and 1094 patients with chronic viral hepatitis B with a delta agent are under the supervision of specialists in the republic. Simultaneous infection with viruses B and D (coinfection) or infection with hepatitis D virus against the background of chronic hepatitis B, carriage of HBsAg (superinfection) is considered as a separate nosological form - delta viral infection (HDV infection), which has features of the course both in acute and in a chronic form. At this time in the republic among patients with chronic viral hepatitis B, the proportion of patients with HDV infection was 17.66% of cases. Among all patients with chronic hepatitis, the proportion of people with chronic hepatitis B with HDV infection was 7.43%. In children, one case of viral hepatitis D (VH D) was registered at the age of 2, 14 and 17 years.

Mixed viral hepatitis in patients occurred to a greater extent due to the combination of hepatitis B and C viruses in 91.05% of cases. Mixed hepatitis B + D + C was detected in 8.58% of patients and combined hepatitis C in HBsAg carriers - in 0.35% (in two patients).

According to the register, with hepatitis B virus monoinfection, liver cirrhosis (LC) and hepatocellular carcinoma (HCC) were established in 1.1 and 0.08% of cases, respectively, with hepatitis B + D these indicators were 16.9 and 0.64% respectively. With triple infection B + D + C, CP developed in 10.4% of cases. This "triple" mixed hepatitis is a potentially severe form of the disease with a high proportion of liver cirrhosis in the early stages of the disease at a young age and the risk of developing hepatocellular carcinoma [1, 11, 12].

The spread of serological markers of viral hepatitis was studied in 148 patients (Table 1). The analysis of the presented studies showed that among conventionally healthy individuals (group I) and patients with CH (group II), there is a high proportion of seropositive individuals for all viral hepatitis. Of the 148 examined, 22 people. (14.86%) serological markers of viral hepatitis were not detected, and in 126 people. (85.14%), serological markers of hepatitis B, C, D, E viruses were determined. Serological markers of hepatitis E (a-HEV IgG) were detected in 32 (21.62%) people. Studies have shown that the majority of apparently healthy individuals have significant infection with serological markers of GV: markers of the hepatitis E virus (a-HEV IgG) in 19.71% of cases, hepatitis C virus (a-HCV) - 12.7% and the virus hepatitis B (HBsAg) - 9.85%. It should be noted that the prevalence of viral hepatitis C is currently increasing throughout the world against the background of a decrease in viral hepatitis B. Previous studies in the Republic of Sakha (Yakutia) showed that the prevalence of HBsAg among the population at the beginning of the 2000s was at a level of 8.3 to 18 , 1% in some areas, a-HCV - from 2.0 to 3.4% [2,3].

However, the presence of antibodies to the hepatitis virus does not allow one to judge the level of infection of the population, since antibodies can either persist for life in convalescents after acute viral hepatitis, or they may not form in persons with immunosuppression [4,13]. Moreover, in more than half of those infected with hepatitis B and C viruses, the course of the disease proceeds in an active form, as evidenced by the results of PCR studies. So, out of 7 HBsAg positive subjects, 5 had HBV DNA (71.4%) and out of 9 a-HCV-positive, 7 had HCV RNA (77.7%). Thus, the results of studies of the conditionally healthy population of Yakutia confirmed the data on a

Table 1

The spread of serological markers of viral hepatitis among different population groups (n-148)

Group	HBsAg		HBV DNA in HBsAg-positive		a-HDV in HBsAg-positive		a-HCV		HCV RNA in a-HCV-positive		a-HEV IgG	
	abs.	%	abs.	%	abs.	%	abs..	%	abs.	%	abs..	%
I group (n-71)	7	9.85	5	71.4	0	-	9	12.7	7	77.7	14	19.71
II group (n-77)	19	24.67	13	68.42	8	42.10	56	72.72	39	50.64	18	23.37
x^2	4.62		0.11		2.5		51.67		0.011		0.292	
p	0.018		0.745		0.114		<0.001		0.918		0.59	
OR*	0.33		1.15		0		0.054		1.526		0.805	
Total n-148	26	17.56	18	69.23	8	30.76	65	43.91	46	70.76	32	21.62

* Odds ratio.

very high infection with hepatitis B and C viruses and their replicative activity. Hepatitis D virus markers have not been found among conventionally healthy individuals.

In the II group of patients with viral hepatitis of various etiology, serological markers of the hepatitis C virus were found with the highest frequency - in 72.7% of cases, while half of the patients (50.6%) had an active replicative phase of the disease, as evidenced by the detected RNA and DNA of viruses. There were 3 times less patients with chronic hepatitis B (24.6%) than patients with hepatitis C (72.7%), but in more than half of the cases the disease was accompanied by B virus replication (HBV DNA) - in 68.4% cases, replication of the hepatitis C virus (HCV RNA) - in 50.6%. Antibodies to the D virus were detected in 42.1% (8 of 19 HBsAg-positive) patients, which confirms the previously obtained data on the extremely high frequency of the spread of HDV infection among the MS (Y) population [2,3,16]. Thus, the studies have shown a very high frequency of detection of the replicative activity of the disease among patients with viral hepatitis C and hepatitis D.

To establish the true picture of infection in the population, it is not enough to determine only serological markers of hepatitis viruses. In order to clarify the diagnosis, specific diagnostic methods are used, such as qualitative PCR. To determine the viral load, to monitor specific treatment with antiviral drugs, quantitative PCR is used. In recent years (2014-2018), during monitoring studies, we have tested the population for CHV by PCR without a preliminary stage of serological diagnosis by ELISA. A total of 645 people were examined. Of these, the group of conditionally healthy individuals (group I) was 440 people, the group of patients with CH of various etiology (group II) - 205. The results of PCR testing are shown in Table. 2.

Among the surveyed conventionally healthy individuals (group I), the proportion of patients with hepatitis C virus was 2.72%, with virus B - 1.81%, with hepatitis D virus - 2.04%. In the group of patients with VH (group II), as in previous studies, a large proportion is accounted for by chronic viral hepatitis C. Thus, hepatitis C virus RNA was detected in 20.48% of patients, hepatitis B virus DNA - in 14.63% sick. The proportion of patients with hepatitis D virus among all surveyed was 11.21%. In the group of patients with hepatitis B virus (30 people), hepatitis D virus was detected in 76.6% of cases (23 people). Among all those examined, hepatitis G virus (HGV RNA) was found in 7.46% of cases. Currently, there is no information in the available literature that the hepatitis G virus independently causes chronic viral hepatitis and associated laboratory changes and morphological changes in the liver [10].

The presence in blood of HDV RNA in 4.96% of the examined, HBV DNA in 5.89% and HCV RNA in 8.37% indicates that the republic should be considered as an endemic territory with a high replicative activity of all hepatitis viruses.

According to the literature, in the world, the number of people infected with the hepatitis G virus can range from 0.75-1.5 billion. and they are associated with hepatitis B and C viruses [8, 15]. In our studies, 22 people. of the 340 investigated, hepatitis G viruses were detected, which accounted for 6.47% of cases. At the same time, in conventionally healthy individuals, this figure was 3.7%, in patients with viral hepatitis with various etiologies - 10.7%. According to foreign authors, among healthy donors, antibodies to the G virus are found in 4.3% of people. [fourteen].

The data obtained during the PCR study indicate that the mono-replicative variant of viral hepatitis among conventionally healthy individuals was found in 85.71% (18 people out of 21) cases, and the mixed-replicative variant of GV - in 14.39% (3 people out of 21). 21) (Table 3).

Mixed-viral hepatitis in group I of conventionally healthy individuals was detected in 3 people, in group II patients - in 14 people. In both groups, the greatest number is accounted for by mixed viral hepatitis B and D (HBV DNA + HDV RNA +).

The analysis of the spread of serological markers of the hepatitis E virus among conventionally healthy individuals and among patients with CVH was carried out. The results obtained indicate that, despite the different modes of transmission, antibodies to hepatitis E virus are found in 37.5% of cases, and mixed hepatitis C + E - 42.6%. Double mixed hepatitis E + B and triple mixed hepatitis

Table 2

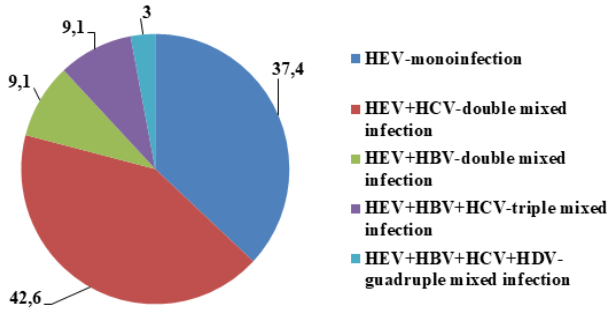
The spread of viruses HBV DNA, HCV RNA, HDV RNA and HGV RNA among different population groups

Study group	ДНК HBV		PHK HCV		PHK HDV		PHK HGV	
	abs.	%	abs.	%	abs.	%	abs.	%
I group (n-440)	8	1.81	12	2.72	9	2.04	7 (+) of 272	2.57
II group (n-205)	30	14.63	42	20.48	23	11.21	15 (+) of 201	7.46
x^2	39.149		57.504		23.054		5.176	
p	<0.001		<0.001		<0.001		0.023	
OR*	0.108		0.109		0.165		0.328	
Total (n-645)	38	5.89	54	8.37	32	4.96	22 (+) of 473	4.65

Table 3

The spread of mono-viral and combined mixed viral hepatitis

	I group (n-440)		II group (n-205)		X^2	p
	abs.	%	abs.	%		
Mono viral infection						
Mono ДНК HBV +	2	11.11	19	23.75	0.74	0.38
Mono PHK HCV +	6	33.33	37	46.25	0.54	0.46
Mono PHK HDV +	4	22.22	9	11.25	0.73	0.39
Mono PHK HGV +	6	33.34	15	18.75	1.01	0.29
Total mono ВГ	18	85.71	80	85.1		
Mixed viral infection						
Mixed ДНК HBV+PHK HCV+	0		2	14.28	0.08	0.77
Mixed ДНК HBV+PHK HDV+	2	66.66	6	42.85	0.01	0.91
Mixed ДНК HBV+PHK HGV+	0		3	21.42	0.00	0.96
Mixed ДНК HCV+PHK HDV +	0		1	7.14	0.77	0.38
Mixed ДНК HCV+PHK HGV +	1	33.34	1	7.14	0.08	0.77
Mixed PHK HDV+PHK HGV+	0		1	7.14	0.77	0.38
Total mixed ВГ	3	14.39	14	14.9		
Total patients	21	14.29	94	14.9		



Frequency of detection of serological markers of parenteral hepatitis B, C, D of enteral hepatitis E

E + B + C were detected with the same frequency - 9.1% of cases each. And in mixed viral hepatitis B + C + D, antibodies to the hepatitis E virus were detected in only 3% of cases (figure).

Conclusions:

1. As of January 2020, according to the republican register, the majority of patients are patients with chronic forms of viral hepatitis B and C - 99.8%, including 49.5% CVH B, 45.5% CVH C, and acute viral hepatitis was recorded in only 0.11% of cases;

2. A high degree of patients with CVH D (17.6%) was revealed among patients with CVH B, since the pathogenetic mechanisms of delta infection have an undeniable connection and dependence on the hepatitis B virus.

3. Among all patients, chronic mixed viral hepatitis was registered in 3.79% of cases and was mainly caused by a combination of hepatitis B and C viruses in 91.05% of cases, hepatitis B, C and D viruses - 8.5%.

4. Monitoring studies of the spread of serological markers of GV among conventionally healthy individuals by ELISA showed markers of hepatitis E virus (a-HEV IgG) in 19.7% of cases, hepatitis C virus (a-HCV) - 12.7%, hepatitis B virus (HBsAg) - 9.8%. Moreover, in these seropositive subjects, replication of virus C (HCV RNA), virus B (HBV DNA) was noted in 77.7%, 71.4%, respectively.

5. PCR monitoring studies of the spread of GV viruses among conventionally healthy individuals detected HCV RNA in 2.7% of cases, HBV DNA - 1.81%, HDV RNA - 2.05%, HGV RNA - 2.57%, among patients VG - 20.4; 14.63; 11.21; 7.46% respectively.

6. In the structure of mixed viral hepatitis, mixed hepatitis E + C (42.6%) was most often found, followed by mixed hepatitis C + G (21.42%), B + C (14.28%), E + B (9.1%). Although in some cases markers of a greater number of GV types were found in patients, which is confirmed by the identification of patients with triple mixed hepatitis E + B + C in 9.1% of cases and fourfold E + B + C + D infection in 9.1% of cases.

Output. Thus, the epidemic situation for GV in Yakutia is unfavorable and is characterized by an extremely high intensity of the epidemic process of parenteral viral hepatitis B, C, D, G and enteral hepatitis E.

This work was supported by the Russian Foundation for Basic Research grant no. 18-415-140005 r_a.

References

1. Абдурахманов Д.Т. Хронический гепатит В и D. Москва: ГЭОТАР-Медиа, 2010: 288. [Abdurakhmanov DT. Chronic hepatitis B and D. Moscow: GEOTAR-Media, 2010: 288. (In Russ.)].

2. Алексеева М.Н. Вирусные гепатиты в Республике Саха (Якутия): дис. … д-ра мед. Наук. СПб., 2002: 285. [Alekseeva MN. Viral hepatitis in the Republic of Sakha (Yakutia): dis. ... doctor of medical sciences. St. Petersburg, 2002: 285. (In Russ.)].

3. Гриненко Л.Е., Алексеева М.Н., Саммель А.М. [и др.]. Клинико-эпидемическая характеристика заболеваемости в городе Якутске и в районах Республике Саха (Якутия) /: сборник научных статей. Якутск, 1995: 55. [Grinenko LE, Alekseeva MN, Sammel AM [et al.]. Clinical and epidemic characteristics of the incidence in the city of Yakutsk and in the regions of the Republic of Sakha (Yakutia): collection of scientific articles. Yakutsk, 1995: 55. (In Russ.)].

4. Кузнецова И.О. Оценка влияния вакцинации против гепатита В подростков, новорожденных детей и лиц из групп риска на активность эпидемического процесса НВ-вирусной инфекции: автореф. дисс. … канд. мед. наук. Москва, 1999: 25. [Kuznetsova IO. Evaluation of the effect of hepatitis B vaccination in adolescents, newborns and people at risk for the activity of the epidemic process of HB virus infection: abstract. diss. … PhD. Moscow, 1999: 25. (In Russ.)].

5. Письмо Федеральной службы по надзору в сфере защиты прав потребителей и благополучия человека от 4 марта 2011 г. № 01/2390-1-32 «О взаимодействии территориальных органов и учреждений Роспотребнадзора с Референс-центром по мониторингу за вирусными гепатитами». URL: https://www.garant.ru/products/ipo/prime/doc/4092267/#review (дата обращения: 01.04.2011). [Letter of the federal service for supervision of consumer rights protection and human well-being of march 4, 2011 № 01/2390-1-32 «On the interaction of territorial bodies and institutions of the Federal Service for Supervision of Consumer Rights Protection and Human Welfare with the Reference Center for Monitoring Viral Hepatitis». URL: https://www.garant.ru/products/ipo/prime/doc/4092267/#review (date of request: 01.04.2011) (In Russ.)].

6. Приказ Минздрава России от 30 октября 2007 г. № 673 О внесении изменений и дополнений в приказ от 27 июня 2001 г. № 229 «О национальном календаре профилактических прививок и календаре профилактических прививок по эпидемическим показаниям». URL: https://www.garant.ru/products/ipo/prime/doc/4085698/ (дата обращения: 01.04.2011). [Order of the Ministry of Health of Russia of October 30, 2007 № 673 On amendments and additions to the order of June 27, 2001 № 229 «On the national calendar of preventive vaccinations and the calendar of preventive vaccinations according to epidemic indications». URL: https://www.garant.ru/products/ipo/prime/doc/4085698/ (date of request: 01.04.2011). (In Russ.)].

7. Слепцова С.С. Парентеральные вирусные гепатиты и их исходы в Республике Саха (Якутия). Москва, 2017: 208. [Sleptsova SS. Parenteral viral hepatitis and their outcomes in the Republic of Sakha (Yakutia). Moscow, 2017: 208. (In Russ.)].

8. Современный взгляд на вирусные гепатиты / под. ред. А.В. Шаброва. Москва: Миклош, 2010: 168. [A modern view of viral hepatitis / under. ed. AV. Shabrov. Moscow: Miklos, 2010: 168. (In Russ.)].

9. Сомова Л.Г. Регистр больных хроническими вирусными гепатитами. URL: https://medgepatit.com/getatit/registr-bolnyh-hronicheskimi-virusnymi-gepatitami/ (дата обращения: 01.04.2011). [Somova LG. Register of patients with chronic viral hepatitis. URL: https://medgepatit.com/getatit/registr-bolnyh-hronicheskimi-virusnymi-gepatitami/ (date of request: 01.04.2011). (In Russ.)].

10. Учайкин В.Ф. Инфекционная гепатология: руководство для врачей. Москва: ГЭОТАР-Медиа, 2014: 608. [Uchaykin VF. Infectious hepatology: a guide for doctors. Moscow: GEOTAR-Media, 2014: 608. (In Russ.)].

11. Бугаева Т.Т., Иванов П.М., Алексеева М.Н. [и др.] Факторы, влияющие на частоту заболевания первичным раком печени в Якутии. *Якутский медицинский журнал.* 2009; 3: 27-29. [Bugaeva TT, Ivanov PM, Alekseeva MN [et al.]. Factors affecting the incidence of primary liver cancer in Yakutia. *Yakut medical journal*. 2009; 3: 27-29. (In Russ.)].

12. Авдеева М.Г, Городин ВН, Кулбужева ИИ [и др.]. Хронический вирусный микст-гепатит: современные клинико-эпидемиологические аспекты. *Эпидемиология и инфекционные болезни.* 2015; Т. 20: 6. 19-25. [Avdeeva MG, Gorodin VN, Kulbuzheva II [et al.] Chronic viral mixed hepatitis: modern clinical and epidemiological aspects. *Epidemiology and Infectious Diseases.* 2015; 6 (20): 19-25. (In Russ.)].

13. Шахгильдян И.В. Парентеральные вирусные гепатиты (эпидемиология, диагностика, профилактика). Москва: ГОУ ВУНМЦ МЗ РФ, 2003: 384. [Shakhgildyan IV. Parenteral viral hepatitis (epidemiology, diagnosis, prevention). Moscow: GOU VUNMTS Ministry of Health of the Russian Federation, 2003: 384. (In Russ.)].

14. Alhetheel A, El-Hazmi MM. Hepatitis G virus in Saudi blood donors and chronic hepatitis B and C patients. *J Infect Dev Ctries.* 2014 Jan; 15; 8 odna tsifra d.b.(1):110-5. DOI:10.3855 / jidc.3796.

15. Chivero ET, Stapleton JT. Tropism of human pegivirus (formerly known as GB virus C/ hepatitis G virus) and host immunomodulation: insights into a highly successful viral infection. *J Gen Virol.* 2015 Jul; 96(Pt 7):1521-32. doi: 10.1099 / vir.0.000086.

16. Semjonov S.I., Savvin R.G., Nikitina S.G., Maximova S.S., Sleptsova S.S. Parenteral viral hepatitis (B, C, D) in the Sakha Republic (Yakutia). *Life Science Journal.* 2014. Т. 11. № 8s. С. 454-458.

17. Symposium report: emerging threats for human health – impact of socioeconomic and climate change on zoonotic diseases in the Republic of Sakha (Yakutia), Russia. *International Journal of Circumpolar Health*, 79:1, DOI: 10.1080/22423982.2020.1715698

TOPICAL ISSUE

T.A. Bairova, O.A. Ershova, S.I. Kolesnikov, L.I. Kolesnikova

FREQUENCIES POLYMORPHISM OF *Q192R* OF THE PARAOXONASE 1 GENE OF DIFFERENT ETHNIC GROUPS OF EASTERN SIBERIA

DOI 10.25789/YMJ.2020.71.19

УДК 575.174.015.3

Paraoxonase 1 (PON1) is an enzyme, which involved in lipid metabolism and biotrans-formation of xenobiotics. It was reported that *Q192R* polymorphism is determining by activity of paraoxonase and differentiated sensitivity to organophosphorus pesticides and the susceptibility to diseases associated with lipid metabolism. The aim of this study was to determine the frequen-cy of alleles and genotypes of the *Q192R* polymorphism of PON1 gene in different populations of Eastern Siberia: Russian, Buryats, Evenks, Tofalars. The frequency of allele R in the popula-tions of Russian, Buryats, Evenks, Tofalars was 0.3; 0.41; 0.38; 0.39, respectively. Evenks, Tofalars and Buryats, belonging to the North Asian small race, form a separate cluster, distanced from the populations of the far Eastern Mongoloids (Japanese, Koreans) and Caucasoid popula-tions.

Keywords: paroxonase, gene, polymorphism *Q192R*, Evenks, Buryats, Tofalars, population.

Introduction. Formation of health of populations is a century's long process that depends on many internal and external factors. Internal factors include gender, age, heredity, etc. External factors are food, lifestyle, climatic, ecological and other factors. The result of the interaction of these factors is the peculiarity of the metabolic characteristics as the result of biocultural adaptation of populations to climatic conditions. Previously conducted studies have shown a lot of many fat and protein in food of indigenous peoples of the North and Siberia. These eating habits have determined the formation of the genotype which compensates for a highly atherogenic diet. The study of genetic diversity of populations is necessary to understand for the ethnogenesis of the populations, for the analysis and forecasting the health of the population and knowledge of adaptation to changing environmental conditions and dietary intake [1, 2, 16].

The gene for paraoxonase 1 (*PON1*) has shared in metabolism of lipid and biotransformation of xenobiotics. Now have described three members of a family of paraoxonase — *PON1, PON2, PON3*. However, only PON1 has high paraoxonase activity, PON3 has very low paraoxonase activity and PON2 doesn't has it [18].

Paraoxonase 1 (PON1) is a protein. She has 354 amino acids with. She's molecular weight is 43 kDa [4]. PON1 prevents the oxidative modification of low density lipoprotein (LDL), hydrolyzes-lipoperoxides, esters, lactones, organophosphorus co-unity, esters of estrogen, numerous exogenous and endogenous esters and cyclic carbonates. These compounds are using in agriculture and industry as medicines and warfare agents (Zarin, Zaman, Tabun) [18].

Carriers of different genotypes of polymorphisms of *PON1* (*Q192R* или *Gln192Arg* (dbSNP: rs662)) have differences in the activity of paraoxonase. That activity differences more than 40 times [13]. T. Bhattacharyya et al (2008) suggest to low activity of *PON1* in carriers *192QQ*-genotype of polymorphism *Q192R* [23].

The prevalence of carriers of the *QQ192*-genotype and *Q*-allele differents among peoples of different races and ethnic groups: *QQ*-genotype is presenting from 3.7% among Nigerians [10] to 55.6% among residents of Finland [11].

The aim was to study the frequency of genotypes and alleles of *PON1 Q192R* in different ethnic groups of Eastern Siberia: Russians, Buryats, Evenks, Tofalars.

Materials and methods. 282 teenagers were included in the study: Russian – 102 (the average age of 15,82±1.06 years), Buryats – 80 (the average age 14,53±1.84 years), Evenks – 65 (the average age of 14.43±10.63 years), Tofalars – 35 (the average age 19,15±9.63 years). All teenagers are living in Eastern Siberia.

Ethnicity was determined by questioning, taking account of guidance on ethnicity of ancestors to the third generation.

These indigenous peoples of Siberia belong to the Mongoloid race. They are part of a small North Asian race, but belong to different anthropological types – Buryats and Evenks belong to the Baikal anthropological type, Tofalars - to Khataghanaian anthropological type. Evenks and Tofalars belong to the small peoples of Russia (Decree of the RF Government. 24 March 2000. № 255).

461 389 Buryats; 38 396 Evenks and 762 Tofalars are living in Russia according to the results of the All-Russian population census (2010).

Now the Tofalars are living in 3 villages. These villages are included in Totalaria, which is locates in the South of the Irkutsk region. Communication with the regional center town of Irkutsk is absent. Communication with the regional center is carried out by small aircraft, communication between settlements is by using horses and copters.

Evenks are living in Katagansky region. There is Katagansky region in

BAIROVA Tatyana Ananyevna – MD, PhD, head of Personalized medicine laboratory of Scientific Centre for Family Health and Human Reproduction Problems. Address: 664003, Irkutsk, Timiryazev str., 16, Ph.num: +7 (3952) 20-73-67; e-mail: tbairova38@mail.ru. **ERSHOVA Oksana Aleksandrovna** – PhD, Research Officer at the laboratory of Personal-ized Medicine, Scientific Centre for Family Health and Human Reproduction Problems. Address: 664003, Irkutsk, Timiryazev str., 16, Ph.num: +7 (3952) 20-73-67; e-mail: oksa-na111088@mail.ru, **KOLESNIKOV Sergei Ivanovich** – Academician of RAS, Scientific Centre for Family Health and Human Reproduction Problems. Address: 664003, Irkutsk, Timiryazev str., 16, Ph.num: +7 (3952) 20-73-67; T/Ф: (395)2 -20-76-36, 20-73-67 e-mail: iphr@sbamsr.irk.ru **KOLESNIKOVA Ljubov Ilyinichna** – Doctor of Medical Sciencens, Professor, Academician of RAS, Scientific Advisor, Scientific Centre for Family Health and Human Reproduction Prob-lems. Address: 664003, Irkutsk, Timiryazev str., 16, Ph.num: +7 (3952) 20-73-67; T/Ф: (395)2 -20-76-36, 20-73-67 e-mail: iphr@sbamsr.irk.ru

Northern of Irkutsk region. This are living 586 Evenks from 1272 Evenks registered in the Irkutsk region.

Adolescents examination at their places of residence (Bajndai (Buryats), Erbogachen (Evenks), Alighger (Tofalars), Upper Gutara (Tofalars) and Nerha (Tofalars)) in 2009 – 2014 years. These villages are selected on the data bases of the All-Russian population census (2010) (Picture 1).

Pic. 1. Map of Irkutsk's region with indication of the territories of the surveyed adolescents

We used ethical principles of World Medical Association Declaration of Helsinki (1964, 2000 ed.)). All study participants informed about the scientific orientation of the study and gave their consent for participation in the work.

We collected blood samples from each teenagers and placed them with anticoagu lancom (6% saline solution ethylenediaminetetraacetic acid; GOST 10652-73) for deoxyribonucleic acid (DNA) analysis. We prepared the genomic DNA with DNA blood kits (OOO «Interlabservice», Russia) according to the recommendations of the manufacturer. Genotyped SNP was performed using RT-PCR on the thermocycler «Terzic» ("DNA-Technology", Moscow).

We used kits for identification SNP-Express «Cardiogenetics» ("Litech", Russia). PCR products were analyzed by horizontal electrophoresis in 3% agarose gel with ethidium bromide (0.05 mg/ml).

To assess compliance of the distribution of genotypes expected the facilitated values used equilibrium hardy-Weinberg equilibrium (online calculator OEGE Hardy-Weinberg Equilibrium). For comparison of frequencies of alleles and genotypes between analiziruet-mymi groups used the criterion χ2. Differences were considered statistically significant at p<0,05. Clustering of populations was performed according to the method of ward (Ward) using measures of the distance of Euclid. For data processing we used methods of mathematical statistics (STATISTICA 8.0).

Results and discussion. Frequency of alleles and genotypes of *Q192R* polymorphism *PON1* gene in the groups shown in table 1. The frequency distribution of alleles and genotypes corresponded to the distribution of Hardy-Weinberg equilibrium.

We were detected all three genotypes in the studied groups. Heterozygous *QR*-genotype and *Q*-allele identified often. The frequency of genotypes and alleles in the studied groups of adolescents didn't differ. The results of pairwise comparisons of the prevalence of the *R*-allele between the studied populations and other world populations (according to the literature) are presented in table 2.

Frequency of *R*-allele is different in populations of the world from 19% in Kenyans [10] to 62% in the Koreans [12]. Data on the prevalence of the *R*-allele in Asian populations is discordant. So if according Suehiro T. and Hong S. H., Japanese and Korean populations is a high frequency of R-allele, the Yamada Y. (Japan) and B. S. Shin (Korea) indicate the opposite of the data for a greater frequency of *Q* allele in this countries. When comparing the frequencies of the *R*-allele in Russians, Buryats, Evenks and Tofalars identified the significant differences with the data of the Koreans (Hong, 2001) and Japanese (T. Suehiro, 2000). Frequency of *R*-allele in adolescents

Table 1

The frequency distribution of alleles and genotypes of *Q192R* polymorphism *PON1* gene in the studied groups

Genotypes and alleles	The frequency (n (%))			
	Russian n – 102	Buryats n – 80	Evenks n – 65	Tofalars n – 35
Genotype *QQ*	47 (46)	24 (30)	26 (40)	13 (37)
Genotype *QR*	49 (48)	46 (57.5)	29 (45)	17 (49)
Genotype *RR*	6 (6)	10 (12.5)	10 (15)	5 (14)
Alleles *Q*	143 (70)	48 (59)	40 (62)	21 (61)
Alleles *R*	61 (30)	32 (41)	25 (38)	14 (39)
Hardy-Weinberg equilibrium, χ^2	2.17	2.78	0.16	0.02

n – number of surveyed.

Table 2

The prevalence of the R allele of the polymorphism *Q192R PON1* gene in different world populations

Populations	N	Allele R	The significance of differences, p				Link
			russian	buryats	evenks	tofalars	
Russian	102	0.3	-	-	-	-	Собственные данные
Buryats	80	0.41	0.091	-	-	-	
Evenks	65	0.38	0.175	0.713	-	-	
Tofalars	35	0.39	0.233	0.867	0.976	-	
Tatars	1116	0.31	0.805	0.069	0.133	0.225	[3]
Iranians	78	0.28	0.815	0.061	0.114	0.162	[15]
Indians	221	0.29	0.879	**0.040***	0.074	0.137	[17]
Mexicans	64	0.51	**0.000***	0.231	**0.046***	0.143	[24]
Greeks	490	0.25	0.172	**0.002***	**0.003***	**0.018***	[22]
Italians	544	0.24	0.088	**0.000***	**0.001***	**0.010***	[8]
Dutch	201	0.32	0.648	0.146	0.285	0.354	[11]
Croats	166	0.23	0.088	**0.001***	**0.002***	**0.010***	[11]
Americans	2553	0.28	0.609	**0.013***	**0.020***	0.069	[9]
Chinese	70	0.4	0.068	0.969	0.792	0.960	[6]
Koreans	988	0.3	0.962	**0.044**	0.081	0.162	[26]
Koreans	123	0.62	**0.000***	**0.002***	**0.000***	**0.000***	[12]
Japanese	2210	0.33	0.397	0.152	0.306	0.394	[7]
Japanese	132	0.6	**0.000***	**0.005***	**0.000***	**0.002***	[4]
Africans	100	0.24	0.221	**0.006***	**0.011***	**0.029***	[5]
Kenya	99	0.19	**0.018***	**0.000***	**0.003***	**0.012***	[10]

Note: p – levels of statistical significance after comparison of the presented populations with the populations of Eastern Siberia using the criterion χ2. * and bold indicates statistically significant differences. n–number of people.

of the studied groups is higher than in Caucasians (Greeks, Italians, Croatians, Americans) and African Americans

Follow to believe that the higher prevalence of the *R*-allele among indigenous populations of Siberia (39-41%), determining the increase in the activity of PON was probably his voice a reaction to evolutionary the indigenous peoples of Siberia stereotype food with a predominance of lipid and protein. So according to E. Thomàs-Moyà (2007), diets with a high fat content could adversely impact on *PON1* gene expression [19].

In contrast, expression of the gene PON1 increases with consumption flavonoids in vegetables and fruits [21], vitamins C and E [27], pomegranate juice [20], and resveratrol. Resveratrol is a natural antioxidant from the group of polyphenols. Resveratrol is also found in the skins of grapes, red wine and other vegetable [25].

Scientists from the Institute Municipal d'Investigació Mèdica saw to a lot of ate of oleic acid in olive oil is associated with an increased level of PON1 in subjects with *QR*- and *RR*- genotypes of *Q192R* polymorphism of the *PON1* [14]. The content of these products in the diet of indigenous peoples of Siberia is very low or absent. Now the food is changing at indigenous peoples of North Asia. The diet of the indigenous peoples of North Asia have a food with a high content of flavonoids. This requires study the effects of flavonoids to biochemical processes, including lipid metabolism for research their influence on the metabolism of the subjects with other genotypes of *Q192R* polymorphism of the *PON1*.

We did a cluster analysis to assess the genetic similarity of the studied populations of Siberia, between themselves and with other world populations. The frequency of *Q192R* polymorphism of the *PON1* in 20 world populations used for this analysis and construction of genetic dendrograms (figure 2).

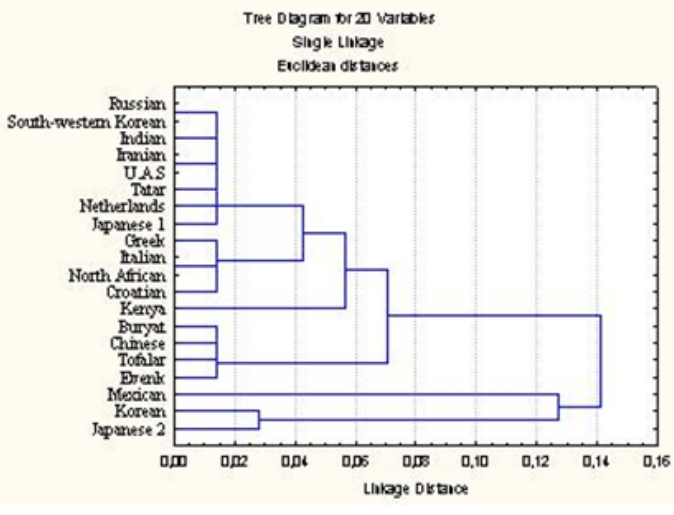


Fig. 2. The genetic dendrogram of *Q192R* polymorphism of the *PON1* of some of the world's population

The gene pools of the Evenks', Buryats', Tofalars' populations are similar and similar to the Chinese from Beijing (China). The Buryats, Tofalars and Evenks are part of the continental Mongoloids. They differ from the far Eastern group of the Pacific Mongoloids (Japanese, Koreans), as well as Caucasians and African Americans.

Conclusion. The *R*-allele of the *Q192R* polymorphism of the *PON1* gene is a lots in the indigenous peoples of North Asia (Buryats, Tofalars, Evenks). Carriage of this allele indicates an increase in paraoxonase 1 activity. Paraoxonase is antioxidant and anti-atherogenic. It prevents the oxidation of lipids in low density lipoproteins (LDL). Carrierly of the *R*-allele of the *Q192R* of the *PON1* is the result of adaptation of the metabolism to a diet with high fat and protein content in the indigenous inhabitants of Northern Asia. The frequency of genotypes and alleles of *Q192R* gene polymorphism *PON1* in the studied populations are the same. This indicates the common origin of modern continental Mongoloids, having different anthropological types small North-Asian race within the Mongoloid race. Caucasian (Russian) are similar to Caucasians living in the Netherlands, Greece, Italy.

References

1. Т.А. Баирова, В.В. Долгих, Л.И. Колесникова и др. Нутрициогенетика и факторы риска сердечно-сосудистой патологии: ассоциативные исследования в популяциях Восточной Сибири. Бюллетень Восточно-Сибирского научного центра Сибирского отделения Российской Академии медицинских наук. 2013; 4(92):87-92. [T.A. Bairova, V.V. Dolgich, L.I. Kolesnokova et al. Nutriciogenetics and risk factors for cardiovascular disease: associative studies in populations of Eastern Siberia./ // Bulletin of the East Siberian Scientific Center of the Siberian Branch of the Russian Academy of Medical Sciences. 2013; 4(92):87-92 (In Russ.)]

2. Окислительный стресс и ген каталазы. О.А.Ершова, Т.А.Баирова, С.И.Колесников и др. Бюллетень экспериментальной биологии и медицины. 2016; 3(161):378-381. [Oxidative Stress and Catalase Gene / O.A.Ershova, T.A.Bairova, S.I.Kolesnikov et al. // Bulletin of Experimental Biology and Medicine. 2016;3(161).378-381 (In Russ.)]

3. Полиморфизм 192Q/R гена параоксоназы 1 у стариков и долгожителей в этнической группе татар. В.В. Паук, И.А. Туктарова, Т.Р. Насибуллин и др. Молекулярная биология. 2007; 4:539-545. [Polymorphism *192Q/R* of the paraoxonase 1 gene in elderly and long-lived in the Tatar ethnic group. V.V.Pauk, I.A.Tuktarova, T.R.Nasibullin et al. Molecular biology. 2007;4:539-545. (In Russ.)]

4. A polymorphism upstream from the human paraoxonase (PON1) gene and its association with PON1 expression. T.Suheiro, T.Nakamura, M.Inoue et al. Atherosclerosis. 2000;150:295–298. (In Eng.) doi.org/10.1016/S0021-9150(99)00379-2

5. Association between Paraoxonase 1 (PON1) Polymorphisms and the Risk of Acute Coronary Syndrome in a North African Population. A.Bounafaa, H.Berrougui, N.Ghalim et al. PLoS ONE. 2015;10(8):e0133719. (In Eng.) doi.org/10.1371/journal.pone.0133719

6. Association of a polymorphism in PON-1 gene with steroid-induced osteonecrosis of femoral head in Chinese Han population Z.Wang, Y.Zhang, X.Kong et al. Diagnostic Pathology. 2013;8:186 (In Eng.) doi.org/10.1186/1746-1596-8-186

7. Association of polymorphisms of paraoxonase 1 and 2 genes, alone or in combination, with bone mineral density in community-dwelling Japanese Y.Yamada, F.Ando, N.Niino et al. J. Hum. Genet. 2003. Vol;48:469–475 (In Eng.) doi.org/10.1007/s10038-003-0063-x

8. CYP17, *GSTP1*, *PON1* and *GLO1* gene polymorphisms as risk factors for breast cancer: an Italian case-control study. C. Antognelli, C. D. Buono, V. Ludovini et al. BMC Cancer. 2009;9:115 (In Eng.) doi.org/10.1186/1471-2407-9-115

9. Evaluation of the paraoxonases as candidate genes for stroke: Gln192Arg polymorphism in the paraoxonase 1 gene is associated with increased risk of stroke. K.Ranade, T.G.Kirchgessner, O.A.Iakoubova et al. Stroke. 2005;36:2346–2350 (In Eng.) doi.org/10.1161/01.STR.0000185703.88944.7d

10. GenBank: http://www.ncbi.nlm.nih.gov.

11. Genetic frequencies of Paraoxonase 1 gene polymorphisms in croatian population. M. Grdic, K.Barisic, L.Rumora et al. Croat. Chem. Acta. 2008;81(1):105-111 (In Eng.)

12. Genetic Variations of Cholesterol Ester Transfer Protein Gene in Koreans. S.H.Hong, Y.R.Kim, J.Song, J.Q.Kim. Human Biology. 2001;6:815-821. (In Eng.) doi.org/10.1353/hub.2001.0083

13. Human paraoxonase-1 (PON1): Gene structure and expression, promiscuous activities and multiple physiological roles. M.Mackness, B.Macknes. Gene. 2015;1:12-21. (In Eng.) doi.org/10.1016/j.gene.2015.04.088

14. Interaction between the Gln–Arg 192 variants of the paraoxonase gene and oleic acid intake as a determinant of high-density lipoprotein cholesterol and paraoxonase activity / M.Tomás, M.Senti, R.Elosua et al. European Journal of Pharmacology. 2001;2-3:121-128 (In Eng.) doi.org/10.1016/s0014-2999(01)01482-0

15. Lack of association between paraoxonase-1 Q192R polymorphism and rheumatoid arthritis in southeast Iran. M.Hashemi, A.K.Moazeni-Roodi, A.Fazaeli et al. Genetics and Molecular Research. 2010;9(1):333-339 (In Eng.) http://www.funpecrp.com.br/gmr/year2010/vol9-1/pdf/gmr728.pdf

16. Oxidative stress and antioxidant defense parameters in different diseases: ethnic aspects / M.A.Darenskaya, S.I.Kolesnikov, L.V.Rychkova et al. // Free Radical Biology & Medicine. 2018;120S1:C. S60 (In Eng.) doi.org/10.1016/j.freeradbiomed.2018.04.199

17. Paraoxonase gene Q192R & L55M polymorphisms in Indians with acute myocardial infarction & association with oxidized low density lipoprotein. R.Lakshmy, D.Ahmad, R. A.Abraham et al. Indian J Med Res. 2010;131:522-529 (In Eng.) https://pdfs.semanticscholar.org/b71c/cd65bbd0f6342e5e4d5f869ff9ff5bc71652.pdf

18. Paraoxonase inhibits high-density lipoprotein oxidation and preserves its functions. A possible peroxidative role for paraoxonase. M.Aviram, M.Rosenblat, C.L.Bisgaier et al. J. Clin. Invest. 1998;8:1581-1590 (In Eng.) doi.org/10.1172/JCI1649

19. Paraoxonase 1 response to a high-fat diet: gender differences in the factors involved / E. Thomàs-Moyà, M. Gianotti, A.M. Proenza, I. Lladо // Molecular Medicine. – 2007 - Vol. 13, № 3-4. P. 203-209 (In Eng.) doi.org/10.2119/2006-00078.thomas-moya

20 Pomegranate juice consumption reduces oxidative stress, atherogenic modifications to LDL, and platelet aggregation: studies in humans and in atherosclerotic apolipoprotein E-deficient mice. M.Aviram, L.Dornfeld, M.Rosenblat et al. The American Journal of Clinical Nutrition. 2000;5:1062-1076 (In Eng.) doi.org/10.1093/ajcn/71.5.1062

21. Quercetin up-regulates paraoxonase 1 gene expression with concomitantprotection against LDL oxidation. M.Gong, M.Garige, R. Varatharajalu et al. Biochemical and Biophysical Research Communication. 2009;4:1001-1004. doi.org/10.1016/j.bbrc.2009.01.015 Relationship of paraoxonase 1 (PON1) gene polymorphisms and functional activity with systemic oxidative stress and cardiovascular risk. T.Bhattacharyya, SJ.Nicholls, EJ.Topol et al. *JAMA*. 2008;299(11):1265-1276 (In Eng.) doi.org/10.1001/jama.299.11.1265

22. Relation of PON1 and CYP1A1 genetic polymorphisms to clinical findings in a cross-sectional study of a Greek rural population professionally exposed to pesticides. A.M.Tsatsakis, A.Zafiropoulos, M.N.Tzatzarakis et al. Toxicology Letters. 2009;186(1):66-72 (In Eng.) doi.org/10.1016/j.toxlet.2008.10.018

23. Relationship of paraoxonase 1 (PON1) gene polymorphisms and functional activity with systemic oxidative stress and cardiovascular risk. T.Bhattacharyya, SJ.Nicholls, EJ.Topol et al. *JAMA*. 2008;299(11):1265-1276 (In Eng.) doi.org/10.1001/jama.299.11.1265

24. Relationship between the paraoxonase (PON1) L55M and Q192R polymorphisms and obesity in a Mexican population: a pilot study. M.Fe.Martínez-Salazar, D.Almenares-López, S.García-Jiménez et al. Genes and Nutrition. 2011;6(4):361–368 (In Eng.) https://doi.org/10.1007/s12263-011-0215-0

25. Resveratrol induces catalytic bioscavenger paraoxonase 1 expression and protects against chemical warfare nerve agent toxicity in human cell lines. B.F.Curtin, K.I.Seetharam, P.Dhoieam et al. Journal of Cellular Biochemistry. 2008;103(5):1524-1535 (In Eng.) doi.org/10.1002/jcb.21543

26. Shin B.S. Paraoxonase gene polymorphism in south-western Korean population. J. Korean Med. Sci. – 2009;24(4);561–566 (In Eng.) https://doi.org/10.3346/jkms.2009.24.4.561

27. Vitamin C and E intake is associated with increased paraoxonase activity. G.P.Jarvik, N.T.Tsai, L.A.McKinstry. Arteriosclerosis, Thrombosis, and Vascular Biology. 2002;22(8):1329-1333 (In Eng.) doi.org/10.1161/01.atv.0000027101.40323.3a

L.N. Afanasyeva, L.S. Burnasheva

DYNAMICS OF ONCOLOGICAL MORBIDITY AND MORTALITY IN THE REPUBLIC SAKHA (YAKUTIA)

DOI 10.25789/YMJ.2020.71.20

УДК 616-006-036.22(571.1/.5+571.6)

The article analyzes the main statistical indicators that characterize the state of cancer morbidity and mortality in the Republic Sakha (Yakutia) and the Russian Federation. Detailed analysis was performed for the regions of the Republic. The main trends of cancer morbidity for individual localizations for 2009-2019 are revealed. The main directions of development of oncologic service in the reduction of mortality of population of the Republic Sakha (Yakutia) from malignant neoplasms and improvement of the quality of life of cancer patients are presented.

Thus, the problem of the growth of oncological pathology remains a priority task of practical health care. In this regard, conducting in-depth epidemiological studies, analyzing territorial, temporal, age-specific prevalence, morbidity and mortality is of particular importance. These data will enable effective screening for cancer. In general, a screening programme is a far more complex public health intervention compared to early diagnosis.

It is necessary to continue work to improve the quality, accessibility and timeliness of medical care in the "oncology" profile, which will affect the increase in the early detection rate, 5-year survival rate, reduce one-year mortality, and accordingly, reduce the mortality rate from malignant neoplasms in the republic. Building up diagnostic capabilities, improving the equipment of medical organizations with highly effective diagnostic equipment, introducing new diagnostic techniques, developing and introducing screening programs, improving the system of training doctors in the general medical network in the "oncology" profile, strengthening anticancer educational and educational work among the population - all this complex of measures is aimed to improve the main indicators of cancer care in the region until 2024 within the framework of the national project "Health".

Keywords: malignant neoplasms, cancer morbidity, Republic Sakha (Yakutia), Russian Federation, screening.

Introduction. The struggle against cancer is one of the global problems of modern society. It directly affects the quality of life of society. More than 12 million new cases of cancer and about 6.2 million deaths are registered annually [6]. According to the WHO, the number of detected cases worldwide will increase by 2050 to 24 million patients, and the number of deaths - to 16 million [7]. More than 450 thousand new cases of malignant neoplasms (MN) of various localizations are registered annually in Russia, more than 80 thousand people die [4, 5]. The largest number of malignant neoplasms is diagnosed in older age groups (60 years and more). About 65% is registered in men and 55% - in women [1-3]. In connection with the available statistics of morbidity and mortality from cancer, the state has adopted a national project "Health", where one of the main tasks is the struggle against cancer.

Research materials and methods. The article analyzes statistical data for 2010-2019 according to Form No. 7 of the federal statistical observation "Information on malignant neoplasms" in the Republic Sakha (Yakutia). All indicators are calculated taking into account the statistical data of the Territorial Body of the Federal State Statistics Service for the

Yakut Republican oncological dispensary, M.K. Ammosov MI NEFU, Yakutsk, Republic Sakha (Yakutia), Russia: **AFANASYEVA Lena Nikolaevna** – PhD (candidate of medical sciences), Ch. doctor, associate professor, lenanik2007@mail.ru; **BURNASHEVA Lyubov' Stepanovna** – PhD (candidate of medical sciences), doctor-methodologist, associate professor, burnashevals@mail.ru.

Republic Sakha (Yakutia) by the average population size. For comparison, data for the Russian Federation were taken from official sources.

Results and discussion. In 2019, 2.719 cases of malignant neoplasms were detected for the first time in the Republic Sakha (Yakutia), including 1.308 (48.1%) in men and 1.411 (51.9%) in women. The incidence rate of malignant neoplasms is increasing in dynamics and amounted to 280.7 per 100 thousand population, which is 7.3% more than in 2018. The increase in this indicator over a 10-year period was 31.3% (Fig. 1).

The highest morbidity rate was found among older people: 65-69 years old - 17.7%, 60-64 years old - 17.5 and 55-59 years old - 14%.

The leading nosologies in the overall structure of oncological morbidity are the following MN: lung (14.7%), breast (10.1), stomach (6.8), skin (except melanoma) (6.6), liver (6.5), kidney (5.4), colon (5.2%), rectum (4.7%), cervix (4.3%), prostate (4.2%).

The incidence rate of malignant neoplasms is higher than the average republican indicator in 13 districts of the republic (Table).

In other regions of the Republic, the morbidity rate is lower than the republican average. Meanwhile, there is an increase in the incidence in comparison with 2018 in the Verkhnevilyuisky district - by 47.1%, Suntarsky - by 28.6, Gorny - by 28.4, Momsky - by 11.9, Kobyaysky - by 8.7%.

In 2019, 25.9% of MN were actively detected, which is 2.7% lower than in 2018, and 3 times more than in 2010 (Fig. 2).

64.9% of the patients, who were actively identified, had I-II stages of the disease (in 2018 - 65.8%, in 2017 - 63.6%). Patients with malignant neoplasms of visual localizations account for 46.1%, which is 36.8% more than in 2018 (in 2018 - 33.7%, 2017 - 38.3).

The maximum indicators of active detection in 2019 were noted in 19 districts: Anabarsky (80%), Tomponsky (64.7), Kobyaysky (60), Verkhnevilyuisky (46.8), Namsky (45.5), Gorny (44), Churapchinsky (42.9), Ust-Maisky (37.5), Vilyuisky (35.4), Bulunsky (35), Olekminsky (34.4), Abyysky (33.3), Aldansky (31.1), Mirninsky (30.1), Zhigansky (30), Verkhoyansky (29.6), Nyurbinsky (28.3), Yakutsk (25.9), Amginsky (25.9%).

The lowest proportion of malignant neoplasms detected actively is recorded in the following districts: Neryungrinsky - 8.3%, Ust-Aldansky - 9.3, Khangalassky - 17.3, Oleneksky - 18.2, Verkhnekolymsky, Ust-Yansky and Eveno-Bytantaysky - 20% each. In Allaikhovsky, Momsky and Nizhnekolymsky districts, there is no active detection of malignant neoplasms.

One of the main indicators determining the prognosis of cancer is the degree of prevalence of the tumor process at the time of diagnosis. Early diagnosis is relevant in all settings and the majority of cancers. In 2019, 46.1% of malignant neoplasms were diagnosed in the early stages of the disease (in I and II), which is 5.8% higher than in 2018. The increase over a 10-year period was 56.4% (Fig. 3).

The early stages of cancer detection at or above the republican average is observed in 13 districts: Anabarsky, Allaikhovsky, Gorny, Verkhnekolymsky, Kobyaysky, Mirninsky, Neryungrinsky, Srednekolymsky, Ust-Aldansky, Ust-Maisky, Eveno-Yytantaysky and in Yakutsk.

A low early detection rate is recorded in Oleneksky - 18.2%, Zhigansky - 20, Oymyakonsky - 26.1, Nizhnekolymsk - 27.3, Momsky - 33.3, Megino-Kangalassky - 36.1, Khangalassky - 36.3, Verkhoy-

Fig. 1. Indicators of the malignancy morbidity in the Republic Sakha (Yakutia) for 2010 - 2019 (per 100 thousand of population)

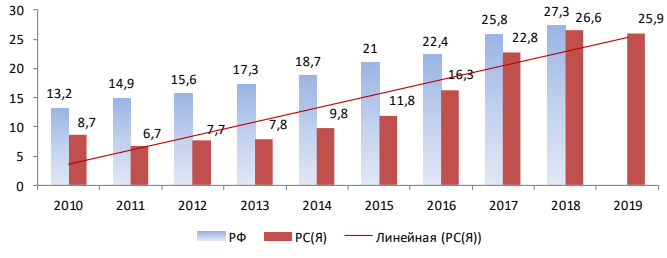


Fig. 2. Indicators of active detection of malignant neoplasms in the Republic Sakha (Yakutia) for 2010 – 2019, (%)

Fig. 3. Indicators of malignancy detection in early stages in the Republic Sakha (Yakutia) for 2010 – 2019, (%)

Table 1

Ranking of regions of the Republic Sakha (Yakutia) with a high level of cancer in 2019

№	Administrative territory, district	Indicator per 100.000 of population	Exceeding the republic average,%
	The Republic Sakha (Yakutia)	280.7	
1	Abyisky	475.2	+69.3
2	Aldansky	431.1	+53.6
3	Allaikhovsky	405.6	+44.5
4	Megino-Kangalassky	353.1	+25.8
5	Neryungrinsky	352.8	+25.7
6	Srednekolymsky	348.4	+24.1
7	Khangalassky	342.4	+22.0
8	Lensky	331.5	+18.1
9	Tomponsky	324.2	+15.5
10	Verkhnekolymsky	318.2	+13.3
11	Oymyakonsky	298.4	+6.3
12	Oleneksky	292.0	+4.0
13	Yakutsk	285.3	+1.6

ansky - 37, Namsky - 37.8, Tomponsky - 38.9, Amginsky - 39.3, Suntarsky - 39.6, Churapchinsky and Ust-Yansky - 40, Aldansky - 40.1, Abyysky - 42.1, Olekminsky – 42. 6, Tattinsky and Lensky - 42.9, Nyurbinsky - 43.5, Verkhnevilyuisky - 44.9, Bulunsky - 45%.

In 2019, in the Republic Sakha (Yakutia), 26% of malignant neoplasms were diagnosed in the presence of distant metastases (stage IV), which is 2.4% less than in 2018, and 25.4% less than in 2010. (Fig. 4).

Fig. 4. Indicators of malignancy neglect in the Republic Sakha (Yakutia) for 2010 – 2019, (%)

In the Eveno-Bytantaisky district in 2019 there were no cases of cancer in the advanced stage. In 14 districts, the neglect rate is lower than the republican average: Allaikhovsky, Aldansky, Verkhnevilyuisky, Vilyuisky, Gorny, Kobyaysky, Mirninsky, Neryungrinsky, Nyurbinsky, Olekminsky, Tattinsky, Churapchinsky, Ust-Aldansky, Ust-Maiksky districts and in Yakutsk. In all other districts, the rate of cancer neglect is higher than the republican average: in Oleneksky - 27.3%, Srednekolymsky and Aldansky - 28, Khangalassky - 28.4, Lensky - 28.6, Namsky - 28.9, Verkhoyansky - 29.6, Zhigansky - 30, Verkhnekolymsky - 30.8, Megino-Kangalassky - 31.5, Abyysky - 31.6, Ust-Yansky and Momsky - 33.3, Suntarsky - 34, Bulunsky - 35, Nizhnekolymsky - 36.4, Anabarsky - 40, Tomponsky - 41.7%, Amginsky - 50, Oymyakonsky - 52.2%.

In 2019, the proportion of patients, who died from malignant neoplasms during the first year after diagnosis was made in patients, registered in the previous year, was 30.7%, which is 5.8% lower than in 2018, and 29.4% lower than in 2010 (Fig. 5).

The one-year mortality rate is lower than the republic average, and is noted in 11 districts: Abyisky, Bulunsky, Vilyuisky, Gorny, Lensky, Mirninsky, Momsky, Neryungrinsky, Tattinsky, Tomponsky and in Yakutsk. In other districts, this indicator remains above the national average: in Anabarsky - 90.9%, Eveno-Bytantaysky - 83.3, Kobyaysky - 54.5, Zhigansky - 52.9, Verkhnekolymsky - 50, Megino-Kangalassky - 50, Amginsky - 47.6, Nyurbinsky - 44.3, Allaikhovsky - 42.9, Nizhnekolymsky - 42.9, Oymyakonsky - 41.7, Verkhoyansky - 41.4, Aldansky - 41.3, Ust-Aldansky - 40.4, Oleneksky - 40, Churapchinsky - 40, Srednekolymsky - 36.8, Namsky - 35.6, Ust-Maisky - 34.6, Khangalassky - 33.8, Suntarsky - 33.3, Ust-Yansky - 33.3, Olekminsky - 31, 9, Verkhnevilyuisky - 31.4%.

At the end of 2019, 12.881 patients were registered in the Republic Sakha (Yakutia). The cumulative prevalence of malignant neoplasms amounted to 1.329.9 per 100 thousand of population, which is almost 7.1% higher than in 2018. The increase over a 10-year period was 38.2% (Fig. 6).

The maximum values of the prevalence of cancer were noted in Neryungrinsky (1910.6), Lensky (1758.1), Aldansky (1670.6), Allaikhovsky (1659.3), Verkhnekolymsky (1566.3), Abyysky (1500.8), Nizhnekolymsky (1468.5), Ust-Maisky (1402.8), Tomponsky (1399.7), Oymyakonsky (1348.6), Mirninsky (1321.9) districts and in Yakutsk (1462.6). In other districts, this indicator is lower than the republic average.

One of the most objective criteria for assessing the effectiveness of anticancer struggle is the survival rate. In 2019, 6455 patients, or 50.1% of all patients with malignant neoplasms under the supervision of an oncological institution, have been registered for 5 years or more, which is 1.4% more than in 2018 (Fig. 7).

High 5-year survival rate (at or above the republic average) are recorded in 17 districts: Amginsky, Allaikhovsky, Verkhnekolymsky, Verkhonyasky, Vilyuisky, Kobyaysky, Lensky, Nizhnekolymsky, Neryungrinsky, Oymyakonsky, Olekminsky, Tomponsky, Churapchinsky, Ust-Aldansky, Ust-Maisky, Eveno-Bytantayskiy and Yakutsk.

In 18 districts, 5-year survival rate below the republic average are recorded in Zhigansky - 34.1%, Verkhnevilyuisky - 36, Srednekolymsky - 41.2, Megino-Kangalassky - 41.7, Mirninsky - 42.6, Oleneksky - 43.2, Tattinsky - 43.4. Namsky - 43.5, Bulunsky - 44.3, Gorny - 45.7, Momsky - 47.4, Aldansky - 48.2, Khangalassky - 48.5, Ust-Yansky - 49.3, Nyurbinsky - 49.4, Suntarsky - 49.8, Anabarsky - 50, Abyysky - 50% districts.

According to official statistics, in the republic in total the number of patients removed from dispensary observation due to death from a malignant neoplasm was 1 213 people, including 648 men (53.4%) and 565 women (46.6%) . The mortality rate was 125.2 per 100 thousand of the population, which is 5.1% lower than in 2018. Over the 10-year period, the mortality rate decreased by 3.1% (Fig. 8).

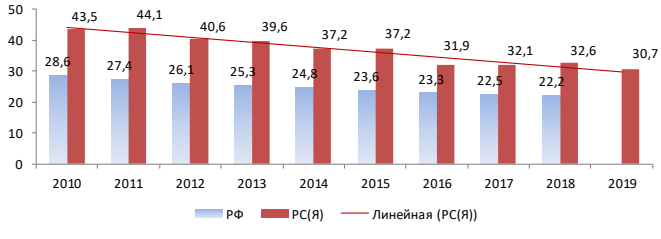


Fig. 5. Indicators of one-year mortality from malignant neoplasms in the Republic Sakha (Yakutia) for 2010 – 2019, (%)

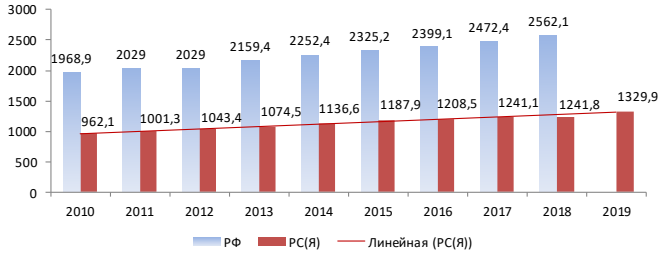


Fig. 6. Indicators of the prevalence of malignant neoplasms in the Republic Sakha (Yakutia) for 2010 - 2019 (per 100 thousand of population)

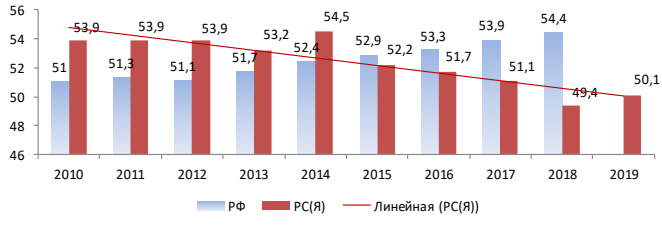


Fig. 7. Indicators of 5-year survival rate of patients with malignancy in the Republic Sakha (Yakutia) for 2010–2019, (%)

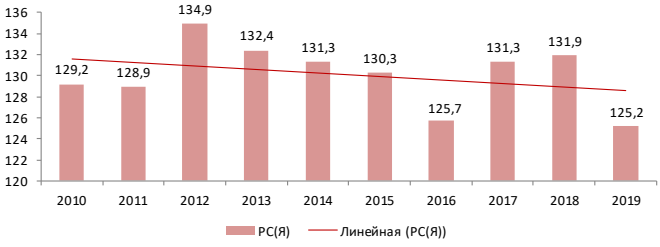


Fig. 8. Indicators of malignancy mortality in the Republic Sakha (Yakutia) for 2010 - 2019, according to data from F.7 of the federal statistical observation (per 100 thousand of population)

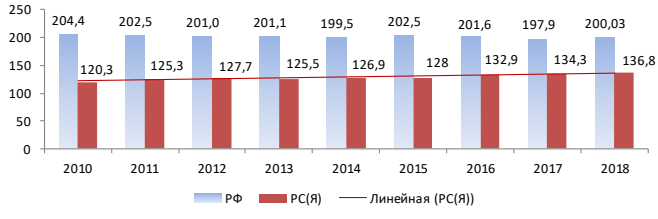


Fig. 9. Mortality rate from malignant neoplasms in the Republic Sakha (Yakutia) for 2010 - 2019 (per 100 thousand of population)

It should be noted that in the Republic Sakha (Yakutia), the mortality rate from cancer for all years of observation, despite its growth, remains lower than the average for the Russian Federation (Fig. 9).

In the mortality structure, malignant neoplasms of the lung (20.6%) are on the 1st place 2nd - stomach cancer (10.1), 3rd - liver cancer (8.1), 4th - pancreatic cancer (6.5), 5th place - breast cancer (5.6%). Mortality from cancer above the republic average is recorded in 21 out of 36 districts - Anabarsky, Aldansky, Allaikhovsky, Verkhnekolymsky, Verkhoyansky, Vilyuisky, Zhigansky, Lensky, Megino-Kangalassky, Nizhnekolymsky, Nyurbinsky, Oymyakonsky, Olekminsky, Oleneksky, Tomponsky, Srednekolymsky, Suntarsky, Khangalassky, Ust-Aldansky, Ust-Yansky and Eveno-Bytantaysky.

Conclusion. In general, in the Republic Sakha (Yakutia) morbidity and mortality rates from malignant neoplasms continue to grow, like all over the world. The incidence rate of cancer in 2019 increased by 7.3% compared to 2018. The growth over a 10-year period is 31.3%. The mortality rate from cancer decreased in comparison with 2018 by 5.1%, in comparison with 2010 - by 3.1%. However, according to Rosstat, in 2018 compared to 2017, there is an increase in the mortality rate by 1.9%. It should be noted that morbidity and mortality rates for all the years of observation remained below the national average.

In dynamics, in the republic there is an improvement in the state of cancer care for the population. So, in 2019, for the first time in recent years, there is a decrease in the one-year mortality rate. This indicator decreased in comparison with 2018 by 5.8%, in comparison with 2010 - by 29.4%. However, the one-year mortality rate in the republic is still higher than the average for Russia. The early detection rate improved, which increased by 5.8% compared to 2018, and by 56.4% compared to 2010. And, on the contrary, there is a decrease in the rate of cancer neglect, which in comparison with 2018 decreased by 2.4%, in comparison with 2010 - by 25.4%.

Thus, the problem of the growth of oncological pathology remains a priority task of practical health care. In this regard, conducting in-depth epidemiological studies, analyzing territorial, temporal, age-specific prevalence, morbidity and mortality is of particular importance. These data will enable effective screening for cancer. In general, a screening programme is a far more complex public health intervention compared to early diagnosis.

It is necessary to continue work to improve the quality, accessibility and timeliness of medical care in the "oncology" profile, which will affect the increase in the early detection rate, 5-year survival rate, reduce one-year mortality, and accordingly, reduce the mortality rate from malignant neoplasms in the republic. Building up diagnostic capabilities, improving the equipment of medical organizations with highly effective diagnostic equipment, introducing new diagnostic techniques, developing and introducing screening programs, improving the system of training doctors in the general medical network in the "oncology" profile, strengthening anticancer educational and educational work among the population - all this complex of measures is aimed to improve the main indicators of cancer care in the region until 2024 within the framework of the national project "Health".

References

1. Гордиенко В.П., Вахненко А.А., Янушевский К.В. Основные показатели заболеваемости и смертности населения от злокачественных новообразований в Дальневосточном федеральном округе. *Социальные аспекты здоровья населения*. 2018:12. [Gordienko VP, Vakhnenko AA, Yanushevsky KV. Main indicators of morbidity and mortality from malignant neoplasms in the far Eastern Federal district. *Social aspects of population health*. 2018:12. (In Russ.)] DOI: https://dx.doi.org/10.21045/2071-5021-2018-64-6-5

2. М-во здравоохранения РФ, Центр информ. технологий и эпидемиол. исслед. в области онкологии в составе Московского НИ онкологич. ин-та; под ред. В. И. Чиссова, В. В. Старинского, Г. В. Петровой. Злокачественные новообразования в России в 2008 году: заболеваемость и смертность. Москва: МНИОИ. 2010: 255. [Ministry of health of the Russian Federation, Center of inform technologies and epidemiol. research in the region of oncology as part of Moscow science-research oncol. institute; ed. by V. I. Chissov, V. V. Starinsky, G. V. Petrova. Malignant neoplasms in Russia in 2008: morbidity and mortality. Moscow: Moscow Oncology research Institute. 2010:255. (In Russ.)]

3. Каприн А.Д., Старинский В.В., Петрова Г.В. Состояние онкологической помощи населению России в 2014 году. Москва: МНИОИ им. П.А. Герцена, филиал ФГБУ «НМИРЦ» Минздрава России, 2015:235. [Kaprin AD, Starinsky VV, Petrova GV. The state of oncological care for the population of Russia in 2014. Moscow: P.A. Gertsen MNIOI, branch of FGBU "NMIRC" of RM of H, 2015:235. (In Russ.)]

4. О состоянии здоровья населения Российской Федерации в 1999 году: Гос. доклад Минздрава РФ, РАМН. Москва: МЗ РФ; РАМН, 1999: 204. [On the state of health of the population of the Russian Federation in 1999: State report of the Ministry of health of the Russian Federation, RAMS. Moscow: Ministry of health of the Russian Federation; the Russian Academy of medical sciences, 1999:204. (In Russ.)]

5. Чиссов В.И., Старинский В.В., Александрова Л.М. [и др.]. Отраслевая программа «Разработка высокотехнологичных методик профилактики, диагностики и лечения, повышающих эффективность медико-социальной реабилитации и качество жизни онкологических больных» и основные итоги ее выполнения в 2001-2005 гг. *Российский онкологич. журнал.* 2006; 4:38-40. [Chissov VI, Starinsky VV, Alexandrova LM [et al.] Industry program "Development of high-tech methods of prevention, diagnosis and treatment that improve the effectiveness of medical and social rehabilitation and quality of life of cancer patients" and the main results of its implementation in 2001-2005. *Russian journal of Oncology*. 2006; 4:38-40. (In Russ.)]

6. Boyle P, Ferlay J. Cancer incidence and mortality in Europe. 2004. *Ann. Oncol.* 2005. 3 (16): 481-488.

7. World Cancer Report. World Health Organization, International Agency for Research on Cancer; eds.: B. W. Stewart, P. Kleihues. Lyon: IARC Press, 2003: 351.

ARCTIC MEDICINE

S.I. Sofronova, V.M. Nikolaev, M.P. Kirillina, A.N. Romanova

CONTRIBUTION OF CERTAIN BIOCHEMICAL AND IMMUNOLOGICAL INDICATORS TO THE DEVELOPMENT OF ARTERIAL HYPERTENSION AND OBESITY IN ARCTIC RESIDENTS

DOI 10.25789/YMJ.2020.71.21

УДК 616-008.9. 1-81(571.56)

A study of the association of certain biochemical and immunological parameters with abdominal obesity and arterial hypertension was carried in indigenous peoples of the North of the Arctic territory of Yakutia. The data obtained show that hypertensive patients have a high frequency of abdominal obesity, high cholesterol due to the atherogenic fraction. A reliable relationship was obtained between the levels of glucose and lipids with waist size and systolic blood pressure. Significant correlation of pro-inflammatory cytokine IL-6 with abdominal obesity in hypertensive patients was revealed.

Keywords: lipids, C-peptide, insulin, pro-inflammatory cytokines, arterial hypertension, abdominal obesity, indigenous peoples of the North.

The mortality rate from circulatory diseases in Russia in 2018 was 46.3%, of which from IHD equaled to 52.6% [3]. One of the main risk factors for the development of cardiovascular complications is arterial hypertension (AH). AH in 2015 caused more than 10 million deaths and 200 million cases of disability [12].

Today, obesity is a global epidemic affecting both adults and children. Numerous researchers have proven the relationship between obesity and insulin resistance. Hyperinsulinemia, in turn, leads to the development of metabolic syndrome and type 2 diabetes. The connection between insulin resistance and the risk of developing cardiovascular disease has been proven in many studies [7, 10, 11, 15, 19]. Change in the traditional way of life, transition to lipid-carbohydrate type of nutrition of the indigenous peoples of the North led to a high prevalence of obesity, especially among women, up to 88%, and cardiovascular pathology among them [2, 4]. Further study of the effect of insulin resistance on obesity and cardiovascular disease among them is important in the prevention of vascular accidents.

Cytokines, in particular interleukin-1 (IL-1), IL-6 are involved in the development of vascular inflammation, thereby being predictors of the progression of cardiovascular diseases [1, 8, 9, 14, 16, 20]. However, the literature provides conflicting data on the effect of anti-inflammatory markers on the development of atherosclerosis, IL-10 in particular. There is evidence of an increase in the level of IL-10 in patients with acute coronary syndrome compared with healthy individuals, significantly correlated with a high risk of death [5]. In another study, patients with acute myocardial infarction had increased levels of IL-2, IL-4, IL-6, interferon gamma (IFNγ) in comparison with healthy patients [21]. Currently, it is unclear whether cytokines affect the development of atherosclerosis and hypertension. The research of the relationship of certain immunological and biochemical indicators with the risk of hypertension remains relevant to this day.

Objective: to analyze the connection of certain biochemical and immunological indicators with abdominal obesity and hypertension in indigenous peoples of the North of the Arctic territory of Yakutia.

Materials and research methods. The collection of material for the study was carried out under expeditionary conditions in the Arctic territory of Yakutia in places of compact residence of indigenous peoples in the villages of Kolymskoye and Andryushkino, Nizhnekolymsky district. A total of 212 people aged 20 to 70 years were examined. Random sampling was formed according to the lists of workers in the village administration. The response was 76%. Kolymskoye village was dominated mainly by the Chukchi, and the Andryushkino village by the Yukagirs. Evens were found equally often in both settlements. The design of the study is presented in the form of "case-control", for which 150 representatives of indigenous peoples of the North (Evens, Yukagirs, Chukchi), which were divided into 2 groups: with AH (case) (n = 73) and without AH (control) (n = 77). The average age of individuals with AH was 53.52 ± 1.12, without AH - 39.62 ± 1.26 years.

Inclusion criteria: representatives of indigenous people (Evens, Chukchi, Yukaghirs).

Exclusion criteria: representatives of non-indigenous nationalities, Yakuts.

The research program included the following sections: a survey on the questionnaire to assess the objective state; informed consent of the respondent to conduct research (according to the Ethical Committee Protocol); anthropometric examination with hip and waist measurement; blood sampling for biochemical studies from the cubital vein in the morning on an empty stomach with a 12-hour abstinence from food. Until analysis the serum was stored in a freezer (-70° C) after centrifugation.

The abdominal obesity (AO) is exposed to the value of the waist measurement (WM) ≥ 80 cm on women, ≥94 cm on (VNOK, 2009).

Laboratory methods of the research included biochemical analysis: blood lipids test total cholesterol (TC), triglycerides (TG), HDL Cholesterol, LDL Cholesterol, glucose test; immunological analysis: determination of C-peptide, insulin, IL-1β, IL-6, IL-4, IL-10, γ-INF.

When judging the incidence of disorders of the blood lipid profile in a population, we used the Russian recommendations of the V revision of Society of cardiology of Russian Federation (VNOK), 2012, into account the European recommendations, 2011. Hypercholesterolemia (HCS) is the level of total cholesterol (TC) ≥ 5,0 mmol/l, the high LDL Cholesterol

Yakut science centre of complex medical problems: **SOFRONOVA Sargylana Ivanovna** – PHD, researcher, ORCID: 0000-0003-0010-9850, sara2208@mail.ru, 89841094825, **NIKOLAEV Vyacheslav Mikhailovich** – PHD, researcher, nikolaev1126@mail.ru, **KIRILLINA Marya Petrovna** – PHD, researcher, kirillinamp@mail.ru, **ROMANOVA Anna Nikolaevna** – MD, director, ORCID: 0000-0002-4817-5315, ranik@mail.ru, 8(4112)32-19-81

level >3,0 mmol/l, the low HDL Cholesterol level <1,0 mmol/l on men; <1,2 mmol/l on women, the hypertriglyceridemia (HTG) is the TG level is ≥1,7 mmol/l; a hyperglycemia (HG) on an empty stomach (a glucose in a blood plasma on an empty stomach ≥ 6,1 mmol/l) or glucose intolerance (a glucose in a blood plasma in 2 hours after glucose loading within ≥7,8 and ≤11,1 mmol/l).

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 2002) according to the instructions for the correct measurement of blood pressure, outlined in the European clinical guidelines for the diagnosis and treatment of hypertension 2017. Hypertension is present at the 140/90 mmHg (2017 ACC/AHA Guideline).

The study was conducted according to Ethics Committee protocol YSC KMP on the respondent's informed consent to the processing of personal data and the study.

Statistical data processing was performed using standard methods of mathematical statistics using the SPSS software package (version 19.0). To define the characteristics, the arithmetic mean (M) and the characteristic's standard error of the mean (m) were calculated. Intergroup differences were evaluated using analysis of variance or non-parametric criteria. Correlation was calculated by Spearman's correlation coefficient. Differences were considered statistically significant at p <0.05.

Results and discussion. This study is exploratory in nature due to the analysis of a small sample of the surveyed population living in the Arctic. The search for correlations of lipid, carbohydrate, hormonal and cytokine profile disorders in individuals with and without AH, as well as its connection with abdominal obesity (AO) was conducted. In the general population, AO was identified in more than half of the respondents (53,3%), including 56.1% in the village of Kolymskoe and 49.5% in the village of Andryushkino. In 72.3% of women, AO was statistically more common than in men (21.4%) (p=0.001). Accordingly, men's average waist size was 84.32±1.50 cm, and women's 90.16±1.47 cm (p=0.01). In individuals with AH, AO was significantly more common than in individuals without it (78.1% and 29.9%, respectively, p = 0.001), confirming the direct relationship between obesity and hypertension.

The analysis of the frequency of lipid and carbohydrate metabolism disorders in Arctic residents, depending on the presence or absence of AH, was conducted. Hypertensive patients had a significantly high incidence of hypercholesterolemia (HCL) compared to normotonics (47.9% versus 29.9%, p = 0.023) due to the increased level of low-density lipoprotein cholesterol (LDL cholesterol) - 57.5% and 42.9 % respectively (p = 0.072). Hypo-α-cholesterinemia (Hypo-α-CS) and hypertriglyceridemia (HTG) also play a significant role in the development of atherosclerosis. Hypo-α-CS was detected in 27.4% of hypertensive patients, and 23.4% of normotonic patients, with no statistically significant differences (p=0.571). There were no significant differences in the frequency of HTG either, with 7.8% in individuals with hypertension and 5.5% in those without it (p=0.570). The frequency of hyperglycemia (HG) was 8.2% in hypertensive patients, 2.6% in normotonic patients (p = 0.126). By gender, we did not obtain significant differences in carbohydrate and lipid metabolism.

A study of the relationship between the average values of blood lipids and glucose with OT and systolic blood pressure (SBP) was conducted. In the general population, a significant correlation was found between OT and average levels of total cholesterol (TC) (r = 0.171, p = 0.036), LDL cholesterol (r = 0.245, p = 0.003), triglycerides (TG) (r = 0.281, p = 0.001), glucose (r = 0.174, p = 0.033), a negative relationship with high density lipoprotein cholesterol (HDL cholesterol) (r = - 0.193, p = 0.018). There is also a direct correlation of SBP with the levels of total cholesterol (r = 0.161, p = 0.049), LDL cholesterol (r = 0.170, p = 0.037), TG (r = 0.224, p = 0.006), glucose (r = 0.221, p = 0.006). A weak negative relationship was obtained with HDL cholesterol (r = -0.064, p = 0.434). As for the study by groups, hypertensive patients showed a strong correlation with the levels of LDL cholesterol (r = 0.233, p = 0.048), TG (r = 0.353, p = 0.002), glucose (r = 0.333, p = 0.004), negative connection with HDL cholesterol (r = -0.246, p = 0.036). The control showed a weak correlation with these blood parameters.

The study of insulin and C-peptide is important because hyperinsulinemia and insulin resistance are one of the triggers for the development of MS. C-peptide, in turn, is a fragment of endogenously produced proinsulin, its level determines the rate of secretion of insulin. We conducted a comparative analysis of the average levels of insulin and C-peptide in residents of the Nizhnekolymsky district in the group of patients with hypertension and the control group (Table 1). The average concentrations of C-peptide and insulin in the group of hypertensive patients were higher compared to normotonics. There were no significant differences between the groups. Significant gender differences were also not found.

A study of the correlation of hormones with the level of SBP and OT was conducted. A direct correlation was established between insulin level and SBP (r = 0.239, p = 0.003), as well as with OT (r = 0.258, p = 0.001). Many studies have also confirmed the association of insulin and C-peptide with SBP [13,17,18]. A weak positive relationship was found with the level of C-peptide, as with the indicator of SBP (r=0.044, p=0.589) and with OT (r=0.124, p=0.129). Of all the parameters of the lipid spectrum, the level of C-peptide had a strong correlation with TG (r= 0.212, p=0.009). The literature also describes the positive relationship of the C-peptide with OT, TG level [6]. In all other cases, we have not identified a reliable link.

Thus, the influence of insulin and C-peptide on abdominal obesity and the risk of hypertension has been confirmed in indigenous peoples of the North.

Many studies have identified the connection of hypertension with inflammation, in particular with pro-inflammatory cytokines. We conducted a comparative analysis of the cytokine content in the general population of the Nizhnekolymsky district (Table 2). It was found that the level of anti-inflammatory cytokine IL-4

Table 1

Average concentrations of C-peptide and insulin in blood plasma in patients with arterial hypertension and healthy people, depending on gender (M ± m)

Group		C-peptide (ng/ml)	p	Insulin (мкЕ/ml)	p
case	total	1.04±0.08	p>0.05	10.19±2.44	p>0.05
	men	0.89±0.08		6.31±1.30	
	women	1.12±0.10		11.67±3.11	
control	total	0.89±0.06		7.94±1.77	
	men	0.75±0.13		5.12±1.18	
		0.89±0.09		9.74±3.45	

Table 2

Average concentrations of proinflammatory cytokines in blood plasma in residents of the Nizhnekolymsky district, depending on the settlement (M ± m)

Indicators	Kolymskoye	Andryushkino	p
IL-1β	1.98 ±0.2	1.59± 0.27	0.001
IL-6	4.82 ±0.51	5.22 ±0.59	0.319
IL-4	2.33 ±0.49	1.53± 0.18	0.017
IL-10	4.47 ± 0.53	5.63 ±0.45	0.000
γ-INF	7.02± 0.44	12.22± 2.90	0.006

Table 3

Average concentrations of proinflammatory cytokines in plasma blood in residents of the Nizhnekolymsky district, depending on the presence and absence of AH (M ± m)

Indicators	Case	Control	p
IL-1β	1.79±0.24	1.61±0.30	0.643
IL-6	5.38±0.70	4.50±0.53	0.318
IL-4	1.74±0.26	1.54±0.14	0.508
IL-10	5.39±0.49	4.67±0.33	0.225
γ-INF	8.30±1.54	8.83±2.76	0.871

is significantly higher in residents of the Kolymskoe village (n=66) than in residents of the Andryushkino village (n=84), and the content of pro-inflammatory cytokine IFN-γ and anti-inflammatory cytokine IL-10 is significantly increased in residents of the village of Andryushkino.

When comparing these blood parameters separately in the case and control, we did not find significant differences (Table 3). The average concentration of pro-inflammatory cytokines IL-1β, IL-6, IL-4, IL-10 in hypertensive patients was higher than in normotonic patients, no significant differences were noted. On the contrary, the average level of IFN-γ was higher in the control compared with hypertonics. There were also no statistically significant differences.

A correlation analysis of cytokines with the level of SBP and OT was carried out. A positive correlation was obtained between OT and IL-6 values in hypertensive patients (r=0.243,p=0.039). In other cases, a clear correlation between cytokines and OT was not obtained. There was also no clear connection between pro-inflammatory markers and the level of SBP.

Conclusion. Thus, in the indigenous peoples of the North - residents of the Arctic suffering from AH, there was an increased content of TC due to the atherogenic fraction, a high rate of abdominal obesity, almost three times higher compared to normotonics. A significant correlation was obtained between the levels of glucose and blood lipids with OT and SBP, proving their contribution to hypertension and metabolic syndrome.

As for the correlation of markers of inflammation with hypertension, we obtained mixed results. The average values of proinflammatory cytokines in individuals with AH were higher compared to normotonics, but there were no significant differences. Out of pro-inflammatory cytokines, significant correlation of IL-6 with abdominal obesity in hypertensive patients was obtained. There is no clear relationship between cytokines and the level of SBP, thereby proving that the immune mechanisms in the formation of AH are subject to further, more thorough study.

References

1. Карагодин В.П., Бобрышев Ю.В., Орехов А.Н. Воспаление, иммунокомпетентные клетки, цитокины - роль в атерогенезе. *Патогенез.* 2014;1(12): 21-35. [Karagodin VP, Bobryshev JuV, Orehov AN. Inflammation, immunocompetent cells, cytokines - a role in atherogenesis. *Patogenez.* 2014; 1(12): 21-35. (In Russ)].

2. Климова Т.М., Федорова В.И., Балтахинова М.Е. Критерии ожирения для идентификации метаболических факторов риска у коренного сельского населения Якутии. *Сибирский медицинский журнал*. 2012;8:110-113. [Klimova TM, Fedorova VI, Baltakhinova ME. Obesity criteria for identifying metabolic risk factors among indigenous rural population of Yakutia. *Sibirskij medicinskij zhurnal.* 2012; 8: 110-113. (in Russ.)].

3. Российский статистический ежегодник. М., 2018. 694 с.

[Russian Statistical Yearbook. *Rossijskij statisticheskij ezhegodnik.*2018: 694p. (in Russ.)].

4. Софронова С.И. Артериальная гипертония и метаболический синдром у коренных малочисленных народов Севера в Якутии. *Якутский медицинский журнал.* 2018;1:14-17. DOI 10.25789/YMJ.2018.61.03 [Sofronova SI. Arterial hypertension and metabolic syndrome in small indigenous people of the North of Yakutia. *Yakutskij medicinskij zhurnal.* 2018. Vol.1. P.14-17. (in Russ.)].

5. Шаленкова М.А., Мухаметова Э.Т., Михайлова З.Д. Роль маркеров некроза и воспаления в прогнозировании острых форм ишемической болезни сердца. *Клиническая медицина*. 2013; 91(11):14-20. [Shalenkova MA, Muhametova JeT, Mikhailova ZD. Role of markers of necrosis and inflammation in predicting acute forms of coronary heart disease. *Klinicheskaya medicina*. 2013; 11: 14-20. (in Russ.)].

6. Andrade R., Gigante D., Oliveira I. and Horta B. C-Peptide and cardiovascular risk factors among young adults in a southern Brazilian cohort. *BMC Endocrine Disorders,* 2018. 18:80. https://doi.org/10.1186/s12902-018-0308-5

7. Beverly J.K, Budoff M.J. Atherosclerosis: Pathophysiology of Insulin Resistance, Hyperglycemia, Hyperlipidemia, and Inflammation. *J Diabetes*. 2020 Feb; 12(2):102-104. doi: 10.1111/1753-0407.12970

8. Bisoendial R, et al. C-reactive protein is a mediator of cardiovascular disease. *Europ Heart J.* 2010; 31(17): 2087–95.

9. Blake G, Ridker P. Inflammatory bio-markers and cardiovascular risk prediction. *J Intern Med.* 2002; 252(4): 283-94.

10. Bloomgarden Z.T. Insulin Resistance, Dyslipidemia, and Cardiovascular Disease. *Diabetes Care.* 2007 Aug; 30(8): 2164-70. doi: 10.2337/dc07-zb08.

11. Eddy D., Schlessinger L., Kahn R. et al. Relationship of Insulin Resistance and Related Metabolic Variables to Coronary Artery Disease: A Mathematical Analysis. *Diabetes Care.* 2009; 32(2): 361-6. doi: 10.2337/dc08-0854

12. Forouzanfar M, Liu P, Roth G, Ng M, Biryukov S, Marczak L et al. Global burden of hypertension and systolic blood pressure of at least 110 to 115 mm Hg, 1990-2015. *JAMA.* 2017; 317(2): 165-182. https://doi.org/10.1001/jama.2016.19043

13. Hirai FE, Moss SE, Klein BE, Klein R. Relationship of glycemic control, exogenous insulin, and C-Peptide levels to ischemic heart disease mortality over a 16-year period in people with older-onset diabetes: the Wisconsin Epidemiologic Study of Diabetic Retinopathy (WESDR). *Diabetes Care*. 2008; 31(3):493–7 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2773445/

14. Koenig W, Lowel H, Baumert J, Meisinger C. C-reactive protein modulates risk prediction based on the Framingham Score: implications for future risk assessment: results from a large cohort study in southern Germany. *Circulation.* 2004; 109: 1349–53.

15. Laakso M., Kuusisto J., Stančáková A. et al. The Metabolic Syndrome in Men study: a resource for studies of metabolic and cardiovascular diseases. *J Lipid Res.* 2017; 58(3): 481–493. DOI 10.1194/jlr.O072629

16. Pai J, et al. Inflammatory markers and the risk of coronary heart disease in men and women. *N Engl J Med.* 2004; 351: 2599–610.

17. Patel N, Taveira TH, Choudhary G, Whitlatch H, Wu WC. Fasting serum CPeptide levels predict cardiovascular and overall death in nondiabetic adults. *J Am Heart Assoc.* 2012; 1(6):e003152. https://doi.org/10.1161/JAHA.112.003152

18. Purohit P, Mathur R. Hypertension association with serum lipoproteins, insulin, insulin resistance and C-peptide: Unexplored forte of cardiovascular risk in hypothyroidism. *North Am J Med Sci.* 2013; 5: 195-201.

19. Reaven G. Insulin Resistance and Coronary Heart Disease in Non diabetic Individuals. *Arterio-*

scler Thromb Vasc Biol. 2012. Aug; 32(8):1754-9. doi: 10.1161/ATVBAHA.111.241885.

20. Ridker PM, Rifai N, Rose L, Buring J, Cook N. Comparison of C-reactive protein and low-density lipoprotein cholesterol levels in the prediction of first cardiovascular events. *N Engl J Med*. 2002; 347:1557–65.

21. Yan W., Song Y., Zhou L. et al. Immune cell repertoire and their mediators in patients with acute myocardial infarction or stable angina pectoris. *Int. J. Med. Sci*. 2017; 14(2): 181-190. DOI: 10.7150/ijms.17119.

L.D. Olesova, N.S. Arkhipova, A.I. Yakovleva, Z.N. Krivoshapkina, E.I. Semenova, A.V. Efremova, L.I. Konstantinova, E.D Okhlopkova, A.A. Grigorieva

AGE-RELATED DYNAMICS OF THE LIPID PROFILE IN THE INDIGENOUS POPULATION OF YAKUTIA

DOI 10.25789/YMJ.2020.71.22

УДК 612.123 (571.56)

The age and gender indicators of lipid metabolism and glucose were analyzed in a cohort of 678 indigenous people aged 20 to 95 years (270 men, 408 women). The frequency of dyslipidemia was studied. Blood lipids in long-livers are close to those of 20-29 year olds. High cholesterol and triglycerides in men occur at the age of 40-49 years, in women 10 years later. In men in all age groups, the glucose content is higher, the antiatherogenic fraction of lipids is lower. The frequency of occurrence of high KA among men decreases from 70 years from 60.0 to 18.9% in long-livers, in women from 40 years from 43.2 to 19.2% in long-livers. The higher is weight, the higher is the level of glucose, triglycerides, cholesterol and lower the level of HDL cholesterol. Changes in the lipid profile in men occur from 30 to 69 years, in women - from 50 to 79 years. The peak incidence of dyslipidemia, obesity and the amount of total cholesterol and glucose occurs at the age of 50-59 years, which classifies this group on the basis of serious metabolic disorders as a risk group for the development of pathologies as an obstacle to surviving to the age of long-livers.

Keywords: dyslipidemia, gender differences, long-livers.

World population ageing is a huge challenge that humanity is facing in our time, but also a great opportunity for a new way of thinking, new technologies, new industries that are transforming the traditional paradigm of aging. In most countries of the world, the growth of the older population has consistently outpaced that of the younger generation. In just 30 years, the number of people over 65 is expected to almost double to 1.6 billion, or 17 per cent of the world population. Therefore, the understanding that a healthy, engaged and productive older population can be an economic boom, rather than an economic and social burden, has been a catalyst for new thinking and new actions that bring the world community together to ensure and maintain healthy longevity [7]. In this regard, the Global Roadmap for Healthy Longevity, launched in 2019 by the U.S. National Academy of Medicine (NAM), will bring together international leaders in science, medicine, healthcare, technology, economics and politics to collect and assess data on strategies to live longer, healthier and more fulfilling lives. By the end of 2020, the initiative will have produced a report that can serve as a priority 10-year action plan adaptable to local conditions. First of all, the events will focus on the social, behavioral and environmental factors of healthy longevity; secondly, on public healthcare; thirdly, on research and development of the basic, clinical, pharmaceutical, social and behavioral sciences; bioengineering; information technology, etc. [6]. The call of Victor Dzau and Joe Ann Jenkins (2019) for active action by gerontologists around the world is of great importance for our country as well. With the increase in retirement age, all measures aimed at preserving health and physical activity at a later stage of life become extremely necessary.

Preserving the health and working capacity of the elderly population is important in the face of extremely cold climate and requires the study of adaptive reserves at the population level for early, donozological diagnostic, determination of risk groups and prevention of pathological conditions. During the recent decades of socio-economic transformation and urbanization, indigenous peoples in the North have increasingly experienced an adaptation breakdown. The shift away from traditional lifestyles and the displacement of protein-lipid diets with high consumption of carbohydrate products is changing the northern type of metabolism. Disturbances in lipid and carbohydrate metabolism are observed [2], which are considered to be the leading risk factors for cardiovascular diseases [5].

The mortality rate of the population of the Republic of Sakha (Yakutia) from the circulatory system diseases takes the first place (45% of all deaths). Rejuvenation of cardiovascular diseases is observed. Among the population of the Far North and Yakutia aged 60 and older, there is practically no data on the specifics of circulatory system diseases, and clinical and epidemiological studies are insufficient [4].

Healthy longevity is based on maintaining health at a young age. Therefore, in this paper we decided to analyze the frequency of lipid profile and glucose disorders in the population of indigenous Yakutia residents from the young age to the age of long-livers.

OLESOVA Lyubov Dygynovna – candidate of Biological Sciences, Leading Researcher laboratory of biochemical research, Yakut Science Centre of Complex Medical Problems, 677010, Yakutsk, Sergelyakhskoe highway, 4, oles59@mail.ru, **ARKHIPOVA N.S.** – nati8692@mail.ru, **YAKOVLEVA Alexandra Ivanovna** – researcher FSBSI "Yakut Science Centre of Complex Medical Problems", sashyak@mail.ru, **SEMENOVA Evgeniya Ivanovna** – candidate of biological sciences, senior researcher laboratory of biochemical research, Yakut Science Centre of Complex Medical Problems, kunsuntar@mail.ru, **KRIVOSHAPKINA Zoya Nikolaevna** – Ph.D., zoyakriv@mail.ru, **EFREMOVA Agrafena Vladimirovna** – Candidate of Biological Sciences, PnD, Senior Researcher, Yakut Science Centre of Complex Medical Problems", a.efremova01@mail.ru, **KONSTANTINOVNA Lena Ivanovna** – researcher laboratory of biochemical research, Yakut Science Centre of Complex Medical Problems, konstanta.l@mail.ru, **OKHLOPKOVA Elena Dmitrievna** – Candidate of Biological Sciences, Leading Researcher leadership laboratory of immunopathology, elena_ohlopkova@mail.ru, **GRIGORYEVA Anastasia Anatolyevna** –researcher Yakut Science Centre of Complex Medical Problems, nastiagrigoryeva@gmail.com, Yakut Science Centre of Complex Medical Problems, 677010, Yakutsk, Sergelyakhskoye Highway, 4 km.

The aim of the study was to determine age and gender-related differences in the lipid profile and glucose in the indigenous population of the Republic of Sakha (Yakutia).

A total of 678 people aged 20 to 97 were surveyed, of whom 270 were men and 408 were women. All those surveyed were divided into age groups [Table 1].

Table 1

Dividing the subjects into age groups

Gender	Age group, years							
	20-29	30-39	40-49	50-59	60-69	70-79	80-89	более 90
men	30	38	29	66	33	38	10	26
women	37	63	62	118	50	34	21	23

Determination of total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), triglycerides (TG) was carried out by the enzymatic method on a "Labio" automatic biochemistry analyzer using "Analyticon" reagents (Germany). LDL-C and VLDL-C were calculated using Friedewald et al. (1972) equation. The atherogenic coefficient (AC) was calculated according to the equation proposed by A.N. Klimov: AC=(C – HDL-C)/HDL-C (Klimov, Nikulcheva, 1999). Malfunction of lipid metabolism was estimated according to three types of dyslipidemia: hypercholesterolemia (TC ≥5.21 mmol/L and TG ≤1.7 mmol/L), hypertriglyceridemia (TC ≤5.21 mmol/L and TG ≥1.7 mmol/L), and combined dyslipidemia (TC ≥5.21 mmol/L and TG ≥1.7 mmol/L).

The statistical processing of data was carried out using the IBM SPSS Statistics 23.0 statistical software package. The methods of parametric and non-parametric statistics were applied. The descriptive method of variation statistics included calculation of average values, standard deviation, median, percentiles. The data in the tables is presented as M(SD) and Me (Q1 and Q2), where M is the average, SD is the standard deviation, Me is the median, Q1 and Q3 are quartiles. Student's t-test was applied in order to estimate intergroup differences of features with continuous distribution. Frequency values were calculated using the contingency tables of descriptive statistics. The significance of multiple group differences when comparing averages was detected by means of the one-way analysis of variance (ANOVA). Interrelationship between values was analyzed through correlation analysis. The probability of the null hypothesis was taken at $p<0.05$.

Results and discussion. The TC levels of long-livers within the reference values had no significant gender-related differences and was lower than in other age groups except for the group of young men aged 20-29 and young women aged 20-29 and 30-39. The differences in the TC content were significant in the groups of men aged 40-49, 50-59, 60-69 years old and among women of only one group aged 50-59 ($p<0.05$), where the average TC value was the highest - 5.65 mmol/L. The gender-related difference in the TC level was observed in the groups of 20-29 and 70-79 years old old: women had higher levels than men ($p<0.05$). The age dynamics of TC levels in men is characterized by a significant increase from 30-39 to 60-69 years old; in women - from 30-39 to 70-79 years old. An average level of TC exceeding 5.2 mmol/L was observed in men from 40 to 69 years old, and in women from 50 to 79 years old (Table 2).

The frequency of the TC level above 5.2 mmol/L has increased with age, reaching the highest values in men in the group of 60-69 years old, in women in the group of 50-59 years old. After those, there has been a decrease in these indicators. In long-livers of both genders, the percentage of occurrence of the increased TC level was much lower, especially in men (Table 3).

As shown in Table 2, high levels of TC (>6.20 mmol/L) among women were detected in 2.7% of youngsters aged 20-29, and among men those were detected in 15.8% of youngsters aged 30-39. In the following groups, there is an increase in the frequency of high levels of TC, reaching the highest percentage among men in the 60-69 years old group (30.3%), and among women in the 50-59 years old group (28%). Then, there is a decrease in the frequency, but the percentage among women remains quite high. In the group of long-livers, the frequency of high TC levels was the lowest compared to other groups. Among long-livers and women the frequency of high TC was 2 times higher.

The average triglyceride levels in long-livers and in all age-groups did not exceed normal values. Its moderate growth starts at the age of 40 in men and 10 years later in women, at the age of 50. A significant gender-related difference in triglyceride content is observed in the 50-59 years old age group: TG levels were higher in women. According to the median, TG levels were higher in men in the first three age-groups. In the following three groups, TG levels were higher in women, while in the 80-89 years old age-group and in long-livers the median was higher in men (Table 2).

High levels of TG were not as common as high levels of TC. In small percentages, high levels of TG were detected in all age groups of men. High levels of TG have not been detected in female long-livers and in the group of 80-89 years old women. In men aged 40-49, the high levels of TG were 3 times more frequent, and on the contrary, in the 60-69 years old group it is 2.3 times more frequent in women (Table 3).

The average value of the antiatherogenic cholesterol fraction (HDL-C) was higher in women of all age groups. The significance of differences was revealed in groups aged 30-39, 80-89 years old and in long-livers (12%, 32% and 20% respectively). Among men, an insignificant increase in HDL-C was observed from 40 to 69 years old (Table 2).

The low levels of HDL-C were detected in all age groups of men, except for the group of 80-89 years old, where it is possible that the results were not quite correct due to the small number of those surveyed. Among women in the age-groups of 40-49, 70-79, 80-89 years old and in the group of long-livers no cases of low HDL-C have been revealed. A quite high percentage of low HDL-C levels was detected among young men aged 20-29, 30-39 years old, and in the age-group of 50-59 years old (6.7%, 13.2% and 7.6% respectively) (Table 3).

The atherogenic cholesterol fraction (LDL-C) had a normal value in all groups. In men, higher values within the reference values were observed in groups from 30-39 to 60-69 years old, which is significantly higher than in long-livers. In women, values were higher in groups from 50-59, 60-69, 70-79 years old. In the group aged 50-59 years old, the difference with the long-livers' values was significant. Gender-related difference was observed in the groups of 30-39, 40-49, 70-79 years old: the content of LDL-C was lower in women (Table 2).

The age-related increase of LDL-C starts in men aged 30-39 years old (7.9%), reaching 27.3% in the group aged 60-69 years old. In women, a high frequency is noted in the group aged 70-79 years old. In the long-livers groups, the rates were much lower than in the other groups (Table 3).

Table 2

Age and gender-related levels of the lipid profile and glucose

M (SD)/ Me (Q_1; Q_3)	Gender	Age groups, years								Cohort
		20-29	30-39	40-49	50-59	60-69	70-79	80-89	> 90	n=678
1	2	3	4	5	6	7	8	9	10	11
Cholesterol, mmol / l										
M (SD)	men	4.29 (0.89)+»	5.11 (0.94)»	5.32 (0.66)*»	5.42 (0.93)*»	5.46 (1.09)*»	4.65 (1.09)+	5.17 (1.08)»	4.67 (0.98)*	5.06 (1.04)
	women	4.50 (0.85)+»	4.76 (0.95)»	5.06 (1.02)»	5.65 (0.95)*»	5.33 (0.96)»	5.43 (1.14)+»	4.99 (1.43)	4.95 (1.02)*	5.18 (1.06)
Me (Q_1; Q_3)	men	4.34 (3.59; 4.96)	4.94 (4.39; 5.95)	5.39 (4.87; 5.59)	5.45 (4.72; 6.09)	5.39 (4.89; 6.36)	4.63 (3.54; 5.41)	5.36 (4.12; 6.14)	4.77 (4.02; 5.04)	4.98 (4.37; 5.72)
	women	4.45 (3.88; 5.05)	4.63 (4.00; 5.33)	4.92 (4.22; 5.70)	5.67 (5.01; 6.35)	5.42 (4.54; 5.88)	5.44 (4.89; 6.14)	4.94 (4.07; 5.96)	4.93 (4.52; 5.34)	5.19 (4.42; 5.87)
Triglycerides, mmol / l										
M (SD)	men	0.86 (0.42)	0.93 (0.32)	1.05 (0.62)	0.91 (0.43)+	1.02 (0.57)	0.87 (0.38)	1.16 (0.64)	0.87 (0.28)	0.94 (0.46)
	women	0.89 (0.40)	0.88 (0.39)»	0.89 (0.43)	1.05 (0.58)+	1.08 (0.55)»	0.96 (0.38)	0.86 (0.24)	0.86 (0.22)	0.96 (0.42)
Me (Q_1; Q_3)	men	0.84 (0.51; 1.14)	0.89 (0.65; 1.16)	0.84 (0.63; 1.28)	0.81 (0.58; 1.07)	0.88 (0.57; 1.44)	0.78 (0.61; 1.08)	0.85 (0.73; 1.57)	0.84 (0.64; 1.00)	0.84 (0.61; 1.13)
	women	0.74 (0.54; 1.13)	0.83 (0.55; 1.06)	0.80 (0.52; 1.12)	0.92 (0.73; 1.30)	1.03 (0.68; 1.13)	0.83 (0.68; 1.11)	0.78 (0.67; 1.01)	0.78 (0.72; 1.55)	0.87 (0.65; 1.12)
HDL cholesterol, mmol / l										
M (SD)	men	1.23 (0.33)	1.17 (0.31)+	1.36 (0.36)	1.36 (0.43)*	1.37 (0.36)	1.27 (0.32)	1.16 (0.26)+	1.19 (0.22)*+	1.28 (0.35) ˚
	women	1.29 (0.33)	1.32 (0.36)+	1.41 (0.32)	1.37 (0.36)	1.32 (0.41)	1.40 (0.31)	1.53 (0.50)+	1.43 (0.39)+	1.37 (0.37) ˚
Me (Q_1; Q_3)	men	1.20 (1.01; 1.28)	1.25 (0.93; 1.33)	1.26 (1.08; 1.65)	1.26 (1.06; 1.65)	1.28 (1.13; 1.49)	1.24 (1.05; 1.54)	1.14 (0.93; 1.36)	1.20 (1.06; 1.31)	1.22 (1.06; 1.52) ˚
	women	1.27 (1.10; 1.43)	1.34 (1.03; 1.48)	1.36 (1.21; 1.61)	1.34 (1.09; 1.59)	1.29 (1.00; 1.64)	1.38 (1.15; 1.64)	1.50 (1.13; 1.68)	1.44 (1.13; 1.60)	1.34 (1.10; 1.59) ˚
LDL cholesterol, mmol / l										
M (SD)	men	2.73 (0.78)	3.42 (0.90)+	3.62 (0.85)*+	3.67 (0.91)*	3.67 (0.99)*	2.98 (0.93)+	3.47 (1.06)	3.06 (0.90)*	3.37 (0.97)
	women	2.79 (0.77)	3.01 (0.86)+	3.23 (0.92)+	3.80 (0.83)*	3.52 (0.83)	3.59 (1.02)+	3.30 (0.91)	3.11 (0.81)*	3.38 (0.93)
Me (Q_1; Q_3)	men	2.60 (2.14; 3.52)	3.33 (2.75; 4.24)	3.43 (3.01; 3.86)	3.60 (3.00; 4.34)	3.55 (2.90; 4.56)	2.74 (2.19; 3.60)	3.15 (2.58; 4.61)	2.99 (2.63; 3.54)	3.32 (2.71; 3.99)
	women	2.81 (2.26; 3.20)	2.99 (2.33; 3.72)	3.17 (2.52; 3.74)	3.85 (3.37; 4.39)	3.42 (2.91; 4.07)	3.50 (2.96; 4.27)	3.28 (2.44; 3.95)	3.00 (2.69; 3.42)	3.39 (2.69; 4.00)
VLDL cholesterol, mmol / l										
M (SD)	men	0.38 (0.19)	0.46 (0.24)	0.45 (0.26)	0.40 (0.19)	0.47 (0.26)	0.41 (0.19)	0.53 (0.29)	0.40 (0.13)	0.43 (0.22)
	women	0.42 (0.21)	0.43 (0.25)	0.41 (0.19)	0.47 (0.20)	0.49 (0.25)	0.44 (0.17)	0.39 (0.10)	0.39 (0.10)	0.44 (0.21)
Me (Q_1; Q_3)	men	0.38 (0.23; 0.49)	0.43 (0.30; 0.58)	0.38 (0.27; 0.55)	0.36 (0.27; 0.46)	0.40 (0.26; 0.66)	0.36 (0.28; 0.51)	0.39 (0.33; 0.71)	0.38 (0.29; 0.45)	0.38 (0.28; 0.52)
	women	0.38 (0.24; 0.52)	0.39 (0.26; 0.52)	0.37 (0.26; 0.52)	0.42 (0.33; 0.60)	0.46 (0.36; 0.55)	0.38 (0.31; 0.50)*	0.37 (0.32; 0.47)	0.37 (0.33; 0.48)*	0.40 (0.30; 0.52)
Atherogenic coefficient										
M (SD)	men	2.68 (0.92)	3.32 (1.15)+	3.22 (1.14)+	3.24 (1.34)+	3.29 (1.30)	2.81 (1.13)	3.67 (1.32)+	3.00 (1.06)	3.13 (1.22) ˚
	women	2.53 (0.92)	2.83 (1.19)+	2.71 (1.04)+	3.28 (1.09)+	3.20 (1.13)	3.03 (1.16)	2.75 (0.84)+	2.47 (0.82)*	2.94 (1.09) ˚
Me (Q_1; Q_3)	men	2.51 (2.20; 3.12)	3.30 (2.20; 4.33)	3.20 (2.45; 4.15)	3.10 (2.10; 4.17)*	3.20 (2.35; 4.40)*	2.50 (1.90; 3.45)	3.80 (2.65; 4.55)	2.95 (2.17; 3.60)	2.90 (2.20; 4.00)
	women	2.30 (2.00; 3.00)	2.67 (2.00; 3.50)	2.60 (2.00; 3.20)	3.10 (2.50; 3.95)	3.00 (2.40; 4.00)	2.75 (2.17; 3.90)	2.70 (2.27; 3.33)	2.10 (1.80; 3.00)	2.80 (2.20; 3.50)
Glucose										
M (SD)	men	5.47 (0.86)*	5.25 (0.95)	5.22 (0.65)	5.56 (0.82)*+	5.81 (0.95)*	5.08 (0.77)+	4.91 (1.12)	4.94 (1.10)*+	5.15 (0.96) ˚
	women	5.15 (0.96)*	5.06 (0.68)*	5.10 (0.77)*	5.27 (0.72)*+	5.52 (1.08)*	4.40 (0.68)+	4.58 (0.56)	4.35 (1.01)*+	5.06 (0.86) ˚
Me (Q_1; Q_3)	men	5.35 (4.80; 5.90)	5.00 (4.60; 5.57)	5.20 (4.80; 5.60)	5.50 (4.95; 5.90)	5.70 (5.05; 6.14)	5.00 (4.67; 5.51)	4.70 (3.80; 6.00)	4.50 (4.30; 5.55)	5.20 (4.75; 5.80) ˚
	women	5.00 (4.72; 5.30)	4.90 (4.60; 5.40)	5.00 (4.57; 5.40)	5.20 (4.80; 5.70)	5.30 (4.80; 5.90)	4.30 (4.07; 4.60)	4.50 (4.20; 4.80)	4.10 (3.70; 4.40)	4.90 (4.50; 5.50) ˚

The end of the table. 2

1	2	3	4	5	6	7	8	9	10	11
Cholesterol + Glucose										
M (SD)	men	9.76 (1.22)	10.37 (ц1.64)*	10.01 (1.76)	10.90 (1.37)*	10.22 (2.79)	9.74 (1.19)	10.08 (1.52)	9.51 (1.57)*	10.21 (1.73)
	women	9.51 (1.48)	9.83 (1.33)*	10.17 (1.52)*	10.75 (1.47)*	10.53 (1.82)*	9.83 (1.50)	9.58 (1.29)	9.02 (1.75)*	10.13 (1.56)
Me (Q_1; Q_3)	men	9.86 (8.78-10.65)	10.51 (9.17-11.19)	10.17 (9.46-11.22)	10.79 (10.24-11.75)	10.58 (9.39-12.19)	9.80 (9.12-10.88)	10.10 (8.71-11.52)	9.15 (8.54-10.55)	10.26 (9.22-11.19)
	women	9.48 (8.66-10.47)	9.41 (8.95-10.83)	9.95 (9.18-11.08)	10.79 (10.03-11.61)	9.93 (9.66-11.77)	9.93 (8.90-10.60)	9.56 (8.57-10.78)	8.72 (8.27-9.67)	10.07 (0.15-11.20)

Note: * - the significance of the differences between these centenarians and other age groups; + - the significance of gender differences in the corresponding age groups, "- the significance of differences between the 1st group and subsequent groups, ˚ - the significance of gender differences in the average indicators and medians of all surveyed.

The increased atherogenic coefficient (AC) was detected in the age-groups of men aged 20-29 and 70-79 years old. In long-livers, AC was 20% higher in men and equal to the upper limit of the norm. Among women, AC slightly exceeded the upper limit of the norm in groups aged 50-59 and 60-69 years old. It should be noted that AC growth in men starts at 30 years old, and in women it starts 20 years later - at 50 years old (Table 2).

According to Table 2, the frequency of increased AC was higher in men except those aged 60-69 years old. For long-livers of both genders, the frequency of increased AC was close to that of the young group: 18.9% for men and 19.2% for women. The high frequency of increased AC was maintained in men from 30 to 89 years old and in women from 50 to 79 years old.

Glucose levels in the group of long-livers were within the norm and were significantly higher in males ($p<0.05$). In all other age groups, glucose content was also higher in men, significantly in the groups of 50-59 and 70-79 years old. In terms of mean value and median, glucose content equal to or higher than 5.5 mmol/L was detected in the age groups of 50-59 years old and 60-69 years old men. Among women, the highest glucose content was 5.30 mmol/L in the group aged 60-69 (Table 2).

In all age groups, the frequency of increased glucose content in men was higher, with an increase in the frequency of occurrence in men from 40 to 69 years old, and women from 50 to 69 years old.

Fig. 1 shows the frequency of occurrence of different types of dyslipidemia depending on age and gender. Hypercholesterolemia is much more common in all age groups, especially in women. Hypertriglyceridemia among women rarely occurs before the age of 60-69 years old, among men it only occurs in the age groups of 70-79 and 80-89 years old. No triglyceridemia has been detected in long-livers of both genders. Combined dyslipidemia in men and women is observed from 30 to 60-69 years old, and more often in men. The peak of dyslipidemia occurs at the age of 50-59.

The sum of cholesterol levels and blood glucose content is a rigid biological constant that is necessary to assess the degree of changes and reversibility of shifts. [3]. In long-livers, the sum of TC and glucose is close to 9 by average and median values, while in women this value is lower than in men. This value was reduced in men due to lower cholesterol levels, and in women due to lower glucose content. Among men, according to the median, values above 10 were detected in groups of 30-39, 50-59, 60-69 years old due to increased glucose levels. The sums were significantly higher than those of long-livers in groups aged 30-39 and 50-59 years old. Among women, the highest sum was detected in the 40-49 age group due to increased cholesterol levels, and in the 50-59 age group due to increased glucose content. At the same time, the sum indicators were significantly higher than those of long-livers in groups from 30-39 to 60-69 years old (Table 2).

Table 3

Frequency of high or low lipids, glucose and their sum (%)

Indicator	Gender	Age group, years								Cohort
			30-39	40-49	50-59	60-69	70-79	80-89	> 90	
Cholesterol, mmol / l										
> 5.21	men	20.0	42.1	57.2	54.5	54.5	34.2	50.0	11.5	41.8
	women	18.9	30.1	37.1	68.7	54.0	52.9	42.8	26.1	46.8
> 6.21	men	-	15.8	14.3	19.7	30.3	7.9	10	3.8	14.7
	women	2.7	9.5	12.3	28.0	18.0	23.5	23.5	8.7	17.3
Triglycerides, mmol / l										
> 1.7	men	3.3	5.3	10.3	6.1	6.1	5.3	20.0	3.8	6.5
	women	2.7	4.8	3.2	6.8	14	5.9	-	-	5.5
HDL cholesterol, mmol / l										
< 0.78	men	6.7	13.2	-	7.6	3.0	5.3	-	3.8	5.7
	women	2.7	1.6	-	2.5	4.0	-	-	-	1.8
LDL cholesterol, mmol / l										
> 4.53	men	-	7.9	10.3	18.2	27.3	7.9	30.0	3.8	12.9
	women	-	3.2	9.7	16.9	12.0	20.6	5.0	4.3	10.3
Atherogenic coefficient										
> 3.0	men	20	55.5	48.5	48.5	31.6	60.0	46.2	18.9	43.7
	women	18.9	31.7	30.6	43.2	42.0	41.2	30.0	19.2	35.2
Glucose										
> 5.5	men	33.3	23.7	31.0	40.9	54.5	23.7	30.0	19.2	33.3
	women	21.6	15.9	19.4	31.4	40.0	8.8	9.5	13.0	23.1
Cholesterol + Glucose										
=10	men	30.0	2.6	31.0	25.8	15.2	23.7	20.0	3.8	19.4
	women	8.1	15.9	22.6	18.6	24.5	29.4	19.0	0.0	18.6
> 10	men	7.0	19.0	12.0	40.0	17.0	10.0	4.0	7.0	42.7
	women	11.0	17.0	22.0	75.0	23.0	10.0	6.0	4.0	41.5
<10	men	14.0	18.0	8.0	9.0	11.0	19.0	4.0	18.0	38.0
	women	23.0	36.0	26.0	21.0	14.0	14.0	11.0	19.0	39.9

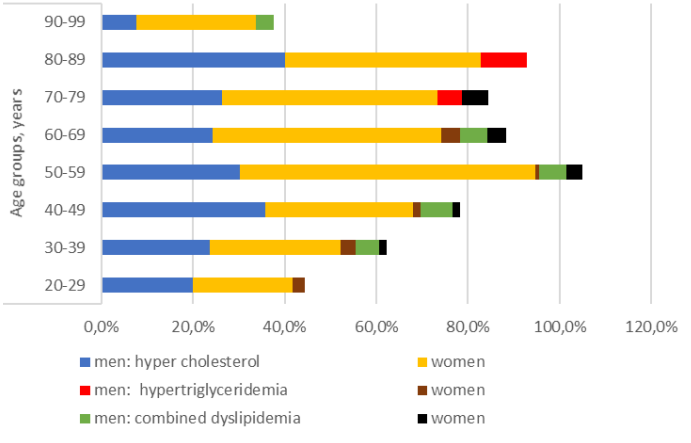


Fig 1. Frequency of different types of dyslipidemia

Illustration 2 shows the percentage of frequency of deviations from the normal sum of TC and glucose levels. According to the median, the highest sum was detected in the age group of 50-59 years old in both men and women. Excess of 10 mmol/L was more common in women (75%) due to increased cholesterol levels, and in men (40%) due to increased glucose levels. In long-livers, the sum of TC and glucose was often reduced, in men due to lower cholesterol levels, and in women due to lower glucose levels. Decreased sum of cholesterol and glucose was more often detected in women of younger age of 20-29, 30-39, 40-49 years old and in men in the age groups of 30-39, 70-79 years old.

The sum of TC and glucose positively correlates with LDL-C and has a positive connection to BMI and AC (Table 4). High AC is associated with excess body weight. Table 3 shows the connection of BMI with lipid profile fractions and glucose. The increase in body weight is accompanied by a significant growth of LDL-C, glucose, TG, TC, reduction of the HDL-C levels and, therefore, AC. Illustration 2 shows that the frequency of obesity increases in men up to 59 years old and in women up to 69 years old. The peak of obesity of both genders falls on the age of 50-59 years old, just as the peak of total cholesterol (TC) and glucose sum frequency exceeding 10 mmol/L falls on the same age range.

Among all parameters associated with lipid metabolism, the antiatherogenic lipid fraction that negatively correlates with cardiovascular diseases is of greater importance [5]. In our research, an increase in the average level of HDL-C in men was detected up to 69 years old with a decrease from 70-79 years old and to the age of long-livers. The age-related change in HDL-C levels in women is different: there is a steady increase, taking quite a significant value in the age of long-livers, which may be due to the influence of estrogen.

The low level of HDL-C in the cohort is more common in men: 5.8% against 1.8% in women. In addition, young men between the ages of 20 and 39 are more likely to have a low level of antiatherogenic cholesterol, which requires a search for causes, since low HDL-C levels are considered the highest risk of cardiovascular diseases [8].

The age-related increase of TG levels and triglyceridemia frequency in men peaks at 40-49 years old, in women it peaks 20 years later. However, there is a sharp decline by 70-79 years old, and triglyceridemia is not present in women over 80 years old at all. TG levels are glucose-coupled, as evidenced by the direct correlation between these values. Dynamics of TG levels by age do not contradict the research results of hypertriglyceridemia prevalence, according to PROMETEUS data conducted in Russia [1].

According to the sum of TC and glucose exceeding a constant ten, it can be concluded that people aged 50 to 59 years old are at the highest risk, since the percentage of obese people surveyed was the highest in both men and women. Under the conditions of severe energy deficiency (e.g. alcoholism or long-term fever), the conjugation of these two components is triggered: glucose content reduction leads to higher cholesterol levels and vice versa, but with a constant ten. Excess of 10 mmol/L indicates severe metabolic disorders [3].

Thus, in contrast to all comparison groups, all lipid spectrum indicators in long-livers were within reference values. Significant gender-related differences among long-livers were found in the levels of HDL-C and glucose: women had higher levels of HDL-C and lower levels of glucose than men ($p<0.05$). The highest frequency of high total cholesterol and triglycerides levels in men was detected at ages 40-49, and in women it was detected 10 years later at ages 50-59. In men, a significant increase in the level of TC begins at 30 and continues until the age of 69, and in women it begins at 30 and stops at 79 years old. In men, the glucose content is higher in all age groups and the antiatherogenic lipid fraction is lower.

Hypercholesterolemia is more common in women, and hypertriglyceridemia in men. At later stages of life, the number of men and women with hypercholesterolemia and hypertriglyceridemia decreases. Hypertriglyceridemia has not been detected among female long-livers. The age-related increase in the antiatherogenic lipid fraction is typical for women, reaching its peak

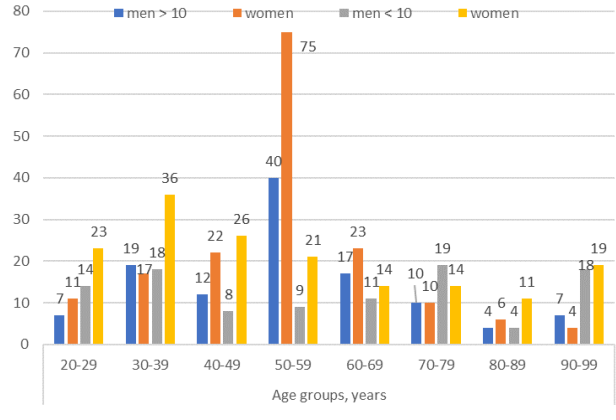


Fig. 2. The frequency of deviations from the sum of total cholesterol and glucose, equal to 10 mmol / l (%).

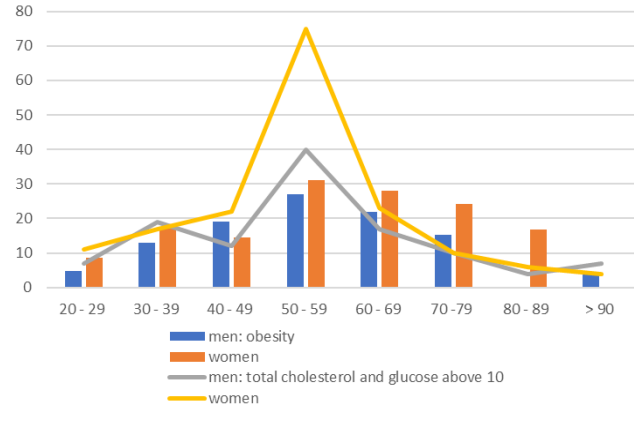


Fig. 3. Frequency of obesity and high total cholesterol and glucose,%.

Table 4

Correlation of BMI, lipids and glucose

	TG	CHL	HDL cholesterol	LDL cholesterol	VLDL cholesterol	KA	Glucose	CHL + Glucose
BMI	0.318** 0.000	0.215** 0.000	-0.244** 0.000	0.255** 0.000	0.338** 0.000	0.241** 0.000	0.336** 0.000	0.186** 0.000
TG		0.298** 0.000	0.354** 0.000	0.285** 0.000	0.944** 0.000	0.564** 0.000	0.278** 0.000	0.217** 0.000
CHL			0.274** 0.000	0.928** 0.000	0.287** -0.000	0.406** -0.000	0.095* -0.014	0.669** 0.000
HDL cholesterol				0.005 0.903	-0.367** 0.000	-0.665** 0.000	-0.192** 0.000	0.108** 0.005
LDL cholesterol					0.248** 0.000	0.604** 0.000	0.126** 0.001	0.612** 0.000
VLDL cholesterol						0.555** 0.000	0.260** 0.000	0.227** 0.000
KA							0.215** 0.000	0.285** 0.000
Glucose								0.642** 0.000

Note: ** - the correlation is significant at the 0.01 level (two-sided)

in long-livers. In men, the content of HDL-C decreases significantly with age, which is the main factor of the higher frequency of dyslipidemia. Changes in the lipid spectrum are associated with obesity, which is more common in men aged 49-69 years old, in women aged 39-69 years old. The higher the weight, the lower HDL-C levels are (-0.244; p<0.000). Changes in the lipid profile in men occur from 30 to 69 years old, and in women from 50 to 79 years old. The peaks of dyslipidemia, obesity, and the sum of TC and glucose occur at the age of 50-59/ Therefore, on the basis of severe metabolic disorders this age group is classified as the group of pathological risk, which is an obstacle to survival to the age of long-livers.

References

1. Карпов Ю.А. От имени участников исследования PROMETHEUS. Распространенность гипертриглицеридемии: новые всероссийские данные. Исследование PROMETHEUS. Кардиология. 2016; 56(7):63-71. [Karpov YuA. On behalf of the PROMETHEUS study participants. The prevalence of hypertriglyceridemia: new all-Russian data. Research PROMETHEUS. Kardiologiya. 2016; 56(7): 63-71. (in Russ)]. DOI: 10.18565/cardio.2016.7.63-71.

2. Козлов А.И. Связанные с потреблением углеводных продуктов нутрициологические и генетические риски развития ожирения у коренных северян. Вопросы питания. 2019; 88(1):5-16. [Kozlov AI. Associated with the consumption of carbohydrate products, nutritional and genetic risks of obesity in indigenous northerners. Vopr. Pitaniya.2019; 88(1):5-16. (in Russ)]. DOI: 10.24411/0042-8833-2019-10001

3. Рослый И.М. Водолажская М.Г. Правила чтения биохимического анализа. Руководство для врачей. 3-е изд., испр. и доп. М: ООО «Медицинское информационное агенство». 2020;112. [Rosly IM, Vodolazhskaya MG. Rules for reading biochemical analysis. Guide for doctors. 3-e izd., ispr. i dop. M: OOO «Medicinskoe informacionnoe agenstvo». 2020;112. (in Russ)].

4. Татаринова О.В. Сердечно-сосудистая патология и ее факторы риска в якутской популяции. Атеросклероз. 2014; 2:61- 69. [Tatarinova OV. Cardiovascular pathology and its risk factors in the Yakut population. Ateroskleroz.2014; 2: 61- 69. (in Russ)].

5. Гуревич, С.А. Уразгильдеева, М.И. Бутхашвили, Л.В. Васина. Эволюция представлений о про- и антиатерогенных свойствах липопротеинов. Атеросклероз и дислипидемии. 2012; 9(4): 53-61. [Gurevich VS., Urazgil'deeva SA., Buthashvili MI., Vasina LV. The evolution of ideas about the pro- and anti-atherogenic properties of lipoproteins. Ateroskleroz i dislipidemii. 2012; 9(4): 53-61.

6. Dzau VJ. Jenkins Jo Ann C. Creating a Global Roadmap for Healthy Longevity. J. Gerontol a Biol Sci Med Sci 74, no. Supplement_1. (November 13, 2019; 4–6. DOI: 10,1093 / герона / glz226 CrossRef

7. Jenkins JC. Disrupt aging: a call to action for gerontologists. Gerontologist. 2017;57(suppl 2): S115–S117. doi:10.1093/geront/gnx079 S115 – S117. DOI: 10,1093 / geront / gnx079 Google ScholarCrossrefPubMedCrossref

8. Urazgildeeva SA., Muzalevskaya MV., Tregubov AV. et al. HDL as a crucial indicator of an early clinical manifestation of coronary atherosclerosis in patients with familial hypercholesterolemia. Abstracts of the 84th European Atherosclerosis Society Congress. Atherosclerosis. 2016;252:e113.

E.Z. Zasimova, A.S. Golderova, E. D. Okhlopkova,
A.A. Zakharova, S. D. Efremova

DYNAMICS OF HORMONE LEVEL IN RIVER TRANSPORTATION WORKERS IN CONDITIONS OF A LONG-TERM TRAVEL

DOI 10.25789/YMJ.2020.71.23

УДК 613.6.027

The labor of river workers under the influence of the factors of the ship's environment causes a significant stress in the body's adaptation systems with a possible disruption of the functional state of the main regulatory systems, leading to a deterioration in health and a decrease in working capacity.

The aim of the study is to evaluate stress-releasing hormones in river dwellers before and after a long voyage in Yakutia. We examined 98 men aged 20 to 68. Venous blood sampling was performed twice: in April and September, before and after the voyage, respectively. The concentration of hormones in the blood serum (free fractions of triiodothyronine (freeT3) and thyroxine (freeT4), thyroid-stimulating hormone (TSH), cortisol and testosterone) were determined by the enzyme immunoassay, and the integral thyroid index (ITI) was calculated using the formula (freeT3 + freeT4 / TSH). The statistical significance of differences in hormone levels was assessed by the Wilcoxon test for two related groups. The results were considered statistically significant when the values of the achieved level of significance p <0.05.

Comparative analysis revealed statistically significant differences, although the median of all hormones, with the exception cortisol levels after a long voyage on a ship, are within normal ranges. Thus, after the voyage, the examined individuals had significantly increased concentrations of TSH, freeT3, freeT4 and testosterone (p = 0.000), as well as decreased levels of cortisol (p = 0.039) and ITI (p = 0.000). An increase in thyroid hormones (freeT3, freeT4 and TSH) in river dwellers after the voyage indicates signs of stress and activation of adaptive mechanisms during a long voyage (3-4 months). However, a low ITI value is an early sign of risk for hypothyroidism. It is known that hypothyroidism reduces the mobilization of compensatory mechanisms under stress. The median concentration of cortisol after the voyage turned out to be below the generally accepted norm (p = 0.039); negative dynamics was revealed in 60.2% of the examined individuals. The concentration of testosterone after the voyage significantly increased 1.48 times (p = 0.000).

Keywords: hormones, TSH, T3, T4, testosterone, cortisol, river workers, Yakutia.

Introduction. The labor of river workers under the influence of the factors of the ship's environment causes a significant stress in the body's adaptation systems with a possible disruption of the functional state of the main regulatory systems, leading to a deterioration in health and a decrease in working capacity [3].

In extreme conditions of long and contrasting voyage turned, great capabilities of the human body can be realized, and its functions change in different ways, depending on what role each of them plays in the general adaptive response of the body [4]. It has been shown that the degree of climatogeographic stress, manifested in the interrelated increase in psycho-emotional stress and the accumulation of stress hormones in the blood, depends on the increase in the geographical latitude of a person's residence and the discomfort of the climate in a given territory [6].

The natural and climatic conditions of the North, which are extreme for humans, cause the activation of functional adaptation mechanisms. One of the homeostatic systems of the body, which makes a significant contribution to northern adaptation, is the thyroid system and its central organ, the thyroid gland. It was shown that the thyroid gland in residents of the northern regions is characterized by an increased functional tone. The boundaries of its adaptive and maladaptive structural and functional rearrangements are determined by the strength and frequency of exposure to thyroid stimulants [1].

With any overloads of the body, including mental ones, the consumption of energy (calories) increases to overcome unfavorable circumstances. Mental stress, like cold and any other, also serves as a condition under which energy expenditure increases. Since all cells of the body can perceive energy only with the help of thyroid hormones (T3 and T4 in free form), it is quite natural that in the case of mental overstrain, the release of thyroid-stimulating hormone (TSH) by the pituitary gland is activated for enhanced stimulation of the formation of T3 and T4 by the thyroid gland [7].

The activation of three endocrine mechanisms (axes): adrenocortical, somatotropic and thyroid, is an adaptation syndrome or stress response.

The aim of this work was to assess the effect of a long voyage on the hormone level of workers in river transport in Yakutia.

Material and research methods. We examined 98 workers of water transport of the Republic of Sakha (Yakutia) before and after the voyage during scheduled periodic medical examinations. The age of the surveyed ranged from 20 to 68 years (average age 46.08 ± 1.35 years): from 20 to 29 years - 13 (13.3%), from 30 to 39 - 20 (20.4%), from 40 under 49 years old - 20 (20.4%), from 50 to 59 years old - 22 (22.4%), from 60 to 69 years old - 23 (23.5%). Of these, 10 were representatives of the indigenous nationality. (Yakuts - 8, small peoples of the North - 2). The non-indigenous population was represented by 88 persons of Russian, Ukrainian, Tatar nationality, etc., of which 36 people. turned out to be natives of Yakutia. Arriving from other regions of Russia and the CIS were 52 people, depending on the length of stay in Yakutia, they were distributed as follows:

ZASIMOVA Ekaterina Zakharovna – physician therapist, early. Department of the Institute of Physical Culture and Sports of the NEFU named after M.K. Ammosov, ekazas15@yandex.ru; **GOLDEROVA Aitalina Semyonovna** – North-Eastern Federal university in the name of M.K. Ammosov, Doctor, Professor, of Medical Sciences hoto68@mail.ru; **OKHLOPKOVA Elena Dmitryevna** − Leading Researcher, PhD of the Department of adaptation mechanisms study of the Yakut Science Centre of complex medical problems, elena_ohlopkova@mail. ru. **ZAKHAROVA Anna Anatolyevna** – Director of the Sports and Recreation Complex of the IFCiS NEFU named after M.K. Ammosov, anna-medsport@mail.ru; **EFREMOVA Svetlana Dmitrievna** - researcher of the Department of adaptation mechanisms study of the Yakut Science Centre of complex medical problems, esd64@mail.ru

up to 1 year - 2 people; up to 5 years old - 1; from 10 to 15 years old - 1; from 15 to 20 years old - 7; 20 years and more - 41 people. To determine changes in the levels of hormones in the blood serum of river sailors, a comparative analysis was carried out during the period of long navigation - in spring and autumn (before and after the voyage).

Blood for research was taken from the cubital vein in the morning on an empty stomach. Laboratory research was carried out under constant internal and external quality control. The concentration of free fractions of triiodothyronine (freeT3) and thyroxine (freeT4), thyroid stimulating hormone, cortisol, and testosterone in the blood serum was determined by enzyme immunoassay using reagent kits from Vector-Best (Novosibirsk, Russia) according to the manufacturer's instructions. The results of the enzyme-linked immunosorbent assay were recorded using a Uniplan photometer (Pikon, Russia).

To characterize the functional state of the thyroid gland, the value of the integral thyroid index (ITI) is of certain interest - the ratio of the levels of the thyroid hormones themselves to their pituitary regulator. The definition of ITI was carried out according to the formula: ITI = (freeT3 + freeT4) / TSH, the norm is 7.04–27.21 [7]. This study was approved by the decision of the local ethics committee at the YSC CMP and was carried out with the informed consent of the subjects in accordance with the ethical standards of the Declaration of Helsinki (2000).

Continuous values were presented as a median (Me) and interquartile range - 25 and 75% percentiles (25 ÷ 75%). When assessing the significance of differences in hormone levels, the Wilcoxon test was used for two related groups. To identify the relationship between the studied indicators, we used the method of correlation analysis of data with the calculation of coefficients and rank correlation of Spearman and Pearson. The results were considered statistically significant when the values of the achieved level of significance p <0.05.

Results and discussion. A comparative analysis of the hormone levels of river carriers before (spring) and after a long voyage (autumn) revealed their significant statistical differences. Table 1 shows the analysis results indicating significant changes in hormones after the voyage compared to the data before the voyage. So, in the examined individuals, the concentrations of TSH, freeT3., freeT4 and testosterone, all differences are statistically significant (Table).

An increase in thyroid hormones (freeT3, freeT4 and TSH) in river dwellers after the voyage indicates signs of stress and activation of adaptive mechanisms during a long voyage (3-4 months). It has been established that iodine-containing thyroid hormones are one of the significant factors of the body's anti-stress system, limiting the activity of the stress system at all levels of the organization. They increase the body's resistance to the action of various stressors [2].

When considering the features of the dynamics of thyroid hormones in age groups, the highest values of the difference (ratio) of hormones before / after sailing on a ship are noted in terms of TSH concentration. The maximum value of the ratio of this hormone is noted in the age group from 40-49 years (4.48 times) and in the group from 25-29 years (3.85 times). Such a difference in TSH level in men of age groups 40-49 years old and 25-29 years old is most likely due to its minimum concentration before sailing on a ship in comparison with other age groups (1.49 ± 0.23 mIU / L) and (1 , 72 ± 0.31 mIU / L), respectively. The minimum ratio of TSH concentration before and after sailing on a ship is observed in the age group 50-59 years old.

ITI - integral thyroid index - is the ratio of thyroid hormones to their pituitary regulator and is normally 7.04 - 27.21. An increase in this index is the earliest sign of hyperthyroidism, while a decrease in ITI is even the initial stages of hypothyroidism. In our study, the ITI tended to decrease: before the voyage on the ship it turned out to be slightly above the lower limit of the norm, and after the voyage it turned out to be below the generally accepted norm (Table 1).

It should be noted that the percentage of persons with normal ITI before the voyage on the ship was more than half - 58.16%, and after the voyage after the voyage it decreased to 37.8%. Only 4.08% had ITI above the norm before the voyage on the ship (Fig. 2). Low ITI values are mostly associated with increased TSH values. Despite the fact that the value of the median content of freeT3 and freeT4 after were within the normal ranges and increased compared to the initial data (before the voyage on the ship), low values of ITI indicate the risk of hypothyroidism.

It is known that the state of hypothyroidism reduces the mobilization of compensatory mechanisms during stressful conditions, which in turn can lead to a breakdown of adaptation mechanisms. An analysis of age characteristics indicates that before the flight, in all age groups, persons with normal or higher ITI prevailed, with the exception of men aged 50-59; 54.5% had low ITI. After the voyage, all groups are dominated by ITI persons below the norm.

In general, it can be argued that the functional state of the thyroid gland in river workers in Yakutia by the end of an almost three-month voyage is characterized by a relative decrease, and long voyages on a ship during several summer months have a negative effect and can provoke the risk of hypothyroidism.

Testosterone concentration after a long voyage significantly increased (p = 0.000) 1.48 times than before the voyage; this increase is possibly associated with an increase in physical activity during the voyage. Testosterone and cortisol are antagonistic steroid hormones. It should be noted that a positive correlation was revealed before the voyage, which was r = 0.330; p = 0.001; however, after the voyage, no correlation was found between these hormones. The level of cortisol in river dwellers after a long voyage (autumn) was significantly lower (p =

Hormone levels in river workers before and after a long voyage

Index, reference values	before sailing on a ship (Spring)	after sailing on a ship (fall)	p….
TSH, 0.3-4.0 mIU / L	1.61 (1.02; 2.58)	2.83*** (1.88; 4.03)	0.000
freeT3, 2,5-7,5 nmol/L	3.45 (2.98; 4.02)	4.73*** (3.95; 5.47)	0.000
freeT4, 10.0 -25.0 nmol/L	9.82 (8.61; 11.96)	14.4*** (9.55; 17.23)	0.000
ITI = freeT3 +freeT4 / TSH 7.04 – 27.21	8.07 (4.98; 13.94)	6.02 (3.82; 8.72)	0.000
Testosterone, 4.5 - 35.4 nmol/L	11.94 (7.92; 17.01)	17.7*** (9.65; 22.63)	0.000
Cortisol, 190.0 – 690.0 nmol/L	705.09 (513.19; 875.89)	573.63* (442.05; 754.85)	0.039

Note. Indicators of medians (Me) and interquartile ranges of 25-75 percentiles are presented; significance of differences in indicators: * -p <0.05; ** - p <0.01; *** - p <0.001; Δ-p <0.1.

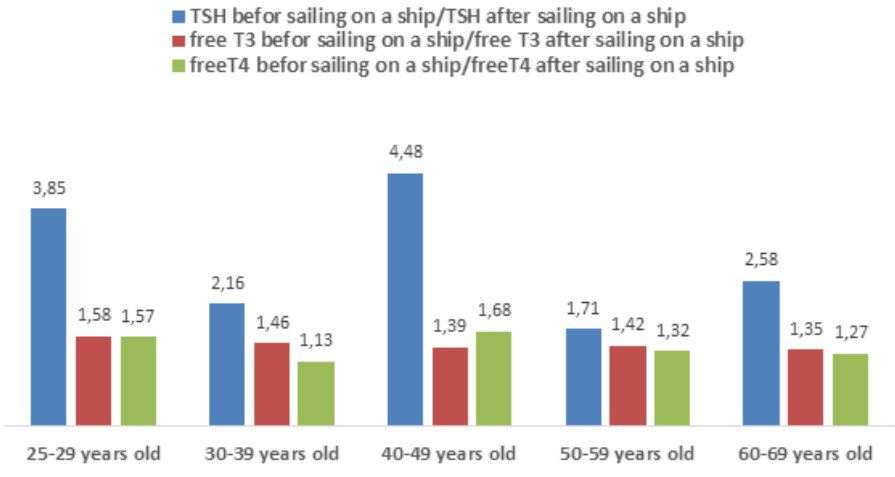


Fig. 1. Ratio of thyroid hormones before sailing / after sailing on a ship in age groups

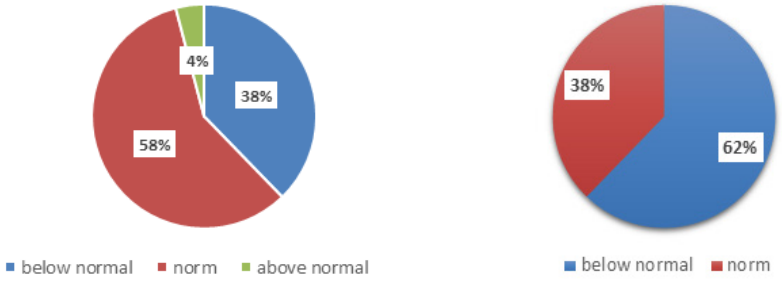


Fig. 2. Levels of the integral thyroid index (ITI) in river workers before the voyage (left) and after the voyage (right)

0.039) than before the voyage (spring); in 60.2% of cases, negative dynamics was revealed. Low cortisol levels in riverine at the end of a long voyage may be associated with the stage of exhaustion during prolonged chronic exposure to stress factors. On the other hand, this condition is associated with seasonal fluctuations in cortisol.

According to L.M. Polyakov [5], the seasonal (circannular) rhythms of cortisol content in residents of these regions differed significantly: in men living in middle latitudes, it was increased in the spring-summer period and significantly decreased in the autumn-winter period, while no seasonal differences in the daily rhythm of the cortisol content were found in northerners.

Conclusions. Thus, the data obtained by us indicate that the rivers of Yakutia as a result of a long voyage (3-4 months) show signs of stress and depletion of adaptation mechanisms.

References

1. Горбачев А.Л. Структурно-функциональные особенности тиреоидной системы человека и его микроэлементный статус в условиях Северо-Востока России. Автореф. дис. ….д-ра биол. наук. 2002;50. [Gorbachev AL. Structural and functional features of the human thyroid system and its microelement status in the North-East of Russia. Avtoref. dis. ….d-ra biol. nauk. 2002;50. 2002:50. (In Russ.).]

2. Городецкая И.В., Гусакова Е.А., Евдокимова О.В. Периферические механизмы стресс-протекторного эффекта йодсодержащих гормонов щитовидной железы. *Физиология.* 2016;6(15):41-51. [Gorodetskaya IV, Gusakova EA, Evdokimova OV. Peripheral mechanisms of the stress-protective effect of iodine-containing thyroid hormones. *Fiziologiya.* 2016;6(15):41-51. (In Russ.).]

3. Кубасов Р.В., Лупачев В.В., Попов М.В. Условия жизнедеятельности экипажа на борту морского судна (обзор литературы). *Вестник Государственного университета Морского и речного флота имени адмирала С.О. Макарова.* 2016; 2 (36):49-56. [Kubasov RV, Lupachev VV, Popov MV. Life conditions of the crew on board a sea vessel (literature review). *Vestnik Gosudarstvennogo universiteta Morskogo i rechnogo flota imeni admirala S.O. Makarova.* 2016; 2(36):49-56. (In Russ.).] DOI: 10.21821 / 2309-5180-2016-8-2-49-56

4. Зайцев В.И., Виноградов С.А. Некоторые теоретические и практические аспекты изучения условий труда на флоте. *Здоровье населения и среда обитания.* 2014;2(251):13-15. [Zaitsev VI, Vinogradov SA. Some theoretical and practical aspects of the study of working conditions in the fleet. *Zdorov'e naseleniya i sreda obitaniya. 2014;2(251):13-15. (In Russ.).]*

5. Поляков Л.М. Суточные и сезонные ритмы содержания кортизола у мужчин, проживающих в высоких и средних широтах. *Сибирский научный медицинский журнал.* 2017;2 (37):92-96. [Polyakov LM. Daily and seasonal rhythms of cortisol content in men living in high and middle latitudes. *Sibirskij nauchnyj medicinskij zhurnal.* 2017;2 (37):92-96. (In Russ.).]

6. Хаснулин В.И., Хаснулина А.В. Особенности психоэмоционального стресса у жителей регионов Севера и Сибири с дискомфортным климатом при высоком и низком содержании гормонов стресса в крови. *Мир науки, культуры, образования.* 2012;5 (36):32-35. [Khasnulin VI., Khasnulina AV. Peculiarities of psychoemotional stress in residents of the regions of the North and Siberia with an uncomfortable climate with high and low levels of stress hormones in the blood. *Mir nauki, kul'tury, obrazovaniya.* 2012;5 (36):32-35.]

7. Fischer S, Strahler J, Markert C, Skoluda N, Doerr JM, Kappert M, Nater UM Effects of acute psychosocial stress on the hypothalamic-pituitary-thyroid (HPT) axis in healthy women. *Psychoneuroendocrinology.* 2019;Sep 6.

A.V. Ivanov, I. D. Ushnitsky, A.V. Yurkevich, D.V. Mikhalchenko, A.A. Ivanova, O.S. Unusyan, T.E. Yavorskaya

CHARACTERISTICS OF RISK FACTORS FOR THE FORMATION AND DEVELOPMENT OF PATHOLOGICAL PROCESSES OF PERIODONTAL TISSUES OF INFLAMMATORY-DESTRUCTIVE CHARACTER IN THE POPULATION LIVING IN THE CONDITIONS OF THE NORTH

DOI 10.25789/YMJ.2020.71.24

УДК 616.31-0:616.31-02

Clinical and physiological study of risk factors for the development of periodontal diseases among the residents of the North is presented.

The presence of biological risk factors related to changes in the composition and properties of oral fluid, as well as a low level of sanitary level, was revealed. Disorders of the biophysical properties of mixed saliva were associated with increased viscosity, reduced salivation rate, preponderance of II and III types of microcristalization, pH acid increase, reduced lysozyme activity, low level of hygiene education. The identified risk factors have a negative effect on the prevalence of periodontal diseases and determine their clinical course.

Keywords: periodontal diseases, oral hygiene, oral fluid, sanitation.

Introduction. Currently, a high level of frequency of periodontal diseases among various population groups is determined [2,3,12,13,16]. The severe natural and climatic conditions of the North have a negative impact on the functional state of the organs and tissues of the oral cavity, as well as the maxillofacial region [8,15]. At the same time, to improve dental care for the population, it is necessary to take into account certain etiological factors in the formation and development of periodontal diseases [1,4,5]. Despite the extensive study of the pathological processes of periodontal tissues of an inflammatory-destructive and metabolic-dystrophic nature, the problems of their treatment [6,7,11] and prevention [9,10,14] remain unresolved. Taking into consideration the above, we studied the risk factors for periodontal diseases, especially since such studies in the Republic of Sakha (Yakutia) have not been previously conducted, which are of great practical importance.

Materials and research methods. A study of the biophysical properties of the oral fluid in the adult population with inflammatory periodontal diseases was carried out with 414 people aged 15-19 years (n = 108) and 35-44 years (n = 306). The clinical study was carried out on the basis of the dental clinic of the Medical Institute of the Federal State Autonomous Educational Institution of Higher Education "North-Eastern Federal University named after M.K. Ammosov ", as well as the dental clinic "Dentalika" (Yakutsk), as well as the clinical diagnostic laboratory of the CBI RS (Y) "Scientific and Practical Center - Phthisiology" (Yakutsk).

To determine the rate of salivation, mixed saliva without stimulation was collected on an empty stomach by spitting into graduated centrifuge tubes for 10 minutes. The collected saliva was hermetically sealed. The salivation rate was determined in ml / min (the optimal indicator is 0.70 ml / min). Microscopy of a drop of dried saliva was carried out according to the method proposed by P.A. Leus (1977). The mineralizing potential of the mixed saliva when reading the preparation, was determined by the nature of crystal formation and distinguished 3 types of microcrystallization. The viscosity of saliva was determined using a viscometer (VK-4) according to the method of T.L. Redinova, A.R. Pozdeeva (1994), (the optimal indicator is 4.16 units). Determination of the pH of the oral fluid was carried out using the apparatus "713 pH Meter" from "Metrohm" (Germany). The optimum pH value in mixed saliva is 6.5 to 7.5. Determination of the content of lysozyme in the oral fluid was carried out according to the method of Bukharin O.V. et al. (1974). The method is based on measuring the FEC-M changes in the optical density of a standardized suspension of micrococcus (strain 2665). The results were expressed using standard lysozyme mg / 100 mg. The state of oral hygiene in the examined age groups was assessed using the index of oral hygiene according to J.C. Green, J.R. Vermillion (IGR-U, OHI-S). The assessment of the social and hygienic status was carried out using a special questionnaire card (Chizhov Yu.V., 2005) with additions, which was approved at the Department of Therapeutic, Surgical, Prosthetic Dentistry and Pediatric Dentistry of the Medical Institute of the Federal State Autonomous Educational Institution of Higher Education "North-Eastern Federal University named after M.K. Ammosov "(2016).

The examination was carried out in accordance with the ethical principles of conducting scientific medical research with human participation, defined by the Declaration of Helsinki by the World Medical Association (1964, ed. 2000), and the requirements set out in the main regulatory documents of the Russian Federation on clinical trials, as well as approved by the Ethics Committee of the Federal

M.K. Ammosov MI NEFU, Yakutsk, Russia: **IVANOV Andrian Vladimirovich** – assistant of the department, andrian_ivanov@mail.ru, **USHNITSKY Innokenty Dmitrievich** –doctor of medical sciences, prof., Head of department, incadim@mail.ru, **IVANOVA Aitalina Alekseevna** – assistant of the department, aitalinalex@mail.ru, **UNUSYAN Onik Sarkisovich** - post-graduate student, onikunusyan@gmail.com; **YURKEVICH Alexander Vladimirovich** – Doctor of Medical Sciences, Dean of the Faculty, Head of Department of FESMU, dokdent@mail.ru; **MIKHALCHENKO Dmitry Valerievich** – Doctor of Medical Sciences, Associate Professor, Head of Department of the Volgograd State Medical University, Ministry of Health of the Russian Federation, karta007@rambler.ru; **YAVORSKAYA Tatiana Evgenievna** – Candidate of Medical Sciences, Associate Professor of the I.N. Ulyanov Chuvash State university, yavorskaya_te@mail.ru.

State Autonomous Educational Institution of Higher Education "North-Eastern Federal University named after M.K. Ammosov". Prior voluntary consent was obtained from all examined.

Statistical processing of the research data was carried out according to the standard methods of variety of statistics, with the calculation of the average value, the root-mean-square error using the Microsoft Excel 2009 application packages (Microsoft Corporation, 2000-2016). The results obtained were grouped according to a set of similar features. When determining statistically significant differences, the values of indicators were compared in groups in pairs using the Student's t test. Correlation analysis of clinical material with determination of Pearson's coefficient (r) was carried out using the SPSS software package, version 22.

Research results. The obtained results of the study determine the presence of some changes in the composition and properties of mixed saliva (Table 1). So, in the age groups 15-19 years old and 35-44 years old, indicators of the salivation rate are interpreted as its decrease (in adolescents 15 years old, the optimal value of the secretion rate in children is 0.40 ml / min, in adults - 0.70 ml / min.). A similar picture can be traced when assessing the average statistical value in the surveyed age groups. Comparative assessment of pH values in the 15-19-year-old group characterizes an unfavorable situation associated with a shift in acid-base balance to the acidic side, where the indicator was at the level of 6.41 + 0.08. At the same time, the pH of mixed saliva in the age group of 35-44 years is within the limits of optimal values, a similar situation is observed when comparing the average statistical indicator of the examined age groups.

It should be noted that local immunity of the oral cavity is of great importance in the formation and development of inflammatory periodontal diseases. Taking into account the above, we studied the content of lysozyme in the oral fluid in the examined age groups of the population. Thus, the data obtained in both age groups are interpreted as a significant decrease in the content of lysozyme in mixed saliva, where the indicators were respectively 3.81 mg / 100 ml and 4.40 mg / 100 ml (the optimal value is 18.1 mg / 100 ml). At the same time, the overall average indicator characterizes a similar situation and the indicator was at the digital level of 4.10 mg / 100 ml. The revealed decrease in the content of lysozyme in the oral fluid in persons living in the harsh natural and climatic conditions of high latitudes is one of the biological factors in the formation and development of pathological processes of periodontal tissues of an inflammatory and destructive nature.

Table 1

Characteristics of the consist and characteristics of the oral fluid in adolescents and adults

Age group	pH (n=261)	Lysozyme mg/100ml (n=165)	Viscosity (unit) (n=423)	Secretion rate ml / min (n=414)	Type of microcrystallization, % (n=414)		
					I тип	II тип	III тип
15-19 age	6.41 ±0.08	3.81 ±0.20[2]	3.08 ±0.08	0.36 ±0.03[2]	12.33 ±0.70[2]	28.46 ±0.58[2]	59.21 ±0.33[2]
35-44 age	6.65 ±0.05[1]	4.40 ±0.19[1]	3.11 ±0.08	0.48 ±0.01[1.2]	8.59 ±0.74[1.2]	45.83 ±0.44[1.2]	45.58 ±0.44[1.2]
Average indicators	6.53 ±0.07	4.10 ±0.20[2]	3.09 ±0.08	0.42 ±0.02[2]	10.46 ±0.73[2]	37.15 ±0.51[2]	52.39 ±0.38[2]

Note: [1] - veraciously significant differences between age groups; [2] - veraciously significant differences in age groups with an average indicator.

The analysis and evaluation of the obtained indicators of the viscosity of the oral fluid in the examined age groups of the population indicate its increase, where they ranged from 3.08 to 3.11 units. (the optimal indicator is 4.16 units). At the same time, in the types of microcrystallization of mixed saliva, certain changes were observed that characterize an unfavorable background. So, in the structure of microcrystallization types, significant predominance of types II and III is determined in comparison with type I (10.46 - 0.73), where the digital values were 37.15 - 0.51 and 52.39 - 0.38%, respectively (p <0.05). Meanwhile, the overall average statistical values of types II and III also characterized their significantly significant prevalence over type I microcrystallization by 26.69 and 41.93%, respectively (p <0.05).

It should be noted that the obtained data on the hygienic state of the oral cavity according to the OHI-S index of Green-Vermillion characterizes an unfavorable situation, except for adolescents and adolescents of 15-19 years old and the group of 20-34 years. So, in age groups, 35-44 and 45-54 years, the indicators vary within the range of digital values from 3.02 to 3.86 and, accordingly, determine the poor hygienic state (Table 2). At the same time, the overall average statistic is also at the level of unsatisfactory oral hygiene.

To the question of the regularity of visits to the dental clinic, 72.92 ± 0.47% of the respondents answered, that the purpose of the treatment was tooth extraction and orthopedic treatment. Meanwhile, annually only 27.08 ± 1.29% of respondents apply for consultation, oral cavity sanitation and dispensary observation. At the same time, the implementation of the recommendations and prescriptions of the doctor in a certain way affects the functional state of the organs and tissues of the oral cavity. So, it was found that 56.43 ± 0.77% always fully comply with the doctor's prescriptions and recommendations, while 25.57 ± 1.32% did not fully comply with the doctor's recommendations and this is associated with a lack of time. In addition, 11.03 ± 1.57% of the respondents noted that the reason for

Table 2

The hygienic state of the oral cavity of the adult population of the Republic of Sakha (Yakutia)

Age group	Number of examined	Dental plaque	Dental calculus	OHI-S Green-Vermillion
15-19	78	1.57±0.10	0.92±0.11	2.49±0.08
20-34	91	1.59±0.08 P<0.05	1.23±0.10 P<0.05	2.82±0.06 P<0.05
35-44	89	2.09±0.08 P<0.02	1.53±0.09 P<0.05	3.62±0.05 P<0.05
45-54	84	2.21±0.08 P>0.05	1.65±0.09 P>0.05	3.86±0.04 P<0.05
Total	342	1.91±0.03	1.33±0.04	3.24±0.02

Note: the degree credibility is calculated by the age group.

non-compliance with medical recommendations is the inability to purchase drugs due to financial difficulties. At the same time, 6.97 ± 1.65% of the respondents did not fully comply with the doctor's recommendations and did not comply with his appointment, considering them inappropriate. Taking into account the above, the study of the quality of teeth cleaning was carried out in the surveyed population groups. Thus, the results of a sociological study characterize an unfavorable situation, which is associated with the fact that 19.30 ± 1.4% of respondents conduct daily morning and evening brushing irregularly, while 6.12 ± 1.6% do not brush their teeth at all, which is confirmed by our data on the unsatisfactory hygienic condition of the oral cavity in the surveyed contingent.

The obtained data of linear correlation according to Pearson characterize the presence of a relationship between the prevalence of periodontal diseases with the rate of salivation (r = 0.61), with the type of microcrystallization (r = 0.39), an increase in the viscosity of the oral fluid (r = 0.35), a decrease in activity lysozyme (r = 0.37) and the level of sanitary culture of the population (r = 0.86). In addition, the analysis revealed the relationship between the hygienic state of the oral cavity and the intensity of the inflammatory process of the periodontal tissues according to the PMA index (r = 0.75).

Conclusion. The revealed features of the properties of the oral fluid associated with an increase in viscosity, a decrease in the rate of salivation, the predominance of II and III types of microcrystallization, a shift in pH to the acidic side, a decrease in the level of lysozyme activity, as well as a low level of health education of the population in combination with other aggressive factors of the internal and external environment can contribute to the formation and development of major dental diseases and are one of the biological risk factors in the population living in the North. The identified risk factors in the examined age groups must be taken into account when improving the provision of medical care, as well as preventing pathological processes in the periodontal tissues of an inflammatory-destructive and metabolic-dystrophic nature.

References

1. Архангельский А.А., Ушницкий И.Д., Иванов А.В., Иванова А.А. Сравнительная характеристика эффективности лечения хронического пародонтита с использованием лечебных паст. *Актуальные проблемы и перспективы развития стоматологии в условиях Севера [Электронный ресурс]: сборник статей межрегиональной научно-практической конференции, посвященной 60-летию ГАУ РС (Я) «Якутский стоматологический специализированный центр»*. Якутск: Издательский дом СВФУ. 2019; 39-43. [Arkhangelsky AA, Ivanov AV, Ushnitsky ID, Ivanova AA. Comparative characteristics of the effectiveness of treatment of chronic periodontitis using therapeutic pastes. *Actual problems and prospects of development of dentistry in the conditions of the North [Electronic resource]: collection of articles of the interregional scientific and practical conference dedicated to the 60th anniversary of the GAU RS (Ya) «Yakut dental specialized center»*. Yakutsk: NEFU Publishing house. 2019; 39-43. (In Russ.)]

2. Ефремов Р.Е., Иванов А.В., Адишеров А.Б., Давыдов И.Е., Ушницкий И.Д., Иванова А.А. Обоснование эффективности применения пасты на основе «Ягель» в лечении хронического пародонтита. *Бюллетень Северного государственного медицинского университета*. 2019; (1):137-139. [Efremov RE, Ivanov AV, Alisherov AB, Davydov IE, Ushnitsky ID. Justification of the effectiveness of the use of paste based on «Yagel» in the treatment of chronic periodontitis. *Bulletin of the Northern state medical University*. 2019; 1:137-139. (In Russ.)]

3. Иванов А.В., Ушницкий И.Д., Иванова О.В., Сивцев М.Е. Частота и структура болезней пародонта у жителей Центральной Якутии. *Актуальные проблемы и перспективы развития стоматологии в условиях Севера Сборник статей межрегиональной научно-практической конференции, посвященной 20-летию стоматологического отделения Медицинского института ФГАОУ ВПО Северо-Восточный федеральный университет имени М.К. Аммосова.* 2016; 46-49. [Ivanov AV, Ushnitsky ID, Ivanova OV, Sivtsev ME. Frequency and structure of periodontal diseases in Central Yakutia. *Actual problems and prospects of development of dentistry in the North Collection of articles of the interregional scientific and practical conference dedicated to the 20th anniversary of the Dental Department of the Medical Institute of the North-Eastern Federal University named after M. K. Ammosov.* 2016; 46-49. (In Russ.)]

4. Иванов А.В. Ушницкий И.Д., Иванова А.А., Юркевич А.В., Седайлова Е.М., Новогодин А.А. Клинико-эпидемиологическая характеристика патологических процессов тканей пародонта воспалительно-деструктивного характера. *Якутский медицинский журнал.* 2018; (1):83-86. [Ivanov AV, Ushnitsky ID, Ivanova OV, Yurkevich AV, Seidalova EM, Novogodin AA. Clinical and epidemiological characteristics of pathological processes, inflammatory-destructive nature of periodontal tissues. *Yakut medical journal.* 2018; 1:83-86. (In Russ.).]

5. Иванов А.В., Ушницкий И.Д., Юркевич А.В., Иванова А.А., Ахременко Я.А., Прокопьев И.А. Клинико-лабораторное обоснование эффективности применения «Ягель» в лечении хронического пародонтита. *Дальневосточный медицинский журнал.* 2019; (1): 40-44. [Ivanov AV, Ushnitsky ID, Yurkevich AV, Ivanova AA, Akhremenko YA, Prokopev IA. Clinical and laboratory substantiation of application efficiency of «Yagel» in the treatment of chronic periodontitis. *Far Eastern medical journal*. 2019; 1:40-44. (In Russ.).]

6. Иванов А.В., Ушницкий И.Д., Ахременко Я.А., Прокопьев И.А. Эффективность применения противомикробных средств комплексном лечении хронического пародонтита. *Методические рекомендации.* Якутск: Издательский дом СВФУ. 2019; 28 с. [Ivanov AV, Ushnitsky ID, Akhremenko YA, Prokopev IA. Effectiveness of antimicrobial agents in the complex treatment of chronic periodontitis. *Methodological recommendations.* Yakutsk: publishing house of NEFU. 2019; 28 p. (In Russ.)]

7. Иванов А.В., Ушницкий И.Д., Иванова А.А., Ахременко Я.А., Унусян О.С., Алишеров А.Б. Способ лечения хронического пародонтита с применением лечебной пасты «Ягель». *Актуальные проблемы и перспективы развития стоматологии в условиях Севера: сборник статей межрегиональной научно-практической конференции, посвященной 100-летию стоматологической службы республики Саха (Якутия).* Якутск: Издательский дом СВФУ. 2020; 182-187.[Ivanov, AV, Ushnitsky ID, Ivanova AA, Akhremenko YA, Unusyan OS, Alisherov AB. Method of treatment of chronic periodontitis with the use of medicinal paste «Yagel». *Actual problems and prospects of development of dentistry in the North: collection of articles of the interregional scientific and practical conference dedicated to the 100th anniversary of the dental service of the Republic of Sakha (Yakutia).* Yakutsk: publishing house of NEFU. 2020; 182-187. (In Russ.)]

8. Иванов А.В., Ушницкий И.Д., Иванова А.А., Ахременко Я.А., Унусян О.С., Алексеев А.С. Способ лечения хронического пародонтита с применением масляного раствора «Ягель». *Актуальные проблемы и перспективы развития стоматологии в условиях Севера. Сб. статей межрегион. научно-практической конференции, посвященной 100-летию стоматологической службы республики Саха (Якутия).* Якутск: Издательский дом СВФУ. 2020; 187-193. [Ivanov AV, Ushnitsky ID, Ivanova AA, Akhremenko YA, Unusyan OS, Alekseev AS Method of treatment of chronic periodontitis with the use of oil solution «Yagel». *Actual problems and prospects of development of dentistry in the North. Sat. articles Mezhregion. scientific and practical conference dedicated to the 100th anniversary of the dental service of the Republic of Sakha (Yakutia).* Yakutsk: publishing house of NEFU. 2020; 188-193. (In Russ.)]

9. Костригина Е.Д., Зюлькина Л.А., Иванов В.В. Современный взгляд на этиопатогенез пародонтита (обзор литературы).*Известия высших учебных заведений. Поволжский регион.* 2017; (3):118-128. [Kostrigina ED, Zyulkina LA, Ivanov VV. Modern view on the etiopathogenesis of periodontitis (literature review). *News of higher educational institutions. Volga region.* 2017; 3:118-128. (In Russ.).]

10. Моисева Н.С., Кунин А.А. Клиническая оценка эффективности применения лечебно-профилактических средств в комплексной профилактике заболеваний пародонта. *Пародонтология.* 2018; (23):19-21. [Moiseyeva NS, Kunin A. A. Clinical evaluation of the effectiveness of the use of therapeutic and prophylactic agents in the complex prevention of periodontal diseases. *Periodontics.* 2018; 23:19-21. (In Russ.)]

11. Сейдалова Е.М., Ушницкий И.Д., Иванов А.В. Клиническая характеристика эффективности применения лечебной пасты «Ягель» в комплексном лечении хронического генерализованного пародонтита. *Актуальные проблемы и перспективы развития стоматологии в условиях Севера. Сборник научно-исследовательских работ студентов стоматологического отделения Медицинского института «Северо-Восточного федерального университета имени М.К. Аммосова»*. 2018; 92-97. [Seidalova EM, Ushnitsky ID, Ivanov AV. Clinical characteristics of the effectiveness of the use of medicinal paste «Yagel» in the complex treatment of chronic generalized periodontitis. *Current problems and prospects of development*

of dentistry in the North. Collection of research papers of students of the dental Department of the Medical Institute «North-Eastern Federal University named after M. K. Ammosov». 2018; 92-97. (In Russ.)]

12. Сувырина М.Б., Ушницкий И.Д., Юркевич А.В., Кобец А.Р., Иванова А.А., Иванов А.В. Частота и структура патологических процессов тканей пародонта воспалительно-деструктивного характера у населения Дальневосточного региона. *Якутский медицинский журнал.* 2018; (3):71-74. [Suvyrina MB, Ushnitsky ID, Yurkevich AV, Kobets AR, Ivanova AA, Ivanov AV Frequency and structure of pathological processes of periodontal tissues of an inflammatory and destructive nature in the population of the far Eastern region. *Yakut medical journal.* 2018; 3:71-74. (In Russ.)]

13. Ушницкий И.Д., Иванов А.В., Ахременко Я.А., Кершенгольц Б.М., Прокопьев И.А., Иванова А.А. Способ лечения пародонтита. Патент №2708624 Рос. Федерации, МПК A61K6/00; заявл. 16.04.2019; опубл. 10.12.2019. [Ushnitsky ID, Ivanov AV, Akhremenko Y A, Kershengolts BM, Prokopev IA, Ivanova AA Method of treatment of periodontitis. Patent 2708624 Russian Federation. decl. 16.04.19; publ. 10.12.19, bul.no. 34. (In Russ.)]

14. Ушницкий И.Д., Иванов А.В., Ахременко Я.А., Кершенгольц Б.М., Прокопьев И.А., Иванова А.А. Способ лечения хронического пародонтита. Патент №2708615 Рос. Федерации, МПК A61K6/00; заявл. 16.04.2019; опубл. 10.12.2019. [Ushnitsky ID, Ivanov AV, Akhremenko Y A, Kershengolts BM, Prokopev IA, Ivanova AA Method of treatment of periodontitis. Patent 2708615 Russian Federation. decl. 16.04.19; publ. 10.12.19, bul.no. 34. (In Russ.)]

15. Tonetti M, Jepsen S., Jin, L. Impact of the global burden of periodontal diseases on health, nutrition and wellbeing of mankind: A call for global action. *Journal of clinical periodontology*. 2017; 45:7-11.

16. Yashika J. Genetics in Periodontics. *British Biotechnology Journal.* 2015; 9:8-13.

SCIENTIFIC REVIEWS AND LECTURES

V.M. Nikolaev, N.K. Chirikova, S.I. Sofronova, E.K. Rumyancev, A.G. Vasileva, A.N. Romanova

PROMISING NATURAL COMPOUNDS AS POSSIBLE MEANS OF PREVENTION AND TREATMENT OF THE NOVEL CORONAVIRUS INFECTION CAUSED BY THE SARS-COV-2 VIRUS

DOI 10.25789/YMJ.2020.71.25

УДК 578.834.11

Every day, researchers are learning more and more about the SARS-CoV-2 virus, the epidemiology of coronavirus infection and its pathogenesis. The search for medicines for the prevention and treatment of this disease is constantly underway. However, to date, there are no specific antiviral drugs against SARS-CoV-2. Nevertheless, recent studies have identified many weaknesses in the life cycle of the virus, allowing the creation of effective targeted drugs.

In the review, we have summarized the accumulated scientific experience of many researchers engaged in the search for plant-derived metabolites with potential action against SARS-CoV-2. Unlike synthetic drugs, herbal antivirals do not require labor-intensive pharmaceutical synthesis and are more affordable and relatively safe. In this paper, a search for promising natural compounds as possible means of prevention and treatment of the novel coronavirus infection caused by the SARS-CoV-2 virus was conducted.

Keywords: SARS-CoV-2, phenolic compounds; terpenoids; alkaloids; lectins; virus life cycle; virus taxonomy; angiotensin-converting enzyme 2; RNA-dependent RNA-polymerase; 3C-like protease; papain-like protease; helicase.

Yakutsk, Russian Federation: **NIKOLAEV Vyacheslav Mikhailovich** - candidate of biological sciences, chief researcher head of the department for studying adaptation mechanisms, Yakut Science Center of Complex Medical Problems (YSC CMP), Nikolaev1126@mail.ru. **CHIRIKOVA Nadezhda Konstantinovna** - Doctor of Pharmaceutical Sciences, professor of the Institute of Natural Sciences of the North-Eastern Federal University named after M.K. Ammosov, hofnung@mail.ru, **SOFRONOVA Sargylana Ivanovna** - candidate of medical sciences, chief researcher - head of the Science-organizational and information-publishing department, Yakut Science Center of Complex Medical Problems (YSC CMP), sara2208@mail.ru. **RUMYANTSEV Egor Konstantinovich** - Junior Researcher, Yakut Science Center of Complex Medical Problems (YSC CMP), tzeentch1993@mail.ru. **VASILYEVA Aina Grigoryevna** - student of the Institute of Natural Sciences, North-Eastern Federal University, kagamisatou@gmail.com. **ROMANOVA Anna Nikolaevna** - Doctor of Medical Sciences, Director of the Yakutsk Scientific Center for Complex Medical Problems (YSC KMP), ranik@mail.ru.

Coronaviruses have been known to mankind since the 1950s. Today, the Coronaviridae family of viruses includes 43 types of RNA-containing viruses, combined into two subfamilies, that infect mammals, including humans, birds and amphibians. There are 7 known coronaviruses that infect humans. Coronavirus in human tissue was first discovered in 1965 in patients with a respiratory viral infection [75, 23, 89]. The virus was called HCoV-229E. Then in 1967, a new coronavirus - HCoV-OC43 was found [51]. Until 2002, human coronaviruses were mainly associated with mild upper respiratory tract infections [52]. In 2002, humanity faced an atypical pneumonia epidemic, caused by the SARS-CoV virus, which began in China and spread to 37 countries, affecting more than 8 thousand people and killing 774. After the outbreak, researchers realized that the coronavirus infection can be transmitted from animals to humans [34, 71, 83]. In 2005, SARS infection was found to be the result of interspecies transmission of the SARS-CoV virus from African palm civets (*Nandinia binotata*) to humans [81].

In 2005, the novel CoV-HKU1 coronavirus was discovered in a 71-year-old man with pneumonia who had returned from Shenzhen, China [85]. In 2004, Van der Hoek et al reported the discovery of a new human coronavirus HCoV-NL63, isolated from a 7-month-old child suffering from conjunctivitis and bronchitis [28].

In 2013, the Middle East Respiratory Syndrome was discovered , a deadly zoonotic pathogen that was first identified in humans in Saudi Arabia and Jordan.

Between April 2012 and December 2019, 2499 laboratory-confirmed cases of MERS-CoV infection were registered in the WHO, including 858 deaths [55]. According to few studies, it has been established that the MERS-CoV virus was transmitted to humans from Arabian camels (Camelus dromedarius) [17].

At the end of 2019, a new outbreak of coronavirus infection (SARS-CoV-2) began in Wuhan, Hubei province (China), which took humanity by surprise [21]. The SARS-CoV-2 virus differs from other coronaviruses in its relatively high virulence and mortality. According to the COVID-19 Dashboard by the Center for Systems Science and Engineering (CSSE) at Johns Hopkins University (JHU), as of July 10, 2020, the outbreak of coronavirus disease caused by SARS-CoV-2 resulted in the death of more than 551,271 people and infection of more than 12 118 667 people worldwide. In Russia, 10 843 people died from this disease and 707 301 were infected. The World Health Organization (WHO) declared the SARS-CoV-2 outbreak a pandemic on March 11. On July 7, 2020, at a press briefing on COVID-19, WHO Director-General Tedros Adhanom Ghebreyesus said that the peak of the pandemic has not passed, rather, the outbreak is gaining momentum. The Javanese pangolin (Manis javanica) is likely to be responsible for the outbreak of the new coronavirus infection [92].

The International Committee on Taxonomy of Viruses, which is responsible for the taxonomic classification of viruses, has assigned the SARS-CoV-2 virus to the kingdom: Orthornavirae; type: Pisuviricota; class: Pisoniviricetes; order: Nidovirales; family: Coronaviridae; genus: Betacoronavirus; subgenus: Sarbecovirus; species: Severe acute respiratory syndrome-related coronavirus [70, 21].

The SARS-CoV-2 virus, obtained from a worker at a seafood market in Wuhan, has a size of 29.9 kilobases. SARS-CoV-2 contains the following proteins: a glycoprotein or S-protein (spike), two structural proteins - E (envelope) and M (membrane), the coronavirus has a nucleocapsid inside which the virus genome and the associated N-protein (nucleocapsid) are hidden, as well as several auxiliary proteins [8].

The spike or S-glycoprotein is a transmembrane protein with a molecular weight of about 150 kDa. S-glycoprotein consists of two subunits: the S1 subunit includes a receptor-binding domain (RBD), and the S2 "spike stem" subunit is required for the virus-induced fusion of infected cells with neighbors and the formation of syncytium [26,41].

Protein M (~25–30 kDa) is the most common structural protein and determines the shape of the viral envelope [58]. Studies have shown that the M-protein exists as a dimer and can assume two different conformations, allowing it to contribute to the curvature of the membrane, as well as bind to the nucleocapsid [58].

Protein E (~8–12 kDa) is the smallest of the major structural proteins. This transmembrane protein has ion channel activity. During the replication cycle, E is abundantly expressed inside the infected cell, but only a small portion is incorporated into the viral envelope [79]. Most of the protein is involved in the assembly and growth of the virus [59].

N-protein is the only protein that binds to the RNA genome [15]. It also participates in the assembly of the virus and its shedding, i.e. to the complete formation of the virion [74,48] (Fig. 1).

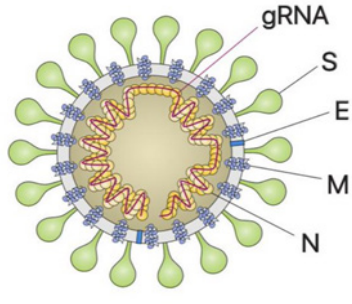


Fig. 1. The structure of the SARS-CoV-2 virus.

In order for the SARS-CoV-2 virus to enter the cell, it is necessary for its spike protein (S-glycoprotein) to bind to the ACE2 (angiotensin converting enzyme 2) protein using RBD. However, for the spike to interact with the ACE2 protein its modification by host cell proteases - furin and transmembrane protease, serine 2 (TMPRSS2) is necessary [69, 27]. The ACE2 protein is expressed in rather large amounts in type I alveolocytes (alveolar type I epithelial cells - AEC I), which is probably what gives the virus the ability to cause pneumonia [54]. However, the expression of this protein, in smaller amounts, is noted in cardiomyocytes, liver cholangiocytes, colon colonocytes, esophageal keratinocytes, epithelial cells of the stomach, ileum and rectum, proximal renal tubules, and urinary bladder [62,86,97]. Therefore, recent studies have shown that SARS-CoV-2 affects not only the lungs, but also the nervous system [56], eyes [22], heart [13], liver [82], kidneys [50] and intestines [95].

Penetration of SARS-CoV-2 into host cells is likely due to endocytosis. After the virus enters the cell, the envelope of the virus fuses with the plasma membrane, and the genomic viral RNA enters the cytoplasm of the host cell. The viral genome consists of 30,000 base pairs that encode a variety of structural and non-structural proteins. Structural proteins, as mentioned, include the membrane protein (M), the spike (S), and the envelope (E). Non-structural proteins - nucleocapsid (N): NSP1-NSP16 (NSP1 (a leader protein, is a potent inhibitor of host gene expression), NSP2 (NSP2 binds to PHB1 and PHB2 proteins, leads to disruption of the host cell life cycle), NSP3 (papain-like protease), NSP4 (interacts with NSP3, participates in viral replication), NSP5 (3-chymotrypsin-like protease "3-CL protease"), NSP6 (restricts the expansion of autophagosomes / lysosomes), NSP11 (its function remains unclear), NSP12 (RNA-dependent RNA polymerase), NSP13 (helicase), NSP14 (N7-methyltransferase), NSP15 (endoribonuclease), NSP16 (2'-O-methyltransferase) are important viral enzymes, while NSP7-NSP10 are regulatory proteins). The SARS-CoV-2 genes orf1a and orf1ab are translated into the pp1a polyprotein and pp1ab polyprotein, respectively [29].

The pp1a and pp1ab proteins are cleaved by proteases (Mpro - major protease, also called 3C-like protease (3CLpro)) and papain-like protease (PLpro) to form 16 non-structural proteins. Some non-structural proteins form the RdRp replication / transcription complex (RNA-dependent RNA polymerase), which use genomic positive RNA as a template. Genomic RNA produced during replication becomes the genome of a new viral particle [96,24]. In coronaviruses, transcription is an intermittent process, which makes it possible to obtain subgenomic RNAs, a process unique to RNA viruses [68]. Subgenomic RNAs obtained as a result of transcription are translated into structural proteins: S - spike protein, E - envelope protein, M - membrane protein, and N - nucleocapsid protein. The proteins of the spike, envelope and membrane enter the endoplasmic reticulum, and the nucleocapsid protein combines with a strand of genomic RNA to form a nucleoprotein complex. They merge into a complete viral particle in the compartment of the endoplasmic reticulum - the Golgi apparatus and are excreted into the extracellular region

through the Golgi apparatus and the vesicle (Fig. 2).

In patients with a new coronavirus infection, the most common symptoms were: fever (91.7%), cough (75.0%), fatigue (75.0%) and diarrhea (39.6%), while hypertension (30, 0%) and diabetes mellitus (12.1%) were the most common comorbidities [93]. The incubation period for SARS-CoV-2 infection was from 3 to 7 days. In 80% of cases, the disease was mild or asymptomatic, in 15% - severe (requiring oxygenation) and in 5% - very severe, critical (requiring mechanical ventilation) [64].

During the disease, the most immunogenic and widely expressed proteins of the virus in the body were S and N proteins. The organism of those infected with the SARS-CoV-2 virus induces IgG antibodies on days 4-14 of the disease. In studies conducted in China, the majority of patients developed: lymphopenia (75.4%) [93] neutrophilia (34.5%) [46], thrombocytopenia (36.2%) [20], a high ratio of neutrophils to lymphocytes was noted (75.8%) [61], a high index of systemic immune inflammation (89.2%) [69], an increased level of C-reactive protein (91.9%) [93], an increase in the level of lactate dehydrogenase (93.2%) and D-dimer (46.4%) [20]. High levels of IL-6 (from 24.6 to 8.4 pg/ml) and IL-8 (from 13.1 to 7.8 pg/ml) were observed in all patients with identified severe and mild forms of the disease [42]. One of the main diagnostic signs of the novel coronavirus infection was developing pneumonia with characteristic changes on a computed tomography of the chest, "ground glass" opacities.

In patients with a severe course of the disease, a high expression of inflammatory cytokines was noted (IL-2, IL-7, IL-10, G-CSF, IP-10, MCP-1, MIP-1A, and TNFα), which was called “cytokine release syndrome "or" cytokine storm "[42].

Every day, researchers are learning more and more about the SARS-CoV-2 virus, the epidemiology of coronavirus infection and its pathogenesis. The search for medicines for the prevention and treatment of this pathology is constantly underway. However, to date, there are no specific antiviral drugs against SARS-CoV-2. Nevertheless, recent studies have identified many weaknesses in the life cycle of the virus, allowing the creation of effective targeted drugs [40,87,47,65].

Some authors have suggested that the drug may be a substance that can prevent the virus from interacting with the receptor protein. In certain articles, special substances are mentioned that can interfere with the interaction of the ACE2

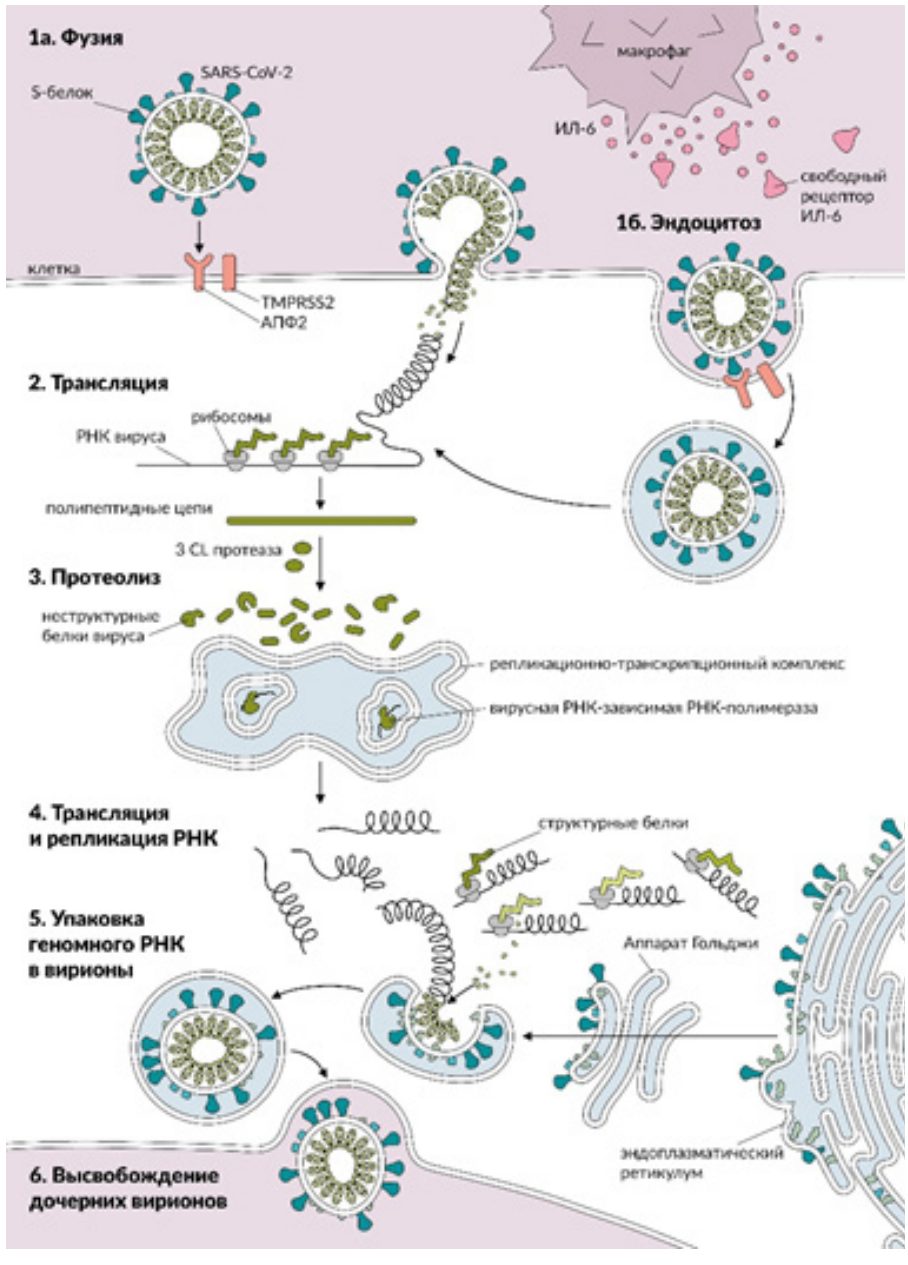


Fig. 2. Life cycle diagram of SARS-CoV-2**.** (ACE2 - angiotensin-converting enzyme 2, IL6 - interleukin 6, 3CL - protease, TMPRSS2 - transmembrane protease, serine 2, S-glycoprotein - SARS-CoV-2 "spike" protein.) *https://empendium.com/ru/image /B33.033_2145*

receptor and the S-glycoprotein of the virus protein, which can help prevent coronavirus infection.

However, the medicine should protect not only from the current outbreak, but also from other infections caused by new varieties of coronaviruses that may appear at any time. For the treatment of coronavirus infection, the best drugs can be those substances that are aimed at blocking the functions of the proteins of the viruses that are necessary for it to carry out its life cycle, which do not change during the evolution of the virus (conservative proteins). One of these proteins are proteases of the SARS-CoV-2 virus: 3CLpro [70,86,31] and PLpro [47]. Additionally, RNA-dependent RNA polymerase (RdRp) is an optimal target due to its crucial role in RNA synthesis, lack of a host homologue, and structural stability [65].

In this paper, we will try to review the accumulated scientific experience of many researchers searching for plant-derived metabolites that have a potential action against SARS-CoV-2. Unlike synthetic drugs, herbal antiviral agents do not require labor-intensive pharmaceutical synthesis and are more readily available. In the modern scientific literature there are a large number of studies devoted to

the study of the antiviral properties of various plant metabolites. The main active components of antiviral agents of plant origin are represented by the following groups of compounds: lectins, alkaloids, terpenoids and phenolic substances.

Lectins (glycoproteins) have the ability to bind carbohydrate residues on the surface of cells, in particular, causing their agglutination. They are widespread among higher plants [77]. Agglutinin *Urtica dioica* (AUD) is a small monomeric plant lectin of 8.7 kDa that has specificity for N-acetylglucosamine and is capable of inhibiting viral replication. There is evidence that AUD inhibits infections with SARS-CoV and SARS-CoV-2 by binding to viral envelope oligosaccharides, thus preventing the interaction of S-glycoprotein viruses with the ACE2 receptor [37,66]. These studies prove that plant lectins can be promising compounds for the development of antiviral agents.

More than 200 extracts of Chinese medicinal plants have been tested for antiviral activity against SARS-CoV. *Lycoris radiata* plant extract had the most powerful antiviral effect. To identify the active component, the *L. radiata* extract was subjected to further fractionation, purification and analysis. The active ingredient in the extract was lycorine. Lycorine is an alkaloid of the indophenanthridine group, which is synthesized in plants from the *Amaryllidaceae family.* The antiviral efficacy of lycorine was rather high (EC50 15.7 ± 1.2 nM), with a selectivity index of more than 900 [43]. However, the authors did not mention lycorine's toxicity at low doses (about 1 mg/kg) in dogs [36]. Recent studies have shown that lycorine is able to effectively inhibit the SARS-CoV-2 virus. According to the authors, lycorine may be a suitable substance for the treatment of SARS-CoV-2, if the therapeutic level of antiviral activity can be achieved without exceeding toxic plasma concentrations [49, 94]. The exact antiviral action mechanism of lycorine remains unclear. Nonetheless, Zhang et al (2020) suggested that lycorine's mechanism of action against SARS-CoV-2 is probably associated with a modulating effect on the molecular mechanisms of the host, and not the virus [94].

Nigella sativa L. is often recommended in foreign countries for the prevention of SARS-CoV-2. Using molecular modeling, it was found that nigellidine, the main alkaloid of *Nigella sativa*, is capable of inhibiting SARS-CoV-2 by acting on the main protease Mpro. At the same time, the compound demonstrated the same performance in comparison with control drugs in clinical trials [9]. Studies have confirmed that alkaloids are suitable candidates for the development of new drugs against SARS-CoV-2.

Terpene metabolites from *Nigella sativa* demonstrated interesting results against SARS-CoV-2. The efficacy of *Nigella sativa* thymoquinone as a protease inhibitor of the SARS-CoV-2 virus was confirmed by molecular docking [57, 32]. Using this method, it has also been proven that three terpenoids (ursolic acid, carvacrol, and oleanolic acid) can serve as potential inhibitors in the regulation of the function of the Mpro SARS-CoV-2 protein [38].

Andrographolide is a labdane diterpenoid, the main active component isolated from the extract of the *Andrographis paniculata* herb - it has a wide spectrum of biological activities, including immune regulation, antiviral, antibacterial, antiparasitic, antitumor and antihyperglycemic effects [35]. Various studies have shown that andrographolid has a wide range of antiviral properties that suppress various viral infections, including the influenza A virus (IAV) [18], human immunodeficiency virus [76], Chikungunya virus CHIKV [84], dengue virus (DENV) [60] and enterovirus D68 (EV-D68) [80]. Andrographolide binds well to key proteins of SARS-CoV-2, including the S-glycoprotein protein, ACE2, 3CL-pro, RdRp, and PLpro, indicating potential efficacy against the novel coronavirus infection [19].

Phenolic compounds are one of the groups of compounds that demonstrate antiviral activity in a number of studies. Flavonoids are key phenolic compounds that are considered promising for the treatment of coronavirus in humans. Cho et al (2013) showed that the flavonoids tomentin A, tomentin B, tomentin C, tomentin D, and tomentin E isolated from the plant *Paulownia tomentosa* have antiviral activity against SARS-CoV [14]. The authors found that all of these substances effectively inhibit the PLpro protease of the SARS-CoV virus due to the presence of a 3,4-dihydro-2H-pyran fragment.

Lin et al (2005) demonstrated a significant inhibitory effect on the 3CLpro protease of the SARS-CoV virus, of plant metabolites (flavanone hesperetin and O-thioglycoside sinigrin) obtained from Isatis indigotica root extract [44]. It should be noted that sinigrin and hesperetin are significantly less cytotoxic.

Flavone luteolin has been proven by many researchers to have broad antiviral properties. Luteolin specifically binds to the S-glycoprotein of the SARS-CoV-2 virus and inhibits its entry into the host cells [91,51]. In addition, luteolin inhibits serine proteases, including the 3CLpro protease [73]. Luteolin has anti-inflammatory properties due to its ability to suppress mast cells, which are the main source of cytokines in the lungs [72].

Myricetin, a flavonol, may be a fairly promising drug, since there is evidence that myricetin strongly binds to the active site of the RdRp replication / transcription complex of the SARS-CoV-2 virus [67] and is an inhibitor of the SARS-CoV virus helicase [49].

A large number of studies using molecular modeling methods have shown that the known flavonol quercetin is a rather potent inhibitor of the 3CLpro protease of the SARS-CoV-2 virus [7]. Moreover, the Gln189 residue plays a key role in binding stabilization. Thus, the mutation replacing Gln189 for alanine did not reduce the enzymatic activity of the 3CLpro viral protease, but reduced the inhibitory ability of quercetin by half [12]. Quercetin and its derivatives have been proposed as potential helicase inhibitors, since they demonstrated good results using the Förster resonant energy transfer method (FRET) [39]. In addition, quercetin has shown the ability to block the entry of SARS-CoV into host cells [90].

Anthraquinones are the most numerous group of natural quinones and are widely represented in plants of the families *Rubiaceae, Rhamnaceae, Fabaceae*. Emodin, an anthraquinone derivative obtained from the plant genera *Rheum* and *Polygonum*, has been shown to be effective in inhibiting the interaction of the S-glycoprotein of viruses (SARS-CoV, SARS-CoV-2) and ACE2 [30,25]. In addition, the ability of emodin to inhibit 11β-hydroxysteroid dehydrogenase, a human enzyme that catalyzes the conversion of cortisol to cortisone and vice versa, has been proven, thereby regulating the effect of glucocorticoids on steroid receptors [63]. A promising substance in the treatment of severe forms of the SARS-CoV-2 viral infection is rhein, a representative of the emodin group. The inhibitory effect of rhein on the interaction of the viral S-glycoprotein SARS-CoV and ACE2 has been proven. In addition, this compound was shown to inhibit the expression of inflammatory cytokines IL-1, IL-2, IL-6, IL-8, IL-12, IL-18, TNF-α, NF-κB, and NALP3 [16].

An ethanol extract of *Psoralea corylifolia* seeds demonstrated high activity in inhibiting the PLpro enzyme of the SARS-CoV virus. As a result of the study of the ethanol extract, six compounds that inhibit PLpro were isolated. Further studies showed that two phenolic compounds: a chalcone derivative - isobachalcone

and coumarin - psoralidin, demonstrated the greatest antiviral activity, since both turned out to be mixed, reversible inhibitors of PLpro by the type I mechanism (i.e. they preferentially bind to a free enzyme rather than a substrate-enzyme complex) [33].

Leptodactylone, a coumarin from *Boenninghausenia sessilicarpa*, according to a few research articles, has a strong protective effect on cells infected with the SARS-CoV virus - the mechanism of action of the substance is unclear [88]. Phenolic compounds (flavonoids, chalcones, anthraquinones, coumarins) can be used as a promising drug against the SARS-CoV-2 infection.

Based on source literature, it is known that herbal remedies of traditional Chinese medicine have a better therapeutic effect in the early stage of the SARS-CoV-2 disease [45], while the use of conventional antiviral drugs (oseltamivir, arbidol and lopinavir / ritonavir) was not effective [10].

Drugs of natural origin, especially extracts from certain organs and parts of plants and their individual compounds are widely used in the treatment of various diseases. A review of scientific literature has shown a high antiviral potential of medicinal plants and their components. Thanks to a safe, integrated approach, they act on specific targets of the SARS-CoV-2 virus (S protein, 3CLpro and PLpro) and strengthen the immune system, therefore, they can become an interesting alternative to antiviral drug therapy.

The vegetation of Yakutia is characterized by a variety of plant species used in allopathic and traditional medicine [2]. The flora of the Republic includes about 2000 species of higher vascular plants, of which more than 230 species are medicinal (157 genera and 55 families) [1]. The combination of various abiotic factors in a single region affected not only the diversity of vegetation in Yakutia, but also the chemical composition of local plants. Features of the climate, light regime, the dominance of the permafrost zone all contribute to the active accumulation of biologically active compounds in plants in a short period of time. The unique qualitative and quantitative composition of local plants provides a prospect for the search for new and effective secondary metabolites with valuable pharmacological effects, including antiviral action.

The flora of Yakutia is rich in plants that accumulate lectins and grow throughout the region (*Allium* sp., *Taraxacum* sp., *Plantago* sp., *Trifolium pratense*, Elytrigia repens, etc.). Raw materials of local plants can become a source of lectins for the creation of new antiviral agents. [5]

Our studies have shown that the most frequently used medicinal plants in Yakutia are species from the families *Asteraceae, Rosaceae* and *Lamiaceae*. We carried out a qualitative analysis of the content of biologically active compounds in medicinal plants of Yakutia. As a result of screening 155 plants from 35 families, it was found that medicinal plants accumulate phenolic and terpene compounds the most. Twelve species of the genus *Artemisia* were characterized as concentrators of flavonoids, namely derivatives of luteolin and quercetin, with a quantitative content ranging from 2.46 mg / g (*A. desertorum*) to 202.67 mg / g (*A. palustris*). Wild plants from the *Lamiaceae* family (*Thymus* sp., *Dracocephalum* sp., *Leonurus* sp., *Scutellaria* sp., *Phlomoides tuberosa, etc.*), *Rosaceae (Geum sp., Potentilla sp., Rubus sp., Cotoneaster lucidus, Chamaehodos erecta,* etc. .) and *Asteraceae (Artemisia* sp*., Gnaphalium*, etc.) are concentrators of phenolic compounds of various structural types and thus are promising plant raw materials for the development of medicines, including antiviral agents.

The works of A.A. Makarov and Samarina V.P. testifies to the high alkaloid content of the local flora, a total of 302 plant species have been studied, of which alkaloids were found in varying amounts in 139 species. The greatest amount of alkaloids accumulates in the following genera: *Veratrum, Aconite, Delphinium, Corydalis, Thermopsis*, etc. [2,3,4]. The high quantitative content of alkaloids in local plants makes it possible to use them as a promising prophylactic agent against SARS-CoV-2 infection.

Thus, biologically active compounds (phenolic compounds, terpenoids, alkaloids, and lectins) have potential activity against SARS-COV-2 and are promising components of new herbal antiviral agents. Unique plants of Yakutia with a high quantitative content of the above compounds can become a source for the creation of antiviral agents, and also as possible means of prevention and treatment of the novel coronavirus infection.

The article was written with the support of the Russian Foundation for Basic Research within the framework of the research project No. 19-09-00361.

References

1. Кузнецова Л.В., Захарова В.И. Конспект флоры Якутии: Сосудистые растения. Новосибирск: Наука. 2012:272. [Kuznetsova LV, Zakharova VI. Abstract of the flora of Yakutia: Vascular plants. Novosibirsk: Science. 2012:272. (In Russ)]

2. Макаров А.А. Лекарственные растения Якутии и перспективы их освоения. Новосибирск: Изд-во Сибирского отделения РАН. 2002.264. [Makarov AA. Medicinal plants of Yakutia and prospects for their development. Novosibirsk: Publishing house of the Siberian Branch of the Russian Academy of Sciences. 2002:264. (In Russ)]

3. Макаров А.А. Биологически активные вещества в растениях Якутии. Якутск: ЯНЦ-СО АН СССР. 1989:156. [Makarov AA. Biologically active substances in plants of Yakutia. Yakutsk: YANTSSO AN USSR. 1989:156. (In Russ)]

4. Макаров А.А. Методы поисков и изучения лекарственных растений. Якутск: Якутское книжное издательство. 1981:66. [Makarov AA. Methods for searching and studying medicinal plants. Yakutsk: Yakutsk book publishing house. 1981:66. (In Russ)]

5. Павловская Н.Е., Гагарина И.Н. Функциональная роль лектинов растений как предпосылка для применения в биотехнологии. *Химия растительного сырья.* 2017. №1: 21-35. [Pavlovskaya NE, Gagarina IN. The functional role of plant lectins as a prerequisite for use in biotechnology. *Himiya rastitel'nogo syr'ya.* 2017;1: 21-35. (In Russ)]

6. Самарин В.П. О суммарном содержании алкалоидов в некоторых растениях Якутии. *Проблемы современной ботаники.* Москва: Наука. 1965: 200-205. [Samarin VP. On the total content of alkaloids in some plants of Yakutia. *Problemy sovremennoj botaniki*. Moscow: *Nauka*. 1965: 200-205. (In Russ)]

7. Abian O, Ortega-Alarcon D, Jimenez-Alesanco A, et al. Structural stability of SARS-CoV-2 3CLpro and identification of quercetin as an inhibitor by experimental screening [published online ahead of print, 2020 Jul 31]. *Int J Biol Macromol*. 2020; S0141-8130(20)33997-0. doi: 10.1016/j.ijbiomac.2020.07.235

8. Astuti I, Ysrafil. Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2): An overview of viral structure and host response. *Diabetes Metab Syndr.* 2020;14(4):407-412. doi:10.1016/j.dsx.2020.04.020

9. Bouchentouf S., Missoum N. Identification of Compounds from Nigella Sativa as New Potential Inhibitors of 2019 Novel Coronasvirus (Covid-19). *Molecular Docking Study*. 2020

10. Cao B, Wang Y, Wen D, et al. A Trial of Lopinavir-Ritonavir in Adults Hospitalized with Severe Covid-19. England: *J Med*. 2020;382(19):1787-1799. doi:10.1056/NEJMoa2001282

11. Cao P, Wu S, Wu T, et al. The important role of polysaccharides from a traditional Chinese medicine-Lung Cleansing and Detoxifying Decoction against the COVID-19 pandemic. *Carbohydr Polym*. 2020;240:116346. doi: 10.1016/j.carbpol.2020.116346

12. Chen L, Li J, Luo C, et al. Binding interaction of quercetin-3-beta-galactoside and its synthetic derivatives with SARS-CoV 3CL (pro): structure-activity relationship studies reveal salient pharmacophore features. *Bioorg Med Chem*. 2006;14(24):8295-8306. doi:10.1016/j.bmc.2006.09.014

13. Chen L, Li X, Chen M, Feng Y, Xiong C. The ACE2 expression in human heart indicates new potential mechanism of heart injury among patients infected with SARS-CoV-2. *Cardiovasc Res*. 2020;116(6):1097-1100. doi:10.1093/cvr/cvaa078

14. Cho JK, Curtis-Long MJ, Lee KH, et al. Geranylated flavonoids displaying SARS-

CoV papain-like protease inhibition from the fruits of Paulownia tomentosa. *Bioorg Med Chem*. 2013;21(11):3051-3057. doi:10.1016/j.bmc.2013.03.027

15. de Haan CA, Rottier PJ. Molecular interactions in the assembly of coronaviruses. *Adv Virus Res*. 2005;64:165-230. doi:10.1016/S0065-3527(05)64006-7

16. de Oliveira PG, Termini L, Durigon EL, Lepique AP, Sposito AC, Boccardo E. Diacerein: A potential multi-target therapeutic drug for COVID-19. *Med Hypotheses*. 2020;144:109920. doi:10.1016/j.mehy.2020.109920

17. de Wit E, van Doremalen N, Falzarano D, Munster VJ. SARS and MERS: recent insights into emerging coronaviruses. *Nat Rev Microbiol*. 2016;14(8):523-534. doi:10.1038/nrmicro.2016.81

18. Ding Y, Chen L, Wu W, Yang J, Yang Z, Liu S. Andrographolide inhibits influenza A virus-induced inflammation in a murine model through NF-κB and JAK-STAT signaling pathway. *Microbes Infect*. 2017;19(12):605-615. doi:10.1016/j.micinf.2017.08.009

19. Enmozhi SK, Raja K, Sebastine I, Joseph J. Andrographolide as a potential inhibitor of SARS-CoV-2 main protease: an in silico approach [published online ahead of print, 2020 May 5]. *J Biomol Struct Dyn*. 2020;1-7. doi:10.1080/07391102.2020.1760136

20. Giannis D, Ziogas IA, Gianni P. Coagulation disorders in coronavirus infected patients: COVID-19, SARS-CoV-1, MERS-CoV and lessons from the past. *J Clin Virol*. 2020;127:104362. doi: 10.1016/j.jcv.2020.104362

21. Gorbalenya A.E., Baker S.C., Baric R.S. et al. The species Severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. *Nat. Microbiol*. 02.03.2020. DOI:10.1038/s41564-020-0695-z.

22. Gupta PC, Kumar MP, Ram J. COVID-19 pandemic from an ophthalmology point of view. *Indian J Med Res*. 2020;151(5):411-418. doi:10.4103/ijmr.IJMR_1369_20

23. Hamre D, Procknow JJ. A new virus isolated from the human respiratory tract. *Proc Soc Exp Biol Med*. 1966;121(1):190-193. doi:10.3181/00379727-121-30734

24. Hilgenfeld R, Anand K, Mesters JR, et al. Structure and dynamics of SARS coronavirus main proteinase (Mpro). *Adv Exp Med Biol*. 2006;581:585-591. doi:10.1007/978-0-387-33012-9_106

25. Ho TY, Wu SL, Chen JC, Li CC, Hsiang CY. Emodin blocks the SARS coronavirus spike protein and angiotensin-converting enzyme 2 interaction. *Antiviral Res*. 2007;74(2):92-101. doi:10.1016/j.antiviral.2006.04.014

26. Hoffmann M, Kleine-Weber H, Pöhlmann S. A Multibasic Cleavage Site in the Spike Protein of SARS-CoV-2 Is Essential for Infection of Human Lung Cells. *Mol Cell*. 2020;78(4):779-784.e5. doi:10.1016/j.molcel.2020.04.022

27. Hoffmann M., Kleine-Weber H.,Schroeder S. et al. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. *Cell*. 2020. pii: S0092-8674(20)30229-4. DOI:10.1016/j.cell.2020.02.052.

28. Hofmann H, Pyrc K, van der Hoek L, Geier M, Berkhout B, Pöhlmann S. Human coronavirus NL63 employs the severe acute respiratory syndrome coronavirus receptor for cellular entry. *Proc Natl Acad Sci USA*. 2005;102(22):7988□7993. doi:10.1073/pnas.0409465102

29. Iftikhar H, Ali HN, Farooq S, Naveed H, Shahzad-Ul-Hussan S. Identification of potential inhibitors of three key enzymes of SARS-CoV2 using computational approach. *Comput Biol Med*. 2020;122:103848. doi:10.1016/j.compbiomed.2020.103848

30. Jahan I, Onay A. Potentials of plant-based substance to inhabit and probable cure for the COVID-19. *Turk J Biol*. 2020;44(3):228-241. Published 2020 Jun 21. doi:10.3906/biy-2005-114

31. Jin Z, Du X, Xu Y, et al. Structure of Mpro from SARS-CoV-2 and discovery of its inhibitors. *Nature*. 2020;10.1038/s41586-020-2223-y. doi:10.1038/s41586-020-2223-y

32. Kadil Y, Mouhcine M, Filali H. In Silico Investigation of the SARS CoV2 Protease with Thymoquinone Major Constituent of Nigella Sativa. *Curr Drug Discov Technol*. 2020;10.2174/1570163817666200712164406. doi:10.2174/1570163817666200712164406

33. Kim DW, Seo KH, Curtis-Long MJ, et al. Phenolic phytochemical displaying SARS-CoV papain-like protease inhibition from the seeds of Psoralea corylifolia. *J Enzyme Inhib Med Chem*. 2014;29(1):59-63. doi:10.3109/14756366.2012.753591

34. King A.M.Q., Adams M.J., Carstens E.B., Lefkowitz E.J. Order — Nidovirales. Virus Taxonomy: Ninth Report of the International Committee on Taxonomy of Viruses. 2012. 784–794. DOI:10.1016/B978-0-12-384684-6.00066-5.

35. Kishore V, Yarla NS, Bishayee A, et al. Multi-targeting Andrographolide and its Natural Analogs as Potential Therapeutic Agents. *Curr Top Med Chem*. 2017;17(8):845-857. doi:10.2174/1568026616666160927150452

36. Kretzing S, Abraham G, Seiwert B, Ungemach FR, Krügel U, Regenthal R. Dose-dependent emetic effects of the Amaryllidaceous alkaloid lycorine in beagle dogs. *Toxicon*. 2011;57(1):117-124. doi:10.1016/j.toxicon.2010.10.012

37. Kumaki Y, Wandersee MK, Smith AJ, et al. Inhibition of severe acute respiratory syndrome coronavirus replication in a lethal SARS-CoV BALB/c mouse model by stinging nettle lectin, Urtica dioica agglutinin. *Antiviral Res*. 2011;90(1):22-32. doi:10.1016/j.antiviral.2011.02.003

38. Kumar A, Choudhir G, Shukla SK, et al. Identification of phytochemical inhibitors against main protease of COVID-19 using molecular modeling approaches. *J Biomol Struct Dyn*. 2020;1-11. doi:10.1080/07391102.2020.1772112

39. Lee C, Lee JM, Lee NR, Kim DE, Jeong YJ, Chong Y. Investigation of the pharmacophore space of Severe Acute Respiratory Syndrome coronavirus (SARS-CoV) NTPase/helicase by dihydroxychromone derivatives. *Bioorg Med Chem Lett*. 2009;19(16):4538-4541. doi:10.1016/j.bmcl.2009.07.009

40. Lee TW, Cherney MM, Liu J, et al. Crystal structures reveal an induced-fit binding of a substrate-like Aza-peptide epoxide to SARS coronavirus main peptidase. *J Mol Biol*. 2007;366(3):916-932. doi:10.1016/j.jmb.2006.11.078

41. Li F. Structure, Function, and Evolution of Coronavirus Spike Proteins. *Annu Rev Virol*. 2016 Sep 29;3(1):237-261. doi: 10.1146/annurev-virology-110615-042301. Epub 2016 Aug 25. PMID: 27578435; PMCID: PMC5457962.

42. Li S, Jiang L, Li X, et al. Clinical and pathological investigation of patients with severe COVID-19. *JCI Insight*. 2020;5(12):138070. Published 2020 Jun 18. doi: 10.1172/jci.insight.138070

43. Li SY, Chen C, Zhang HQ, et al. Identification of natural compounds with antiviral activities against SARS-associated coronavirus. *Antiviral Res*. 2005;67(1):18-23. doi:10.1016/j.antiviral.2005.02.007

44. Lin CW, Tsai FJ, Tsai CH, et al. Anti-SARS coronavirus 3C-like protease effects of Isatis indigotica root and plant-derived phenolic compounds. *Antiviral Res*. 2005;68(1):36-42. doi:10.1016/j.antiviral.2005.07.002

45. Liu C. X. Wang, Yu-Li; Zhang, Hong-Bing et al. Attach importance to research and development of Chinese materia medica based on prevention and control needs of SARS-CoV-2 infection. *Chinese Traditional and Herb Drugs*. 2020, 6(51): 1361-1374, 20200328.

46. Liu X, Zhang R, He G. Hematological findings in coronavirus disease 2019: indications of progression of disease. *Ann Hematol*. 2020;99(7):1421-1428. doi:10.1007/s00277-020-04103-5

47. Macchiagodena M, Pagliai M, Procacci P. Identification of Potential Binders of the Main Protease 3CLpro of the COVID-19 via Structure-Based Ligand Design and Molecular Modeling. *Chem Phys Lett*. 2020;750:137489. doi:10.1016/j.cplett.2020.137489

48. Malik YA. Properties of Coronavirus and SARS-CoV-2. *Malays J Pathol*. 2020;42(1):3-11.

49. Mani JS, Johnson JB, Steel JC, et al. Natural product-derived phytochemicals as potential agents against coronaviruses: A review. *Virus Res*. 2020;284:197989. doi:10.1016/j.virusres.2020.197989

50. Martinez-Rojas MA, Vega-Vega O, Bobadilla NA. Is the kidney a target of SARS-CoV-2?. *Am J Physiol Renal Physiol*. 2020;318(6):F1454-F1462. doi:10.1152/ajprenal.00160.2020

51. Maurya VK, Kumar S, Prasad AK, Bhatt MLB, Saxena SK. Structure-based drug designing for potential antiviral activity of selected natural products from Ayurveda against SARS-CoV-2 spike glycoprotein and its cellular receptor. *Virusdisease*. 2020;31(2):179-193. doi:10.1007/s13337-020-00598-8

52. McIntosh K, Becker WB, Chanock RM. Growth in suckling-mouse brain of "IBV-like" viruses from patients with upper respiratory tract disease. *Proc Natl Acad Sci U S A*. 1967 Dec;58(6):2268-73. doi: 10.1073/pnas.58.6.2268. PMID: 4298953; PMCID: PMC223830.

53. McIntosh K, Kapikian AZ, Turner HC, Hartley JW, Parrott RH, Chanock RM. Seroepidemiologic studies of coronavirus infection in adults and children. *Am J Epidemiol*. 1970;91(6):585-592. doi:10.1093/oxfordjournals.aje.a121171

54. McIntosh K., Perlmanin S. Coronaviruses, Including Severe Acute Respiratory Syndrome (SARS) and Middle East Respiratory Syndrome (MERS). *Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases*. Eighth Edition. Bennett J.E., Dolin R., Blaser M.J. (eds.). Elsevier Inc. 2014; 1928–1936.

55. Memish ZA, Perlman S, Van Kerkhove MD, Zumla A. Middle East respiratory syndrome. *Lancet*. 2020;395(10229):1063-1077. doi:10.1016/S0140-6736(19)33221-0

56. Moriguchi T, Harii N, Goto J, et al. A first case of meningitis/encephalitis associated with SARS-Coronavirus-2. *Int J Infect Dis*. 2020;94:55-58. doi:10.1016/j.ijid.2020.03.062

57. Narkhede RR, Pise AV, Cheke RS, Shinde SD. Recognition of Natural Products as Potential Inhibitors of COVID-19 Main Protease (Mpro): In-Silico Evidences. *Nat Prod Bioprospect*. 2020;1-10. doi:10.1007/s13659-020-00253-1

58. Neuman BW, Kiss G, Kunding AH, et al. A structural analysis of M protein in coronavirus assembly and morphology. *J Struct Biol*. 2011;174(1):11-22. doi:10.1016/j.jsb.2010.11.021

59. Nieto-Torres JL, Dediego ML, Alvarez E, et al. Subcellular location and topology of severe acute respiratory syndrome coronavirus envelope protein. *Virology*. 2011;415(2):69-82. doi:10.1016/j.virol.2011.03.029

60. Panraksa P, Ramphan S, Khongwichit S,

Smith DR. Activity of andrographolide against dengue virus. *Antiviral Res*. 2017;139:69-78. doi:10.1016/j.antiviral.2016.12.014

61. Ponti G, Maccaferri M, Ruini C, Tomasi A, Ozben T. Biomarkers associated with COVID-19 disease progression [published online ahead of print, 2020 Jun 5]. *Crit Rev Clin Lab Sci*. 2020;1-11. doi:10.1080/10408363.2020.1770685

62. Qi F, Qian S, Zhang S, Zhang Z. Single cell RNA sequencing of 13 human tissues identify cell types and receptors of human coronaviruses. *Biochem Biophys Res Commun*. 2020;526(1):135-140. doi:10.1016/j.bbrc.2020.03.044

63. Robson B. COVID-19 Coronavirus spike protein analysis for synthetic vaccines, a peptidomimetic antagonist, and therapeutic drugs, and analysis of a proposed achilles' heel conserved region to minimize probability of escape mutations and drug resistance. *Comput Biol Med*. 2020;121:103749. doi:10.1016/j.compbiomed.2020.103749

64. Rokni M, Ghasemi V, Tavakoli Z. Immune responses and pathogenesis of SARS-CoV-2 during an outbreak in Iran: Comparison with SARS and MERS. *Rev Med Virol*. 2020;30(3):e2107. doi:10.1002/rmv.2107

65. Shannon A, Le NT, Selisko B, et al. Remdesivir and SARS-CoV-2: Structural requirements at both nsp12 RdRp and nsp14 Exonuclease active-sites. *Antiviral Res*. 2020;178:104793. doi:10.1016/j.antiviral.2020.104793

66. Sharma N, Muthamilarasan M, Prasad A, Prasad M. Genomics approaches to synthesis plant-based biomolecules for therapeutic applications to combat SARS-CoV-2 *Genomics*. 2020;112(6):4322-4331. doi:10.1016/j.ygeno.2020.07.033

67. Singh S, Sk MF, Sonawane A, Kar P, Sadhukhan S. Plant-derived natural polyphenols as potential antiviral drugs against SARS-CoV-2 via RNA-dependent RNA polymerase (RdRp) inhibition: an in-silico analysis. *J Biomol Struct Dyn*. 2020;1-16. doi:10.1080/07391102.2020.1796810

68. Sola I, Almazán F, Zúñiga S, Enjuanes L. Continuous and Discontinuous RNA Synthesis in Coronaviruses. *Annu Rev Virol*. 2015;2(1):265-288. doi:10.1146/annurev-virology-100114-055218

69. Sternberg A, Naujokat C. Structural features of coronavirus SARS-CoV-2 spike protein: Targets for vaccination. *Life Sci*. 2020;257:118056. doi:10.1016/j.lfs.2020.118056

70. Su S, Wong G, Shi W, et al. Epidemiology, Genetic Recombination, and Pathogenesis of Coronaviruses. *Trends Microbiol*. 2016;24(6):490-502. doi:10.1016/j.tim.2016.03.003

71. Tang Q, Song Y, Shi M, Cheng Y, Zhang W, Xia XQ. Inferring the hosts of coronavirus using dual statistical models based on nucleotide composition. *Sci Rep*. 2015 Nov 26;5:17155. doi:10.1038/srep17155. PMID: 26607834; PMCID: PMC4660426.

72. Theoharides TC, Conti P. Dexamethasone for COVID-19? Not so fast. *J Biol Regul Homeost Agents*. 2020;34(3):10.23812/20-EDITORIAL_1-5. doi:10.23812/20-EDITORIAL_1-5

73. Theoharides TC. COVID-19, pulmonary mast cells, cytokine storms, and beneficial actions of luteolin. *Biofactors*. 2020;46(3):306-308. doi:10.1002/biof.1633

74. Tooze J, Tooze S, Warren G. Replication of coronavirus MHV-A59 in sac- cells: determination of the first site of budding of progeny virions. *Eur J Cell Biol*. 1984;33(2):281-293.

75. Tyrrell DA, Bynoe ML. Cultivation of viruses from a high proportion of patients with colds. *Lancet*. 1966;1(7428):76-77. doi:10.1016/s0140-6736(66)92364-6

76. Uttekar MM, Das T, Pawar RS, et al. Anti-HIV activity of semisynthetic derivatives of andrographolide and computational study of HIV-1 gp120 protein binding. *Eur J Med Chem*. 2012;56:368-374. doi:10.1016/j.ejmech.2012.07.030

77. Van Damme, E., Peumans, W., Pusztai, A., Bardocz, S, Handbook of Plant Lectins: Properties and Biomedical Applications. John Wiley & Sons 1998.

78. van der Hoek L, Pyrc K, Jebbink MF, et al. Identification of a new human coronavirus. *Nat Med*. 2004;10(4):368-373. doi:10.1038/nm1024

79. Venkatagopalan P, Daskalova SM, Lopez LA, Dolezal KA, Hogue BG. Coronavirus envelope (E) protein remains at the site of assembly. *Virology*. 2015;478:75-85. doi:10.1016/j.virol.2015.02.005

80. Wang D, Guo H, Chang J, et al. Andrographolide Prevents EV-D68 Replication by Inhibiting the Acidification of Virus-Containing Endocytic Vesicles. *Front Microbiol*. 2018;9:2407. Published 2018 Oct 8. doi:10.3389/fmicb.2018.02407

81. Wang M, Yan M, Xu H, et al. SARS-CoV infection in a restaurant from palm civet. *Emerg Infect Dis*. 2005;11(12):1860-1865. doi:10.3201/eid1112.041293

82. Wang Y, Liu S, Liu H, et al. SARS-CoV-2 infection of the liver directly contributes to hepatic impairment in patients with COVID-19 [published online ahead of print, 2020 May 11]. *J Hepatol*. 2020;S0168-8278(20)30294-4. doi:10.1016/j.jhep.2020.05.002

83. Weiss SR, Leibowitz JL. Coronavirus pathogenesis. *Adv Virus Res*. 2011;81:85-164. doi:10.1016/B978-0-12-385885-6.00009-2

84. Wintachai P, Kaur P, Lee RC, et al. Activity of andrographolide against chikungunya virus infection. *Sci Rep*. 2015;5:14179. Published 2015 Sep 18. doi:10.1038/srep14179

85. Woo PC, Lau SK, Chu CM, et al. Characterization and complete genome sequence of a novel coronavirus, coronavirus HKU1, from patients with pneumonia. *J Virol*. 2005;79(2):884-895. doi:10.1128/JVI.79.2.884-895.2005

86. Xu H, Zhong L, Deng J, et al. High expression of ACE2 receptor of 2019-nCoV on the epithelial cells of oral mucosa. *Int J Oral Sci*. 2020;12(1):8. Published 2020 Feb 24. doi:10.1038/s41368-020-0074-x

87. Xu Z., Peng C., Shi Y. et al. Nelfinavir was predicted to be a potential inhibitor of 2019-nCov main protease by an integrative approach combining homology modelling, molecular docking and binding free energy calculation. *BioRxiv*. 28.01.2020. DOI:10.1101/2020.01.27.921627.

88. Yang QY, Tian XY, Fang WS. Bioactive coumarins from Boenninghausenia sessilicarpa. *J Asian Nat Prod Res*. 2007;9(1):59-65. doi:10.1080/10286020500382397

89. Yeager CL, Ashmun RA, Williams RK, et al. Human aminopeptidase N is a receptor for human coronavirus 229E. *Nature*. 1992;357(6377):420-422. doi:10.1038/357420a0

90. Yi L, Li Z, Yuan K, et al. Small molecules blocking the entry of severe acute respiratory syndrome coronavirus into host cells. *J Virol*. 2004;78(20):11334-11339. doi:10.1128/JVI.78.20.11334-11339.2004

91. Yu R, Chen L, Lan R, Shen R, Li P. Computational screening of antagonists against the SARS-CoV-2 (COVID-19) coronavirus by molecular docking. *Int J Antimicrob Agents*. 2020;56(2):106012. doi: 10.1016/j.ijantimicag.2020.106012

92. Zhang C, Zheng W, Huang X, Bell EW, Zhou X, Zhang Y. Protein Structure and Sequence Reanalysis of 2019-nCoV Genome Refutes Snakes as Its Intermediate Host and the Unique Similarity between Its Spike Protein Insertions and HIV-1. *J Proteome Res*. 2020;19(4):1351-1360. doi:10.1021/acs.jproteome.0c00129

93. Zhang JJ, Dong X, Cao YY, et al. Clinical characteristics of 140 patients infected with SARS-CoV-2 in Wuhan, China. *Allergy*. 2020;75(7):1730-1741. doi:10.1111/all.14238

94. Zhang YN, Zhang QY, Li XD, et al. Gemcitabine, lycorine and oxysophoridine inhibit novel coronavirus (SARS-CoV-2) in cell culture. *Emerg Microbes Infect*. 2020;9(1):1170-1173. doi:10.1080/22221751.2020.1772676

95. Zhou J, Li C, Liu X, et al. Infection of bat and human intestinal organoids by SARS-CoV-2. *Nat Med*. 2020;26(7):1077-1083. doi:10.1038/s41591-020-0912-6

96. Ziebuhr J. The Coronavirus Replicase. In: Enjuanes L. (eds) Coronavirus Replication and Reverse Genetics. *Current Topics in Microbiology and Immunology*, 2005: vol 287. Springer, Berlin, Heidelberg. https://doi.org/10.1007/3-540-26765-4_3

97. Zou X, Chen K, Zou J, Han P, Hao J, Han Z. Single-cell RNA-seq data analysis on the receptor ACE2 expression reveals the potential risk of different human organs vulnerable to 2019-nCoV infection. *Front Med*. 2020; 14(2):185-192. doi:10.1007/s11684-020-0754-00754-0

DOI 10.25789/YMJ.2020.71.26
УДК 618.3-06

A.A. Vorobyev, M.S. Selikhova, M.S. Yakovenko

MODERN VIEW ON THE PROBLEM OF SYMPHYSIOPATHIA OF PREGNANCY

Information about the frequency of occurrences of symphysiopathia is contradictory and registered in the range from 0.03 to 2.8%. Currently, there are no standards for the diagnosis of "symphysitis" and the correctness of the terminology of this pathology is being discussed. Clinically manifested diastasis more than 10 mm is accompanied by pain. A number of authors consider young age, insufficient physical activity, smoking, calcium deficiency, and hypomagnesemia to be risk factors for the manifestation of symphysiopathy, but this information is based on individual observations and small series of cases. For the diagnosis of symphysiopathy, it is recommended to use x-ray diagnostic methods, including CT and MRI, ultrasound, but there is no comparative data on their informative value. In the literature there is some information about the methods of treatment of this pathology and indications for surgical delivery. Thus, there is currently need for randomized controlled trials to study the epidemiology, etiology, pathogenesis and diagnosis of symphysiopathy during pregnancy and childbirth, the development of pregnancy management tactics, the method and timing of optimal delivery.

Keywords: symphysitis, symphysiopathy, pain, pubis joint diastasis, risk factors, calcium and magnesium deficiency, pregnancy management tactics, delivery.

For a long time, the syndrome involving pain and diastasis of the pubic bones was considered as an inflammatory process of the pubic symphysis-symphysis. The theory about the inflammatory genesis of this pregnancy complication has not stood the test of time, so the term "symphysitis", still the most common in Russia, is outdated. Nevertheless, pain in the pubic joint is the most frequent and defining feature of this problem (but not the only one), so "symphysiopathy" seems to be the most appropriate of the existing terms [10].

Modern literature on symphysiopathy is limited to describing individual observations and small series of cases. Evidence-based research with a good design and a large number of observations not enough. The least studied data are on the biomechanical causes of symphysiopathy, changes in laboratory parameters, methods of diagnosis and prognosis of this disease.

To generalize and systematize the information published in the scientific literature on modern views on the epidemiology, etiology, pathogenesis and diagnosis of symphysiopathy during pregnancy and childbirth, a search for publications in databases pubMed, pubMed central, Google Scholar и elibrary by keywords: pubic symphysis diastasis, symphysis pubis separation, symphysis rupture, perupartum pubic symphysis diastasis, perupartum complications pubic symphysis, pregnancy pubic symphysis complications, delivery symphysis pubis separation, disjonction pubienne and also by their Russian equivalent.

The work was conducted with literature published over the past 5 years. Materials published earlier than 2015 were included in this review if they contained important data that was not reflected in newer publications.

Information about the frequency of occurrences of symphysiopathia is contradictory and registered in the range from 0.03 to 2,8% [3,4,13,14]. Such differences are explained by the lack of a single definition of symphysiopathy, when the dysfunction of the pubic joint without diastasis and with its presence are not differentiated. Local pain is the main manifestation of symphysitis. According to the information of the clinical Protocol "Normal pregnancy" of the Ministry of health of the Russian Federation (2019) in normal pregnancy, pubic pain is observed in 0.03-3% of cases [12].

However, according to Jain S et al. [31], pain in the symphysis is detected in 22-37. 5% of pregnant women, and in 5% they are strongly pronounced. So, diastasis in the area of the womb does not occur in all cases [30]. According to a number of researchers, the frequency of diagnosed symphysiopathy may depend on the clinical alertness of treating physicians, increasing with close attention to the problem, and, conversely, decreasing if pain and diastasis of the symphysis after childbirth are perceived as a transient postpartum sign [4, 5]. The increase in the distance in the pubic joint during pregnancy is physiological.

According to H. Cicek and co-authors [21], the distance between the pubic bones can increase up to 7-8 mm without causing symptoms, with the average size of the increase being 4.8 mm [20]. According to J. J. Shawla and co-authors [20], the increase in distance during pregnancy is observed in 59-94% of patients and begins at the beginning of the 1st trimester, so the width of the symphysis can increase by 9-139% from the original.

The expansion of the symphysis and sacroiliac joints together increase the diameter of the pelvic outlet in the supine position with the limbs separated by 1.5-2.0 cm [5]. According to Sergeeva O.P. [15], the width of the symphysis did not change significantly with the progression of pregnancy, amounting to 7.2–1.5 mm at the period of 20-24 weeks, and 7.1–2.0 mm at the period of 37-40 weeks. Diastasis of the pubic joint is considered symptomatic of an increase in the distance of the symphysis with a width of more than 10 mm [10, 11, 21, 26, 29, 30].

Various sources mention many risk factors for developing symphysiopathy, but all of these factors indicated in studies describing one or a series of cases are not confirmed by statistical analysis and require further study. It is necessary to take into account the occurrence of data on the role of mineral metabolism disorders in the development of symphysiopathy and consider the factors leading to these conditions in

VOROBYEV Aleksandr Aleksandrovich – Doctor of medical Sciences, Professor, scientific Secretary Of the academic Council of Volgmu, Honored scientist of the Russian Federation, head of the Department of operative surgery and topographic anatomy, Volgograd state medical University, e-mail: cos@volgmed.ru тел: 8-909-380-66-13. Russia, Volgograd, 1 Rokossovsky str., Morphological building of Volgsmu. **SELIKHOVA Marina Sergeevna** – Doctor of medical Sciences, Professor of the Department of obstetrics and gynecology, Volgograd state medical University, e-mail: selichovamarina@yandex.ru, тел: 8-960-867-48-48, Clinical base: GUZ "Clinical hospital of emergency medical care No. 7", obstetric and gynecological Department, 400002, Russia, Volgograd, Kazakhskaya str., 1; tel. 41-49-58, 1 Kazakhskaya str., Volgograd, Russia, 400002; tel. 41-49-58, **YAKOVENKO Marina Sergeevna** – resident of the Department of obstetrics and gynecology INFO, Volgograd state medical University, e-mail: marina.yak.070@mail.ru тел: 8-909-378-12-03.

more detail, since mineral metabolism disorders in pregnant women may have different origins.

A new view on the causes of symphysiopathy in pregnant women was presented By U. V. Mozgovaya et al. [10]. According to the results of research, the majority (more than 80%) of pregnant women with symphysiopathy have urinary tract infections, chronic and first detected during pregnancy. Due to urinary tract infections, there is a decrease in reabsorption of calcium and magnesium in the renal tubular system, which is manifested by increased excretion of them in the urine and leads to a disturbance of mineral metabolism [1, 3, 9, 10].

The most characteristic signs of symphysiopathy are pain in the pubic joint. According to the work of E. V. Ananyev [1], in every fifth case they are combined with pain in the lower spine, in the sacroiliac joint , in the pelvic joints (pelvic ring), in accordance with this, there are two clinical variants - "pure" and "mixed"[1]. Also, symphysiopathies are clinically manifested by violations of static and dynamic functions of the lower limbs and pelvis, positive symptoms of Trendeleburg, Laseg, Patrick-Faber (in every second case) [1], local edema of soft tissues. Pain in the pubic joint increases when trying to stand up, walking, bending in the hip joints, when changing the position of the body in bed and climbing the stairs. Sometimes the pain syndrome can be so severe that patients cannot walk or stand [29].

In the standing position and when changing the position of the body, clicks in the symphysis may be felt, and some patients have dysuric disorders [24]. In a clinical study, local pain in the symphysis is detected, and in the presence of a sufficiently wide diastasis, the defect is determined palpatively in the pubic joint. A defect in the symphysis can also be detected during vaginal examination [21]. Pain during compression of the large trochanter and the inability to actively Flex the hip joints in the supine position are a pathognomonic sign of symphysiopathy [23].

The severity of symptoms is often not correlated with the width of the diastasis [20, 1]. For an objective analysis of the condition and control in dynamics, the pain severity assessment is used on a visual-analog scale (VAS), a walking test, in which the time for which the pregnant woman has covered a distance of 5 m is detected, and ultrasound-signs of symphysiopathy.

In the work of D. I. Yemelyanova [4] devoted to the clinical and anamnestic characteristics of women diagnosed with symphysiopathy during pregnancy in the third trimester, significant risk factors for violations of bone mineral density during pregnancy were identified: young age, insufficient physical activity, Smoking. The last two factors are modified, so they can be eliminated. These results contradict the results of O. p. Sergeeva [15], in which the main factor affecting the width of the pubic joint and the development of symphysis was the age of a woman: in the pregnant age group of 16-25 years, the width of the pubic joint was significantly lower than in the group older than 26 years [2, 4, 15, 29].

For a long time, the main method of diagnosing symphysiopathy was pelvic radiography in direct projection, which visualized diastasis between the pubic bones [24, 28]. To diagnose combined sacroiliac injuries and vertical instability, pelvic radiography was used, performed in the patient's standing position on one leg (Flamingo pose). A sign of vertical instability is the presence of a vertical displacement of one of the pubic bones [6].

Currently, for the diagnosis of symphysiotomy most commonly used ultrasound. According to the authors, the accuracy of ultrasound is not inferior to radiography, and also has significant advantages: the lack of radiation load and the ability to perform a study of pregnant women at the patient's bed, easy reproducibility [2, 6, 18, 26].

At suspicion on pathology of symphysis ultrasound involves measuring the width of the symphysis at the upper edge of the pubic bone in the supine position, and defining the vertical displacement of the pubic bones using the chamberlain method. the essence of this technique is as follows: the patient in the supine position bends the straight leg to an angle of 40° in the hip joint, while the same half of the pelvic ring is lowered, at this point the displacement of the pubic bones relative to each other is evaluated [29]. The measurement of diastasis alone was not informative enough, since it was not possible to find a reliable connection between the degree of diastasis of the pubic bones, on the one hand, and complaints and clinical symptoms, on the other. The authors Logutova L. S., Chechneva M. A., Cherkasova N. U. [8] carried out work on the search for the most significant ULTRASOUND signs of symphysiopathy in pregnant women, as one of the variants of the syndrome of pelvic girdle pain associated with pregnancy. ((PPGP- pregnancy-related pelvic girdle pain). The authors determined that the discriminant model that includes variables as a double contour of the pubic bone, changing form and Hypo-echogenicity of the upper ligament, as well as Hypo-echogenicity of the anterior ligament, correctly classified norm and symphysiopathy in 95% of cases. The model, which used the hypoechoic section or reverberation in the symphysis and the thickness of the anterior ligament as variables, correctly classified 91% of cases, and the analysis of only diastasis between the pubic bones, correctly classified norm and symphysiopathy in only 56% of cases, which is not much more than a random choice [1, 2, 7].

According to D. I. Yemelyanova [4], the analysis of the pubic joint using an ultrasound sensor is not enough, since it is necessary to assess the state of the bone tissue, for this purpose, she recommends using the method of ultrasonic densitometry. In last years, more and more modern methods such as CT and MRI are used to diagnose symphysiopathy, which allow us to obtain more detailed information, especially in cases involving the sacroiliac joints [6, 27]. MRI allows not only to identify and measure the width of the diastasis, but also to assess the condition of the surrounding soft tissues, in particular the ligamentous apparatus of the symphysis and bone marrow, which is necessary in the case of differential diagnosis with inflammatory processes [19]. Sometimes radioisotope scanning may be required for differential diagnosis with inflammatory ineoplastic processes.

So, there is no unified approach to the treatment and control of patients with symphysiopathy.

Studies by E. V. Ananyev [1] have shown that combined therapy, including physiotherapy (UFOs) and behavioral therapy (performing exercises and wearing a special bandage) are more effective than using these methods separately. In addition, it was found that the effect of therapy decreased after two weeks after discontinuation of treatment, which, according to the author, indicates the feasibility of continuous therapy until delivery. According to a number of researchers, an important role is played by the supply of microelements to the body of a pregnant woman. So, magnesium plays an important role in the formation of the normal structure of connective tissue, and violation of magnesium homeostasis is one of the etiological factors of symphysiopathy [17]. According to other data, calcium

rather than magnesium ions affect flexible fibers: Ca2+ is necessary for the active centers of elastases, Ca2+ also stabilizes the structure of microfibrils (in particular, through interactions with fibrillin-1 and microfibrile-bound glycobelk-1) [22]. Ca2+ plays a great role in supporting normal bone mineral density, which makes calcium deficiency one of the main factors in the development of symphysiopathy along with hypomagnesemia. In the work of N. Y. Cherkasova [16], the effectiveness of treatment of symphysitis with magnesium, calcium and the use of UFOs was evaluated. The results showed that the inclusion of trace elements in combination with UFOs led to a decrease in the severity of clinical symptoms of symphysiopathy and a significant reduction in the width of the pubic joint diastasis by 28% compared to data before treatment. Similar results were obtained in the study of E. V. Mozgova et al. [11].

One of the most difficult questions to resolve when detecting clinical manifestations of symphysitis in a pregnant woman is the choice of delivery method. Predicting the risk of delivery via the natural birth canal in women with symphysiopathy is based on the severity of the dysfunction of the pubic joint .

Mild and medium severity of pubic joint dysfunction (classification of E. A. Ananieva [1], based on the severity of pain syndrome) without obstetric and extragenital pathology do not require operative delivery, severe degree is an indication for cesarean section. Children born to women with pubic joint dysfunction did not differ on the Apgar scale and anthropometric indicators from children born to the control group [1]. In their work, L. S. Logutova et al. [8] concluded that with the total area of structural changes of the interlobular fibroblast disk (based on the echo pattern of the pubic joint) up to 50%, delivery via the natural birth canal does not increase the risk of injury to the pubic joint in childbirth. When area changes from 50 to 80% prognosis is based on the degree of changes in the pubic joint, if the structure changes by more than 80 %, the risk of rupture of the pubic joint in childbirth through the natural birth canal increases.

Conclusion. Thus, at present, it should be recognized that the problem of symphysiopathy in pregnancy is not sufficiently studied. The inflammatory cause of this disease has not been confirmed and the etiology remains unknown, which does not allow us to identify risk factors for its manifestation. Symphysiopathy is not an urgent condition, but the development of pain in a pregnant woman affects the quality of her life and is often an indication for operative delivery in a young patient. All this dictates the need for randomized studies with a good evidence base to study epidemiology, etiology, pathogenesis and diagnosis, treatment approaches and the development of clear criteria for choosing the method of delivery.

References

1. Ананьев Е.А. Оптимизация диагностики, тактики ведения беременности и родов при дисфункциях лонного сочленения: дис. … канд. мед. наук. Москва; 2012. [Anan'ev EA. Optimization of diagnostics, tactics of pregnancy and childbirth management in the case of pubic joint dysfunction. *Dissertation of the candidate of medical Sciences*. Moscow; 2012. (In Russ.)]

2. Дедуль А.Г. Клинические и биохимические маркеры симфизиопатии у беременных и методы ее коррекции: дис… канд. мед. наук. Санкт-Петербург; 2013. [Dedul' AG. Clinical and biochemical markers of symphysiopathy in pregnant women and methods of its correction. *Dissertation of the candidate of medical Sciences*. Sankt-Peterburg; 2013. (In Russ.)]

3. Дедуль А.Г., Мозговая Е.В. Повышенная экскреция кальция и магния как причина нарушения минерального обмена у беременных с симфизиопатией. *Журнал акушерства и женских болезней*. 2009;5: 100. [Dedul' AG, Mozgovaya EV. Increased excretion of calcium and magnesium as a cause of impaired mineral metabolism in pregnant women with symphysiopathy. *ZHurnal akusherstva i zhenskih boleznej*. 2009; 5: 100. (In Russ.)]

4. Емельянова Д. И. Клинико-анамнестические особенности женщин с диагнозом симфизиопатии при беременности в III триместре с позиции определения плотности костной ткани. *Вятский медицинский вестник*. 2015;4:12-15. [Emel'yanova DI. Clinical and anamnestic features of women diagnosed with symphysiopathy during pregnancy in the third trimester from the position of determining bone density. *Vyatskij medicinskij vestnik*. 2015;4:12-15. (In Russ.)]

5. Капанджи А.И. Позвоночник. Физиология суставов. Москва: ЭКСМО;2009. [Kapandzhi A.I. Spine. Physiology of the joints. Moscow: EKSMO; 2009. (In Russ.)]

6. Клипфель И.В., Калыгина Н.А., Емельянова Н.Б. Возможности ультразвукового исследования в диагностике дисфункции лонного сочленения. *Вестник Челябинской областной клинической больницы*. 2016; 1(31):64-67. [Klipfel' IV, Kalygina NA, Emel'yanova NB. Possibilities of ultrasound examination in the diagnosis of pubic joint dysfunction . *Vestnik CHelyabinskoj oblastnoj klinicheskoj bol'nicy*. 2016; 1(31):64-67. (In Russ.)]

7. Логутова Л.С., Чечнева М.А., Черкасова Н.Ю. Значимые ультразвуковые признаки симфизиопатии у беременных. *Тезисы VII Съезда ассоциации специалистов ультразвуковой диагностики в медицине*. 2007; 1:101. [Logutova LS, CHechneva MA, CHerkasova NYU. Significant ultrasound signs of symphysiopathy in pregnant women. *Tezisy VII S"ezda associacii specialistov ul'trazvukovoj diagnostiki v medicine*. 2007; 1:101. (In Russ.)]

8. Логутова Л.С., Чечнева М.А., Черкасова Н.Ю. Возможности прогнозирования риска родоразрешения через естественные родовые пути у женщин с симфизиопатией. *Российский вестник акушера-гинеколога*. 2016;3:32-37. [Logutova LS, CHechneva MA, CHerkasova NYU. *Possibilities of predicting the risk of delivery through the natural birth canal in women with symphysiopathy*. Rossijskij vestnik akushera-ginekologa. 2016;3:32-37. (In Russ.)]

9. Mozgovaya E.V. Infekcii mochevyvodyashchih putej u beremennyh [Urinary tract infections in pregnant women]. Gestacionnye simfiziopatii: metodicheskie rekomendacii. [Of gestational simpozioane: guidelines] . Sankt-Peterburg: N-L, 2008, pp .40.

10. Мозговая Е.В., Дедуль А.Г., Опарина Т.И. Новый взгляд на причины развития симфизиопатии у беременных и поиск эффективных методов лечения. *Журнал акушерства и женских болезней*. 2010;1:71-83. [Mozgovaya EV, Dedul' AG, Oparina TI. A new look at the causes of symphysiopathy in pregnant women and the search for effective treatment methods. *ZHurnal akusherstva i zhenskih boleznej*. 2010;1:71-83. (In Russ.)]

11. Мозговая Е.В., Дедуль А.Г., Опарина Т.И Оценка эффективности и безопасности терапии препаратами кальция у беременных с симфизиопатией. *Журнал акушерства и женских болезней*. 2013;62:10-16. [Mozgovaya EV, Dedul' AG, Oparina TI. Evaluation of the effectiveness and safety of calcium therapy in pregnant women with symphysiopathy. *ZHurnal akusherstva i zhenskih boleznej* . 2013;62:10-16. (In Russ.)]

12. Нормальная беременность. Клинические рекомендации МЗ РФ. Москва: Изд-во стандартов; 2019. [Normal'naya beremennost'. Clinical recommendations MZ RF. Moscow; 2019. (In Russ.)]

13. Савельева Г.М. Акушерство: учебник. Москва: Изд-во Медицина; 2008. [Savel'eva GM. Obstetrics: textbook. Moscow: Medicina; 2008. (In Russ.)]

14. Савельева Г.М., Сухих Г.Т., Серов В.Н. Акушерство. Национальное руководство. 2-е издание. перераб. и доп. Москва : ГЭОТАР-Медиа; 2018. [Savel'eva GM, Suhih GT, Serov VN. Obstetrics. National leadership. 2th ed. Moscow: GEOTAR-Media; 2018. (In Russ.)]

15. Сергеева О.П. Состояние лонного сочленения при беременности и в послеродовом периоде по данным эхографии. *Казанский медицинский журнал*. 2003;84(4):261-263. [Sergeeva OP. The state of the pubic joint during pregnancy and in the postpartum period according to echography. Kazanskiĭ medicinskiĭ zhurnal. 2003;84(4):261-263. (In Russ.)]

16. Черкасова Н.Ю. Прогнозирование риска материнского травматизма у беременных с патологией лонного симфиза: дис… канд. мед. наук: Москва; 2016. [CHerkasova N.YU. Predicting the risk of maternal injuries in pregnant women with pathology of the pubic symphysis. *Diss. Candidate of medical Sciences*. Moscow; 2016. (In Russ.)]

17. Чурилина А.В. К вопросу о роли магния в формировании дисплазии соединительной ткани. *На подмогу педиатру*. 2010;5(26):97-100. [CHurilina AV, Moskalyuk ON, CHalaya LF. On the role of magnesium in the formation of connective tissue dysplasia. *Na polmogu pediatru*. 2010;5(26):97-100. (In Russ.)]

18. Bjorklund K. et al: Sonographic assessment of symphyseal joint distention during pregnancy and post partum with special. *Acta Obstet Gynecol Scand*. 2009; 79(4): 269–275.

19. Brandon C.L. Jacobson J.A. Low I.K. Pubic bone injuries in primiparous women: magnetic resonance imaging in detection and differential

diagnosis of structural injury. *Ultrasound Obstet Gynecol.* 2012; 39(4):444-451. DOI: 10.1002/uog.9082.

20. Shawla J.J. Arora D. Sandhu N. Pubis symphysis diastasis: a case series and literature review. *Oman Med J.* 2017; 32(6):510-514. DOI: 10.5001/omj.2017.97.

21. Çıçek H. Keskın H. Tuhanıoğlu Ü. Simultaneous disruption of the pubic symphysis and sacroiliac joint during vaginal birth. *Case Rep Orthop*. 2015; 2015:1-5. DOI: 10.1155/2015/812132.

22. Clarke A. W., Weiss A. S. Microfibril-associated glycoprotein-1 binding to tropoelastin: multiple binding sites and the role of divalent cations. *Eur. J. Biochem.* 2004; 14:3085–3090.

23. Erickson D. Low J. Shumway J. Management of postpartum diastasis of the pubic symphysis. *Orthopedics.* 2016; 39(2):367-369.DOI: 10.3928/01477447-20160307-02.

24. Gabriel A.O. Oladire O. Jovita D.A. Transcutaneous electrical nerve stimulation (TeNS) in the management of peripartium diastasis symphysis pubis (dSp): cases report. *Ind J Physiotherap Occup Therap.* 2015; 9(3):221-225. DOI: 10.5958/0973-5674.2014.00001.X.

25. Herren C. Sobjttke R. Ringe M. Peripartum pubic symphysis separation - current strategies in diagnosis and therapy and presentation of two cases. *Injury.* 2015; 46(6):1074-1080.

26. Hierholzer C. Ali A. Toro arbelaez J.B. Traumatic disruption of pubis symphysis with accompanying posterior pelvic injury after natural childbirth. *Am J Orthop (Belle Mead NJ).* 2007; 36:167-170.

27.Jain S. Eedarapalli P. Sawdy R. Symphysis pubis dysfunction: a practical approach to management. Obstet Gynaecolog. 2011;24(3):153-158. DOI: 10.1576/toag.8.3.153.27250.

28. Khorashadi L. Petscavage J.M. Richardson M.I. Postparum symphysis pubis diastasis. *Radiolog Case Rep.* 2011; 6(3):1-3. DOI: 10.2484/rcr.v6i3.542.

29. Laadioui M. Slimani W. Alaoui F. Disjonction symphysaire apre un accouchement par voie basse dystocique: a propos d'un cas. *Pan African Med J.* 2014; 17:33. DOI: 10.11604/pamj.2014.17.33.3441.

30. Shnaekel K.I. Magnan E.F. Ahmadi S. Pubic symphysis rupture and separation during pregnancy. *Obstet Gynecol Surv.* 2015; 70(11):713-718. DOI: 10.1097/OGX.0000000000000247.

31. Jain S. Eedarapalli P. Jamjute P. Symphysis pubis dysfunction: a practical approach to management. *Obstet Gynaecolog.* 2011; 24(3):153-158. DOI: 10.1576/toag.8.3.153.27250

A.A. Shvaikovskaya, S.Y. Zhanaeva, A.V. Evsyukova, M.A. Tikhonova, K.V. Danilenko, L.I. Aftanas

BRAIN NEUROTROPHIC FACTOR (BDNF) AND ITS DIAGNOSTIC SIGNIFICANCE WHEN MEASURED IN BLOOD: ANALYTICAL REVIEW

DOI 10.25789/YMJ.2020.71.27

УДК 612.8.04

Brain neurotrophic factor (BDNF) is one of the key indicators of neurogenesis and neuroplasticity. The concentration of BDNF in the blood is positioned as a potential biomarker of the development and dynamics of neuropsychiatric diseases. However, the extent to which its concentration in the blood reflects its content in the brain is not clear; a literature analysis on this matter has not been performed.

This review provides basic information about the biological role, content and regulation of BDNF in the body (chapter I), about the concentration of BDNF in the blood in neuropsychiatric diseases (chapter II), and analyzes the correlation of BDNF content in the brain and blood (chapter III, the main one).

In 5 out of 6 studies on intact animals identified as a result of the literature search, a statistically significant positive correlation between the concentration of BDNF in the brain and blood is reported. However, the correlation coefficient is variable and generally not high – from 0.40 to 0.83. There were no during-life studies performed in human. Some post-mortem studies have reported a significant association between BDNF indices values in the brain and in the periphery. Thus, the question of the extent to which BDNF in the blood is diagnostically valuable remains unanswered.

Keywords: human, brain-derived neurotrophic factor (BDNF), neuropsychiatric diseases, brain, blood, correlation between brain and blood

SHVAIKOVSKAYA Anna Andreevna – student, V. Zelman Institute of medicine and psychology Novosibirsk State University, 630090, Novosibirsk, Russia, a.a.shvaikovskaya@mail.ru, 983-309-7089; **ZHANAEVA Svetlana Yakovlevna** – PhD, Institute of Physiology and Basic Medicine, 630117, Novosibirsk, Russia, djsja@mail.ru, (383)373-02-38; **EVSIUKOVA Aleksandra Vladislavovna** – student, V. Zelman Institute of medicine and psychology Novosibirsk State University, 630090, Novosibirsk, Russia, sashulya2011a@yandex.ru, 953-775-9188; **TIKHONOVA Maria Aleksandrovna** – PhD, head of the laboratory of experimental models of neurodegenerative processes, Institute of Physiology and Basic Medicine, 630117, Novosibirsk, Russia, mara-tikh@mail.ru, (383)373-02-19; **DANILENKO Konstantin Vasilevich** – MD, Vice-Director for scientific and clinical research, Institute of Physiology and Basic Medicine, 630117, Novosibirsk, Russia, kvdani@mail.ru, (383)334-89-70; **AFTANAS Liubomir Ivanovich** – MD, Professor, Academician of the Russian Academy of Sciences, Director of the Institute of Physiology and Basic Medicine, 630117, Novosibirsk, Russia, liaftanas@gmail.com, (383)335-98-55.

I. Biological role and content of BDNF in the human body

Biological role of BDNF. The brain-derived neurotrophic factor (BDNF) was discovered in 1982 [9] and is currently one of the most well-known and studied representatives of the neurotrophin family of proteins. It is produced in various organs and systems, but it plays a special role in the nervous system, providing the processes of neurogenesis and neuroplasticity. BDNF has its neurotrophic effect when binding to two different types of receptors: the mature form of BDNF interacts primarily with a receptor from the tyrosine kinase group – TrkB (synthesis and differentiation of new neurons), and the proforma – with a receptor from the family of tumor necrosis factor receptors – p75 (triggering apoptosis).

Previously, it was believed that human neurogenesis in the cortex ends in the early postnatal period [65]. Currently, it is known that less active processes of neurogenesis continue throughout life (in particular, in the subventricular zone of the lateral ventricles of the brain and the hippocampus), but their noticeable weakening is observed with age [65] and in neuropsychiatric diseases [42]. In the hippocampus of adults, newly formed neurons have high plasticity and appear to play a key role in learning, emotion formation mechanisms, and memory consolidation, supporting cognitive functions throughout life [28; 36]. At the same time, it is BDNF and the closely related serotonergic 5-HT system of the brain that is assigned a key role in maintaining the processes of neurogenesis (migration, development, differentiation, synaptogenesis), maintaining the viability of neurons and their adaptation to changing external influences, not only in early

ontogenesis, but also in the adult brain.

Investigations of transcriptional and translational mechanisms that control neurotrophin expression have made it possible to understand the regulatory pathways of neurogenesis and neuroplasticity. It is assumed that disturbances in the synthesis, processing or transport of BDNF can lead to various neurological and mental disorders, such as: bipolar affective disorder, depression, eating behavior disorders, schizophrenia, Alzheimer's disease, epilepsy, Huntington's chorea, Rett syndrome [16; 59].

BDNF content in the human body. To determine the concentration of BDNF in the brain, including post-mortem, Western immunoblot [39] or enzyme immunoassay (ELISA) [27; 37] is used. A high level of mRNA (matrix RNA) of the BDNF gene encoding the BDNF precursor protein was detected in such brain structures as the cerebral cortex, hippocampus, hypothalamus, thalamus, striatum, transparent septum cavity in the brain, dorsal spinal root ganglia, olfactory bulb [69]. In the adult human brain, BDNF is expressed mainly in neurons; in addition, it is found in astrocytes and microglia cells in both healthy and non-healthy individuals, as well as in T-cells and macrophages of perivascular infiltrates of the brain [45]. Outside of the Central nervous system, BDNF is found in saliva, urine, lacrimal fluid, serum and plasma, white blood cells (T- and B-lymphocytes, monocytes, eosinophils), platelets, vascular endothelium, smooth muscle and striated muscles; it is found in the thymus, liver, spleen, heart, retina, prostate, kidneys [7; 21; 30; 33; 54].

Circulating BDNF exists in two different forms: platelet-bound and free, with total BDNF measured in serum and only the unbound portion in plasma [68]. The concentration of BDNF in serum is about 100-200 times higher than in plasma, it is calculated in values of the order of 10^3-10^4 pg/ml and varies significantly between individuals (the difference is 10-50 times) [49]; the reasons for inter-individual variability are not clarified. The serum concentration of BDNF is maximal in the morning (at 8:00 a.m.), with a gradual decrease of 3-4 times during the day, and minimal at midnight [49]. In addition to daily fluctuations, there are distinct seasonal fluctuations in humans: the concentration of serum BDNF increases in the spring-summer period and decreases in the autumn-winter period [56]. Due to the high variability, the norms for the content of BDNF in the blood have not been developed. It should be noted that the relatively high coefficients of variation found for some commercial biochemical kits for serum / plasma BDNF assay may also contribute to the variability [4].

Regulation of BDNF in the human body. The *BDNF* gene is localized on chromosome 11 in the p14-region and comprises 12 exons [69]. The expression of the *BDNF* gene is mainly controlled by 9 tissue-specific promoters, ensuring its regulation at the transcription level [43].

In the endoplasmic cell network, a precursor protein pre-pro-BDNF is synthesized, cleaved to form pro-BDNF, which is transported to the Golgi complex, where it is stored as vesicles and converted to BDNF by proteolysis intracellularly, or in the process of anterograde transport along axons to presynaptic terminals [20]. Maintaining a balance between pro-BDNF and BDNF is an important factor in regulating processes in the brain, since pro-BDNF has a greater affinity for the p75 receptor than for TrkB, activating pro-apoptotic processes [41].

The expression of BDNF transcripts is regulated not only by internal factors, but also by environmental factors. One of these factors is stress, which can significantly change the expression of BDNF and the pro-BDNF/BDNF ratio [47]. After stress or physical load, there is an immediate increase in BDNF concentration in blood plasma [25].

II. BDNF in neuropsychiatric diseases

BDNF in depression. Numerous studies show that BDNF is closely involved in the pathophysiological mechanisms of depression, is associated with changes in neuroplasticity indices in this disease and with the efficacy of antidepressant therapy [2]. In comparison with healthy controls, the concentration of BDNF in the blood of patients with depression is usually lower on average [55] and depends on the severity of depression – with more severe depression, the level of BDNF is usually (but not always) lower [38]. Literature data on changes in peripheral BDNF concentration as a result of antidepressant treatment of depression are contradictory: one of the meta-analyses indicates no changes in serum BDNF concentration (6 studies [63]), the other – an increase in serum/plasma concentration (20 studies [44]), mainly due to groups of patients who were treated with sertraline. A decrease in depression scores during treatment is positively correlated with an increase in blood BDNF concentration according to meta-analysis data, however, this correlation is not strong [22].

The results of our own study did not confirm the significance of BDNF as a biomarker for evaluating the efficacy of treatment of non-seasonal unipolar depression [unpublished data]. The concentration of BDNF in the blood serum did not change significantly following a triple chronotherapy (Wilcoxon test) (Figure 1A), which led to an antidepressant response in 37.1% of patients [3]. There was no association between BDNF and psychometric scales. The correlation between the initial values of BDNF and HDRS-17 was the closest to significance: a lower concentration of BDNF corresponded to a greater severity of depression (p=0.19, Spearman test; Figure 1B), which corresponds to the literature data [38]. The BDNF concentration was measured in samples in a single run by multiplex solid-phase analysis on a analyzer MILLIPLEX Luminex 200 (Merck KGaA, Germany) using a reagent kits from Merck.

Taken together, the results do not yet allow us to conclude whether peripheral

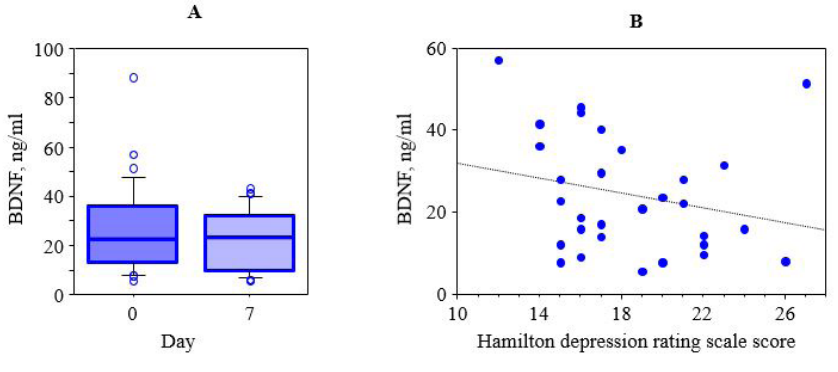


Fig. 1. A change of serum BDNF levels in 31 depressed patients undergoing triple chronotherapy (diagram A). Diagram B shows correlation between baseline depression score and BDNF concentration (one outlier removed); a regression line is indicated; the correlation does not attain the level of statistical significance (p=0.19).

blood BDNF is suitable for use as a biomarker for depression.

BDNF in neurodegenerative diseases. *Alzheimer's disease.* The development of Alzheimer's disease leads to a decrease in the number of neurons and their functions, which causes a deterioration in the axonal transport of BDNF. It has been shown that patients with Alzheimer's disease have a reduced concentration of BDNF in serum and plasma compared to healthy persons [26], while some studies have revealed positive correlations between the level of BDNF and the stage of the disease, which gives the authors reason to believe that BDNF can be a predictor of the rate of the disease progression [8; 34]. According to another analytical review, a significant decrease in peripheral BDNF levels was found only in patients with Alzheimer's disease with low values on the short mental status scale (MMSE), while the authors show that in the early stages of Alzheimer's disease, the concentration of BDNF in the blood may increase, and the decrease occurs only in the later stages of Alzheimer's disease [48]. The relationship between decreased levels of BDNF in the brain and the development of cognitive deficits and dementia in Alzheimer's patients compared to healthy controls was found [32; 61].

Parkinson's disease. The association of BDNF with the TrkB receptor plays an important role in the long-term maintenance of the nigrostriatal pathway, and its change may contribute to the occurrence and progression of Parkinson's disease [35]. A decrease in the concentration of BDNF and the number of TrkB receptors found in some studies in patients may be associated with neurodegeneration of dopaminergic neurons, while an increase in the level of BDNF and TrkB in later stages may be caused by the use of the drug levodopa. All this suggests that BDNF concentration and TrkB expression can be used as biomarkers for early diagnosis of Parkinson's disease and for treatment correction [35].

Amyotrophic lateral sclerosis (ALS). The expression of *BDNF* and *NTRK2* mRNA encoding TrkB receptors, as well as the content of BDNF and TrkB in lymphocytes are greatly reduced in patients with ALS, which allows differentiating ALS from other neurodegenerative motor diseases even at an early stage [46]. Serum BDNF levels may not differ in healthy individuals and individuals with diagnosed ALS, but BDNF concentrations differ between groups of patients with different phenotypic forms of ALS, which differ in prognosis and quality of life [13]. Activation of signaling pathways via BDNF/TrkB stimulates neuronal survival and resistance to toxic damage, so BDNF potentiation is considered to prevent the progression of neurodegenerative diseases [14]. In the case of ALS, however, there is evidence that increased BDNF / TrkB signaling contributes to increased glutamate excitotoxicity and motor neurone death in the early stages of the disease [51]. The data obtained demonstrate the high significance of BDNF for the diagnosis and treatment of ALS, but the results are not yet fully consistent, and the role of BDNF in the pathogenetic mechanism of ALS requires further clarification.

BDNF in epilepsy. BDNF is synthesized in the body of granular cells of the hippocampal dentate gyrus, and is transported anterogradely along axons (mossy fibers) to presynaptic terminals in the CA (*Cornu Ammonis*) areas [66]. Abnormal growth of mossy fibers in the dentate gyrus instead of CA-areas leads to the formation of excitatory synapses, creating electrical circuits with the ability to synchronize and generate epileptic seizures. Often these seizures are pharmacoresistant and are stopped only by resection of the epileptogenic focus [1]. BDNF can play a proepileptic role by potentiating excitatory synapses for epileptogenesis. Consequently, the frequency and severity of seizures, especially in a chronically damaged hippocampus, can be regulated by the total BDNF concentration. Increased levels of neurotrophins, including BDNF, may be associated with the initiation of epileptogenesis in the hippocampus after injury [58].

Two human BDNF polymorphisms have been identified that may be associated with epilepsy: Val66Met and Cys270Thr. Val66Met is associated with loss of neuronal function, decreased transmission of electrical stimuli along axons and dendrites, and impaired regulation of neural interaction activity. Cys270thr polymorphism is directly associated with idiopathic temporal lobe epilepsy and convulsions [66].

In studies involving control groups of healthy people and patients with temporal lobe epilepsy, completely opposite results were found: BDNF levels in the blood can be lower in people with epilepsy than in the control group [57], and higher [31]. This can be explained by the fact that, based on the excitatory effects of BDNF, an increase in its concentration may predispose to the occurrence of seizures [10], whereas a decrease in BDNF concentration may represent an adaptive mechanism for minimizing convulsions in a long-term damaged hippocampus [58]. In general, an increase in the activity of *BDNF* mRNA, and hence in the expression of BDNF, occurs during epileptogenesis, namely when the granular cells of the hippocampus are excited [31].

III. BDNF coherence in the brain and periphery. Direct measurement of BDNF in vivo in the human brain is practically impossible. Is it valid to judge the state of the brain by measuring BDNF in peripheral biological fluids (blood, urine, saliva)? The third chapter is devoted to the analysis of scientific literature on this issue. We searched for studies that provide information on the coherence of the BDNF content in the brain and periphery. The search was performed in the PubMed database using relevant links to other papers in refereed articles, including review articles [16;18; 23; 62].

Animal studies. A total of 11 studies were found (Table 1) in which BDNF (or its forms) were measured simultaneously in brain and in blood (serum, plasma, blood cells) or saliva (in 1 study). Almost all of these studies were performed on rats; only one of them also used mice and pigs, and another only used mice (studies # 3 and # 1, respectively, in Table 1). The first 3 studies [rows # 1-3 in Table 1] were aimed directly at finding a correlation between the brain and blood, in 6 the correlation was studied after experimental exposure (in 3 – with the control group [## 4, 5, 6] and in 3 – without it [## 3, 7, 8]), and 3 shows data on association, but not correlation [## 9, 10, 11].

In 5 of the first 6 studies in intact groups (that is, excluding groups with experimental exposure in studies # 4-6), positive correlations were found between the content of BDNF in the brain and blood (the correlation coefficient varies from 0.40 to 0.83). Less consistent are the results of correlation analysis in groups with exposure (rows 4-8 in Table 1): the correlation was often absent during the period of exposure (electroconvulsive therapy, administration of immunotoxin, ketamine, simulation of ischemic stroke) or was even reversed (row 8 of Table 1), which is explained by the possible inertia of reflection of brain BDNF expression in the circulating blood.

Association studies (studies in which the results of correlation analysis were not reported; rows 9-11 in Table 1) brought even more contradictory results: in groups of rats with exposure (injection of kainic acid, inducing rheumatoid

Correlation of BDNF concentration in the brain and blood in animals

Article	Materials	Methods	Results
in observational studies			
[67]	Mice (serum, brain, and spinal cord segments)	2 groups: the group that received 125I-BDNF injection in PBS with 1% bovine serum albumin (BSA) and a group that received only 125I-PDGF; measurement of 125I-BDNF after 0-5-10-20-30-45-60 min	Positive correlation between 125I-BDNF concentrations in the brain, cervical segments of the spinal cord, and blood serum in both groups
[40]	Rats (serum, platelets and brain)	4 groups of different ages: newborns, 3 weeks, 2 months, 2 years	Positive correlation between the content of BDNF (and BDNF mRNA) in the brain and blood serum in each age group
[17]	Mice, rats, pigs (whole blood, plasma, brain tissue –hippocampus and frontal cortex)	No intervention	Positive correlation between BDNF concentrations in blood and brain in rats and pigs
in interventional studies			
[24]	Rats (serum and brain –hippocampus and frontal cortex	3 groups: groups exposed to ECT (electroconvulsive therapy) once or five times and a control group	Positive correlation between BDNF concentrations in the brain and serum in the control group (without intervention) and a week later in all groups after the end of the ECT
[11]	Rats (serum and brain – hippocampus, frontal and parietal cortex, and striatum)	2 groups: a group that received immunotoxin once (192IgG-saparin) and control group (PBS)	Positive correlation between serum and parietal cortex BDNF concentrations 15 days after exposure in both groups; no correlation was found between other brain regions and blood
[5]	Wistar and Wistar-Kyoto rats (serum and brain – hippocampus, frontal cortex, neocortex and cerebellum)	2 groups: with ECT (electroconvulsive therapy), which were studied 3-7-15 days after exposure, and the control group	Positive correlation between BDNF concentrations in serum, hippocampus, and frontal cortex only in Wistar rats, and only 1 day after ECT
[6]	Rats (plasma and brain tissue –prefrontal cortex, hippocampus, amygdala)	40-minute ketamine infusion (10 mg / kg) in different ways: intraperitoneal, subcutaneous, intravenous bolus; measurement of BDNF after 3-5-15-30-45-60-90-120-180-240 minutes after exposure	BDNF concentration in the blood (which increased after administration of ketamine in different ways) did not correlate with its concentration in the brain (which did not change)
[64]	Rats (blood lymphocytes and brain)	Simulation of ischemic stroke (colorant + laser); measurement of BDNF after 6h-1d-3d-14d after exposure	Strong positive correlation for proBDNF 6 h and 1 day after ischemic stroke; negative correlation for mBDNF 1 day after ischemic stroke
asociation studies (not correlations)			
[53]	Rats (plasma, cerebrospinal fluid and brain – cerebellum, hippocampus, striatum, amygdala, frontal cortex)	2 groups: with kainic acid injection and with saline solution injection (control); measurement of BDNF 1.5-5-3-6-12-24 h after injection	No association: increased BDNF content in the brain and normal – in plasma in the experimental group (in the cerebrospinal fluid concentration was below the measurement threshold)
[19]	Rats (serum and brain –hippocampus and frontal cortex)	2 groups: with induced rheumatoid arthritis and intact control	Negative association: increased BDNF content in the blood and decreased – in the brain in the experimental group
[29]	Rats (plasma, saliva and brain – hippocampus, prefrontal cortex)	2 groups: rats in social isolation for 8 weeks and a control group	Positive association: reduced BDNF concentrations in plasma, saliva, and brain in the experimental group

arthritis, social isolation), changes i BDNF content (compared to the contr group) in the brain and blood were nc always unidirectional. However, the valu of associative research for the goal of thi review is low since the presence of a association does not necessarily imply a correlation.

The first study in Table 1, performed on mice that were injected with labeled BDNF, looks interesting methodologically. Labeled BDNF was detected almost immediately after administration – in the brain, spinal cord, and blood serum, which is why the authors conclude that BDNF easily passes through the blood-brain barrier in mice [67]. In mice, BDNF is abundantly expressed in the brain, but, unlike in rats and humans, it is not detected in the blood [17; 52].

Studies in humans. BDNF poorly passes through the blood-brain barrier in humans, so its concentration in the brain may not correlate with the concentration in the blood [15]. According to the literature search, there were no lifetime studies of BDNF simultaneously in the brain and blood in humans. Post-mortem studies suggest, however, that there is a correlation between brain and peripheral BDNF in humans.

A correlation was found between the expression of *BDNF* mRNA in tissues (in the prefrontal cortex, hippocampus, quadriceps femoris) and blood obtained in 121 patients with bipolar disorder after autopsy [12].

In patients with depression, reduced BDNF concentration and reduced *BDNF* mRNA expression were found in post-mortem samples of both the brain and blood [62]; the results of the actual correlation analysis were not reported. This result is consistent with the fact that a lower lifetime BDNF concentration in the blood during depression is associated with a smaller hippocampus volume at post-mortem study [60].

In patients with Alzheimer's disease, the concentration of BDNF post-mortem is significantly reduced both in the brain (in the hippocampus, frontal, temporal, and parietal cortex), as well as in the cerebrospinal fluid and blood in comparison with the control group, and reflects the degree of disease progression [50].

Conclusion. The concentration in the blood of the brain neurotrophic factor BDNF, which is important for the processes of neurogenesis and neuroplasticity in the brain, reflects its content in the brain to varying degrees, which follows from a review of correlation studies in animals (varying power size, on average – moderate), and indirect data from postmortem research in humans. It is necessary to perform an in vivo human study to solve the question of the diagnostic significance of measuring BDNF in the blood.

Financial support. *The study was supported by budgetary funding for applied scientific research in 2016 (results related to Figure 1) and basic scientific research (theme # AAAA-A16-116021010228-0).*

References

1. Копачев Д. Н. и др. Склероз гиппокампа: патогенез, клиника, диагностика, лечение. *Журнал «Вопросы нейрохирургии» имени НН Бурденко*. 2016; 80(4):109-116. [Kopachev DN et al. Hippocampal sclerosis: pathogenesis, clinical picture, diagnosis, treatment. *ZHurnal «Voprosy nejrohirurgii» imeni NN Burdenko*. 2016; 80(4):109-116. (In Russ.)].

2. Левчук Л.А. Роль BDNF в патогенезе неврологических и психиатрических расстройств. *Современные проблемы науки и образования.* 2018; 6:58. [Levchuk LA. The role of BDNF in the pathogenesis of neurological and psychiatric disorders. *Sovremennye problemy nauki i obrazovaniya*. 2018; 6:58. (In Russ.)]

3. Danilenko K.V., Lebedinskaia M.Y., Gadetskaia E.V. et al. A 6-day combined wake and light therapy trial for unipolar depression. *Journal of affective disorders*. 2019; 259:355-361.

4. Polacchini, A., Metelli, G., Francavilla, R. et al. A method for reproducible measurements of serum BDNF: comparison of the performance of six commercial assays. *Scientific reports,* 2015; 5:17989.

5. Kyeremanteng, C., James, J., Mackay, J. et al. A study of brain and serum brain-derived neurotrophic factor protein in Wistar and Wistar-Kyoto rat strains after electroconvulsive stimulus. *Pharmacopsychiatry.* 2012; 45(06):244-249.

6. Le Nedelec, M., Glue, P., Winter, H. et al. Acute low-dose ketamine produces a rapid and robust increase in plasma BDNF without altering brain BDNF concentrations. *Drug delivery and translational research*. 2018*;* 8(3): 780-786.

7. Yang, B., Ren, Q., Zhang, J. C. et al. Altered expression of BDNF, BDNF pro-peptide and their precursor proBDNF in brain and liver tissues from psychiatric disorders: rethinking the brain–liver axis. *Translational psychiatry*. 2017*;* 7(5): e1128-e1128.

8. Angelucci, F., Spalletta, G., Iulio, F. D. et al. Alzheimer's disease (AD) and Mild Cognitive Impairment (MCI) patients are characterized by increased BDNF serum levels. *Current Alzheimer Research*. 2010; 7(1):15-20.

9. Barde Y. A., Edgar D., Thoenen H. Purification of a new neurotrophic factor from mammalian brain. *The EMBO journal*. 1982; 1(5):549-553.

10. Binder, D. K., Croll, S. D., Gall, C. M. et al. BDNF and epilepsy: too much of a good thing? *Trends in neurosciences*. 2001; 24(1):47-53.

11. Angelucci, F., Gelfo, F., De Bartolo, P. et al. BDNF concentrations are decreased in serum and parietal cortex in immunotoxin 192 IgG-Saporin rat model of cholinergic degeneration. *Neurochemistry international*. 2011; 59(1):1-4.

12. Stenz, L., Zewdie, S., Laforge-Escarra, T. et al. BDNF promoter I methylation correlates between post-mortem human peripheral and brain tissues. *Neuroscience research*. 2015; 91:1-7.

13. Tremolizzo, L., Pellegrini, A., Conti, E. et al. BDNF serum levels with respect to multidimensional assessment in amyotrophic lateral sclerosis. *Neurodegenerative Diseases*. 2016; 16(3-4):192-198.

14. Kowiański, P., Lietzau, G., Czuba, E. et al. BDNF: a key factor with multipotent impact on brain signaling and synaptic plasticity. *Cellular and molecular neurobiology*. 2018; 38(3):579-593.

15. Lu, B., Nagappan, G., Guan, X. et al. BDNF-based synaptic repair as a disease-modifying strategy for neurodegenerative diseases. *Nature Reviews Neuroscience*. 2013; 14(6):401-416.

16. Benarroch E. E. Brain-derived neurotrophic factor Regulation, effects, and potential clinical relevance. *Neurology.* 2015; 84(16):1693-1704.

17. Klein, A. B., Williamson, R., Santini, M. A et al. Blood BDNF concentrations reflect brain-tissue BDNF levels across species. *Journal of Neuropsychopharmacology*. 2011; 14(3): 347-353.

18. Polyakova, M., Schroeter, M. L., Elzinga, B. M. et al. Brain-derived neurotrophic factor and antidepressive effect of electroconvulsive therapy: systematic review and meta-analyses of the preclinical and clinical literature. *PLoS one*. 2015; 10(1): e0141564.

19. Pedard, M., Demougeot, C., Prati, C. et al. Brain-derived neurotrophic factor in adjuvant-induced arthritis in rats. Relationship with inflammation and endothelial dysfunction. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*. 2018; 82:249-254.

20. Giacobbo, B. L., Doorduin, J., Klein, H. C. et al. Brain-derived neurotrophic factor in brain disorders: focus on neuroinflammation. *Molecular neurobiology*. 2019; 56(5):3295-3312

21. Fujimura, H., Altar, C. A., Chen, R. et al. Brain-derived neurotrophic factor is stored in human platelets and released by agonist stimulation. *Thrombosis and haemostasis*. 2002; 87(4):728-734.

22. Brunoni A. R., Lopes M., Fregni F. A systematic review and meta-analysis of clinical studies on major depression and BDNF levels: implications for the role of neuroplasticity in depression. *International Journal of Neuropsychopharmacology*. 2008; 11(8):1169-1180.

23. Castrén E., Kojima M. Brain-derived neurotrophic factor in mood disorders and antidepressant treatments. *Neurobiology of disease*. 2017; 97:119-126.

24. Sartorius, A., Hellweg, R., Litzke, J. et al. Correlations and discrepancies between serum and brain tissue levels of neurotrophins after electroconvulsive treatment in rats. *Pharmacopsychiatry*. 2009; 42(6):270-276.

25. de Assis G. G., Almondes K. M. Exercise-dependent BDNF as a modulatory factor for the executive processing of individuals in course of cognitive decline. A systematic review. *Frontiers in psychology*. 2017; 8:584.

26. Ng, T. K. S., Ho, C. S. H., Tam, W. W. S. et al. Decreased serum brain-derived neurotrophic factor (BDNF) levels in patients with alzheimer's disease (AD): A systematic review and meta-analysis. *International journal of molecular sciences*. 2019; 20(2):257.

27. Duman R.S., Monteggia L. M. A neurotrophic model for stress-related mood disorders. *Biological psychiatry*. 2006; 59(12):1116-1127.

28. Moon J., Lee S. T., Kong I. G. et al. Early diagnosis of Alzheimer's disease from elevated olfactory mucosal miR-206 level. *Scientific reports*. 2016; 6(1):1-9.

29. Nakagawa, Y., To, M., Saruta, J. et al. Effect of social isolation stress on saliva BDNF in rat. *Journal of oral science*. 2019; 61(4):516-520.

30. Quinlan, M. G., Almey, A., Caissie, M. et al. Estradiol and striatal dopamine receptor antagonism influence memory system bias in the female rat. *Neurobiology of learning and memory*. 2013; 106:221-229.

31. Zhand, A., Sayad, A., Ghafouri-Fard, S. et al. Expression analysis of GRIN2B, BDNF, and IL-1β genes in the whole blood of epileptic patients. *Neurological Sciences*. 2018; 39(11):1945-1953.

32. Gan K. J., Silverman M. A. Dendritic and axonal mechanisms of Ca2+ elevation impair BDNF transport in Aβ oligomer–treated hippocampal neurons. *Molecular biology of the cell*. 2015; 26(6):1058-1071.

33. Hashimoto K. Molecular mechanisms of the rapid-acting and long-lasting antidepressant actions of (R)-ketamine. *Biochemical Pharmacology*. 2020; 177:113935

34. Buchman, A. S., Yu, L., Boyle, P. A. et al. Higher brain BDNF gene expression is associated with slower cognitive decline in older adults. *Neurology*. 2016; 86(8):735-741.

35. Huang Y., Huang C., Yun W. Peripheral BDNF/TrkB protein expression is decreased in Parkinson's disease but not in Essential tremor. *Journal of Clinical Neuroscience*. 2019; 63:176-181.

36. Boldrini, M., Fulmore, C. A., Tartt, A. N. et al. Human hippocampal neurogenesis persists throughout aging. *Cell stem cell*. 2018; 22(4):589-599. e5.

37. Galland, F., Negri, E., Da Ré, C. et al. Hyperammonemia compromises glutamate metabolism and reduces BDNF in the rat hippocampus. *Neurotoxicology*. 2017; 62:46-55.

38. Caldieraro, M. A., Vares, E. A., Souza, L. H. et al. Illness severity and biomarkers in depression: using a unidimensional rating scale to examine BDNF. *Comprehensive psychiatry*. 2017; 75:46-52.

39. Chen, B., Dowlatshahi, D., MacQueen, G. M. et al. Increased hippocampal BDNF immunoreactivity in subjects treated with antidepressant medication. *Biological psychiatry*. 2001; 50(4);260-265.

40. Karege F., Schwald M., Cisse M. Postnatal developmental profile of brain-derived neurotrophic factor in rat brain and platelets. *Neuroscience letters*. 2002; 328(3):261-264.

41. Libman-Sokołowska M., Drozdowicz E., Nasierowski T. BDNF as a biomarker in the course and treatment of schizophrenia. *Psychiatr. Pol*. 2015; 49(6):1149-1158.

42. Lima S. M. A., Gomes-Leal W. Neurogenesis in the hippocampus of adult humans: controversy "fixed" at last. *Neural regeneration research*. 2019; 14(11):1917.

43. McGregor C. E., English A. W. The role of BDNF in peripheral nerve regeneration: activity-dependent treatments and Val66Met. *Frontiers in cellular neuroscience*. 2018; 12:522.

44. Zhou C., Zhong J., Zou B. et al. Meta-analyses of comparative efficacy of antide-

pressant medications on peripheral BDNF concentration in patients with depression. *PLoS One*. 2017; 12(2).

45. Murer M.G., Yanb Q., Raisman-Vozari R. Brain-derived neurotrophic factor in the control human brain, and in Alzheimer's disease and Parkinson's disease. *Progress in Neurobiology*. 2001; 63:71-124. DOI: 10.1016/s0301-0082(00)00014-9

46. Sadanand, A., Janardhanan, A., Vanisree, A. J. et al. Neurotrophin expression in lymphocytes: a powerful indicator of degeneration in Parkinson's disease, amyotrophic lateral sclerosis and ataxia. *Journal of Molecular Neuroscience*. 2018; 64(2):224-232.

47. Novkovic, T., Mittmann, T., Manahan-Vaughan, D. BDNF contributes to the facilitation of hippocampal synaptic plasticity and learning enabled by environmental enrichment. *Hippocampus*. 2015; 25(1):1-15.

48. Kim, B.Y., Lee, S.H., Graham, P.L. et al. Peripheral brain-derived neurotrophic factor levels in Alzheimer's disease and mild cognitive impairment: a comprehensive systematic review and meta-analysis. *Molecular neurobiology*. 2017; 54(9):7297-7311

49. Begliuomini, S., Lenzi, E., Ninni, F. et al. Plasma brain-derived neurotrophic factor daily variations in men: correlation with cortisol circadian rhythm. *Journal of Endocrinology*. 2008; 197(2):429-436.

50. Du, Y., Wu, H. T., Qin, X. Y. et al. Postmortem brain, cerebrospinal fluid, and blood neurotrophic factor levels in Alzheimer's disease: a systematic review and meta-analysis. *Journal of Molecular Neuroscience*. 2018; 65(3):289-300.

51. Pradhan J., Noakes P. G., Bellingham M. C. The role of altered BDNF/TrkB signalling in amyotrophic lateral sclerosis. *Frontiers in cellular neuroscience*. 2019; 13:368.

52. Radka, S.F., Hoist, P.A., Fritsche, M. et al. Presence of brain-derived neurotrophic factor in brain and human and rat but not mouse serum detected by a sensitive and specific immunoassay. *Brain research*. 1996; 709(1):122-130.

53. Lanz, T. A., Bove, S. E., Pilsmaker, C. D. et al. Robust changes in expression of brain-derived neurotrophic factor (BDNF) mRNA and protein across the brain do not translate to detectable changes in BDNF levels in CSF or plasma. *Biomarkers*. 2012; 17(6):524-531.

54. Corey, S., Abraham, D. I., Kaneko, Y. et al. Selective endovascular cooling for stroke entails brain-derived neurotrophic factor and splenic IL-10 modulation. Brain Research. 2019; 1722: 146380. DOI: 10.1016/j.brainres.2019.146380

55. Molendijk, M. L., Spinhoven, P., Polak, M. et al. Serum BDNF concentrations as peripheral manifestations of depression: evidence from a systematic review and meta-analyses on 179 associations (N= 9484). *Molecular psychiatry*. 2014; 19(7):791-800.

56. Molendijk M. L., Haffmans J. P., Bus B. A. et al. Serum BDNF concentrations show strong seasonal variation and correlations with the amount of ambient sunlight. *PloS one*. 2012; 7(11).

57. Chen, S.F., Jou, S.B., Chen, N.C. et al. Serum Levels of Brain-Derived Neurotrophic Factor and Insulin-Like Growth Factor 1 Are Associated With Autonomic Dysfunction and Impaired Cerebral Autoregulation in Patients With Epilepsy. *Frontiers in neurology*. 2018; 9:969.

58. Shetty A.K., Zaman V., Shetty G.A. Hippocampal neurotrophin levels in a kainate model of temporal lobe epilepsy: a lack of correlation between brain-derived neurotrophic factor content and progression of aberrant dentate mossy fiber sprouting. *Journal of neurochemistry*. 2003; 87(1):147-159.

59. Hosang, G.M., Uher, R., Keers, R. et al. Stressful life events and the brain-derived neurotrophic factor gene in bipolar disorder. *Journal of affective disorders*. 2010; 125(1-3):345-349.

60. Sun H.S., Kennedy P.J., Nestler E.J. Epigenetics of the depressed brain: role of histone acetylation and methylation. *Neuropsychopharmacology*. 2013; 38(1):124-137.

61. Tanila H. The role of BDNF in Alzheimer's disease. 2017.

62. Cattaneo, A., Cattane, N., Begni, V. et al. The human BDNF gene: peripheral gene expression and protein levels as biomarkers for psychiatric disorders. *Translational psychiatry*. 2016; 6(11):e958-e958.

63. Arumugam, V., John, V.S., Augustine, N. et al. The impact of antidepressant treatment on brain-derived neurotrophic factor level: An evidence-based approach through systematic review and meta-analysis. *Indian journal of pharmacology*. 2017; 49(3):236

64. Luo, H.Y., Rahman, M., Bobrovskaya, L. et al. The Level of proBDNF in Blood Lymphocytes Is Correlated with that in the Brain of Rats with Photothrombotic Ischemic Stroke. *Neurotoxicity research*. 2019; 36(1):49-57.

65. DeCarolis, N. A., Kirby, E. D., Wyss-Coray, T. et al. The Role of the Microenvironmental Niche in Declining Stem-Cell Functions Associated with Biological Aging. *Cold Spring Harbor Perspectives in Medicine*. 2015; 5(12):a025874.

66. Tongiorgi E., Domenici L., Simonato M. What is the biological significance of BDNF mRNA targeting in the dendrites? *Molecular neurobiology*. 2006; 33(1):17-32.

67. Pan, W., Banks, W. A., Fasold, M. B. et al. Transport of brain-derived neurotrophic factor across the blood–brain barrier. *Neuropharmacology*. 1998; 37(12):1553-1561.

68. Walsh J.J., Tschakovsky M.E. Exercise and circulating BDNF: Mechanisms of release and implications for the design of exercise interventions. *Appl Physiol Nutr Metab*. 2018; 43(11):1095-1104. DOI: 10.1139/apnm-2018-0192.

69. West A.E., Pruunsild P., Timmusk T. Neurotrophins: transcription and translation. *Neurotrophic factors. Springer, Berlin, Heidelberg*. 2014; 67-100.

POINT OF VIEW

E.P Ammosova, T.M. Klimova, A.I. Fedorov, M.E. Baltakhinova, R.N. Zakharova

RESEARCHING CONNECTIONS OF PRO12ALA POLYMORPHIC MARKERS OF THE *PPARγ2* GENE WITH METABOLIC DISORDERS IN YAKUTIA'S INDIGENOUS POPULATION

DOI 10.25789/YMJ.2020.71.28

УДК 616-01/09; 575.875

This research aimed to study the association of the pro12Ala polymorphic markers of the PPARγ2 gene with metabolic syndrome and its components in the indigenous inhabitants of Yakutia. We have examined 228 DNA samples of representatives of the Sakha (Yakut) nationality, permanently residing in the village of Berdigestyakh in the Gorny Ulus in Yakutia. Carriers of the Ala allele were quite often observed in the studied group (21.8%). Of these, the Pro/Ala genotype was established in 61 cases, and Ala/Ala in 2 cases. This distribution is generally not very typical for Asian populations.

The results of the study did not reveal statistically significant associations of polymorphic markers of the PPARγ2 gene (rs 1801282) with metabolic disorders.

Keywords: PPARγ2 gene, Pro12Ala polymorphic markers, obesity, metabolic syndrome, multiple metabolic risk factors, population, genetics, dyslipidemia, indigenous population, Yakutia, North.

Introduction. Peroxisome proliferation activating receptors (PPARs) are involved in the control of intracellular metabolism and energy homeostasis. Thus, one of the isomers, PPARγ2, affects adipogenesis, increases the sensitivity of tissues to insulin. It also participates in glucose and lipid metabolism, plays a role in the development of arterial hypertension. Therefore, it is actively studied in the context of factors involved in the development of metabolic syndrome (MS) . One of the common mutations of the *PPARγ2* gene is a single nucleotide substitution of cytosine for a guanine in codon 12 (exon B) (rs1801282). This which results in the replacement of proline for alanine (*Pro-12Ala*) in the PPARγ2 protein [9].

Studies have shown significant differences in the prevalence of the Ala allele among representatives of different ethnic groups. According to different authors, the frequency of carriage of the Ala allele of the *PPARγ2* gene among Asian and African populations is quite low, and amounts to 1-5%, while among European populations it reaches 20% (Table 1) [3, 4, 6, 9, 10, 11, 14- 17, 20, 21]. The data on the association of the Pro12Ala polymorphic markers of the PPARγ2 gene with the development of MS, cardiovascular diseases (CVD), and type 2 diabetes mellitus (T2DM) are contradictory. According to several meta-analyses, the presence of the Ala allele reduces the risk of developing type 2 diabetes and insulin resistance, while the Pro allele, on the contrary, increases the risk of developing the disease [17-19]. Similar results were obtained in the study of the Russian population: carriers of the Pro allele and Pro/Pro genotype had a higher risk of developing MS [2]. But at the same time, there are large studies that showed the association of the Ala allele with a high body mass index, insulin sensitivity, and the risk of developing coronary heart disease [5, 13, 22]. Ruei Zhang et al., as a result of a meta-analysis involving 10 large studies, found no association with MS, but confirmed the association of the Ala allele with bodyweight, abdominal obesity, and hypercholesterolemia [7]. Thus, the role of PPARγ2 in the development of diseases associated with metabolic disorders is still ambiguous.

The study **aimed** to search for the association of the Pro12Ala polymorphic markers of the PPARγ2 gene with metabolic syndrome and its components in the indigenous inhabitants of Yakutia.

Materials and Methods. We have examined 228 DNA samples of representatives of the Yakut nationality, permanently residing in the village of Berdigestyakh of the Gorny Ulus in Yakutia. The average age of the participants was 44±17 years. The research project was approved by the local committee on bioethics of the Yakutsk Scientific Center for Complex Medical Problems (Extract from Protocol No. 39 dated June 26th, 2014). All participants signed informed voluntary consent to participate in the study.

The criteria of the International Diabetes Federation (IDF, 2005) were used to verify the metabolic syndrome [12]. All participants were examined according to a single program, which included: anthropometric examination according to the standard method, analysis of body composition using the Tanita SSC 330 Body Composition Analyzer (Japan), two-fold blood pressure (BP) measurement, and fasting venous blood sampling. The content of glucose, total cholesterol (TC), triglycerides, high-density lipoproteins (HDL cholesterol) was determined using the CardioChek PA Analyzer (USA). Patients

AMMOSOVA Elena Petrovna – Candidate of Medical Sciences, Leading Researcher at the NEFU Medical Institute's Scientific Research Center, Associate Professor of the Department of Pharmacology and Pharmacy at M.K. Ammosov North-Eastern Federal University, 677000, 27 Ulitsa Oiunskogo, Yakutsk, Russia. Tel.: +79644243908, e-mail: ammosovael@mail.ru. ORCID: 0000-0002-7973-6103. **KLIMOVA Tatiana Mikhailovna** – Candidate of Medical Sciences, Associate Professor at Department of Pharmacology and Pharmacy of M.K. Ammosov North-Eastern Federal University's Medical Institute, Senior Researcher at Department of Epidemiology of Chronic Noncommunicable Diseases of the Yakutsk Scientific Center for Complex Medical Problems, Yakutsk, Russia, Tel.: +79142336724, e-mail: biomedykt@mail.ru. ORCID: 0000-0003-2746-0608. **FEDOROV Afanasy Ivanovich** – Candidate of Biological Sciences, Senior Scientist at Research Laboratory of Molecular Medicine and Human Genetics of M.K. Ammosov North-Eastern Federal University's Medical Institute, 677000, Yakutsk, 27 Ulitsa Oiunskogo, Russia. Tel.: +79142332966, e-mail: fedorow@yandex.ru. ORCID: 0000-0002-7973-6103.**BALTAKHINOVA Marina Egorovna** – General Practitioner, Aurora Medical Clinic, Yakutsk, 20/1 Ulitsa Poyarkova, Russia. Tel.: +79142710634, e-mail: bmeg@rambler.ru. ORCID: 0000-0003-0986-7754. **ZAKHAROVA Raisa Nikolaevna** – Candidate of Medical Sciences, Leading Researcher at M.K. Ammosov North-Eastern Federal University Medical Institute's Research Center, 677000, Yakutsk, 27 Ulitsa Oiunskogo, Russia. Tel.: +79142223002, e-mail: prn.inst@mail.ru. ORCID: 0000-0002-1395-8256.

with these disorders also included persons receiving specific drug treatment for these conditions. Multiple metabolic risk factors were established in the presence of 2 or more of 4 risk factors: high blood pressure, hypoalphacholesterolemia, hypertriglyceridemia, and hyperglycemia. The following criteria were used to diagnose obesity: body mass index (≥30 kg/m2); waist-to-height ratio (height≥0.5m); the size of the waist according to the IDF criteria (IDF1) for European populations (more than 80 cm in women and 94 cm in men) [12]. Metabolic syndrome was diagnosed in 36 individuals, which accounted for 16.4% of the total number of those examined. Among women, MS occurred significantly more often than among men – 20.7% and 7.2%, respectively (Fisher's exact test, p = 0.017).

For SNP genotyping, we used TaqMan probes specific to the regions containing the SNPs of interest. Samples and primers were developed using PREMIER Biosoft's Beacon Designer 8 software suite. FAM and R6G dyes were used as reporters, and BHQ-1 was used as a quencher. The real-time polymerase chain reaction was performed in a CFX96 system from Bio-Rad. The volume of the reaction mixture was 25 μl. Each reaction was performed in triplicate. Reaction conditions: activation stage was carried out at 95°C for 3 minutes, course of one cycle consisted of three temperature-time segments: 95°C (30 sec), 54°C (20 sec), and 72°C (20 sec). The total number of cycles was 40.

The verification of the correspondence of the distribution of genotypes according to the Hardy–Weinberg principle was carried out using an online calculator at https://wpcalc.com/en/ equilibrium-hardy-weinberg/ [8]. Statistical analysis of the data was performed in the IBM SPSS STATISTICS 22 package. When comparing groups depending on the type of data, we used the Mann-Whitney and Pearson χ2 tests. The critical value of the level of statistical significance of differences (p) was taken as equal to 5%.

Results and Discussion. The distribution of alleles and genotypes of polymorphic markers of the PPARγ2 gene (rs1801282) in the group of Yakut people is shown in Table 1. The distribution of genotypes in subgroups is consistent with the Hardy–Weinberg principle.

Carriage of the Ala allele was quite often observed in the studied group (21.8%). Of these, the Pro/Ala genotype was established in 61 cases, and Ala/Ala in 2 cases.

This distribution is generally not very typical for Asian populations. Closer data on the Ala allele prevalence among Asian populations were obtained in the populations of Uighurs (11%) and Kazakhs (9%) (Table 2) [10].

Considering the limited number of cases of Ala/Ala genotype carriage, the Pro/Ala (n = 61) and Ala/Ala (n = 2) variants were combined into one group for further analysis.

When comparing subgroups formed by the carriage of individual alleles and genotypes, no statistically significant differences were found in the average levels of metabolic parameters (Table 3). Close-to-critical p values (significance levels) were obtained only for fasting glucose.

Further analysis of the frequency of metabolic risk factors depending on the carriage of alleles and genotypes also

Table 1

Distribution of Alleles and Genotypes of Polymorphic Markers of the *PPARγ2* Gene (rs1801282) in the Group of People of Yakut Nationality*

Group	n (%)			p
	Allele			
	Pro		Ala	
Men Women	71 (79.8) 155 (77.5)		18 (20.2) 45 (22.5)	0.665
Both Genders	226 (78.2)		63 (21.8)	
20-39 years old 40-59 years old 60 years and older	91 (76.5) 92 (78.6) 43 (81.1)		28 (23.5) 25 (21.4) 10 (18.9)	0.783
Genotype				
	Pro/Pro	Pro/Ala	Ala/Ala	
Men Women	54 (75) 111 (71.2)	17 (23.6) 44 (28.2)	1 (1.4) 1 (0.6)	0.669
Both Genders	165 (72.4)	61 (26.8)	2 (0.9)	
20-39 years old 40-59 years old 60 years and older	83 (69.2) 68 (73.1) 34 (77.3)	28 (30.8) 24 (25.8) 9 (20.5)	0 (0) 1 (1.1) 1 (2.3)	0.505

*The distribution of genotypes in all groups is consistent with the Hardy–Weinberg principle; p is the achieved level of statistical significance of differences when comparing groups using the Pearson χ2 test.

Table 2

Allele Frequency of *PPARγ2* Gene (rs1801282) in Different Populations

Authors, Year of Publication	Country	Races / Ethnic Groups	n	Gender	Ala Allele Frequency (95% CI)
Memisoglu et al., 2003	USA	Europeans	2142	Women	0.13 (0.11; 0.14)
Evans et al., 2001	Germany	Europeans	568	Both Genders	0.14 (0.11;0.17)
Frederiksen et.al, 2002	Denmark	Europeans	1951	–«–	0.14 (0.13;0.16)
Deeb et al., 1998	Finland	Europeans	1306	–«–	0.15 (0.13;0.16)
Hara et al, 2000	Japan	Japanese	541	–«–	0.04 (0.02;0.06)
Lei et al, 2000	Taiwan	Asians	310	–«–	0.04 (0.02; 0.06)
Lin-Lin Li et.al., 2008	China	Khanty	102 80 111	–«–	0.05 0.09 0.11-
E. Shyong Tai et al., 2017	China	Chinese Malays Indians	2730 740 568	–«–	0.037 (0.032; 0.042) 0.032 (0.023; 0.040) 0.119 (0.100; 0.139)
Data from this study, 2017	Russia	Yakuts	228	–«–	0.218 (0.170; 0.263)

showed no statistically significant associations of polymorphic markers of the PPARγ2 gene (rs 1801282) with metabolic disorders in the indigenous population of Yakutia (Table 4).

Thus, the study did not reveal an associative relationship between polymorphic markers of the PPARγ2 gene (rs 1801282) with metabolic parameters and the frequency of metabolic disorders among the indigenous population of Yakutia.

Conclusion. Information on the relationship between polymorphic markers of the PPARγ2 gene (rs 1801282) with the development of metabolic disorders is extremely contradictory. There is a hypothesis that evolutionarily the Pro allele played a key role in the human body in the processes of efficient utilization of food and maintaining energy balance under conditions of hunger. In modern conditions of unbalanced nutrition and physical inactivity, the Pro allele plays a negative role in increasing the risk of developing diseases associated with metabolic disorders [17- 19]. At the same time, according to other researchers, carriers of the Ala allele are more at risk of diseases associated with impaired carbohydrate and lipid metabolism [5, 13, 22]. Scientists account the conflicting results of numerous large studies on the nature of the relationship between polymorphic markers of the PPARγ2 gene (rs 1801282) and metabolic parameters and risk factors. Noteworthy is the high – not typical for Asian populations – frequency of Ala allele carriage in the group of Yakut people (Table 1). In early studies together with foreign colleagues in this ethnic group, the following metabolic features were identified: increased metabolic rate, features of the ratio of lipid fractions of blood, adaptive seasonal dynamics of thyroid hormones. This supports the hypothesis of the presence of the so-called "northern" type of metabolism [1, 15, 16]. The high frequency of Ala allele prevalence among the Yakut population may be a reflection of adaptive selection. In this regard, further study of genes associated with the northern type of metabolism in the indigenous inhabitants of Yakutia is of significant scientific interest.

Table 3

Anthropometric and Metabolic Characteristics of Respondents Depending on the Allelic Variant and Genotypes of Polymorphic Markers of the *PPARγ2* Gene (rs 1801282)

Indicators	Genotype	Me (Q1;Q3)	p	Allele	Me (Q1;Q3)	p
SBP, mm Hg	Pro/Pro	115.6 (108.3; 130)	0.604	Pro	116(107.3; 130)	0.662
	Pro/Ala и Ala/Ala	117.6 (105; 140)		Ala	117.6 (105; 140)	
DBP, mm Hg	Pro/Pro	76.3 (70; 82.5)	0.396	Pro	76.6(70; 85)	0.493
	Pro/Ala и Ala/Ala	77.3 (69; 90)		Ala	77.3 (69; 90)	
TC, mmol/L	Pro/Pro	4.4 (3.5; 5.4)	0.550	Pro	4.3(3.5; 5.2)	0.672
	Pro/Ala и Ala/Ala	4.3 (3.5; 5.5)		Ala	4.3 (3.5; 5.0)	
TC HDL, mmol/L	Pro/Pro	1.7 (1.4; 2.2)	0.998	Pro	1.7(1.4; 2.2)	0.942
	Pro/Ala и Ala/Ala	1.6 (1.4; 2.1)		Ala	1.6 (1.4; 2.1)	
Triglycerides, mmol/L	Pro/Pro	0.9 (0.7; 1.0)	0.713	Pro	0.88(0.76; 1.0)	0.814
	Pro/Ala и Ala/Ala	0.8 (0.7; 1.0)		Ala	0.85 (0.73; 1.0)	
TC LDL, mmol/L	Pro/Pro	2.3 (1.6; 3.8)	0.745	Pro	2.3(1.6; 3.8)	0.832
	Pro/Ala и Ala/Ala	2.3 (1.7; 3.5)		Ala	2.3 (1.7; 3.5)	
Fasting Glucose, mmol/L	Pro/Pro	4.9 (4.5; 5.5)	0.056	Pro	5.0(4.5; 5.5)	0.123
	Pro/Ala и Ala/Ala	5.1 (4.8; 5.5)		Ala	5.1 (4.8; 5.5)	
Body Mass, kg	Pro/Pro	59.7 (52.1; 70.7)	0.442	Pro	59.8 (52.5; 70.8)	0.524
	Pro/Ala и Ala/Ala	61.8 (53.6; 72.1)		Ala	61.8 (53.6; 72.1)	
Body Mass Index, kg/m^2	Pro/Pro	23.3 (20.8; 27.3)	0.279	Pro	23.5 (20.9; 27.5)	0.373
	Pro/Ala и Ala/Ala	24 (21.8; 29.3)		Ala	24 (21.8; 29.3)	
Waist Circumference, cm	Pro/Pro	85.7 (77.7; 96)	0.490	Pro	85.9 (77.6; 96.2)	0.542
	Pro/Ala и Ala/Ala	88.2 (77.3; 96.5)		Ala	88.2 (77.3; 96.5)	
Waist Circumference / Height (cm)	Pro/Pro	0.53 (0.48; 0.62)	0.645	Pro	0.56 (0.47; 0.61)	0.663
		0.56 (0.47; 0.61)		Ala	0.54 (0.48; 0.62)	

Note: p is the achieved level of statistical significance of differences when comparing groups using the Mann–Whitney test; data are presented as median and interquartile range in Me format (Q1; Q3).

This research was carried out within the framework of the basic part of the state assignment of the Ministry of Education and Science of the Russian Federation on the topic of "Genome-Wide Studies of the Gene Pool of the Indigenous Population of the Arctic Coast of Yakutia" (state registration number FSRG-2020-0017).

References

1. Климова Т.М., Федорова В.И., Балтахинова М.Е. Метаболические факторы риска хронических неинфекционных заболеваний у коренного сельского населения Якутии. *Экология человека*. 2013; 2:3-7. [Klimova TM, Fedorova VI, Baltakhinova ME. Metabolic risk factors chronic non-communicable diseases among indigenous rural population of Yakutia. *Ekologiya cheloveka*. 2013; 2: 3-7. (In Russ.].

2. Мкртумян А.М., Бирюкова Е.В., Маркина Н.В. Молекулярно-генетические особенности, характер метаболизма глюкозы и функция эндотелия у больных метаболическим синдромом русской популяции. *Сахарный диабет*. 4; 2008: 26-30. [Mkrtumyan AM, Biryukova EV, Markina NV. Molecular genetic features, the features of glucose metabolism and endothelial function in patients with metabolic syndrome of the Russian population. *Saharnyj diabet*. 2008; 4: 26-30. (In Russ.].

3. Deeb SS, Fajas L, Nemoto M et al. A Pro-12Ala Substitution in pparg2 Associated with Decreased Receptor Activity, Lower Body Mass Index and Improved Insulin Sensitivity. *Nat. Genet.* 1998; 20: 284–7. DOI: 10.1038/3099.

4. Evans D, Wolf AM, Nellessen U et al. Association between Polymorphisms in Candidate Genes and Morbid Obesity. *Int. J. Obes.* 2001; 25: S19–S21. DOI: 10.1038/sj.ijo.0801690.

5. Anke Tönjes, Markus Scholz, Markus Loeffler et.al. Association of Pro12Ala Polymorphism in Peroxisome Proliferator-Activated Receptor γ With Pre-Diabetic Phenotypes. *Diabetes Care*. 2006; 29: 2489–2497.

6. Laura Frederiksen, Kasper Brødbaek, Mogens Fenger et al. Comment: Studies of the Pro12Ala Polymorphism of the PPAR-G Gene in the Danish Monica Cohort Homozygosity of the Ala Allele Confers a Decreased Risk of the Insulin Resistance Syndrome. *J. Clin. Endocri-*

Table 4

Frequency of Metabolic Syndrome and Its Components Depending on the Allelic Variant and Genotypes of Polymorphic Markers of the *PPARγ2* Gene (rs 1801282)

Genotype	MS Component, n (%)		χ^2, p	Allele	MS Component, n (%)		χ^2, p
	Yes	No			Yes	No	
High Blood Pressure							
Pro/Pro	58 (35.2)	107 (64.8)	χ=2.887 p=0.089	Pro	85 (37.9)	139 (62.1)	χ=1.839 p=0.175
Pro/Ala и Ala/Ala	29 (46.5)	32 (52.5)		Ala	29 (47.5)	32 (52.5)	
Hypoalphacholesterolemia							
Pro/Pro	13 (8)	150 (92)	χ=0.141 p=0.707	Pro	19 (8.5)	205 (91.5)	χ=0.067 p=0.796
Pro/Ala и Ala/Ala	6 (9.5)	57 (90.5)		Ala	6 (9.5)	57 (90.5)	
Hypertriglyceridemia							
Pro/Pro	12 (7.4)	151 (92.6)	χ=0.071 p=0.790	Pro	16 (7.1)	208 (92.9)	χ=0.048 p=0.827
Pro/Ala и Ala/Ala	4 (6.3)	59 (93.7)		Ala	4 (6.3)	59 (93.7)	
Fasting Hyperglycemia							
Pro/Pro	38 (23.5)	124 (76.5)	χ=0.071 p=0.790	Pro	50 (22.5)	172 (77.5)	χ=0.000 p=0.992
Pro/Ala и Ala/Ala	14 (22.6)	48 (77.4)		Ala	14 (22.6)	48 (77.4)	
Obesity							
Pro/Pro	31 (19.5)	128 (80.5)	χ=0.597 p=0.440	Pro	45 (20.5)	174 (79.5)	χ=0.382 p=0.536
Pro/Ala и Ala/Ala	15 (24.2)	47 (75.8)		Ala	15 (24.2)	47 (75.8)	
Abdominal Obesity (IDF_1)							
Pro/Pro	86 (54.8)	71 (45.2)	χ=0.931 p=0.335	Pro	123 (56.4)	95 (43.6)	χ=0.602 p=0.438
Pro/Ala и Ala/Ala	39 (61.9)	24 (38.1)		Ala	39 (61.9)	24 (38.1)	
Abdominal Obesity (IDF_2)							
Pro/Pro	90 (57.3)	67 (42.7)	χ=0.707 p=0.400	Pro	128 (58.7)	90 (41.3)	χ=0.464 p=0.496
Pro/Ala и Ala/Ala	40 (63.5)	23 (36.5)		Ala	40 (63.5)	23 (36.5)	
Multiple Metabolic Factors							
Pro/Pro	29 (17.6)	136 (82.4)	χ=0.707 p=0.400	Pro	40 (17.7)	186 (82.3)	χ=0.284 p=0.594
Pro/Ala и Ala/Ala	13 (20.6)	50 (79.4)		Ala	13 (20.6)	50 (79.4)	
Metabolic Syndrome							
Pro/Pro	28 (17.3)	134 (82.7)	χ=0.172 p=0.679	Pro	38 (17.2)	183 (82.8)	χ=0.201 p=0.654
Pro/Ala и Ala/Ala	12 (19.7)	49 (80.3)		Ala	12 (19.7)	49 (80.3)	

Note: χ2* is the Pearson's chi-squared test, p is significance level.

nol. Metab. 2002; 87: 3989–92. DOI: 10.1210/jcem.87.8.8732.

7. Ruyi Zhang, Jiao Wang, Rui Yang et al. Effects of Pro12Ala Polymorphism in Peroxisome Proliferator-Activated Receptor-γ2 Gene on Metabolic Syndrome Risk: A Meta-Analysis. 2014; Feb. 1; 535 (1): 79-87 DOI: 10.1016/j.gene.2013.07.087.

8. Equilibrium Hardy-Weinberg [Online]. [cited 2017 June 15]. Available from: https://wpcalc.com/en/equilibrium-hardy-weinberg/.

9. Paracchini V, Pedotti P, Taioli E. et al. Genetics of Leptin and Obesity: A HuGE Review. *American Journal of epidemiology.* 2005; 2 (162): 101-114. DOI: 10.1093/aje/kwi174.

10. Lin-Lin Li, Xiao-Li Ma, Jian-Xin Ran et al. Genetic Polymorphism of Peroxisome Proliferator-Activated Receptor-g2 Pro12Ala on Ethnic Susceptibility to Diabetes in Uygur, Kazak and Han Subjects. *Clinical and Experimental Pharmacology and Physiology.* 2008; 35: 187–191. DOI: 10.1111/j.1440-1681.2007.04796.x

11. Asli Memisoglu , Frank B Hu, Susan E Hankinson et al. Interaction Between a Peroxisome Proliferator-Activated Receptor G Gene Polymorphism and Dietary Fat Intake in Relation to Body Mass. *Hum Mol Genet*. 2003; 12: 2923–9. DOI: 10.2337/diacare.26.10.2915.

12. International Diabetes Federation: IDF Consensus Worldwide Definition of the Metabolic Syndrome [Article Online], 2006. URL: http://www.idf.org/webdata/docs/IDF_Meta_def_fi nal.pdf (accessed 15 August 2012).

13. Anahita Mansoori, Maryam Amini, Fariba Kolahdooz, Ensiyeh Seyedrezazadeh Obesity and Pro12Ala Polymorphism of Peroxisome Proliferator-Activated Receptor-Gamma Gene in Healthy Adults: A Systematic Review and Meta-Analysis. *Annals Nutrition and Metabolism*. 2015; 67: 104–118. DOI: 10.1159/000439285.

14. Lei HH, Chen MH, Yang WS et al. Peroxisome Proliferator-Activated Receptor G 2 Pro12Ala Gene Variant Is Strongly Associated With Larger Body Mass in the Taiwanese. *Metabolism.* 2000; 49: 1267–70. DOI: 10.1053/meta.2000.9517.

15. Stephanie B Levy, William R Leonard, Larissa A Tarskaia et al. Seasonal and Socioeconomic Influences on Thyroid Function Among the Yakut (Sakha) of Eastern Siberia. *American Journal of Human Biology*. 2013; 25(6): 814-820. DOI: 10.1002/ajhb.22457.

16. Leonard WR, Levy SB, Tarskaia LA et al. Seasonal Variation in Basal Metabolic Rates among the Yakut (Sakha) of Northeastern Siberia. *American Journal of Human Biology.* 2014; 26(4): 437-445. DOI: 10.1002/ajhb.22524.

17. Laura Frederiksen, Kasper Brødbaek, Mogens Fenger et al. Studies of the Pro12Ala Polymorphism of the PPAR-Gene in the Danish Monica Cohort: Homozygosity of the Ala Allele Confers a Decreased Risk of the Insulin Resistance Syndrome. *The Journal of Clinical Endocrinology & Metabolism*. 2002; 87(8): 3989–3992.

18. Hebe N Gouda, Gurdeep S Sagoo, Anne-Helen Harding et.al. The Association Between the Peroxisome Proliferator-Activated Receptor-g2 (PPARG2) Pro12Ala Gene Variant and Type 2 Diabetes Mellitus: A HuGE Review and Meta-Analysis. *American Journal of Epidemiology*. 2010; 6 (171): 645-655. DOI: 10.1093/aje/kwp450.

19. Altshuler D, Hirschhorn JN, Klannemark M et.al. The Common PPAR Gamma Pro12ala Polymorphism Is Associated with Decreased Risk of Type 2 Diabetes. *Nature Genetics*. 2000; Sep; 26(1): 76-80. DOI: 10.1038/79216.

20. Hara K, Okada T, Tobe K et al. The Pro-12Ala polymorphism in PPAR G2 May Confer Resistance to Type 2 Diabetes. *Biochem. Biophys. Res. Commun*. 2000; 271: 212–16. DOI. org/10.1006/bbrc.2000.2605.

21. Yue Qi, Jing Liu, Wei Wang et al. The Hind III Polymorphism in the Lipoprotein Lipase Gene Predicts Type 2 Diabetes Risk Among Chinese Adults. *Clinica Chimica Acta*. 2011; №13-14 (412): 1229-1233.

22. Zhijun Wu, Yuqing Lou, Wei Jin et al. The Pro12Ala Polymorphism in the Peroxisome Proliferator-Activated Receptor Gamma-2 Gene (PPARγ2) Is Associated with Increased Risk of Coronary Artery Disease: A Meta-Analysis. PLoS ONE. 2012; 7 (12): DOI:10.1371/journal.pone.0053105.

S.A.Galkin, O.V. Roshchina, A.G. Peshkovskaya

EFFECT OF COMORBID DEPRESSIVE DISORDER ON WORKING MEMORY AND FUNCTIONAL BRAIN ACTIVITY IN ALCOHOL-DEPENDENT PATIENTS

DOI 10.25789/YMJ.2020.71.29

УДК 616.89-008

Objective: to study changes in the activity of the cerebral cortex during the task on working memory in alcohol-dependent patients with and without comorbid depressive disorder.

Materials and methods: 97 patients were examined before treatment: 67 patients with alcohol dependence and 30 patients with comorbidity, alcohol dependence and depression. EEG was registered at rest, as well as when performing a cognitive test for working memory (Corsi test).

Results: lower values of working memory volume were found in alcohol-dependent patients with comorbid depressive disorder compared to patients suffering only from alcohol dependence. In patients with comorbidity of alcohol dependence and depressive disorder, only beta activity in the posterior cortex changes during the task on working memory.

Conclusion: the presence of comorbid depressive disorder in people with alcohol dependence leads to a significant decrease in the volume of working memory, which is accompanied by differences in the activation of the cortex.

Keywords: depression, alcohol dependence, working memory, electroencephalography.

Introduction. The impact of chronic alcohol use on the brain has long been a subject of increased interest. Failure to control alcohol use, repeated relapse, and continued alcohol use, despite negative physical, psychological, and social consequences, are not only diagnostic criteria for the disorder, but may also indicate impaired ability to make rational decisions [5]. This is partly due to the fact that patients with alcohol dependence have disorders in a wide range of cognitive domains [6].

Previous studies have reported working memory deficits in patients with alcohol dependence [12, 14]. Working memory is one of the higher mental functions that provides temporary storage and management of information, as well as influences other cognitive functions, such as inhibitory control and goal-oriented behavior [2]. As shown by neuroimaging studies, the prefrontal and parietal cortex are involved in the functioning of working memory. For example, when performing a task on working memory, there is an increase in activity in the dorsolateral prefrontal cortex, posterior parietal cortex, and cerebellum in a healthy control group [17].

One of the factors contributing to the development of cognitive disorders in alcohol dependence is depression [9]. It is known that depressive disorders are quite often observed in patients with alcohol dependence. Many studies have shown that 15-28% of all alcohol-dependent patients have comorbid mood disorders [4]. However, the potential cumulative effects of alcoholism and depression on the cognitive level have not yet been studied in detail. Many studies have shown that the onset of depression is associated with a decrease in the number of neurons in the hippocampus [18]. This area of the brain is important for normal learning and memory [10]. Thus, it is assumed that the presence of comorbid depressive disorder in alcohol-dependent patients significantly impairs the functioning of working memory, which undoubtedly affects both the course and remission of alcohol dependence.

Most previous research has focused on identifying structural abnormalities in the brain in patients with various mental disorders, including alcohol dependence and depression. However, neuroanatomic changes can not fully explain the violations of various cognitive functions. Currently, quantitative electroencephalography (EEG) methods are increasingly used to assess neurophysiological changes in mental disorders. The technical features of this technique allow us to detect patterns of cortical activation with high time resolution, both at rest and during various tasks. The cortex is known to play a key role in complex cognitive functions such as memory, attention, thinking, and so on [15]. Since the diagnostic criteria for alcohol dependence and depression include cognitive deficits, more and more researchers are seeking to identify the nature of this deficits and investigate the brain structures involved in these disorders.

The aim of the study was to study changes in the activity of the cerebral cortex during the task on working memory in alcohol-dependent patients with and without comorbid depressive disorder.

Materials and methods of research. The study was conducted in accordance with the principles of the Helsinki Declaration of the world medical Association. All patients gave written informed consent to participate in the study and data processing, which was approved by the local ethics Committee at the research Institute of Mental health (Protocol No. 114).

On the basis of the 4th Department (Department of addictive conditions) of the clinic of the research Institute of mental health of the Tomsk nimc, 97 patients were examined before treatment: 67 patients with an established diagnosis of alcohol dependence (F10.2 according to ICD-10) and 30 patients with comorbidity of alcohol dependence and affective disorder: dysthymia (F34.1-43.4%), bipolar affective disorder (F31.3 - 30%), depressive episode (F32.0-1 - 13.3%) or recurrent depressive episode (F33.0-1 - 13.3%) (Table 1).

Based on the literature data on various profiles of electrophysiological indicators in right-handed and left-handed people, the group of study participants was selected right-handed according to the questionnaire of lateral signs. Diagnostic evaluation and clinical qualification of disorders was performed using diagnostic criteria of ICD-10 and a set of standardized psychometric tools. Inclusion criteria: established diagnosis of alcohol dependence and / or comorbidity of alcohol dependence and affective disorder according to ICD-10, age 20-55 years, voluntary consent to participate in the study. Exclusion criteria: the presence

GALKIN Stanislav A. – PhD Student, Mental Health Research Institute, Tomsk NRMC. ORCID iD 0000-0002-7709-3917.**ROSHCHINA Olga V.** – Researcher, Mental Health Research Institute, Tomsk NRMC. ORCID iD 0000-0002-2246-7045.**PESHKOVSKAYA Anastasia G.** – Researcher, Mental Health Research Institute, Tomsk NRMC. ORCID iD 0000-0002-3951-395X.

of chronic somatic diseases in the acute stage, severe organic brain disorders, traumatic brain injuries of any severity, mental retardation, epilepsy, pregnancy, refusal to study.

EEG was recorded using a computer electroencephalograph "Neuropolicy". The electrodes were arranged in accordance with the international system 10-20 (Fig. 1). The combined electrodes located on the ear lobes (A1 and A2) were used as a reference, and the grounding electrode was used in the Fpz lead. The resistance of the electrodes did not exceed 10 kOm. The parameters of the LVF and LVF were 0.5 Hz and 30 Hz, respectively, and the quantization frequency was 250 Hz. EEG was recorded (background recording) with the eyes open for at least 2 minutes, as well as when the subjects performed a cognitive test. Artifact correction was performed by zeroing the independent EEG components. In addition, the automatic procedure excluded from further analysis epochs in which excessively large potentials (more than 100 mV), high-amplitude slow oscillations in the range from 0.53 to 1 Hz (amplitude greater than 50 mV) or high-amplitude fluctuations in the range from 20 to 30 Hz (amplitude greater than 35 mV). The signals were processed using fast Fourier transform, and the values of absolute spectral power (mV2) θ - (4 - 7 Hz), α - (8 - 13 Hz) and β - (14 - 30 Hz) rhythms were analyzed. The duration of the analyzed EEG section was equal to the duration of the entire epoch (sample).

The working memory level was evaluated using the Corsi block-Tapping test. On the computer screen, 9 cubes appear, which in turn light up in yellow. The task of the research participant is to remember and reproduce this sequence. The sample begins with a sequence of two cubes, and if the answer is correct, the length of the sequence increases. The test is terminated if two consecutive erroneous reproductions of the sequence occur.

Statistical data processing was performed using the Statistica 10 program. Data is presented as Median [Q1; Q3]. Verification of agreement with the normal distribution law was performed using the Shapiro - Wilk test. The obtained data did not follow the normal distribution law. The nonparametric Mann-Whitney U-test was used to evaluate differences between two independent samples (patients with alcohol dependence versus patients with comorbidity) and the Wilcoxon W-test to evaluate differences between dependent samples (background versus test). The differences were considered statistically significant at a significance level of $p<0.05$.

Results. The clinical characteristics of the studied groups of patients are presented in Table.

Data analysis showed statistically significantly higher values on the cgi-s scale (p=0.00063) and HDRS (p=0.00041) in patients with comorbidity of alcohol dependence and affective disorder compared to patients suffering only from alcohol dependence.

Table 1

Gender and age characteristics of the study sample

Group	Gender				Age Me [Q1; Q3]
	men		women		
	%	abs.	%	abs.	
Patients with alcohol dependence (n=67)	73.1	49	26.9	18	45 [37; 50]
Patients with comorbidity (n=30)	63.3	19	36.7	11	46 [39; 49]

Analysis of the Corsi test results showed statistically significantly lower values of working memory in alcohol-dependent patients with comorbid depressive disorder compared to patients suffering only from alcohol dependence (4 [4; 5] vs. 5 [4; 6], p = 0.028). The dynamics of changes in the bioelectric activity of the brain in the studied groups of patients is shown in Fig. 2.

Statistical analysis of EEG data revealed a statistically significant decrease in the spectral power of the alpha rhythm in the Central (p=0.0001), parietal (p=0.0002) and left temporal cortex (p=0.0015) during the task on working memory in patients suffering only from alcohol dependence. In alcohol-dependent patients with comorbid depressive disorder, no statistically significant changes in the alpha rhythm were found (p>0.05).

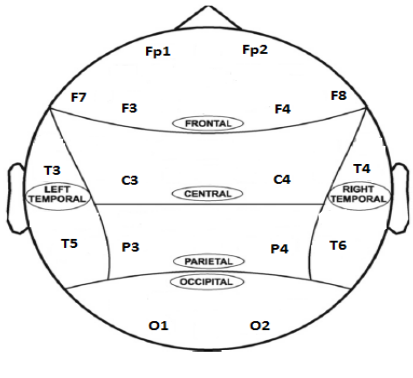


Fig. 1. Scheme of the EEG

When analyzing changes in the spectral power of the beta rhythm, a statistically significant increase in beta activity was found when performing the task on working memory in the occipital (p=0.0001 and p=0.0012), right (p=0.0001 and p=0.0003) and left (p=0.0007 and p=0.004) temporal cortex in patients with alcohol dependence and patients with comorbidity of alcoholism and depression.

Statistically significant changes in the spectral power of the theta rhythm during the cognitive test were found only in the group of patients with alcohol dependence. Data analysis revealed a statistically significant increase in theta power in the task for working memory in the frontal cortex (p=0.0035) in alcohol-dependent patients.

Discussion. Thus, our study evaluated the effect of comorbid depressive disorder in patients with alcohol dependence on the level of working memory and brain activity during the performance of a cognitive task.

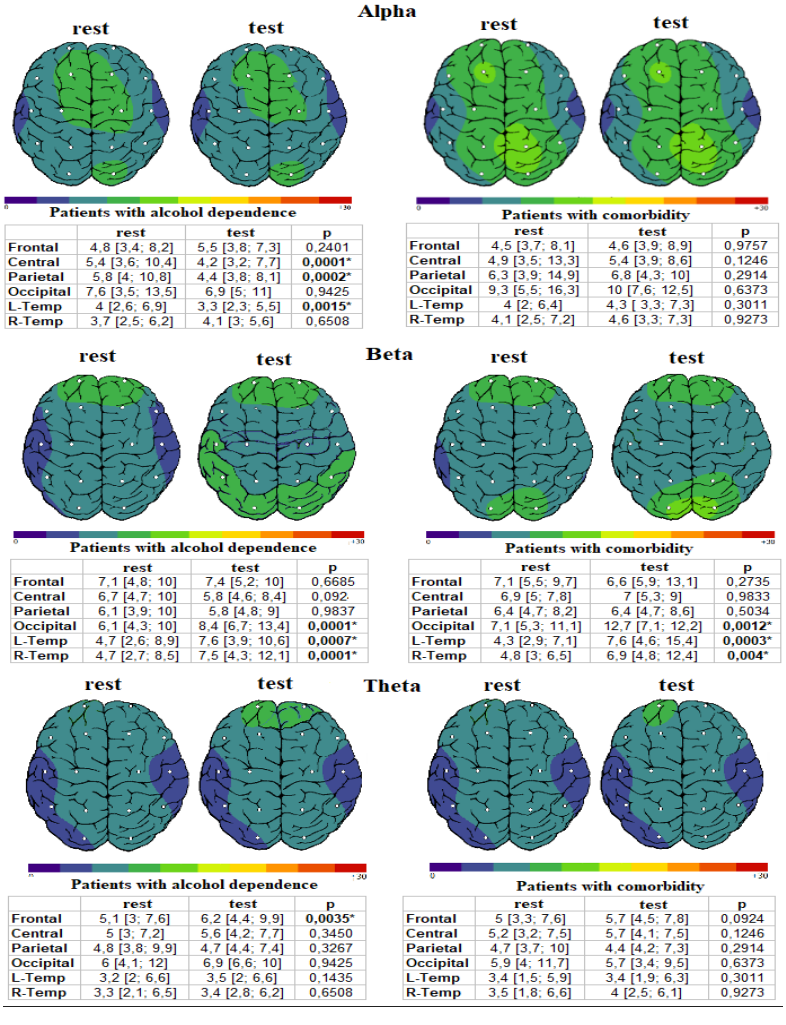
As many studies show, people with alcohol dependence show a deficit of working memory, while a decrease in working memory was observed even during long-term remission [7]. Similar results were obtained in studies on patients with depressive disorders [3, 13]. A number of authors have found violations of many cognitive functions, including attention, information processing speed, memory and Executive functions in the presence of concomitant affective disorder in alcohol-dependent patients [9]. However, some researchers indicate that the presence of depressive symptoms in alcohol-dependent individuals did not significantly affect cognitive functions, including working memory [11]. Our data also showed lower values of working memory in alcohol-dependent patients with concomitant depressive disorder compared to patients suffering only from alcohol dependence. Currently, understanding the neural basis of working memory functioning remains an urgent task. A number of researchers have found a deterioration in working memory in people with damage to the prefrontal cortex [20]. Earlier studies have shown that neurons in the posterior parietal cortex also provide working memory functions [22]. The balance of neurotransmitters in the functioning of the brain is also important [1]. It has been shown that a deficit of dopamine and

Table 2

Clinical features of the study sample

Prescription	Patients with alcohol dependence	Patients with comorbidity	Significance level p
prescription of alcohol dependence (years)	15 [6; 22]	13 [5; 20]	0.131
age of depressive disorder (years)	-	5 [2; 10]	-
AUDIT	28 [22; 34]	26 [20; 29]	0.097
CGI-s	4 [4; 5]	5 [5; 6]	0.00063*
HDRS	3 [2; 5]	12 [10; 15]	0.00041*

Note. p - level of statistical significance when comparing groups using the Mann-Whitney U-test



Patients with alcohol dependence

	rest	test	p
Frontal	4,8 [3,4; 8,2]	5,5 [3,8; 7,3]	0,2401
Central	5,4 [3,6; 10,4]	4,2 [3,2; 7,7]	**0,0001***
Parietal	5,8 [4; 10,8]	4,4 [3,8; 8,1]	**0,0002***
Occipital	7,6 [3,5; 13,5]	6,9 [5; 11]	0,9425
L-Temp	4 [2,6; 6,9]	3,3 [2,3; 5,5]	**0,0015***
R-Temp	3,7 [2,5; 6,2]	4,1 [3; 5,6]	0,6508

Patients with comorbidity

	rest	test	p
Frontal	4,5 [3,7; 8,1]	4,6 [3,9; 8,9]	0,9757
Central	4,9 [3,5; 13,3]	5,4 [3,9; 8,6]	0,1246
Parietal	6,3 [3,9; 14,9]	6,8 [4,3; 10]	0,2914
Occipital	9,3 [5,5; 16,3]	10 [7,6; 12,5]	0,6373
L-Temp	4 [2; 6,4]	4,3 [3,3; 7,3]	0,3011
R-Temp	4,1 [2,5; 7,2]	4,6 [3,3; 7,3]	0,9273

Patients with alcohol dependence

	rest	test	p
Frontal	7,1 [4,8; 10]	7,4 [5,2; 10]	0,6685
Central	6,7 [4,7; 10]	5,8 [4,6; 8,4]	0,092
Parietal	6,1 [3,9; 10]	5,8 [4,8; 9]	0,9837
Occipital	6,1 [4,3; 10]	8,4 [6,7; 13,4]	**0,0001***
L-Temp	4,7 [2,6; 8,9]	7,6 [3,9; 10,6]	**0,0007***
R-Temp	4,7 [2,7; 8,5]	7,5 [4,3; 12,1]	**0,0001***

Patients with comorbidity

	rest	test	p
Frontal	7,1 [5,5; 9,7]	6,6 [5,9; 13,1]	0,2735
Central	6,9 [5; 7,8]	7 [5,3; 9]	0,9833
Parietal	6,4 [4,7; 8,2]	6,4 [4,7; 8,6]	0,5034
Occipital	7,1 [5,3; 11,1]	12,7 [7,1; 12,2]	**0,0012***
L-Temp	4,3 [2,9; 7,1]	7,6 [4,6; 15,4]	**0,0003***
R-Temp	4,8 [3; 6,5]	6,9 [4,8; 12,4]	**0,004***

Patients with alcohol dependence

	rest	test	p
Frontal	5,1 [3; 7,6]	6,2 [4,4; 9,9]	**0,0035***
Central	5 [3; 7,2]	5,6 [4,2; 7,7]	0,3450
Parietal	4,8 [3,8; 9,9]	4,7 [4,4; 7,4]	0,3267
Occipital	6 [4,1; 12]	6,9 [6,6; 10]	0,9425
L-Temp	3,2 [2; 6,6]	3,5 [2; 6,6]	0,1435
R-Temp	3,3 [2,1; 6,5]	3,4 [2,8; 6,2]	0,6508

Patients with comorbidity

	rest	test	p
Frontal	5 [3,3; 7,6]	5,7 [4,5; 7,8]	0,0924
Central	5,2 [3,2; 7,5]	5,7 [4,1; 7,5]	0,1246
Parietal	4,7 [3,7; 10]	4,4 [4,2; 7,3]	0,2914
Occipital	5,9 [4; 11,7]	5,7 [3,4; 9,5]	0,6373
L-Temp	3,4 [1,5; 5,9]	3,4 [1,9; 6,3]	0,3011
R-Temp	3,5 [1,8; 6,6]	4 [2,5; 6,1]	0,9273

Fig. 2. Spectral power of EEG rhythms. Note. p - level of statistical significance when comparing groups using the Wilcoxon W-test

norepinephrine significantly reduces the activity of the prefrontal cortex and, as a result, leads to a decrease in working memory [23].

It is assumed that the activation of neurons involved in the functioning of working memory is carried out in the theta rhythm range [16]. Many authors have found an increase in the power of the theta rhythm during the task on working memory [8, 16]. We also obtained a significant increase in theta power in the frontal cortex during the task on working memory, however, these changes were observed only in the group of alcohol-dependent patients without concomitant depressive symptoms. We suggest that the absence of significant changes in theta activity during the test in patients with comorbid alcohol dependence and depression may be a consequence of impaired functioning of neurons that provide working memory functions. However, in the group of alcohol-dependent patients with depressive disorders, we found no changes in the spectral power of the alpha rhythm, which indicates a decrease in the degree of cortical activation on the cognitive stimulus. Changes in high-frequency activity (beta rhythm) in both groups of patients during the task on working memory are accompanied by an increase in beta power in the posterior cortex. Many researchers associate the activity of the posterior cortex with certain executive functions that include not only working memory, but also attention [19]. It was also found that the function of storing information is provided in the posterior regions of the brain, and retrieval is provided in the prefrontal cortex [19, 21]. Thus, it can be assumed that the presence of depressive symptoms in alcohol-dependent patients leads to a violation of activity in the prefrontal cortex (the absence of changes in the theta rhythm in response to the activation of working memory) while maintaining the functioning of the posterior cortex. In turn, this can affect the amount of working memory in the form of impaired information retrieval, while maintaining the ability to remember and store data.

Conclusion. Thus, our study expands the available data on the impact of depressive disorder on cognitive function in individuals with alcohol dependence. The presence of comorbidity, depression and alcohol dependence leads to more severe violations of the function of working memory, which is accompanied by neurophysiological changes in the cerebral cortex. The results obtained do not claim to be pathogenetic markers, and their application in clinical practice is unlikely to be possible at the present time. However, our findings highlight the importance of research on cognitive functions, including working memory, in psychiatry and addiction medicine. In addition, we believe that further research is needed to identify therapeutic targets for improving the treatment of these disorders. Since it is known that the presence of depressive disorders in people with alcohol dependence significantly increases therapeutic resistance to traditional methods of treatment, as well as increases the risk of relapse. Effects on cognitive function can overcome therapeutic resistance and/or reduce the risk of relapse.

The study was carried out with the financial support of the Administration of

the Tomsk Region and the Russian Foundation for Basic Research No. 19-413-7.

References

1. Ветрилэ Л.А., Невидимова Т.И., Давыдова Т.В., Захарова И.А., Савочкина Д.Н., Галкин С.А., Бохан Н.А. Аутоантитела к нейромедиаторам дофамину, норадреналину, серотонину, глутамату и ГАМК при коморбидном течении депрессии и алкогольной зависимости. *Патологическая физиология и экспериментальная терапия*. 2019; (4):5-12. [Vetrile LA, Nevidimova TI, Davydova TV, Zakharova IA, Savochkina DN, Galkin SA, Bohan NA. Autoantibodies to the neurotransmitters dopamine, noradrenaline, serotonin, glutamate and GABA in the comorbid course of depression and alcohol dependence. *Patologicheskaja fiziologija i jeksperimental'naja terapija*. 2019; (4):5-12. (In Russ.).]

2. Ларионова Е.В. Частота спектрального пика альфа-активности у лиц, ранее употреблявших каннабиноиды, в состоянии спокойного бодрствования и при выполнении арифметического задания. *Журнал высшей нервной деятельности*. 2016; 6:690-697. [Larionova EV. Frequency of the spectral peak of alpha activity in individuals who previously used cannabinoids, in a state of calm wakefulness and when performing an arithmetic task. *Zhurnal vysshej nervnoj dejatel'nosti*. 2016; 6:690-697. (In Russ.).]

3. Галкин С.А., Пешковская А.Г., Симуткин Г.Г., Васильева С.Н., Рощина О.В., Иванова С.А., Бохан Н.А. Нарушения функции пространственной рабочей памяти при депрессии легкой степени тяжести и их нейрофизиологические корреляты. *Журнал неврологии и психиатрии им. С.С. Корсакова.* 2019; (10):56-61. [Galkin SA, Peshkovskaya AG, Simutkin GG, Vasilieva SN, Roshchina OV, Ivanova SA, Bohan NA. Violations of the function of spatial working memory in mild depression and their neurophysiological correlates. Zhurnal *nevrologii i psihiatrii im. S.S. Korsakova*. 2019; 10:56-61. (In Russ.).]

4. Понизовский П.А., Гофман А.Г. Депрессия у больных с алкогольной зависимостью. *Журнал неврологии и психиатрии им. С.С. Корсакова*. 2015; (7):146-150. [Ponizovsky PA, Hoffman A. G. Depression in patients with alcohol dependence. Zhurnal *nevrologii i psihiatrii im. S.S. Korsakova*. 2015; (7):146-150. (In Russ.).]

5. Шушпанова Т.В., Мандель А.И., Бохан Н.А., Бадыргы И.О., Новожеева Т.П., Счастный Е.Д., Солонский А.В., Грущенко Н.Ф., Удут В.В., Шушпанова О.В., Маркова Е.В., Князева Е.М. Роль нейроэндокринных факторов в формировании алкогольной зависимости и экологии человека в различных этнических популяциях: новые подходы терапии. *Якутский медицинский журнал*. 2019; (4):113-118. [Shushpanova TV, Mandel AI, Bokhan NA, Badyrgy IO, Novozheeva TP, Schastny ED, Solonsky AV, Grushchenko NF, Udut VV, Shushpanova OV, Markova EV, Knyazeva EM. the Role of neuroendocrine factors in the formation of alcohol dependence and human ecology in various ethnic populations: new approaches to therapy. *Jakutskij medicinskij zhurnal*. 2019; (4):113-118. (In Russ.).]

6. Sachdeva A, Chandra M, Choudhary M, Dayal P, Anand KS. Alcohol-Related Dementia and Neurocognitive Impairment: A Review Study. *Int. J. High. Risk. Behav. Addict*. 2016; (3):e27976. doi: 10.5812/ijhrba.27976.

7. Bates ME, Buckman JF, Nguyen TT. A role for cognitive rehabilitation in increasing the effectiveness of treatment for alcohol use disorders. *Neuropsychol. Rev.* 2013; (1):27-47. doi: 10.1007/s11065-013-9228-3

8. Berger B, Griesmayr B, Minarik T. Dynamic regulation of interregional cortical communication by slow brain oscillations during working memory. *Nat. Commun.* 2019; (1):4242. doi: 10.1038/s41467-019-12057-0

9. Bernardin F, Maheut-Bosser A, Paille F. Cognitive impairments in alcohol-dependent subjects. *Front. Psychiatry*. 2014; 5:78. doi:10.3389/fpsyt.2014.00078

10. Sawyer K, Corsentino E, Sachs-Ericsson N, Steffens DC. Depression, hippocampal volume changes, and cognitive decline in a clinical sample of older depressed outpatients and non-depressed controls. *Aging. Ment. Health*. 2012; (6):753-762. doi: 10.1080/13607863.2012.678478

11. Uekermann J, Daum I, Schlebusch P, Wiebel B, Trenckmann U. Depression and cognitive functioning in alcoholism. *Addiction.* 2003; (11):1521-1529. doi: 10.1046/j.1360-0443.2003.00526.x

12. Lechner WV, Day AM, Metrik J, Leventhal AM, Kahler CW. Effects of alcohol-induced working memory decline on alcohol consumption and adverse consequences of use. *Psychopharmacology (Berl).* 2016; (1):83-88. doi: 10.1007/s00213-015-4090-z

13. Li M, Feng L, Liu X, Zhang M, Fu B, Wang G, Lu S, Zhong N, Hu B. Emotional working memory in patients with major depressive disorder. *Journal of International Medical Research.* 2018; (5):1734-1746. doi: 10.1177/0300060518758225

14. Day AM, Kahler CW, Ahern DC, Clark US. Executive functioning in alcohol use studies: A brief review of findings and challenges in assessment. *Current Drug. Abuse Reviews*. 2015; 8:26-40.

15. Funahashi S. Working Memory in the Prefrontal Cortex. *Brain Sci.* 2017; (5):49. doi: 10.3390/brainsci7050049

16. Hsieh LT, Ranganath C. Frontal midline theta oscillations during working memory maintenance and episodic encoding and retrieval. *Neuroimage.* 2014; 85:721-729. doi: 10.1016/j.neuroimage.2013.08.003

17. Loeb FF, Zhou X, Craddock K. Reduced Functional Brain Activation and Connectivity During a Working Memory Task in Childhood-Onset Schizophrenia. *J. Am. Acad. Child. Adolesc. Psychiatry*. 2018; (3):166-174. doi:10.1016/j.jaac.2017.12.009

18. Mechawar N, Savitz, J. Neuropathology of mood disorders: do we see the stigmata of inflammation? *Transl. Psychiatry*. 2016; 6: e946. doi: 10.1038/tp.2016.212

19. Eriksson J, Vogel EK, Lansner A, Bergström F, Nyberg L. Neurocognitive Architecture of Working Memory. *Neuron*. 2015; (1):33-46. doi: 10.1016/j.neuron.2015.09.020

20. Petruo VA, Mückschel M, Beste C. On the role of the prefrontal cortex in fatigue effects on cognitive flexibility - a system neurophysiological approach. *Sci. Rep.* 2018; 8:6395. doi: 10.1038/s41598-018-24834-w

21. Preston AR, Eichenbaum H. Interplay of hippocampus and prefrontal cortex in memory. *Curr. Biol.* 2013; (17):764-773. doi: 10.1016/j.cub.2013.05.041

22. Rawley JB, Constantinidis C. Neural correlates of learning and working memory in the primate posterior parietal cortex. *Neurobiol. Learn. Mem*. 2009; (2):129-138. doi: 10.1016/j.nlm.2008.12.006

23. Xing B, Li YC, Gao WJ. Norepinephrine versus dopamine and their interaction in modulating synaptic function in the prefrontal cortex. *Brain Res.* 2016; 1641:217-233. doi: 10.1016/j.brainres.2016.01.005

EXPERIENCE EXCHANGE

Y.I. Khabarova, Z.N. Alekseeva, T.K. Davidova, O.V. Tatarinova

ANALYSIS OF THE COGNITIVE IMPAIRMENT OFFICE OF THE NEURODEGENERATIVE DISEASES CENTER OF THE CLINIC OF THE YAKUT SCIENCE CENTRE OF COMPLEX MEDICAL PROBLEMS ACTIVITY

DOI 10.25789/YMJ.2020.71.30

УДК 616.831

Abstract. The article analyzes the data of a study of 114 patients who applied for an initial consultation to the Cognitive impairment office of the Neurodegenerative Diseases Center of the Clinic of the Yakut Science Centre of complex medical problems in 2018-2019. The age of patients varied from 24 to 90 years. Structure of cognitive impairment assessed by Montreal Cognitive Assessment (MoCA) and the modified Hachinski ischemic scale. A correlation between cognitive impairment with age and educational level was established. Various neurodegenerative diseases have been diagnosed.

Keywords: cognitive impairment, cognitive impairment office, neurodegenerative diseases, MoCA, visual-constructive sphere, delayed reproduction.

Introduction: The prevalence of cognitive impairment (CI) with no dementia reaches 15–20%. It has been shown that with aging, the incidence rate of CI progressively increases: from 20% in people 60–69 years old to more than 40% in the group with age over 85 years old [2].

Neurodegenerative diseases (NDD) are a heterogeneous group of chronic fatal diseases of the nervous system, which are characterized by progressive death of brain neurons. The most common NDDs are Alzheimer's disease (AD) and Parkinson's disease (PD). Currently, there are more than 45 million people around the world, principally the elderly who suffer from various types of NDD. This makes NDD the most important medical and social problem in society [5].

KHABAROVA Yuliya Ilinichna – neurologist, head of neurological department, Federal state non-budget scientific institution hospital YSC CMP, junior researcher of Laboratory of neurodegenerative diseases, Yakut Science Centre of Complex Medical Problems, 677010, Yakutsk, Sergelyakhskoe Highway, 4 km. E-mail: september062007@mail.ru; **ALEKSEEVA Zinaida Nikolaevna** – clinical psychologist, junior researcher Laboratory of neurodegenerative diseases, Yakut Science Centre of Complex Medical Problems, 677010, Yakutsk, Sergelyakhskoe Highway, 4 km. E-mail: gzinaida@mail.ru; **DAVYDOVA Tatyana Kimovna** – PhD, Head of the Center of Neurodegenerative Diseases of the 677010, Yakutsk, Sergelyakhskoe Highway, 4 km. E-mail: tanya.davydova.56@inbox.ru: **TATARINOVA Olga Viktorovna** – MD, PhD, Deputy Head, Republican Hospital №3, Leading Researcher of Laboratory of clinical-population and medico-social researches, Yakut Science Centre of Complex Medical Problems, 677010, Yakutsk, Sergelyakhskoye Highway, 4 km. E-mail: tov3568@mail.

In Russia, the problem of NDD is very relevant, and in particular, this applies to those diseases that are accompanied by CI. According to the State Statistics Committee, in 2007 about 20 million elder people lived in Russia. Based on the age and gender structure of our population, average prevalence rates (about 6-7% of the elderly population) and the structure of dementia in most European countries, the estimated number of patients with dementia in our country is 1.5 million, including about 1 million patients with Alzheimer's disease [4].

Now this necessary to search for new pathogenetic approaches to the treatment of NDD using the latest achievements in biomedical sciences. Early diagnosis of NDD is crucial in there. Specific markers of the disease in peripheral tissues in NDD now actively researching but so far, no such method has been introduced into clinical practice [5].

One of the provisions for solving this problem is the early identification of patients with CI at the primary care level (outpatient clinics). At the same time, it is very difficult for a neurologist to establish a diagnosis of neurodegenerative disease with CI: this requires a thorough examination, collection of medical history, usage of neuropsychological scales, interpretation of the results of neuroimaging and other medical information. Thus, patients with CI can receive sufficient primary medical care in specialized CI offices organized in outpatient clinics.

The CI office of the Neurodegenerative Diseases Center of the Clinic of the Yakut Science Centre of complex medical problems (YSC CMP) has been operating since 2018. The purposes of this office are to identify pre-dementive CI in neurodegenerative, autoimmune and other diseases in people over 18 years of age and correction with the help of pharmacological and non-pharmacological treatment (cognitive training) in the conditions of this office or their further direction if there are indications for the hospital phase of treatment.

The objective of the study: To identify the degree of CI and its structure in primary and secondary dementing neurodegenerative diseases.

Materials and research methods: The material of the study is the data of medical outpatient records of 114 patients who applied for an initial consultation to the office of cognitive disorders for the period 2018-2019, at the age of 24 to 90 years.

To analyze the structure of CI, we used data on individual Montreal Cognitive Assessment (MoCA) neuropsychological testing domains: visual-constructive and executive sphere, naming, attention, speech, abstraction, delayed reproduction, orientation. Also, we used the Modified Hachinski Ischemic Scale (Modified Hachinski Ischemic Scale), which applicable for vascular CI;

2. Somatic and neurological examination, neuroimaging by magnetic resonance imaging (MRI) [3];

3. For statistical processing of the research results, the software package “IBM SPSS Statistics 23” was used. Based on the mismatch of the Gaussian distribution by the normal Kolmogorov-Smirnov test, we calculated the median (Me), quartile (Q1, Q3) indicators, used the nonparametric comparison method - Mann-Whitney criterion, and Spearman rank correlation analysis. The statistical significance was taken at $p < 0.05$

Inclusion criteria:

1. Persons over 18 years of age;
2. A subjective and objective decrease in cognitive functions;

Exclusion criteria:

1. Persons under the age of 18;
2. Absence of cognitive decline;
3. Presence of physical restrictions that impede the usage of neuropsychological scales;

Results and discussion: Before the study, all patients filled out consent to the processing of personal data and informed consent to the study.

A study of the medical records of 114 patients in the CI office demonstrated that the number of women (n = 70; 61.4%) was more than men (n = 44; 38.6%). The total average age was 65.2 ± 13.2 years. The average age of individuals without CI was 59.3 ± 15.8 years, with CI - 66.5 ± 12.3. According to the results of the examination in 82.5% of patients presence of CI has been noted, 3.5% revealed a late stage of CI (dementia) and were referred to a psychiatrist, and 17.5% were within the age limits. The median value according to the results of the MoCA - 21 points, which indicates a significant decrease in cognitive functions and indicates a delayed visit for consultative help to a neuropsychologist or neurologist. A similar problem and the percentage of detection of CI in about 90% of patients were noted in the reports of such cognitive disorder offices [7,8].

In ethnic composition representatives of indigenous nationality prevailed (71.1%). The percentage of Caucasians was 28.9%. The average age of women - 66.1 ± 12.6 years, of men – 63.8 ± 14.1 years. Most patients were aged 60 and over and accounted for 73.7% of the total, the remaining 26.3% were in the range of 24 to 59 years. The youngest patient at the age of 24 who suffered from post-traumatic encephalopathy complaint about a subjective decrease in memory, which was not confirmed by the examination. Most of the patients live in the city of Yakutsk (82.5%) and 17.5% lived in other regions of the republic. One patient was left-handed (n = 1), all other was right-handed (n = 113). Analysis of educational status showed that people with higher education amounted to 57.9%, with secondary special - 22.8%, secondary - 13.2%, and lower than average - 6.1% of patients. The percentage of patients who studied in Sakha-speaking and Russian-speaking schools was almost equal: 49.1% and 50.9, respectively. The low level of education was associated with low indicators of the overall score of CI by the MoCA test (p = 0.007). In the structure of CI, the degree of education was most significantly correlated with low rates in the domains of visual-spatial and executive skills (p = 0.008), speech (p = 0.001), and abstraction (p = 0.004). A low level of education prevailed among the rural population, which affected the severity of the CI in the latter population compared with urban residents (p = 0.025), who mostly had an education higher than secondary special.

We also evaluated the structure of CI based on the performing of the MoCA test in each age group (Table 1).

Table 1 shows that there was an age-specific decline in cognitive functions. All median values are lower than MoCA normal (N≥26). During the assessment of the cognitive defect structure, this is obvious that functions "attention" and "orientation" remain relatively preserved up to 80 years. The most preserved function is "naming" which indicates the relatively enough function of visual gnosis in elder age. According to our data, speech function is significantly reduced starting with 70 years. The function of delayed reproduction which reflects long-term memory begins to decline from the age of 50. Also, the study showed a correlation between low MoCA test scores with age (p=0.009). Decline of speech functions (p=0.009), delayed reproduction (p=0.016) and orientation (p=0.038) was noted with increasing age.

By the references, the average score for the MoCA test is very variable depending on age and level of education. According to sources, the average values on the MoCA test were higher than those we identified [6,9,12], which is explained that patients of CI office requested with significant memory impairment. Also, the study of domain-specific features is very rare in the literature. Nevertheless, it is showed that the "orientation" domain is enough feasible, and its deterioration indicates the severity of CI, and the "memory" and "executive functions" domains are more difficult to perform, which consistent with our data [22]

Statistical processing showed that the differences by age and gender were insignificant (p = 0.393) and we compared and studied gender differences in performing MoCA domains (Table 2).

As shown in Table 2, significant gender differences were noted only for the "speech" domain, where the rate was higher for women than for men (p = 0.036). Also, in the analysis of the relationship between the results of MoCA and the education level among women, a correlation between the low level of education and decline in "speech" (p = 0.013) and "naming" (p = 0.048) domains were recognized. Among men, significant correlations with educational levels were not found. Obtained information could be associated with the prevalence of wom-

Table 1

Age differences in MoCA test domains performing (Me (Q1;Q3))

Age (years)	24-40 лет (n=7)	41-50 лет (n=9)	51-60 лет(n=14)	61-70 лет(n=35)	71-80 лет(n=39)	81-90 лет(n=10)
MoCA score	25 (24;26)	24 (17.5;26.5)	22.5 (17.75;28)	22 (17;25)	20 (15;24)	20 (18.5; 23.25)
Domains (Me (Q1;Q3))						
Visual-constructive and executive skills	4 (3;5)	4 (1;5)	4.5 (3;5)	4 (2;5)	4 (2;5)	4 (3.5)
Naming	3 (3;3)	3 (3;3)	3 (3;3)	3 (3;3)	3 (3;3)	3 (3;3)
Attention	6 (5;6)	6 (5.5;6)	6 (4.75;6)	6 (5;6)	6 (4;6)	5.5 (5;6)
Speech	2 (2;3)	2 (0;3)	2 (1.75;3)	2 (1;3)	1 (1;2)	1 (0.75;2.25)
Abstraction	1 (0;1)	0 (0;1.5)	1 (0;1.25)	0 (0;1)	0 (0;1)	0.5 (0;1)
Delayed reproduction	3 (2;4)	3 (0;4.5)	1.5 (0;4)	2 (0;3)	1 (0;3)	0.5 (0;2.25)
Orientation	6 (6;6)	6 (5.5;6)	6 (6;6)	6 (5;6)	6 (5;6)	6 (4.75;6)

Table 2

Gender differences in MoCA test domains performing (Me (Q1;Q3))

	Female (70)	Male (44)	Difference significance (p)
MoCA score	22 (18;25)	19.5 (16;24)	p=0.106
Domains (Me (Q1;Q3))			
Visual-constructive and executive skills	4 (2;5)	4 (2;5)	p=0.708
Naming	3 (3;3)	3 (3;3)	p=0.792
Attention	6 (5;6)	6 (4;6)	p=0.412
Speech	2 (1;3)	1 (1;2)	p=0.036
Abstraction	0 (0;1)	0 (0;1)	p=0.148
Delayed reproduction	2 (0;3)	1 (0;3)	p=0.101
Orientation	6 (5;6)	6 (5;6)	p=0.192

en in the study group. On the other hand, there is evidence of greater preservation of speech fluency and verbal memory in women in references, where the authors tie this with the difference in the rate of glucose metabolism in the temporal lobe in women and call this form of the cognitive reserve [16,27]. Among statistically non-significant indicators, it is necessary to note that the overall score on the MoCA test for men was lower in comparison with women. Lower rates of attention and delayed reproduction were more often detected in men, which reflects the trend to more severe CI among them. Besides, patients were equally poor at tasks on the "abstraction" domain regardless of gender. Sources indicate conflicting data regarding gender differences associated with CI. Thus, earlier studies note that women have more intense cognitive decline [19,28], but later researches show a greater severity of cognitive dysfunction in men [13,23]. Perhaps gender is not a determining factor, and this requires an analysis of additional characteristics.

The obtained information allowed us to identify and clarify the degree of CI and conduct a differential diagnosis between primary and secondary dementing diseases. 82.5% (n = 94) of patients had various neurodegenerative and vascular diseases of the brain, and cognitive abnormalities were detected (n = 90), and some patients had unspecified CI that required further study. Cognitive functions within the age limits were detected in 17.5% (n = 20) patients (Table 3).

Alzheimer's disease (AD) is a primary dementing disease. According to table 3, patients with AD were identified in 11.7% (n = 11). To confirm this diagnosis in all patients with suspected AD MRI of the brain had been performed. In MRI, atrophy of the deep sections of the temporal lobes (hippocampus) of varying severity was found. According to literature, in AD there are no pathognomonic signs of brain MRI and the diagnosis established based on clinical and neuropsychological studies. Frontotemporal dementia (FTD) was detected in 5.3% (n = 5) of patients with CI. FTD also refers to primary dementing diseases. The incidence is 8.9 cases per 100 thousand people per year [17, 26]. The peak incidence occurs at the age of 55–65 years (the average age of clinical debut is about 58 years). Neuroimaging was performed for all patients and atrophy of the frontotemporal region of the cerebral cortex of varying degrees was found. Levy body dementia (LBD) diagnosed in 2.1% (n = 2) of the cases. The rates of LBD in different studies varies greatly even with similar diagnostic criteria, which reflects its complexity [1]. On average, it can be concluded that LBD accounts for approximately 10-15% of all cases of dementia [1,21]. Early and more accurate detection of LBD is necessary to predict the course of the disease, to prompt prescription of effective pharmacotherapy, and optimal tactics to avoid the adverse effects of antipsychotic therapy [1]. In 4.2% (n = 4) Hakim-Adams syndrome (normotensive hydrocephalus, NTH) was established. Available epidemiological data remain controversial, partly due to differences in diagnostic criteria and an insufficient number of population studies. Despite this, it can be assumed that the number of cases of primary and secondary NTH will increase, most likely due to an increase in the quality of medical care, wider introduction of neuroimaging, and an increase in life expectancy [14]. Currently, NTH is more often regarded as a disease of the elderly at the age of 60–80 years [18].

A preliminary diagnosis of "neurodegeneration with brain iron accumulation" (NBIA) was established in one woman with a pathognomic MRI picture. She complained to decreased cognition, assessment of CI on the MoCA scale was the maximum score (29). Neurological extrapyramidal and pyramidal symptoms were not found in the patient. According to the MRI of the brain in T2-weighted images, a hypo-intense signal was found in the basal ganglia, which is described as a characteristic symptom of the "tiger eye", indicating extracellular accumulation of iron in these structures of the brain. Currently, the patient is undergoing further examination for aceruloplasminemia. A man with cognitive deficiency was diagnosed with progressive supranuclear

Table 3

Cognitive disorders distribution by nosology

Nosology, %	Amount of patients (n=114)	CI (n=90)
Alzheimer's disease	11 (11.7)	11 (100)
Frontotemporal dementia	5 (5.3)	5 (100)
Lewy body dementia	2 (2.1)	2 (100)
Progressive supranuclear palsy	1 (1.1)	1 (100)
Neurodegeneration with brain iron accumulation	1 (1.1)	0
Normotensive hydrocephalus	4 (4.3)	4 (100)
Parkinson's disease	18 (19.1)	15 (83.3)
Vascular CI	38 (40.4)	36 (94.7)
Unspecified CI	14 (14.8)	14 (100)
Cognitive function in the age limits	20 (17.5)	0

palsy (PSP). According to various epidemiological studies, PSP is found at least 20 times less often than PD, but slightly more often than multisystem atrophy; the average frequency of occurrence is about 5 cases per 100,000 [15, 20]. Usually, PSP diagnoses to middle-aged and elderly people [10].

19.1% (n = 18) of patients were visited the office of cognitive disorders with an established diagnosis of Parkinson's disease (PD) and CI was detected in 83% of cases. The onset of CI is always accompanied by worsening of the condition, a decrease in the quality of life, increased pressure to the caregivers, and an increase in the cost of treatment [11].

40.4% (n = 38) were patients with vascular diseases of the brain. CI was found in 92% (n = 35) of them. Post-stroke CI diagnosed for 23.7% (n = 19). According to meta-analysis, post-stroke dementia observed in 1 out of 10 patients, and in 1 out of 3 among patients with the second stroke. Also, some authors consider that in 1 out of 10 patients, dementia occurred before a stroke [24, 25]. For all patients of this group, the Hachinski ischemia score was performed, and all patients showed a tendency to vascular dementia (above 8 points). The following risk factors for cerebrovascular diseases were identified: arterial hypertension in 100% of patients, atrial fibrillation in 15.8%, atherosclerosis of brachiocephalic vessels in 50%, and diabetes mellitus in 26% of patients. As we see, all patients had risk factors for the development of vascular diseases of the brain, which led to cognitive decline. According to the neuroimaging (MRI of the brain), including series of the T2 weighted image and FLAIR, leukoaraiosis and postischemic cysts of different sizes and locations were found.

Unspecified CI share is 14.8% (n = 14) and this is associated with insufficient examination and the lack of dynamic observation due to the absence in further visits. In our opinion, medical care for patients with NDD with CI at the primary care level is complicated by the provision of this service on a cost-reimbursable basis. This caused by the absence in the register of services provided by the Territorial Fund of Compulsory Health Insurance as well as the lack of awareness of neurodegenerative diseases that are accompanied by CI and the possibility of slowing down the neurodegenerative process with early intervention by a neuropsychologist. Late visits of patients may also be associated with the lack of specialized offices for CI in clinics.

Findings:

1. For the period of activity of the CI office of the Neurodegenerative Diseases Center of the Clinic of the Yakut Science Centre of complex medical problems (YSC CMP) from 2018-2019 CI was diagnosed in 82.5% of patients.

2. A correlation between CI with age and educational level has been established. The domain "naming" remains the best-preserved with age, and the indicators for the "speech", "delayed reproduction" and "orientation" domains decline significantly with age.

3. Unspecified CI share is 14.8% (n = 14) and this is associated with insufficient examination and the lack of dynamic observation due to the absence in further visits.

4. The cost-reimbursable character of CI office services has influenced and restricted visits number.

Conclusion. Inclusion of the CI office services into the register of services provided by the Territorial Fund of Compulsory Health Insurance with the aim of early diagnostics of cognitive disorders in various neurodegenerative and vascular diseases of the brain due to population aging in recent years and demand for cognitive counseling could help to solve the medical and social problem of NDD.

References

1. Левин О.С., Васенина Е.Е., Чимагомедова А.Ш. [и др.]. Деменция с тельцами Леви. Обозрение психиатрии и медицинской психологии им. В.М. Бехтерева. 2018;2:11-21 [Levin O.S., Vasenina E.E., Chimagomedova A.S. [et al.]. Dementia with Lewy bodies. *Obozrenie psihiatrii i medicinskoj psihologii im. V.M. Behtereva*. 2018; 2:11-21. (In Russ.)]
https://doi.org/10.31363/2313-7053-2018-2-11-21

2. Екушева Е.В. Когнитивные нарушения – актуальная междисциплинарная проблема. *РМЖ*. 2018;12(1):32-37 [Ekusheva E.V. Cognitive impairment — relevant interdisciplinary problem. *RMJ*. 2018;12(1):32-37 (In Russ.)] https://www.rmj.ru/articles/nevrologiya/Kognitivnye_narusheniya_aktualynaya_meghdisciplinarnaya_problema/#ixzz65cQY8DOn

3. Федосеева И.Ф., Попонникова Т.В., Галиева Г.Ю. [и др.]. Клинический случай редкого нейродегенеративного заболевания с накоплением железа в мозге, тип 4, у ребенка 15 лет. *Российский вестник перинатологии и педиатрии*. 2019;64(5):109-113 [Fedoseeva I.F., Poponnikova T.V., Galieva G.Yu. [et al.]. Clinical case of a rare neurodegenerative disease with iron accumulation in the brain, type 4, in a 15-year-old child. *Rossijskij vestnik perinatologii i pediatrii*. 2019. 64 :(5). P.109–113 (in Russ)]. DOI: 10.21508/1027– 4065–2019–64–5–109–113

4. Левин О.С., Васенина Е.Е. Диагностика и лечение когнитивных нарушений и деменции. Методические рекомендации. Москва. 2015:80. [Levin O.S., Vasenina E.E. Diagnosis and treatment of cognitive impairment and dementia. Metodicheskie rekomendacii. Moscow. 2015: 80 (in Russ)] https://www.03book.ru/upload/iblock/318/31809f2643f7421d60b7dd3eefa0d723.pdf

5. Нейродегенеративные заболевания: от генома до целостного организма. В 2-х томах. Том 2 / Под ред. М.В. Угрюмова. Москва: Научный мир. 2014: 848 с. [Neurodegenerative diseases: from the genome to the whole organism. In 2 volumes. Vol. 2 / Ed. M.V. Ugryumov. Moscow: Nauchnyj mir. 2014:848 (in Russ)] https://kpfu.ru/staff_files/F986520275/2014_Disfunkciya_perifericheskih_vozbudimyh_struktur.pdf

6. Остроумова Т.М., Парфенов В.А. Остроумова О.Д. Артериальная гипертензия и когнитивные нарушения: взгляд с позиций доказательной медицины. *Неврология, нейропсихиатрия, психосоматика*. 2017;9(4):70-76 [Ostroumova T.M., Parfenov V.A., Ostroumova O.D. Hypertension and cognitive impairment: the standpoint of evidence-based medicine. *Nevrologija, nejropsihiatrija, psihosomatika*. 2017;9(4):70-76. (In Russ)] DOI: http://dx.doi.org/10.14412/2074-2711-2017-4-70-76

7. Пизова Н.В., Данилова М.В. Когнитивные нарушения в клинической практике и анализ работы кабинета нарушений памяти в Ярославле (амбулаторный прием). *Неврология, нейропсихиатрия, психосоматика*. 2014:32-37 [Pizova N.V., Danilova M.V. Cognitive impairments in clinical practice and analyzing the work of a memory room in Yaroslavl (an outpatient reception). *Nevrologija, nejropsihiatrija, psihosomatika*. 2014:32-37 (In Russ)] DOI: http://dx.doi.org/10.14412/2074-2711-2014-3-32-37

8. Яхно Н.Н., Преображенская И.С., Захаров В.В. [и др.] Распространенность когнитивных нарушений при неврологических заболеваниях (анализ работы специализированного амбулаторного приема). *Неврология, нейропсихиатрия, психосоматика*. 2012:30-34 [Yakhno N.N., Preobrazhenskaya I.S., Zakharov V.V. [et al.] Prevalence of cognitive impairments in neurological diseases: analysis of the activities of a specialized outpatient reception office. *Nevrologija, nejropsihiatrija, psihosomatika*. 2012: 30-34 (In Russ)].

9. Сизова Ж., Лапидус Н., Богатырева Л. Коррекция когнитивных нарушений при артериальной гипертензии. *Врач*. 2013; 4:28-31 [Sizova J., Lapidus N., Bogatyreva L. Correction of cognitive impairment in arterial hypertension. *Vrach*. 2013;4:28-31(In Russ)]

10. Чечеткин А.О., Федотова Е.Ю., Иванова-Смоленская И.А. [и др.] Случай прогрессирующего надъядерного паралича с кортикобазальным синдромом. *Нервные болезни*. 2009; 2:38-43 [Chechetkin AO, Fedotova E.Yu., Ivanova-Smolenskaya IA [et al.] A case of progressive supranuclear palsy with corticobasal syndrome. *Nervnye bolezni*. 2009;2:38-43 (In Russ)]

11. Шипилова Н.Н., Титова Н.В., Катунина Е.А. Проблема немоторных симптомов при болезни Паркинсона и влияние дофаминергической терапии на их коррекцию. *РМЖ*. 2018; 4(11):85-90 [Shipilova N.N., Titova N.V., Katunina E.A. The problem of non-motor symptoms of Parkinson's disease and the effect of dopaminergic therapy on their correction. *RMJ*. 2018; 4 (11):85-90 Russ)] https://www.rmj.ru/articles/nevrologiya/Problema_nemotornyh_simptomov_pri_bolezni_Parkinsona_ivliyanie_dofaminergicheskoy_terapii_na_ih_korrekciyu/#ixzz6O9f8HaBk

12. Malek-Ahmadi M., Powell J. J., Belden C. M. [et al.] Age- and education-adjusted normative data for the Montreal Cognitive Assessment (MoCA) in older adults age 70–99. *Aging, Neuropsychology, and Cognition*. 2015; 22(6):755–761. doi:10.1080/13825585.2015.1041449

13. Zaninotto P, David Batty G., Allerhand M. [et al.] Cognitive function trajectories and their de-

terminants in older people: 8 years of follow-up in the English Longitudinal Study of Ageing. *J Epidemiol Community Health*. 2018:1–10. doi:10.1136/jech-2017-210116

14. Relkin N., Marmarou A., Klinge P. [et al.] Diagnosing – idiopathic normal-pressure hydrocephalus. *Neurosurgery*. 2005; 57(3):4-16.

15. Dickson D.W., Rademakers R., Hutton M.L. Progressive supranuclear palsy: pathology and genetics. *Brain Pathol.* 2007;17:74-82

16. Sundermann E.E., Maki P.M., Rubin L.H. [et al.] Female advantage in verbal memory: Evidence of sex-specific cognitive reserve. *Neurology.* 2016; 87(18):1916–1924. doi: 10.1212/WNL.0000000000003288

17. Ferrari R., Hernandez D.G., Nalls M.A. [et al.] Frontotemporal dementia and its subtypes: a genome-wide association study. *Lancet Neurol.* 2014; 13:686–99.

18. Gallia G.L., Rigamonti D., Williams M.A. The diagnosis and treatment of idiopathic normal pressure hydrocephalus. *Nat. Clin. Pract. Neurol.* 2006; 2:375-381.

19. C Proust-Lima, H Amieva, L Letenneur [et al.]. Gender and education impact on brain aging: a general cognitive factor approach. *Psychol Aging.* 2008; 23(3):608–620. doi: 10.1037/a0012838

20. Litvan I. Atypical Parkinsonian Disorders: Clinical and Research Aspects. Totowa, N.J., 2005.

21. McKeith I. DLB and PDD: the same or different? Is there a debate? *Int Psychogeriatr.* 2009; 21:220-224.

22. Freitas S., Prieto G., Simoes M. R. [et al.] Scaling Cognitive Domains of the Montreal Cognitive Assessment: An Analysis Using the Partial Credit Model. *Archives of Clinical Neuropsychology.* 2015; 30:435–447.

23. McCarrey A.C., An Y., Kitner-Triolo M.H. [et al.] Sex differences in cognitive trajectories in clinically normal older adults. *Psychol Aging.* 2016; 31:166–75.

24. Pendlebury S.T., Rothwell P.M. Prevalence, incidence, and factors associated with pre-stroke and post-stroke dementia: a systematic review and meta-analysis. *Lancet Neurol.* 2009; 8:1006–1018.

25. Pendlebury ST. Stroke-related dementia: rates, risk factors and implications for future research. *Maturitas.* 2009;64:165–171

26. Pressman P.S., Miller B.L. Diagnosis and management of behavioral variant frontotemporal dementia. *Biol Psychiatry.* 2014; 75:574–581.

27. Shafto M.A., Burke D.M., Stamatakis E.A. [et al.] On the tip-of-the-tongue: neural correlates of increased word-finding failures in normal aging. *Journal of Cognitive Neuroscience.* 2007; 19:2060–2060.

28. Wen H, Zhang Z, Niu F, Li L. The application of Montreal cognitive assessment in urban Chinese residents of Beijing. *Zhonghua Nei Ke Za Zhi.* 2008;47(1):36 –39.

A. A. Balandin, L. M. Zheleznov, I. A. Balandina, V. A. Balandin, D. K. Garmaeva

DOI 10.25789/YMJ.2020.71.31

УДК 616.831.47-053.8-073.914.343

AGE LINEAR DIMENSIONS OF THE HUMAN THALAMUS

The aim of the study was to determine the age-related morphometric features of the thalamus in mesocrane men in young, old and senile age using magnetic resonance imaging data. The analysis of the results of morphometric study of the thalamus using the method of magnetic resonance imaging of 73 mesocrane men aged 27 to 83 years without diseases and injuries of the Central and peripheral nervous system, who do not have a history of alcohol and drug dependence, with a predominance of the right hand (right-handed), with no signs of pathology of the brain departments detected during the study. The transverse, longitudinal and vertical dimensions of the thalamus were determined. In the time period from young to old age, mesocrane men showed a decrease in the parameters of all linear sizes of the thalamus ($p<0.001$). In the young, elderly, and senile ages in the left hemisphere, there is a tendency for the predominance of parameters of the transverse, longitudinal and vertical dimensions of the thalamus in comparison with the right hemisphere ($p>0.05$). The results obtained can be used as a reference for the age-related anatomical norm of the size of the thalamus in mesocrane men in young, elderly, and senile ages and will be useful for radiologists when conducting magnetic resonance imaging studies of brain structures.

Keywords: thalamus, age, morphometry, MRI, mesocephalic.

BALANDIN Anatoliy Alexandrovich – Senior lecturer of the Department of Normal, Topographic and Clinical Anatomy, Operative Surgery, E.A.Vagner Perm State Medical University, +79129822508, e-mail: balandinnauka@mail.ru, Perm, Russia, ORCID icon http://orcid.org/0000-0002-3152-8380, **ZHELEZNOV Lev Mihajlovich** – doctor of medical Sciences, Professor of the Department of human anatomy, rector Kirov State Medical University, +7332640976, e-mail: lzm-a@mail.ru, Kirov, Russia. ORCID icon http://orcid.org/0000-0001-8195-099, **BALANDINA Irina Anatolyevna** – Doctor of Medical Sciences, Professor, Head of the Department of Normal, Topographic and Clinical Anatomy, Operative Surgery, E.A.Vagner Perm State Medical University, +79124993580, e-mail: balandina_ia@mail.ru, Perm, Russia. ORCID icon http://orcid.org/0000-0002-4856-9066, **BALANDIN Vladimir Alexandrovich** – Methodologist of the Department of Normal, Topographic and Clinical Anatomy, Operative Surgery, E.A.Vagner Perm State Medical University, +79194630499, e-mail: balandin.ru@mail.ru, Perm, Russia. ORCID icon https://orcid.org/0000-0001-5142-7117, **GARMAEVA Darima Kyshektovna** – Doctor of Medical Sciences, Professor, Head of the Department of Normal an Pathological Anatomy, Operative Surgery with Topographic Anatomy and Forensic Medicine, M.K.Ammosov NEFU Medical Institute Docent, +79142349680, e-mail: dari66@mail.ru, Yakutsk, Republic Sakha (Yakutia), Russia.

Introduction. Morphofunctional studies of human brain structures have been given a huge amount of scientific work by both domestic and foreign scientists. One of the" gray cardinals» who oversee a considerable number of functions performed by the brain is the thalamus. The thalamus is a paired organ located in the intermediate brain, near the III ventricle, under the corpus callosum. Due to the huge number of afferent-efferent connections with other structures of the brain, researchers consider it to be the "main connecting node". Of particular interest to doctors and scientists of various specialties are information about the age-related anatomy of the thalamus, since researchers have found that the thalamus not only participates in involuntary and voluntary movements, is an analyzer of ascending information of all types of sensitivity, in the primary processing of visual information, regulates circadian rhythms, but also actively participates in the work of human cognitive functions [9 – 15]. In scientific publications of Russian scientists, a curious fact is noted that in acute circulatory disorders of areas located in the zone of the intermediate brain, the doctor faces a diverse clinical picture, including multiple neurological symptoms: from paresis to aphasia [5, 8].

The transmission of thalamocortical information is necessary for the normal functioning of the cortex, and therefore an understanding of the principles of thalamic retransmission will help clarify the main approaches to its work. Research in recent years shows that the principle of the thalamus does not lend itself to simple logic, but reflects a sig-

nificant regional plasticity of the information flow, both from lower and higher-located brain structures, which allows us to consider the thalamus as a global multi-modal network that connects different regions of the brain [11, 15]. Scientists have established the relationship between the decrease in activity of brain microstructures due to the restructuring of its morphofunctional system «microglia – neural networks» with the manifestations of aging, such as the weakening of cognitive, social and physical abilities [14]. Researchers believe that the prefrontal cortex is associated with the area of higher Executive functions. However, in recent years, there has been causal evidence that the mediodorsal nucleus of the thalamus is also a key structure in various cognitive processes, in particular, the rapid introduction of new information during training into working memory or adaptive decision-making in primates and rodents. This information correlates with the fact that age-related changes, as well as with pathology where there are no direct lesions of the prefrontal cortex, cause cognitive and social disorders [4, 8, 10, 14].

In the scientific literature, there is information about the anatomical characteristics and structural component of some parts of the brain, taking into account the specific period of postnatal ontogenesis of a person and in various pathologies [4, 6, 7].

The possibility of using such modern research methods as magnetic resonance imaging in the lifetime diagnosis of brain diseases imposes new requirements for the level of detailed knowledge of specialists about the parameters of specific anatomical formations, taking into account the gender, age and typological features of the subject [1, 2, 3]. It should be noted that information about the morphometric features of the thalamus in the age aspect, revealed by magnetic resonance imaging, is very sparse and incomplete.

The aim of the study was to establish age – related morphometric features of the thalamus in mesocrane men in young, old and senile age using magnetic resonance imaging data.

Materials and methods of research. The work is based on the analysis of the results of magnetic resonance imaging studies of 73 men who were examined in the Department of radiation diagnostics of the state Autonomous health institution of the Perm region "City clinical hospital №4". The age of the subjects ranged from 27 to 83 years inclusive. The research was approved by the ethics Committee of the Perm state medical University named after academician E. A. Wagner (№. 10 dated 22.11.2017). the Criteria for inclusion of subjects in the study: young, elderly or senile age of the patient; absence of a history of diseases and injuries of the Central and peripheral nervous system, as well as alcohol and drug dependence; craniotype – mesocrane; predominance of the right hand (right-handed); absence of signs of brain pathology detected during the study. All patients consented to magnetic resonance imaging, which was performed only for indications.

Craniometry was performed on the extreme protruding points on the axial section in 3D reconstruction mode. The study sample was made up of subjects with medium-sized skulls with a head index value from 75.0 to 79.9. Magnetic resonance imaging was performed on a 1.5 T Brivo 335 device (General Electric – GE Healthcare, USA). Scanning was performed natively with a slice thickness of 5 mm, followed by postprocessor reconstructions in T2 mode using sharpness filters. All patients were divided into three groups according to their age category. Group I included 27 young patients (from 27 to 42 years). Group II included 25 elderly subjects (from 61 to 68 years). Group III consists of 21 men aged 75 to 83 years. Morphometric study of the thalamus included the determination of their linear dimensions: cross – distance from the most remote points (medial and lateral) of the thalamus in axial projection, the longitudinal – distance from the most remote points (front and rear) in axial projection and a vertical dimension – distance from the most distant points (upper and lower) in the frontal projection. Statistical processing of the obtained results was performed using the STATISTICA V.6.0 software system. Results were presented as values of the arithmetic mean (M), relative error (m), maximum and minimum values, coefficient of variation, and median. The reliability of differences in average values was evaluated using the student's parametric t-test. The critical level of significance when testing statistical hypotheses was considered equal to 0.05, and a confidence interval was determined, $p<0.01$, indicating differences between the relative frequencies of the attribute values.

Results and discussion. The maximum transverse size of the thalamus is established in young men. This indicator is 14.10±0.30 mm in the left hemisphere of the brain and 14.09±0.25 mm in the right hemisphere. The minimum cross-sectional size of 13.43±0.41 mm in the left hemisphere and 13.40±0.30 mm in the right hemisphere was found in the senile age period.

The maximum longitudinal size of the thalamus, as well as the transverse one, was detected in the subjects at a young age. In the right hemisphere, the length of the thalamus at a young age reaches

Morphometric characteristics of the left and right thalamus in mesocephalic men according to magnetic resonance imaging data in different age periods (n=73)

Thalamus	Age	M±m	Max	Min	σ	Cv	Me
Transverse size of the thalamus, mm							
Right	Young	14.09±0.25	17.60	12.05	1.67	0.20	15.20
	Elderly	13.46±0.35	16.45	10.85	1.81	0.24	12.30
	Senile	13.40±0.30	16.20	10.25	1.95	0.28	12.90
Left	Young	14.10±0.30	17.65	12.20	1.67	0.20	15.25
	Elderly	13.62±0.35	16.55	10.80	1.82	0.24	12.30
	Senile	13.43±0.41	16.30	10.25	1.95	0.28	12.95
Longitudinal size of the thalamus, mm							
Right	Young	21.03±0.41	24.05	18.70	2.05	0.24	21.65
	Elderly	20.26±0.38	23.50	17.25	1.95	0.19	20.25
	Senile	19.81±0.40	22.80	16.25	1.95	0.19	19.60
Left	Young	21.12±0.41	24.20	18.80	2.05	0.24	21.80
	Elderly	20.41±0.46	23.60	17.25	1.95	0.19	20.20
	Senile	19.87±0.41	22.80	16.40	1.95	0.19	19.65
Vertical size of the thalamus, mm							
Right	Young	16.08±0.36	19.15	13.20	1.79	0.21	15.50
	Elderly	15.60±0.38	18.65	12.10	1.95	0.24	15.30
	Senile	15.50±0.41	18.50	11.75	1.95	0.25	15.15
Left	Young	16.12±0.36	19.30	13.25	1.79	0.21	15.55
	Elderly	15.61±0.38	18.70	12.20	1.95	0.24	15.30
	Senile	15.54±0.41	18.55	11.85	1.95	0.25	15.15

21.03±0.41 mm, in the left – 21.12±0.41 mm. The smallest size limit is set for senile men. The length of the right thalamus is 19.81±0.40 mm, and the left thalamus is 19.87±0.41 mm.

The largest vertical size of the thalamus is also found in young people, similar to the two linear sizes listed above. It reaches 16.08±0.36 mm in the right hemisphere and 16.12±0.36 mm in the left hemisphere. The smallest vertical size of the thalamus was also observed in old age, which is 15.50±0.41 mm in the right hemisphere and 15.54±0.41 mm in the left hemisphere of the brain (table).

When comparing the parameters of the transverse, longitudinal and vertical dimensions of the thalamus, established in young and old age, there are no statistically significant age differences in indicators (p>0.05), but there is a tendency to decrease in all linear sizes from young to old age. When comparing the studied linear sizes of the thalamus in young and old age, statistically significant differences in parameters with their predominance in young age were established (p<0,001).

Thus, in the right hemisphere, the transverse size of the thalamus decreases by 4.31% in old age and by 4.54% in old age. In the left hemisphere, the decrease in this indicator in the elderly is 3.60%, in the senile – 4.75%.

The longitudinal size of the thalamus in the right hemisphere decreases by 3.80% in old age, and by 6.16% in old age. In the left hemisphere, this indicator becomes less by 3.48% in old age, and by 6.29% in old age.

The vertical size in the right hemisphere of the brain decreases by 3.08% in old age, and by 3.74% in old age. In the left hemisphere, the vertical size index decreases by 3.27% in old age, and by 3.73% in old age.

It should be noted that when comparing the linear sizes of the thalamus with each other in all the age periods studied, there is a tendency for the predominance of parameters in the left hemisphere without a statistically significant difference in indicators (p>0,05).

Conclusion.

In the time period from young to old age in mesocrane men, there was a decrease in the parameters of all linear sizes of the thalamus (p<0,001).

In the young, elderly and senile ages in the left hemisphere, there is a tendency for the predominance of parameters of the transverse, longitudinal and vertical dimensions of the thalamus in comparison with the right hemisphere (p>0,05).

The results obtained can be used as a reference for the age-related anatomical norm of the size of the thalamus in mesocrane men in young, old and senile ages and will be useful for radiologists when conducting magnetic resonance imaging studies of brain structures.

References

1. Араблинский А.В. Компьютерная и магнитно-резонансная томография в диагностике острых заболеваний головного мозга. *Медицинский алфавит*. 2010; 2(19): 21-24. [Arablinski AV. Computer and magnetic resonance imaging in the diagnosis of acute brain diseases. *Medicinskij alfavit.* 2010; 2(19): 21-24. (In Russ.)]

2. Бирюков А.Н., Медведева Ю.И., Хазов П.Д. Возрастно-половые аспекты МРТ-каллозометрии. *Вестник Санкт-Петербургской медицинской академии последипломного образования*. 2011; 3(4): 56-63 [Biryukov AN, Medvedeva YuI, Hazov P.D. Age and gender aspects of MRI-callosometry. *Bulletin of the st. Petersburg medical academy of postgraduate education*. 2011; 3(4): 56-63. (In Russ.)]

3. Евзиков Г.Ю., Морозов С.П., Терновой С.К., Синицын В.Е. Значение функциональной магнитно-резонансной томографии головного мозга в хирургии внутримозговых патологических образований области центральных извилин. *Нейрохирургия*. 2004; (4): 27-29. [Evsikov GYu, Morozov SP, Ternovoy SK, Sinitsyn VE. Value of functional magnetic resonance imaging of the brain in surgery of intracerebral pathological entities of the region of the Central convolutions. *Nejrohirurgiya*. 2004; (4): 27-29. (In Russ.)]

4. Ишунина Т.А. Корковые и подкорковые структуры мозга человека при старении и болезни Альцгеймера. *Морфологические ведомости*. 2011; (4): 83-98 [Ishunina TA. Cortical and subcortical human brain structures in aging and in Alzheimer's disease. *Morphological Newsletter.* 2011; (4): 83-98. (In Russ.)]

5. Королев А.А., Суслова Г.А. Таламус-синдром у постинсультных больных с расстройствами движений. *Международный журнал прикладных и фундаментальных исследований.* 2011; (5): 96 [Korolev AA, Suslova GA. Thalamus syndrome in post-stroke patients with movement disorders. *International journal of applied and fundamental research*. 2011; (5): 96 (In Russ.)]

6. Кудренко А.С., Семченко В.В., Степанов С.С. Морфометрическое исследование нейрональной и глиальной популяции ядер вентрального таламуса у плодов и новорожденных в условиях хронической плацентарной недостаточности. *Морфологические ведомости*. 2009; (1-2): 29-32. [Kudrenko AS, Semchenko VV, Stepanov SS. Morphometric research neuronal and glial populations of kernels ventral thalamus at fetus and newborns in conditions of chronic placentary insufficiency. *Morphological Newsletter.* 2009; (1-2): 29-32 (In Russ.)]

7. Моргун В.В., Черненко О.Г., Маловичко И.А., Руденко С.А., Малышева Т.А., Сильченко В.П., Вербова Л.Н. Медуллобластомы мозжечка у детей: клинико-морфологические сопоставления *Паталогія.* 2017; 14(39): 31-37 [Morgun VV, Chernenko OG, Malovichko IA, Rudenko SO, Malysheva TA, Sil'chenko VP, Verbova LM. Medulloblastomas of the cerebellum in children: clinicopathologic comparison. *Pathology*. 2017; 14(39): 31-37. (In Russ.)] doi: 10.14739/2310-1237.2017.1.97171

8. Перепелица С.А., Тумилович Т.А., Щербакова А.А. Постинсультный таламический синдром (обзор). *Общая реаниматология.* 2019; 15(5): 88-105 [Perepelitsa SA, Tumilovich TA, Shcherbakova AA. Post-Stroke Thalamic Syndrome (Review). *General resuscitation.* 2019; 15(5): 88-105. (In Russ.).]

9. Esther A. Pelzer, Corina Melzer, Lars Timmermann et al. Basal ganglia and cerebellar interconnectivity within the human thalamus. *Brain Structure and Function*. 2017; 222(1): 381–392. doi: 10.1007/s00429-016-1223-z

10. Zakaria Ouhaz, Hugo Fleming, Anna S. Mitchell. Cognitive Functions and Neurodevelopmental Disorders Involving the Prefrontal Cortex and Mediodorsal Thalamus. *Frontiers in Neuroscience.* 2018; 12(33). doi: 10.3389/fnins.2018.00033

11. Zita Rovó, István Ulbert, László Acsády. Drivers of the Primate Thalamus. *J Neurosci*. 2012; 32(49): 17894–17908. doi: 10.1523/JNEUROSCI.2815-12.2012

12. Wallace DJ, Fitzpatrick D, Kerr JND. Primate Thalamus: More Than Meets an Eye. *Current Biology*. 2016; 26(2): 60-61. doi: 10.1016/j.cub.2015.11.025.

13. Gent TC, Bassetti C, Adamantidis AR. Sleep-wake control and the thalamus. *Current Opinion in Neurobiology.* 2018; 52(32):188-197. doi: 10.1016/j.conb.2018.08.002

14. Satoh A., Imai SI., Guarente L. The brain, sirtuins, and ageing. *Nature Reviews Neuroscience.* – 2017; 18(6): 362–374. doi: 10.1038/nrn.2017.42.

15. Kai Hwang, Maxwell A. Bertolero, William B. Liu, Mark D'Esposito. The Human Thalamus Is an Integrative Hub for Functional Brain Networks. *J Neurosci.* 2017; 37(23): 5594–5607. doi: 10.1523/JNEUROSCI.0067-17.2017

CLINICAL CASE

E.V. Egorova, V.V. Shabalin, D.V. Dmitrenko, A.A. Usoltseva, K.D. Yakovleva

TAKAYASU ARTERITIS, MANIFESTED BY CONVULSIVE SYNCOPE: A CLINICAL CASE

DOI 10.25789/YMJ.2020.71.32

УДК 616.13-002-06:616-009.24

Takayasu arteritis (TA) has different clinical manifestations from asymptomatic to severe disease and can be complicated by neurological symptoms, but convulsive attacks in such patients are rarely registered. The presented clinical case demonstrates the difficulties of differential diagnosis of epileptic seizures and convulsive syncope in a rare course of Takayasu arteritis in a 19-year-old girl who treated recurrent paroxysms with impaired consciousness, convulsions and falls, motor automatism in the hands, starting with darkening of the eyes during verticalization, which were regarded as pharmacoresistant epileptic attacks. Clinical examination showed severe arterial hypotension, and further examination revealed the diagnosis of non-specific aorto-arteritis.

Keyword: Takayasu disease, non-specific aortoarteritis, epilepsy, convulsive syncope, arterial hypertension, clinical observation.

Introduction. Differential diagnosis of epileptic seizures with non-epileptic paroxysms often causes serious difficulties for the doctor. A variety of conditions that are observed in somatic, neurological, psychiatric disorders, and even normal physiological conditions in healthy children imitate epilepsy [1, 2, 4].

Takayasu arteritis (TA), which has many synonyms ("non-specific aortoarteritis", "aortic arch disease", "lack of pulse disease"), is a chronic granulomatous panarteritis with a predominant lesion of the aorta and its large branches [9, 10]. The incidence of at is 1-2 per 1 million inhabitants in Japan, in Europe it is significantly lower and varies from 0.4 to 1.5 per 1 million people. The highest prevalence of at (about 40 per 1 million people) is registered in Japan, in Europe – from 4.7 to 33 per 1 million people, in the United States – 0.9 per 1 million [11]. The disease debuts mainly in girls and women younger than 40 years. At leads to simultaneous damage to the aorta and its numerous branches. The process involves subclavian and carotid arteries (70-90%), less often – the arch and descending part of the aorta, renal and pulmonary arteries (30-50%), even less often – the abdominal aorta, the ventral trunk, mesenteric, common iliac and coronary arteries (10-30%). Isolated lesions of the pulmonary, hepatic, or iliac artery are extremely rare. It is possible to damage not only large, medium, but also small vessels in at, in particular the arteries that supply the retina and optic nerve [15]. According to the nature and localization of vascular lesion, at can wear various clinical "masks" or their combinations [13, 14].

Etiology. The cause of the disease has not yet been established. It is believed that there is a genetic predisposition to the development of an autoimmune inflammatory process in elastic-type vessels. Thus, patients with TA were found to carry the *HLA-B*52 genotype, which was confirmed in various cohorts and in several ethnic groups [12]. The role of infection in the pathogenesis of at cannot be excluded. In particular, tuberculosis is often found among patients with at. In addition, the onset of the disease may be associated with viral or streptococcal infections, rheumatoid fever, and rheumatoid arthritis [7].

Pathophysiology. Typical for at segmental granulomatous inflammation of the aorta and its branches, defined in 50% of cases, begins in adventitia in the outer layers of the media, as well as in the vasa vasorum region. Microscopic examination of granulomas reveals clusters of lymphocytes, plasma, and reticular cells, with neutrophils and giant multinucleated cells present to a lesser extent. As the disease progresses, granuloma fibrosis develops, sclerosis and media tears, endothelial proliferation leading to narrowing of the vessel lumen. In the intima, media and processes of neovascularization. Intima due to the proliferation and migration of fibroblasts, as well as the accumulation of polysaccharides, thickens, there is a deposition of fibrin on its surface, which can lead to thrombosis of the vessel. The muscular layer of the artery atrophies, is replaced by connective tissue and is compressed by a wide fibrous intima and a thickened adventitia. If the inflammatory process progresses faster than the formation of connective tissue, an aneurysm is formed in the artery wall [7, 14].

Vascular damage to the brain leads to ischemia and can lead to a variety of neurological manifestations, including headache, confusion, dementia, convulsions, meningitis, encephalitis, stroke, visual impairment, and fainting [5, 6, 8]. Although 42 to 80% of patients with at have symptoms of Central nervous system (CNS) damage, convulsive seizure as a primary manifestation is rare [6].

We present a clinical case of TA debuting with convulsive syncope in a 19-year-old girl of Asian origin.

Clinical example. Patient H., 19 years old, a resident of the Republic of Tyva, went to consult a neurologist-epileptologist of the Neurological center for epileptology, neurogenetics and brain research of prof. V. F. Voino-Yasenetsky Krassmu in October 2018 with complaints of repeated paroxysms with impaired consciousness, convulsions and falling to the left, with the establishment of the eyeballs to the right and facial asymmetry, motor automatism in the hands, starting with darkening in the eyes. Frequency of paroxysms up to 8 times a day. Parox-

EGOROVA Ekaterina Alexandrovna – PhD student of medical genetics and clinical neurophysiology, V.F. Voyno-Yasenetsky Krasnoyarsk State Medical University, Krasnoyarsk. E-mail: Volka-katya@mail.ru ORCID:0000-0001-7248-4946, **SHABALIN Vladimir Viktorovich** – Ph. D., associate Professor, Department of therapy. E-mail: vlshabalin@yandex.ru ORCID: 0000-0001-8002-2362, **DMITRENKO Diana Viktorovna** – MD, Ph. D., head of the Department of medical genetics and clinical neurophysiology, V.F. Voyno-Yasenetsky Krasnoyarsk State Medical University, Krasnoyarsk. mart2802@yandex.ru ORCID:0000-0003-4639-6365, **USOLTSEVA Anna Alexandrovna** – the resident, laboratory assistant, Department of medical genetics and clinical neurophysiology, V.F. Voyno-Yasenetsky Krasnoyarsk State Medical University, Krasnoyarsk a.usoltseva@list.ru ORCID:0000-0002-9678-6719, **YAKOVLEVA Kristina Dmitrievna** – the resident, laboratory assistant, Department of medical genetics and clinical neurophysiology, V.F. Voyno-Yasenetsky Krasnoyarsk State Medical University, Krasnoyarsk Kris_995@mail.ru ORSID: 0000-0002-2728-5830

ysms are registered mainly in the state of wakefulness against the background of psychoemotional and physical exertion, with verticalization. In the waking state, paroxysms are stopped in the sitting position with the head down.

From anamnesis: ill since 2016, when there were headaches, coordination disorders in the morning when getting out of bed. According to the MRI of the brain, a volume formation was detected in the right temporal region, which was considered a glial tumor (figure 1). The patient underwent surgery: removal of a diffuse tumor of the right temporal lobe. According to histological research, the morphological picture and immunophenotype correspond to the immunoreactive-inflammatory process. Liquor analysis by PCR to HSV 1, 2, 6 types, EBV, CMV, negative. ELISA for tick-borne encephalitis virus: negative. Analysis for cancer markers (AFP, CA-125, CA-19-9, CA-15-3, REA, HCG): negative. In the General blood test, anemia of a mild degree was registered (the etiology is not specified). According to MRI data of the brain after surgical treatment: cystic fibrotic changes in the right temporal region (figure 2). Within a year after the surgical treatment, the patient felt satisfactory.

In 2017, one year after surgical treatment, morning attacks with verticalization with tonic tension and tremor of the extremities were registered for the first time. Deterioration of health after 6 months, in the form of an increase in the frequency and severity of paroxysms registered in wakefulness up to 7-8 times a day, a decrease in exercise tolerance, verticalization. The patient was examined at the place of residence. According to repeated EEG data: epileptiform activity was not registered. However, the patient was diagnosed with symptomatic epilepsy of the Jackson type with frequent attacks. Condition after removal of a diffuse tumor of the right temporal lobe of the brain. In therapy, valproic acid medications are prescribed 900 mg/day without clinical effect. The subjective tolerance of antiepileptic drug (AED) is satisfactory. According to neuroimaging data, cystic-glia changes in the right temporal region were detected without dynamics in comparison with the previous study. According to the MR angiogram of the intracranial arteries, there was an asymmetry of blood flow along the intracranial segments of the internal carotid arteries. Hypoplasia of the left vertebral artery (D>S).

The hereditary history of epilepsy is not burdened.

Objectively: on the skin of the left thigh, left forearm hyperpigmented spots with clear uneven contours that do not protrude above the skin surface. In the lumbar region, a rounded spot of "gray" color, on the neck, a spot of "coffee with milk" color with smooth clear contours, slightly protruding above the skin surface. Multiple rounded, oval areas of tissue paper-like skin about 1.0 cm in diameter on the skin of the trunk and limbs.

In neurological status: clear consciousness, oriented in place, time, self. Estensione. CNs: pupils D=S, photoreactions live, eye slits D=S, movement of the eyeballs in full, no nystagmus. The face is symmetrical. Slight deviation of the tongue to the left, pharyngeal reflex is called, swallowing is saved. Motor sphere: muscle tone is normal. Muscle strength in the left leg 3.5-4 points, in the hand, 4-4. 5 points. Anisoreflexia on the left. Clear disorders of sensitivity does not give. In the Romberg position, shakiness, heel test and calcaneal knee test are performed. There are no meningeal signs. There are no pelvic abnormalities.

At the time of examination, the patient with verticalization registered tachycardia up to 140 beats per minute, followed by a decrease in blood pressure of 80/50 mm Hg, expressed General weakness, hyperhidrosis.

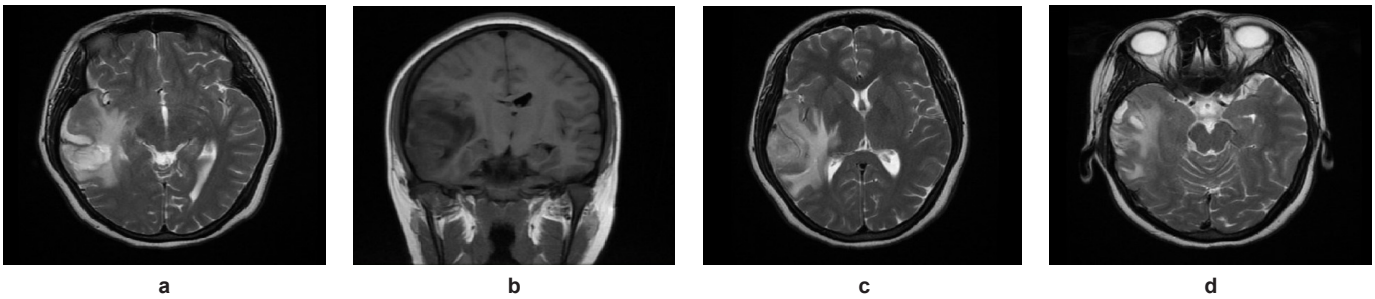
According to the additional investigation data:

Holter ECG monitoring: without pathology

EEG: ictal and interictal epileptiform activity was not registered.

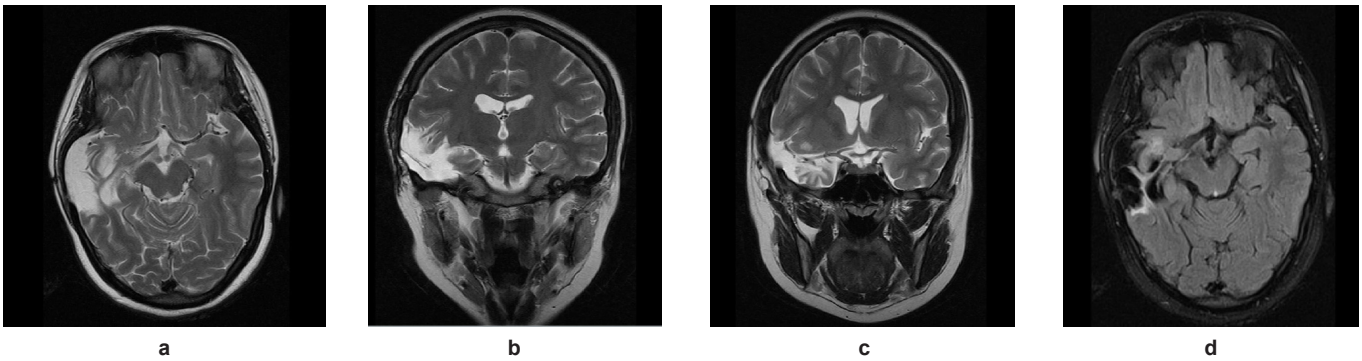
The level of valproic acid in the blood is in the sub-therapeutic range-44.3 mcg/ml (reference range 50-100 mcg/ml).

When repeated examination in 2 days, the patient has paroxysm with fluctuation of the level of consciousness, clones in the muscles of the face on the right, and withdrawal of the right hand to the side when trying to verticalize. The patient is



a b c d

Fig. 1. MRI of the brain of patient H., 19 years old before surgical treatment: a) T1 VI-mode, coronary projection, b) FLAIR-mode, axial projection



a b c d

Fig. 2. MRI of the brain of patient H., 19 years old after surgery: a) T2 VI-mode, coronary projection, b) T2 VI-mode, axial projection;

not available to the contact for a short time, then follows the instructions, and answers questions slowly for a few seconds. Arterial pressure on the hands is not defined. Against the background of medication correction, the patient retains arterial hypotension-40/0 mm Hg. art., consciousness is clear, critical, answers questions. Ambulance was called, and the patient was admitted to the neurological Department of the city hospital.

The extended survey revealed:

DS with brachiocephalic artery CDC: critical stenosis of segment I of the right subclavian artery, collateral blood flow in segment II - III. Critical stenosis of both common carotid arteries. Collateral blood flow along the internal carotid artery, external carotid artery, and vertebral artery on both sides. Occlusion of segment I of the left subclavian artery, collateral blood flow in segment II - III. Collateral blood flow in the arteries of the upper extremities on both sides. Collateral blood flow along the middle cerebral artery, anterior cerebral artery on both sides (figure 3).

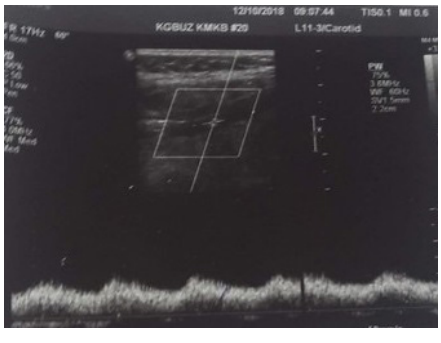


Fig. 3. Duplex scanning with CDC of brachiocephalic arteries of patient H., 19 years old: a) the right common carotid artery (critical stenosis), b) right internal carotid artery (critical stenosis)

MSCT of the neck vessels: the walls of the common carotid arteries are thickened circularly on both sides throughout, with thread-like contrast of their lumen (critical stenosis). Vascular lumen from the bifurcation level (external and internal carotid arteries) is preserved. The plexiglass trunk and the initial sections of the subclavian artery, and the mouth of the right vertebral artery are narrowed (uncritical stenosis).

Blood tests showed accelerated ESR of 40-25 mm / h, anemia-hemoglobin 95 g/l, elevated C-reactive protein (CRP) 9.81 mg/l:

Considering the criteria of the American College of rheumatologists for the diagnosis of Takayasu arteritis (1990), as well as the criteria for the diagnosis of Takayasu arteritis in childhood, *EULAR/PRINTO/PRES (2010)* the patient was diagnosed with:

(M31.4) Non-Specific aortoarteritis (Takayasu's disease) type I, activity of St III, chronic course, critical stenosis of segment 1 of the right subclavian artery, occlusion of the left subclavian artery, critical stenosis of both common carotid arteries, hypotension syndrome.

Complications: frequent syncopal States on the background of transient arterial ischemia, anoxic convulsive attacks.

Anemia of a mild chronic disease. Myxomatous degeneration of the mitral valve flaps with regurgitation 1 art

Treatment with prednisone 40 mg / day was prescribed, the daily dose of valproates was reduced to 250 mg / day. Against the background of GCS therapy, the frequency of convulsive syncopations and positive laboratory dynamics decreased. According to repeated EEG data: no epileptiform activity was registered.

The patient was consulted at the scientific center of cardiovascular surgery named after A. N. Bakulev; Nasonova Federal state medical university. The operation is planned after the process activity has decreased. Takes GCS (prednisone) at a dosage of 20 mg/day, it is planned to reduce the daily dose to 5 mg. Methotrexate 10 mg/week was added to the therapy. Against this background, there is an improvement in health: the patient can sit, stand, walk without developing syncopation. Convulsive syncopations persist up to 1 time a day, mainly after sudden movements.

Discussion. TA is a chronic vasculitis, with predominant damage to the aorta and its main branches (brachiocephalic, carotid, subclavian, vertebral and renal arteries), as well as to the coronary and pulmonary arteries. The main pathology is an inflammation that leads to stenosis, occlusion of the arteries, or the formation of an aneurysm. This disease is also called "pulse-free disease" because of the difficulty in detecting pulsation in the peripheral vessels. The etiology of the disease is still insufficiently studied.

The clinical picture of the disease is heterogeneous and depends on the vessels involved in the pathological process. There are three stages of TA, in the initial stage of the disease, general symptoms of systemic inflammation prevail. Due to the non-specific clinical manifestations, the diagnosis is often delayed until the next stage – vascular insufficiency. The second stage may be devoid of any signs of inflammation and manifest as hypertension due to renal artery stenosis, retinopathy, aneurysm or aortic dilation, congestive heart failure, postural vertigo, amaurosis, transient ischemic attacks, and stroke. The third stage is the rest stage. Collateral blood circulation develops against the background of the chronic nature of the disease, neurological manifestations, despite their relatively late onset, are not uncommon in TA [8].

While convulsive syncopation as the initial clinical picture of TA is rarely registered [6] a number of other neurological manifestations, including headache, dizziness, confusion, dementia, meningitis, encephalitis, stroke, visual impairment and intracranial aneurysms are registered quite often [5, 8]. Many pathological conditions can be manifested by convulsive syncopation, but not every paroxysm with impaired consciousness and convulsions is an epilepsy.

In the presented clinical case, the girl's convulsive syncope was regarded as an epileptic attack, and antiepileptic therapy was not effective. Due to the lack of other clinical manifestations, it took more than a year to diagnose TA. This clinical case is a reminder of how important it is to carefully collect anamnesis and physical examination of the patient with a long video electroencephalographic study [3]. Correct and timely diagnosis allows you to choose the appropriate therapy treatment to stop the pathogenetic process and will improve the quality of life of the patient.

Conclusion. Thus, misdiagnosing epilepsy remains a serious clinical problem. We presented this clinical case to emphasize two conclusions: the importance of a detailed analysis of the medical history and an interdisciplinary approach for timely diagnosis and appropriate treatment.

References

1. Айвазян С.О. Не эпилептические пароксизмальные состояния, имитирующие эпилепсию у детей. *Эпилепсия и пароксизмальные состояния.* 2016; 4: 23-33. [Ayvazyan S. O. Non-epileptic paroxysmal states that mimic epilepsy in children // *Epilepsy and paroxysmal states.* 2016; 4: 23-33. (In Russ.).] DOI: 10.17749/2077-8333.2016.8.4.023-033.

2. Дмитренко Д.В., Донцева Е.А., Строцкая И.Г. Клиническое наблюдение пароксизмальной некинезиогенной дискинезии у 41-летней женщины. *Уральский медицинский журнал = Ural Medical Journal*. 2016; 10: 21-25. [Dmitrenko D. V., Dontsova E. A., Stotskaya I. G. Clinical observation of paroxysmal kinesiogenic dyskinesia in a 41-year-old woman. *Ural medical journal = Ural Medical Journal*. 2016; 10: 21-25. (In Russ.).]

3. Шнайдер Н.А. Видеомониторинг электроэнцефалографии при эпилепсии. *Сибир-*

ское медицинское обозрение. 2016; 2: 93-105. [Schneider N. A. Videomonitoring of electroencephalography in epilepsy. *Siberian medical review.* 2016; 2: 93-105 (In Russ.).]

4. Arrhythmogenic convulsive syncope in neurological practice: a case report. Dmitrenko DV., Strotskaya IG., Artyukhov IP. [at el]. *International Journal of Biomedicine*. 2018; 1: .65-68. DOI: 10.21103/Article8(1) CR1.

5. Bilateral ocular ischemia induced blindness as a presenting manifestation of Takayasu arteritis: a case report. Pallangyo P, Epafra E, Nicholaus P. [at el] *J Med Case Rep*. 2017;11(1):153. DOI: 10.1186/s13256-017-1330-3

6. Correlation of neurological manifestations of Takayasu's arteritis with cerebral angiographic findings. Kim HJ, Suh DC, Kim JK, [at el] *Clin Imaging*. 2005;29(2):79-85. DOI: 10.1016/j.clinimag.2004.04.026

7. J.L. Espinoza, S. Ai , I. Matsumura. New Insights on the Pathogenesis of Takayasu Arteritis: Revisiting the Microbial Theory. *Pathogens.* 2018; 7(3). DOI: 10.3390/pathogens7030073

8. Intracranial and extracranial neurovascular manifestations of takayasu arteritis. Bond K.M., Nasr D., Lehman V. [at el] *AJNR Am J Neuroradiol*.2017;38(4):766-772. DOI: 10.3174/ajnr.A5095.

9. Keser G, Aksu K, Direskeneli H. Takayasu arteritis: an update. *Turk J Med Sci*. 2018;48(4):681-697. DOI: 10.3906 / sag-1804-136.

10. Kim ESH, Beckman J. Takayasu Arteritis: Challenges in Diagnosis and Management. *Heart.*2018 ;104 (7):558-565. DOI: 10.1136/heartjnl-2016-310848

11. Onen F, Akkoc N. Epidemiology of Takayasu arteritis. *Presse Med*.2017;46(7-8 Pt 2): e197-e203. DOI: 10.1016/j.lpm.2017.05.034

12. Relationship of HLA-B*51 and HLA-B*52 alleles and TNF-α-308A/G polymorphism with susceptibility to Takayasu arteritis: A meta-analysis. Chen S, Luan H, Li L. [at el] *Clin. Rheumatol.*2017; 36:173–181. DOI: 10.1007/s10067-016-3445-0.

13. Takayasu Arteritis Mistaken for Epilepsy: A Case Presenting With Convulsive Syncope. Pallangyo P, Misidai N, Hemed N. [at el] *Journal of Medical Cases.*2020; 11; 37-40. DOI: 10.14740/jmc3424.

14. Takayasu's arteritis - a comprehensive review. Setty HSN, Vijaykumar JR, Nagesh CM. [at el]. *J Rare Dis Res Treat.* 2017;2(2):63-68. DOI: 10.29245/2572-9411/2017/2.1048

15. Tian G., Chen Q., Wang W. Takayasu arteritis presenting as isolated anterior ischemic optic neuropathy. *Int Ophthalmol*.2018; 38(2):823-828. DOI: 10.1007/s10792-017-0

S.N. Alekseeva, V.B. Egorova, I.P. Govorova,
V.M. Konstantinova, N.I. Douglas, S.G. Ksenofontova

CLINICAL CASE OF CONGENITAL HEART DISEASE: AORTA ARC BREAK

DOI 10.25789/YMJ.2020.71.33

УДК 616.132.14

ALEKSEEVA Sargylana Nikolaevna – Candidate of Medical Sciences, head of the department of newborns pathology №1, Republican hospital №1 – National Centre of medicine Address: 677000, Yakutsk, Sergelyakhsky str., 4. Mob. Tel. 8-964-422-89-09, E-mail: sargylanao@mail.ru, **EGOROVA Vera Borisovna** – Candidate of Medical Sciences, Associate Professor of the Department of Pediatrics and Pediatric Surgery,Medical Institute of the M.K. Ammosov North-Eastern Federal University. Addresss: 677018, Yakutsk, ul. Oyunsky, 27. Mob. Tel. 8-914-101-90-33, E-mail: veraborisovna@yandex.ru, ORCID: 0000-0003-3051-5251, **GOVOROVA Isabella Prokopyevna** – candidate of medical sciences, doctor of the department of ultrasound testing Republican hospital №1 – National Centre of medicine. Address: 677000, Yakutsk, Sergelyakhsky str., 4. e-mail: iz-govorova@mail.ru, Mob. 8-914-288-37-99, **KONSTANTINOVA Valentina Mikhailovna** – Candidate of Medical Sciences, Head of the Department of Ultrasound, Republican hospital №1 – National Centre of medicine. Address: 677000, Yakutsk, Sergelyakhsky str., 4. e-mail: konstavm@mail.ru, Mob. 8-914-272-67-25, **DOUGLAS Natalia Ivanovna** – MD, Head of the Department of Obstetrics and Gynecology, Medical Institute of the M.K. Ammosov North-Eastern Federal University. Addresss: 677018, Yakutsk, ul. Oyunsky, 27. e-mail: nduglas@yandex.ru. Mob. 8-924-662-67-22; Fax: 8-4112-36-30-46; **KSENOFONTOVA Sardana Gavrilyevna** – postgraduate student of the Department of Pediatrics and Pediatric Surgery of the Medical Institute of the M.K. Ammosov North-Eastern Federal University. Address: 677018, Yakutsk, ul. Oyunsky, 27. e-mail: sardafn4ik91@gmail.com. Mob. 8-962-738-98-93.

Cardiovascular disease is one of the main causes of death, regardless of age. The neonatal mortality prevails in the structure of infant mortality, accounting for more than 60% of all deaths in the first year of life. Congenital malformations are the second leading cause of infant mortality. Congenital malformations of the heart and cardiovascular system account for 47% (12.2 per 10 thousand live births) of all causes of death from malformations [1].

Interruption of the aortic arch is a rare critical congenital heart disease, the frequency of which is 0.02 per 1000 newborns, 0.4% among all congenital heart defects and about 1% among critical congenital heart defects [2]. The defect develops as a result of a violation of the formation of the aortic arch at 5-7 weeks of fetal development. With a reduced ejection of blood from the left ventricle to the upward aorta and in the presence of a defect in the ventricular septum (DVS) and obstruction of the exit tract of the left ventricle or increased blood flow to the pulmonary artery, the structures of the embryonic heart from which the aortic arch develops are in their infancy and can be completely closed. From the point of view of embryology, the three segments of the aortic arch are of different origin, which makes AAI possible in different places with different frequencies [4].

In this article, we present a clinical case of AAI in a child born in 2019 and which was examined at the Perinatal Center of “Republican Hospital №1 - National Medical Center”. We do not have clear evidence of the etiology of intrauterine formation of atresia of the aortic arch, we can only assume the combined nature of the adverse factors (infectious factors, toxic effects of nicotine) that led to the development of critical congenital heart and vascular disease in a child.

In clinical practice, the majority of patients die due to the rapid development of a critical condition, which is characterized by acute cardiac output deficiency, rapid progression of heart failure, oxygen starvation of tissues with the development of decompensated metabolic acidosis and impaired functions of vital organs. The correct management tactics for pregnant women with cardiac pathology in the fetus, including timely and accurate diagnosis, careful dynamic monitoring with the assessment of anatomical, functional and hemodynamic parameters, adequate drug therapy, helps reduce mortality, the incidence of complications and improve the results of surgical correction of congenital heart defect [3]

Keywords: critical heart defects, interruption of the aortic arch, newborns.

Introduction. In the neonatal period, there is a certain circle of congenital heart defects that can potentially threaten the life of the child. These are the so-called critical defects in which the provision of cardiac surgery is indicated in the early days, and sometimes in the first hours of a child’s life [3].

One of these defects is the interruption of the aortic arch, which is characterized by the absence of one of the segments of the aortic arch with a violation of its continuity and the ductus-dependent nature of the blood flow along the descending aorta. Aortic arch interruption is the most severe defect among the entire spectrum of obstructive lesions of the aortic arch.

The frequency of this fate is about 0.4% among all congenital heart diseases (CHD) and 1% among newborns with critical CHD [2].

As a critical ductus-dependent defect, AAI is characterized by the development of a critical state in the first days of a child's life, as a rule, it requires intensive therapy and, without surgical intervention, leads to death in the neonatal period. The death of a newborn without treatment is associated with the physiological closure of the ductus arteriosus and occurs within 4-10 days after birth. The defect develops as a result of disturbances in the formation of the aortic arch at 5 7 weeks of fetal development. Isolated AAI is extremely rare, as a rule (up to 97% of cases) this pathology is combined with other CHD. For the survival of patients with AAI, the early development of powerful collaterals is necessary.

The **aim** of this study is to present the clinical case of CHD with interruption of the aortic arch in a newborn.

Materials and research methods. A pro- and retrospective analysis of the medical records of the newborn with PDA, which was being examined and treated in the Department of Pathology of Newborns and Premature Children No.1, "Republican Hospital №1 - National Medical Center", was carried out.

The department conducted a thorough in-depth examination. Laboratory (clinacal blood and urine analysis, biochemical blood analysis, blood PCR, blood linked immunosorbent assay), and instrumental studies (chest x-ray, computed tomography of the lungs, ultrasound, electrocardiogram, ECHO-kardiogram) were performed.

Results and discussion. Clinical case. The child from the fifth pregnancy, the second birth. Mother 30 years old, with a burdened obstetric and gynecological history (chronic nicotine intoxication, chronic pyelonephritis without exacerbation, chronic colpitis, chronic bronchitis, the carrier of the herpes simplex virus). The first pregnancy in 2006 ended in timely delivery, the next three pregnancies ended in medical abortion.

This pregnancy proceeded against the background of the threat of interruption. In the second trimester, according to the ultrasound of the fetus, signs of polyhydramnios were revealed, at 32 weeks of gestation - signs of intrauterine infection of the fetus.

Childbirth occurred at 40 weeks of gestation, spontaneously, in a head presentation. At the same time, polyhydramnios up to 1500 ml was noted, amniotic fluid green OPV green, with a smell.

A girl was born with a body weight of 2250 g, a body length of 48 cm, a head circumference of 33 cm, a chest circumference of 32 cm. Mass-growth coefficient 46.87 (norm 60-80), (MGC = Newborn's body weight (g) / body length newborn (cm). Corresponding to 3 degrees of malnutrition, body weight deficit of 31% or more. Apgar score 8/8 points.

The condition at birth was regarded as moderate due to malnutrition. The child was transferred from the maternity ward to the intensive care unit, and the regimen of open resuscitation system was prescribed. On the second day of life, the development of negative dynamics was noted: worsening of well-being, the appearance of increasing shortness of breath, pallor of the skin, gross systolic murmur along the left edge of the sternum, a decrease in saturation to 80-82%. The girl was transferred to tube feeding.

According to the results of ECHO-KG, the diagnosis was made: congenital heart disease (CHD); coarctation of the aorta in a typical site (CoA); multiple atrial septal defect (ASD). Pulmonary hypertension (PH) I degree.

On the fourth day of life, the child was sent and hospitalized with a ambulance flight to the Perinatal Center of Republican Hospital №1 - National Medical Center, the intensive care unit of anesthesiology for the provision of high-tech medical care (State Health Development Program of the Russian Federation, approved by Order of the Government of the Russian Federation No. 2511-r from December 24, 2012).

The condition at admission is serious, due to heart failure, decompensated metabolic acidosis. On examination, the child retained consciousness, a cry of moderate strength, a forced position, a disproportionate physique, malnutrition. The head is round in shape, a large fontanel is 2.5 * 2.5cm in size. Spontaneous motor activity is reduced, muscle hypotension, decreased reflexes, with anxiety, tremor of the hands is noted. The skin is clean, acrocyanosis, cyanosis of the nasolabial triangle. Mucous, dry, bright red, pink pharynx.

Weakened breathing, wheezing in the lungs, shortness of breath with a breathing rate of 88 per minute. Heart sounds are rhythmic, gross systolic murmur at all points, heart rate of 148 beats per minute. Blood pressure: left arm 59/24 mm Hg; right hand 62/31 mm Hg; left leg 68/38 mm Hg; right leg 67/40 mmHg

Feeding through a tube of 35.0 ml of expressed breast milk, does not spit up. The abdomen is soft, painless on palpation, not swollen, intestinal motility is heard. Liver +1.0 cm, spleen not palpable. The umbilical wound is epithelized.

In a clinical blood test, moderate leukocytosis with a shift in the formula to the left, thrombocytopenia. In the analysis of urine, inflammatory and other changes were not detected. An increase in creatinine and total bilirubin was noted in a biochemical blood test. According to the gas composition of the blood, decompensated metabolic acidosis was detected. Blood electrolytes are within normal limits. Revealed IgG antibodies to cytomegalovirus infection (CMV). Blood PCR for infection was negative.

When conducting instrumental studies on ultrasound of the abdominal organs, pathology was not detected. On neurosonography - moderate hyperechoogenicity of the periventricular zone. On the chest x-ray there was an increase in the shadow of the heart in transverse dimensions. When conducting an ECG: sinus rhythm, heart rate of 166 beats per minute, the electrical axis is deflected to the right, violation of intraventricular conduction.

On ECHO-KG with color Doppler mapping: heart rate of 133 beats per minute, coarctation of the aorta / interruption of the aortic arch cannot be ruled out. Bicuspid aortic valve. Atrial septal defect 0.4-0.5 cm of the secondary part. The ventricular septal defect is perimembranous 0.4-0.5 cm. Fetal arterial duct 0.36-0.5 cm. The trunk extension of pulmonary artery 1.18 cm, right ventricle 1.0 cm, right atrium 2.09 cm, left atrium 1.4-1.7 cm. Regurgitation on the tricuspid valve I degree, on the mitral valve I degree. Myocardial hypertrophy of right ventricle 0.34 cm. Estimated pressure in right ventricle right ventricle 40.0 mm Hg. Functional discharge - 79% (Fig. 1-2)

Computed tomography images of the chest cavity with intravenous bolus contrast showed a complete break of the aortic arch, open ductus arteriosus, and pulmonary hypertension (expansion of the pulmonary artery). (Fig. 3-4)

Figure 4. RCT. 3D reconstruction. Division of the ascending aorta into the left and right carotid arteries. Discharge of the subclavian arteries from the descending aorta. Dilation of the pulmonary artery, maintaining blood flow in the descending aorta due to the wide arterial duct

Based on clinical and instrumental studies, the child was given a clinical diagnosis: main: Cardiac disorders in the perinatal period. Interruption of the aortic arch. Associated: ventricular septal defect, atrial septal defect. Intrauterine

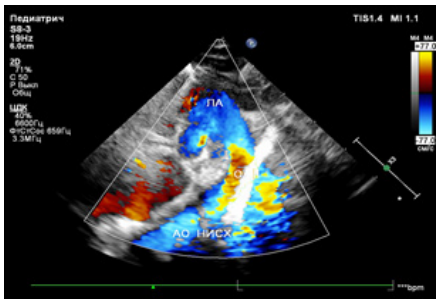


Fig. 1. In the CDC mode, visualization of the filling of the descending aorta through the patent ductus arteriosus, the absence of an aortic arch in a typical place

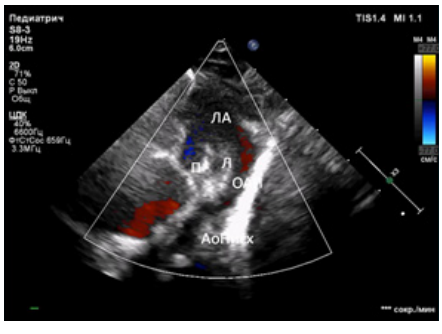


Fig. 2. Discharge from the pulmonary artery of a wide arterial duct, passing into the descending aorta

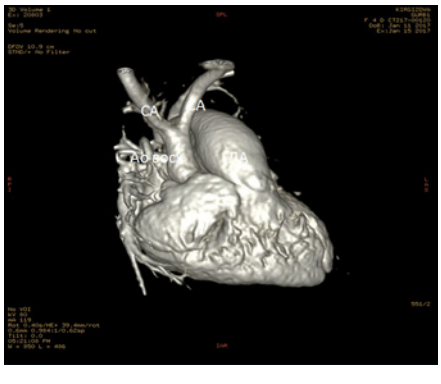


Fig. 3. RCT. 3D reconstruction. Dilated trunk of the pulmonary artery. Division of the ascending aorta into right and left carotid arteries

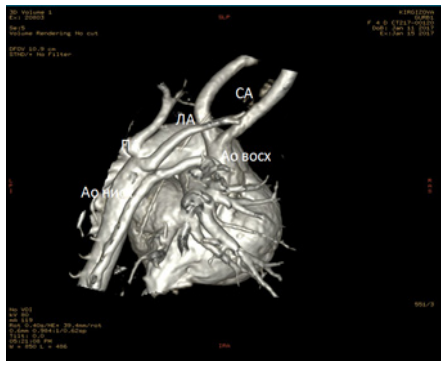


Fig. 4. RCT. 3D reconstruction. Division of the ascending aorta into the left and right carotid arteries. Discharge of the subclavian arteries from the descending aorta. Dilation of the pulmonary artery, maintaining blood flow in the descending aorta due to the wide arterial duct

growth retardation, degree 3 malnutrition.

From the moment of admission to the department, antibiotic therapy was administered intravenously (Ampiside); antifungal (diflucan); immunomodulatory, normalizing intestinal microflora (bifidumbacterin); hemostatic (sodium ethamylate, vikasol) and parenteral nutrition in accordance with the neonatal protocol. Also, on the basis of ECHO-KG, a vasodilator Vazoprostan intravenously No.4 was prescribed.

During his stay in the hospital, the condition of the child was moderate, the child was urgently sent to hospitalization to provide high-tech medical care in the "Academician E.N. Meshalkin National Medical Research Center" in Novosibirsk.

Conclusion. For successful cardiosurgical correction of CHD, the main tasks are the development of prenatal diagnosis, including critical heart defects. A timely diagnosis involves a number of important points of ante- and postnatal observation, such as identifying risk factors for giving birth to a child with CHD, fetal ultrasound screening, fetal ECHO-KG, signs of CHD during the initial examination of the newborn, and dynamic monitoring in the first days of life.

Timely diagnosis of PDA in this patient, conservative treatment for preoperative stabilization of the child's condition - his hemodynamics, metabolic disorders, correction of disorders from other organs and systems, allowed to positively solve the question of the timing of surgical treatment, as well as arrange transportation to a specialized cardiac surgery center in Novosibirsk.

References

1. Бокерия Е.Л. Перинатальная кардиология: настоящее и будущее. Часть I: врожденные пороки сердца. *Российский вестник перинатологии и педиатрии*. 2019; 64(3):5-10. [Bokeriya EL. Perinatal cardiology: present and future. Part I: Congenital Heart Diseases. *Rossijskij vestnik perinatologii i pediatrii.* 2019; 64(3):5-10. (In Russ.).]

2. Затикян Е.П. Кардиология плода. *М.: Триада-Х*. 2009; 216 с. [Zatikyan E.P. Fetal cardiology. *M.: Triada-X*. 2009; 216. (In Russ.).]

3. Саидова В.Т., Сабирова Е.М., Немировская Е.М., Миролюбов Л.М. Раннее выявление критических врожденных пороков сердца у новорожденных с помощью NT-proBNP. *Практическая медицина*. 2013; 6(75):144-146. [Saidova VT, Sabirova DR, Nemirovskaya EM, Mirolyubov LM. Early detection of critical congenital heart defects in infants using NT-proBNP. *Prakticheskaya medicina.* 2013; 6(75): 144-146. (In Russ.).]

4. Соколов А.А., Марцинкевич Г.И. Сопоставление результатов фетальной и постнатальной эхокардиографии. *Российский вестник перинатологии и педиатрии.* 2014; 59(3): 66-70. [Sokolov AA, Marcinkevich GI. Comparison of the results of fetal and postnatal echocardiography. *Rossijskij vestnik perinatologii i pediatrii.* 2014; 59(3):66-70. (In Russ.).]

5. Ялынская Т.А. Перерыв дуги аорты: диагностика порока методом мультидетекторной компьютерной томографии. *Променева діагностика, променева терапія.* 2012; 4: 17-20. [Yalynskaya TA. Aortic arch interruption: diagnosis of malformations using multi-detector computed tomography. *Promeneva diagnostika, promeneva terapiya.* 2012; 4: 17-20. (In Russ.).]

Error notification

Dear readers of the “Yakut Medical Journal”, we would like to inform you that the article “Register of births as the most important tool for forecasting obstetric complications and reducing of perinatal loss level” (authors Bulshiy I.S., Douglas N.I., Burtseva T.E., Pavlova T.Y., Borisova E.A., Savvina N. V., Rad Y.G.), published in №1 (69) 2020., DOI 10.25789 / YMJ.2020.69.25, p.103-104, due to technical errors made by the author inadvertently - the reference numbers in the text did not match the numbers of the sources in the list of references - WITHDRAWN.

The editors apologize to the readers for the situation.