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Contents

YAKUT MEDICAL JOURNAL 2(46)2014

MEDICAL-GENETIC SERVICE OF THE POPULATION REPUBLIC OF SAKHA (YAKUTIA)

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Medical-genetic consultation RH№1-NCM

The main objective of the medical-genetic service (MGS) is prevention of hereditary and congenital diseases, decrease of mortality rate and disability. The medical-genetic assistance to the population represents prevention, diagnostics and treatment of hereditary pathology.

As the first structural unit in practical health care of the republic an office of medical genetics within the City Clinical hospital named after S. Ordzhonikidze was established in 1989 by the order of the minister of MH RS(Y) Mestnikov I.I. and by initiative of the Chief pediatrician Grigoryeva A.N.

Since 1993 the medical-genetic consultation (MGC) joined the Maternal health care centre (the head Nogovisyna Anna Nikolaevna), since 1998 it became the subdivision of the National center of medicine, with modern equipment for development of all types of laboratory diagnostics of hereditary pathology. Since 1999 invasive methods of prenatal diagnostics, since 2000 DNA diagnostics of monogenic hereditary diseases were elaborated. In 2000 a group of monitoring of congenital developmental anomalies was founded, the Republican register of congenital and hereditary pathology of RS(Ya) was introduced. The order of Ministry of Health of RS (Y) № 01-8/4-112 of 28.03.2001 was issued. "About further development of medical-genetic service in RS (Y)".

In the following years the consulting assistance to the population, laboratory diagnostics was actively developed (biochemical, cytogenetic, molecular and genetic), monitoring of congenital defect in the republic was introduced, the department of prenatal diagnostics (the head Sukhomyasova Aytalina Lukichna) was founded. Within the priority national Health projects expanded mass inspection of newborns on five hereditary diseases (phenylketonuria, congenital

hypothyrosis, adrenogenital syndrome, mucoviscidosis, galactosemia) were realized since 2006, prenatal diagnostics of developmental disorder on the basis of a new algorithm since 2011.

In 2010 the staff structure of MGC was expanded to 47,5 units. Within the framework of the National project and the Modernization of health care of RS(Y) the MGC facilities were improved significantly (expert ultrasonic equipment, sets for cytogenetic researches, molecular and genetic technologies.

Close work with the Scientific research institute of medical genetics of Tomsk Scientific Center SD RAMS since 1993 as well as appropriate personnel training allowed to create the MGC at a high professional level.

Now the MGC is a structure of the Prenatal center of the State Budgetary Institution RS (Y) "Republican hospital №1 - National center of medicine". The MGC incorporates a medical-genetic consulting department, a prenatal diagnostics department, an in-patient department, a group of monitoring of congenital developmental disorders, laboratories of molecular genetics, prenatal diagnostics, biochemical and cytogenetic laboratories.

The principle of organizing the medical-genetic assistance in RS (Ya) is based on its availibility to all inhabitants of the republic, co-operating with other services, first of all pediatric, obstetric and gynecologic, therapeutic, neurologic, etc. One of important forms of rendering the medico-genetic assistance in the republic is mobile consulting service together with research associates of the department of molecular genetics of CMP SD RAMS to the regions of Yakutia for the purpose of active identification of the patients needing the specialized aid. This type of the medical-genetic consultation was effective if considering the huge territory of Yakutia, accumulation of hereditary pathology in certain areas. In total over the last 10 years 79335 people (67155 families) have been examined in MGC including 37220 (47,0) rural inhabitants. In 1748 (2,6%) cases the consultations were conducted during the mobile departures to the areas of accumulation of hereditary pathology. The families burdened with monogenic hereditary pathology have been recommended to undergo thorough examination in the medico-genetic consultation.

Annually more than 3 thousand patients address to MGC for clarifying the diagnosis, 2 thousand pregnant women of risk group for forecasting health of their future posterity. In total for 2004-2013 37286 patients were examined. Among them children amounted to 20,7%. 38,7% were presented by the group of the women who had addressed to MGK for the purpose of prenatal diagnostics. Identification of congenital defect of a fetus, ultrasonic markers of

chromosomal anomalies, congenital and hereditary pathology in a family are considered the most frequent causes of addressing pregnant women.

The majority of patients addressed for the medical-genetic consultation have been directed by other experts for clarifying the congenital and hereditary pathology. The great number of patients (14699 people (39,4%) were directed from departments RH№1-NCM. 2879 people addressed for prospective consultation and for clarifying the diagnosis. The active medical-genetic consultation was carried out at 1878 people (5,0%) from the group of high risk.

The MGK formation in the structure of a leading multi-faceted medical institution of the republic provides high level of rendering medical assistance. Consultations of a geneticist and medical-genetic methods of laboratory diagnostics are conducted not only to patients and families addressed to MGC, but also to in-patients and patients from consultative departments of State Budgetary Institution RS (Ya) RH№1-NCM, it amounting to 39,4% addresses.

The medical-genetic consultation is up-dated by introduction of the automated Republican register of congenital and hereditary pathology, foundation of automated workplaces for doctors geneticists.

Modern methods of laboratory diagnostics of congenital and hereditary pathology are joined together in MGC. The technologies of genetic analyses installed in MGC are subject to all population of the republic that increase the efficiency of medical-genetic consultation. When selecting methods and types of diagnostics a range of the most frequent pathology in the region , its remoteness from the Federal centers is considered.

Now the MGC has the following equipment: a ScanRI scanner microchip (Perkin Elmer, Finland), AxioScop research class luminescent microscopes (Carl Zeiss, Germany), (Japan) with the software for cytogenetic and molecular and cytogenetic researches (G-banding, FISH, CGH) (Applied Spectral Imaging, the USA), light microscopes for cytogenetic researches, DNK-amplifiers (Bio-Rad, the USA), an amplifier for PCR in real time (Bio-Rad, the USA), registration system of images, centrifuges, microcentrifuges, thermostats, a nanospectrophotometer, a spectrophotometer, an equipment for neonatal screening, an automatic biochemical analyzer.

The opening of the Department of molecular genetics of the Yakut scientific center RAMS and RS(Ya) Government (at present YSC CMP RAMS) with laboratories of hereditary pathology, molecular and population genetics in 2002 became a significant stage in the MGC development in Yakutia. The practical help of research associates DMG in consultation is invaluable in cases difficult for diagnostics.

In 2013 the new division as a part of the Clinic NEFU named after M. K. Ammosov - Laboratory of genomic medicine (the head Maximova Nadezhda Romanovna) was established, that has made great contribution for further scientific development in practical health care for the purpose of improvement of the medical-genetic help to the population of Yakutia.

Main findings of scientific work of the department of molecular genetics YSC CMP SD RAMS for 2002 - 2013.

Genetic-demographic researches showed that for the majority of ethnic groups of the Republic of Sakha (Yakutia) positive marriage assortativity regarding birthplaces and couple's nationality is characteristic, while international marriages are widespread in settlements, mainly between indigenous people. Characteristics of vital indicators at women of the Yakut nationality in three age groups are given. Temporal variations of vital parameters and components of differential mortality and fertility are shown. For the age groups studied in this research in the content of total selection the greatest contribution is made by the component, connected with differential fertility (over 72%). The decrease in a share of differential mortality in the content of natural selection along with development of medicine can result in accumulation and fixing of negative genes in the population, i.e. increase in genetic burden (Kucher, etc., 2010).

The clinic, epidemiology and molecular reasons of autosomal - dominant myotonic dystrophy in Yakutia have been investigated. The introduction of molecular and genetic methods (PCR) in practice of medical-genetic consultation in RS (Y) has allowed not only to diagnose MD1 in informative families, but to carry out differential diagnostics with diseases with a similar phenotype as well. At present (till 2013) in the Republican genetic register of hereditary and congenital pathology of RS (Y) 202 patients with MD1 are registered, 185 patients have been consulted. (Sukhomyasova, 2005).

According to the republican Register of hereditary and congenital pathology the whole range of monogenic pathology amounts 104 nozologies including 46 diseases with AD t.n., AR pathology of 34 nozologies, 12 nozologies of X-linked-recessive and 10 X-linked dominant pathologies, 10 nozologies of syndromes with unknown t.n. The hereditary tainted and congenital pathology has been estimated, the monitoring of genetic health of the populations of the Republic of Sakha (Yakutia) has been carried out. When comparing hereditary tainted city and rural populations, higher indices on all types of inheritance are noted at rural people. Basic frequency of congenital defect of the central nervous system and chromosomal pathology at newborns of the Republic of Sakha (Yakutia) has been detected, the average frequency of

chromosomal pathology in the republic not exceeding the all-Russian. In industrial regions (Aldan, Lensk, Neryungrinsky, Mirninsky) the prevalence of Down syndrome moderately exceeds the average indices all over the republic as well as the high prevalence of this disease is revealed (in 2-3 times) in agricultural areas: Ust-Aldansky, Churapchinsky, Vilyuisky (Nogovisyna, etc., 2007).

The screening of entire haploid genome at 39 Yakut patients and 39 their relatives from 33 unrelated families has been carried out. Huber with coauthors in 2005 described 25 various mutations in *CUL7* gene at patients with 3-M syndrome from countries of the Mediterranean and Europe, but among Yakut patients it was caused by the only mutation not described earlier in *CUL7* gene. The population prevalence of the mutation 4582insT in *CUL7* gene in the Yakut population amounted to 1,5%, and the prevalence of heterozygotic carriage was 3%. The carriage of the mutation 4582insT in *CUL7* gene at Evens, Evenks, Yukaghirs from RS(Ya), Buryats from Buryatia, Russians from the Tomsk region was carried out, the mutation wasn't found among them. The patent of RF №2315310 "A way of diagnostics of 3-M a syndrome in the Yakut population" was obtained . Date of registration was 20.01.2008. The method of DNA diagnostics, algorithm of medical-genetic consultation and prenatal diagnostics is elaborated. 3M syndrome at Yakuts is as a synonym with the number #273750 *yakut short stature syndrome* in the international base of the National scientific biotechnology institute of the USA (NCBI) in the section OMIM (Online Mendelian Inheritance in Men) (Maximova, etc., 2007, 2008, 2010)

The clinical-genealogical description of a new syndrome of idiopathic short stature with cone dysfunction, atrophy of optic nerve atrophy and Pelger-Huet anomaly at the Yakuts has been presented and its prevalence in the Yakut population has been studied. This gene has been mapped for the first time and the mutation causing the syndrome of idiopathic short stature with cone dysfunction, atrophy of optic nerves and Pelger-Huet anomaly of leukocytes has been identified. The new syndrome was named SOPH syndrome (Short stature, Optic nerve atro syndrome with Cone dysfunction, Optic atrophy and Pelger-Huet anomaly). The SOPH syndrome is included into the international base of National scientific biotechnology institute of the USA (NCBI) in the section OMIM (Online Mendelian Inheritance in Men) with number #614800.

Mechanisms of accumulation of ethnospecific hereditary disease forms (syndrome OPMD, YSN, SCOP) in populations of Yakutia on the basis of haplotype construction in gene loci of the studied diseases by means of microsatellite markers have been considered. The age of

the identified mutations in CUL7 and NAG genes in the Yakut population (Maximova, etc., 2010) has been estimated.

For the first time 5 ethnospecific Yakut hereditary diseases (YHD) by two criteria determined: spinal cerebral ataxia type 1, ocular pharyngeal myodistrophy, myotonic dystrophy, methemoglobinemy, 3M-syndrome (the Yakut nanizm). First, their prevalence is much higher than in the world population, and two diseases - spinal cerebral ataxia type 1 and enzymopenic methemoglobinemy have been referred to the world centers of accumulation of hereditary diseases. Secondly, for each of them the characteristics of molecular nature have been revealed distinguishing them from similar phenotypes in other populations, the latter ones noted incomparably rarely (Puzyrev, 2008).

For the first time in the population of Yakuts by means of the disbalance analysis on coupling and the SSCP analysis with subsequent sequencing of samples with modified flexibility the molecular and genetic reason of the hereditary congenital deafness which is caused by a mutation of splicing donor site IVS1+1G>A of GJB2 (Cx26) gene and, according to the international OMIM catalog (Online Mendelian Inheritance in Men) it is classified as allelic option of autosomal-recessive deafness type 1A (ARG 1A). The prevalence of ARG 1A amounted to 16,2 per 100000 Yakut population, and the frequency of heterozygous carriage of the mutation IVS1+1G>A varies from 3,8 to 11,7% among indigenous people of Yakutia (Evens, Evenks, Dolgans, Yakuts). The findings of the research testify to the local accumulation of the mutation of splicing site of GJB2 (Cx26) gene and characterize the region of Eastern Siberia as the largest in the world "endemic center" of IVS1+1G>A distribution (Barashkov, 2010).

The DNA diagnostics has been introduced by employees DMG in practice of health care of medical-genetic consultation RH№1-NCM:

1. The new PCR method of 3-M syndrome diagnostics has been elaborated, in 2008 the patent RF "The method of 3-M syndrome diagnostics in the Yakut population" by №2315310 from January 20, 2008 was obtained. This method of DNA diagnostics is introduced into the practice of medico-genetic consultation, the prenatal diagnostics is carried out, the register of patients with 3M syndrome is created.

2. For the purpose of fast molecular and genetic diagnostics of ocular pharyngeal myodistrophy the method of direct DNA diagnostics of OPMD by means of amplification of trinucleotide gene locus by the PCR method and the electrophoresis in 8% polyacrylamide gel

(PAAG) has been introduced. The republican register of patients and members of families with OFMD is created.

3. The method of direct DNA diagnostics of Kennedy disease by means of amplification of trinucleotide site of AR gene by the PCR method and the electrophoresis in 1% agarose gel in practical health care in the laboratory of medico-genetic consultation of Republican hospital №1-NCM has been introduced. The DNA direct method of diagnostics allows to clarify the clinical diagnosis, to carry out differential and pre-symptomatic diagnostics, to reveal the heterozygous carriage among women for the purpose of early prevention and prenatal diagnostics of the disease in the burdened families and at the population level.

Monographs:

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2 . Fedorova S. A. Yakuts: comparison of genetic and historical reconstruction / under the editorship of P. B. Konovalov//Ethnogenesis and culturogenesis in the Baikalsk region. 2011 . - Ulan-Ude: Publishing house of BNTs of the Siberian Branch of the Russian Academy of Science. – P.151-176.

3 . Genomic and health in the developing world. Collection. Ed. by D. Kumar. N. - Y. University Press Press, 2012. :

- Kononova S.K. Bioethical aspects of genetics and genomics in Yakut (Siberia) of in Genomics and Health in the Developing World edited by D.Kumar/S.K. Kononova, S.A.Fedorova, and Elza K. Khusnutdinova//Chapter 120, Oxford University Press: New York, P.1426-1430.

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- Fedorova S. Khusnutdinova E. Villems R. MtDNA and Y-chromosomal variation in populations of Sakha (Yakutia)//Collection Genomic and health in the developing world. Ed. by D. Kumar. N. - Y. University Press Press, 2012. P.1269-1280.

A list of the patents, useful models obtained by research associates DMG:

1 . The patent for invention of RF №2315310 "The method of 3-M syndrome diagnostics in the Yakut population". Maximova N. R., Nogovisyna A.N. Sukhomyasova A.L. Date of registration 20.01.2008

2 . The patent for invention No. 244863 "The way of detecting 17 mutations of GJB2 b GJB6 genes at hereditary non-syndrome deafness. Barashkov, etc. Date of registration 20.04.2012 of SI: URAN Institute of biochemistry and genetics of the Ufa Russian Academy of Sciences scientific center, URAMS the Yakut scientific center of complex medical problems SD RAMS.

Other types of scientific production:

- OMIM (Online Mendelian Inheritance in Men). 3M syndrome at the Yakuts is noted as a synonym with the number #273750 *yakut short stature syndrome* in the international base of national institute of biotechnology of the USA (NCBI);

- OMIM (Online Mendelian Inheritance in Men) with number #614800 *shot stature, optic nerve atrophy, and Pelger-Huet anomaly* (SOPH syndrome) is included into the international base of national institute of biotechnology of the USA (NCBI);

- the mutation of splicing donor site IVS1+1G>A of GJB2 gene (Cx26) according to the international OMIM catalog (Online Mendelian Inheritance in Men), is classified as allelic option of autosomal-recessive deafness type 1A (ARD 1A).

The educational and scientific laboratory "Genomic Medicine" of the Clinic of MI NEFU was organized by the order of the rector in September, 2011.

Purpose: Improvement of health quality of the population in the Republic of Sakha (Yakutia) and the Russian Federation on the basis of introduction of high medical technologies and quality promotion of personnel training in the field of medical genetics.

Tasks: - Planning and organization of scientific and educational and research works in the field of medical genetics;

- Development of innovative educational programs, projects and educational and methodical complexes in the field of medical genetics;

- Introduction of findings of scientific researches and experimental development in the educational process;

- Organization of scientific and practical conferences, seminars, meetings, consultations on problems of medical genetics at the present stage;

- Assistance in preparation and professional development of scientific and pedagogical staff development in the field of medical genetics;

- Inclusion of students into research and scientific practical activities as well as application of comprehensive high medical technologies;

Principal directions of research work of the laboratory:

- Clinical-genealogic and molecular and genetic study of hereditary and congenital pathology at the people of the Republic of Sakha (Yakutia);

- Studying of molecular and genetic bases of multifactor and infectious pathology;

- Biochemical and molecular and genetic aspects of hereditary interchange pathology;
- Principles of the organization of genetic screening in the Republic of Sakha (Yakutia).

For the development of the Yakut science and upgrading strategy of medical education for hi-tech purposes in the Yakutsk state university the scientific and educational center «Genetic Health of the Population» on the basis of the tripartite Contract № 620-12/08 «About joint activity on the organization of research work between the Yakut state university, the Yakut scientific center SD RAMS RS(Y) RH№ 1-NCM MH» was estimated in 03.12.2008. The director of the SEC is the Professor, Dr. of Medicine Petrova Palmyra Georgiyevna, . Problems of the SEC include quality upgrading of scientific and pedagogical staff, increase of effectiveness of staff training for science, education and other branches of medicine; development of educational, scientific and innovative activity; creation of modern laboratory base for educational, research and productive activity.

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Clinical Description of a Rare Autosomal Recessive Syndrome in the Yakut Children

Gurinova E.E., Maksimova N.R., Sukhomyasova A.L.

ABSTRACT

The article describes the clinical signs (symptoms) of a rare storage disease in eleven Yakut infants. All the children were from unrelated families. The disease is very severe, leading to the disability and death at an early age. Diagnostics using the most complicated and advanced biochemical methods hasn't produced results.

Keywords: hereditary metabolic disease, lysosomal diseases, storage disease, Yakuts, Hurler-like phenotype.

INTRODUCTION

Inherited metabolic disorders (IMD) occupy a prominent place in the inherited human pathology. This place is defined as a large number of IMD, presently known, and a large number of patients with HMD, occurring in all populations are heavy and in most cases fatal manifestations [2].

Currently, more than 700 forms IMD. The vast majority of these diseases are caused by mutations in genes encoding enzymes, but also in this class include defects transport and signaling proteins. According to biochemical classification IMD divided into 22 groups according to the type of the damaged pathway (aminoacidopatologies, organic aciduria, etc.) or because of its localization in a particular compartment within the cell (lysosomal, mitochondrial and peroxisomal diseases) [3].

Clinical manifestations of the IMD is so diverse that there is no medical specialization, which would not have had to do with their specific spectrum of the IMD. The practical significance of the study is determined by purely medical aspects, the need to develop methods for diagnosis, treatment and prevention of these diseases that pose a serious challenge to modern health care. On the other hand, the IMD - the typical " biochemical " mutation of the human genome - is a powerful tool for the study of normal human metabolism, characterized by great complexity and a large number of "white spots " on your map [1]. Biochemical methods aimed at identifying the biochemical phenotype of the organism . The levels at which the measured phenotype can be different , from the primary gene product (polypeptide chains) to the end of metabolites in the urine or sweat . Therefore, biochemical

methods are extremely diverse, and their importance in the diagnosis of hereditary diseases is increasing.

Due to the variety of biochemical methods used in the laboratory diagnosis of hereditary diseases, the use of these methods must be defined system. We surveyed the proband or a member of his family is unrealistic to eliminate all genetic disease that could be in sight in the survey . If we apply the possible number of methods of diagnosis, then every survey will be a very time- consuming and lengthy . That's why the original scheme of the survey is based on the clinical picture of the disease, genealogical data and biochemical strategies that allow us to determine the further course of the survey based on the phasing out of certain classes of diseases. In addition , a modern diagnosis of hereditary diseases is unthinkable without the genetic research [4].

Lysosomal storage diseases (LSD) now includes 45 different shapes. LSD are rare hereditary diseases. The frequency of individual forms is from 1 : 40,000 to 1 : 1,000,000 , and the total frequency of LSD estimated 1: 5000 - 1: 7000 newborns. Clinical manifestations are extremely diverse LSD from the earlier, manifesting fetal hydrops, to the lungs, benign forms that manifest themselves in the 5-6 months of age and did not significantly affect the quality and duration of life. In the presence of the expressed clinical polymorphism LSD characterized by steadily progressive course and lead to early disability and premature death [1].

MATERIALS AND METHODS

Data on the patients were obtained from the National Register of hereditary genetic and congenital disorders Medical -Genetic Counseling of Perinatal Center State Budget Institution Republic of Sakha (Yakutia) “Republican Hospital №1-National Medical Center”. Survey was conducted clinical geneticist 11 infants with the same clinical picture Hurler - like disease. Also, all children were examined by doctors of other specialties (cardiologist, pulmonologist, neurologist, orthopedicist, audiologist, ophthalmologist).

RESULTS AND DISCUSSION

In the Medical -Genetic Counseling Department in Yakutsk from 2006 were counseling 11 children with Hurler-like phenotype: 7 girls and 4 boys. Eight children died before the 1,5 years old (including two boys) and one child died two years. All the children were from Yakut unrelated families. Parents are young.

Genealogical history of intermarriage was denied, but in most cases, both parents were natives of Vilyuiskaya region. Also a history of the two families, children from his first marriage were healthy. In one family, two children had Hurler-like phenotype, and died in infancy from

cardio-respiratory failure. In the same family of siblings sick children.

Medical history of that half of the children born from first pregnancy. During pregnancy birth defects, particularly heart defects, none of these children have not been identified (ultrasound investigation). There is information about visualization ventriculomegaly on II stage prenatal screening in one case.

All the children were born at term, full-term. Apgar score an average of 7-8 points.

Most of the children from birth or from a very early age (about 2 months) had so-called stigm disembriogenesis: heavy touch cheeks, nose, noisy breathing, on the hands all the fingers are slightly bent. Almost all children with the late neonatal period became concerned acute respiratory infections.

Onset of the disease was noted in an average of 4-5 months of age in the form of bronchial obstruction: noisy breathing, shortness of breath, coughing, high body temperature, heartbeat on the background of acute respiratory infection. Such acute conditions since the beginning tended to more frequent and each time the child's condition worsened, the increase of heart failure. Condition of patient became serious on valvular heart disease - first with mitral and tricuspid valves 1-2 degrees, moderate pulmonary hypertension. During 3-4 months disease is progressing, failure of the heart valves become 3-4 degrees and significant pulmonary hypertension.

All patients have similar clinical features:

- Dysplastic physique. In the dynamics of the delay in height and weight. With the progression of the disease forced position - sitting, lying. Long as the impossibility of lying on his back and on his abdomen.
- Face: gradual change like gargoilizm; hydrocephalic head closer to the hydrocephalic form, enlarged parietal tuber, low growth of hair on the forehead, hypertelorism, thick and thick eyebrows, long eyelashes, periorbital edema of the eyelids, wide noseband, a short nose with open nostrils forward, firm to the touch cheeks and nose, macroglossia.
- Skin: thick, relatively tight skin
- Skeletal: skeletal deformities, very short neck, chest wall deformity: barrel-shaped or bell-shaped, the shortening of the chest, stiff, wide aperture, mainly thoracolumbar kyphosis, lumbar spine, stiffness progression of small and large joints to contraction, claw –hand deformities, deepening palmar furrows.
- Respiratory system: the presence of bronchial obstruction, noisy breathing, shortness of breath increase, auscultation hard breathing, various dry and moist rales, the need for oxygen therapy.

- Cardiovascular system: tachycardia, hypertension, systolic murmur auscultation, birth heart defects, heart failure.

- Gastrointestina: enlarged abdomen due to hepatosplenomegaly, inguinal, umbilical, hernia non-operated (50%), without breaking the chair.

- Central nervous system: psychomotor retardation, development delay.

All children were conducted routin laboratory tests of blood and urine. Of the research results should be noted ultrasound investigation: hepatosplenomegaly, nephromegaly (3 cases). X-ray examinations showed signs of bone-destructive changes of the skeleton.

Echocardiography seven children after birth were identified congenital heart defects. One of them interventricular defect and atrial septal defect was detected on the 10 day, the remaining in the first six months of life. Five children was recommended surgery of CHD. Of these, four children were operated on, but one child died in the postoperative period, and the 5th child arrived. During echocardiography in all children were diagnosed with heart valve failure: the manifestation of the disease 1-2 degrees in the later stages - to 3 - 4 degrees, also showed signs of pulmonary hypertension at the beginning of a minor and in the end to marked degree.

As a result were excluded congenital hypothyroidism, cystic fibrosis, deficiency of alpha-1-antitrypsin, a chromosomal abnormality.

Medical history and clinical signs of possible suspect storage disease with Hurler-like phenotype. Due to the impossibility of challenging diagnosis in our respublic, analyzes and extracts children were sent to the federal Centers for enzyme diagnostic since 2006. In 2011, there were also investigated using dried blood spots on mucopolysaccharidoses types I and VI in the metabolic laboratory of the University Medical Center Hamburg (Germany). In 2013, one child was able to hold full-time counseling followed by laboratory diagnosis (enzymatic diagnosis, culture of skin fibroblasts to lysosomal storage diseases) in Department of Pediatrics Osaka University Graduate School of Medicine (Japan). Parallel biochemical study conducted in Lysosomal Storage Disease Section of Medical Department University of Cambridge (United Kingdom). Four out of eleven children were in a hospital examination of the federal medical centers (Moscow) like Institute of Pediatrics and Pediatric Surgery, Children's Clinical Hospital and National center of the healthy children.

During these years were excluded mucopolysaccharidoses I, II, III, IVB, VI, VII types , GM -1, 2- gangliosidosis , Gaucher's disease , mucolipidosis, disease Niemann -Pick type C / H / A, a hereditary amyloidosis, congenital disorder of glycosylation type I a, fucosidosis, mannosidosis, sialidosis, Krabbe, Fabry, Pompe. Despite the in-depth , sophisticated and modern

survey, accurate diagnosis for these children have not exhibited. But at the moment in Osaka University Graduate School of Medicine continues further research of this disease – the full genome sequencing for identification of the gene.

CONCLUSION

In conclusion, on the basis of clinical, genetic and instrumental and laboratory data described unverified rare recessive lysosomal disease with rapid progression and fatal in infancy. In this connection it's necessary to clarify the early diagnosis, which is made possible to due the full genome sequencing conducted in Japan.

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Clinical Characteristics of Tuberous Sclerosis in Children

Pshennikova G.M., Ozhegov P.S., Noyev D.D.

ABSTRACT

This article is devoted to the question of rare hereditary disease from the phakomatoses group with autosomal dominant type of inheritance. It's Bourneville- Pringle`s disease. There are discussed the questions of this pathology prevalence, variable clinical picture, modern diagnosing procedure. The results of examination of children with tuberous sclerosis are presented.

Keywords: Bourneville-Pringle's disease, tuberous sclerosis, clinical manifestations.

INTRODUCTION

Bourneville-Pringle's disease (synonym: tuberous sclerosis, epiloyya, central neyronomatoz, neurocutaneous syndrome type of Bourneville, syndrome seborrheic adenoma, seizures and mental retardation) - a hereditary disease characterized by systemic lesions of visceral organs, bones, eyes, skin, nervous system (for by disrupting the proliferation, migration and differentiation of glial cells), pathologic substrate which is hamartomatous proliferation of various localization [1]. Frequency of pathologies in newborns varies from 1:6000 to 1:10000, among the adult population is 1:20,000 - 1:100,000 .

Disease affects all races, men and women are equally likely to get sick. Type of inheritance - autosomal dominant, with more than 75 % of cases are caused by new mutations, variable expressivity, incomplete penetrance gene. At the genetic level the disease is caused by mutations inactivating one of the genes TSC1 (34 - th portion of the long arm of chromosome 9 - 1/ 3 of the cases), or TSC2 (13th portion of the short arm of chromosome 16 - 2/ 3 of the cases) encoding hamartin protein synthesis (130kD) and tuberin (180kD), respectively. Hamartin - tuberin complex plays a key role in regulating of cell growth [6 - 9, 12].

Normally, TSC1 and TSC2 - natural genes tumor suppressors, their damage activates the signal transmission R13K/Akt/mTOR that underlies the pathogenesis of TS. MTOR inhibitors - pathogenetic basis targeted systemic therapy of TS [3, 10]. In 1999 E.S. Roach proposed diagnostic criteria for TS [11] (Table 1)

Table 1

Diagnostic criteria of tuberous sclerosis

Primary signs	Secondary signs
Facial angiofibroma or fibrous plaques on the forehead	Multiple grooves in tooth enamel
Nontraumatic periungual fibroma	Hamartomatous rectal polyps *
Hypopigmented spots (>3)	Bone cysts **
Area of "shagreen skin"	Migration paths in the white matter of the brain
Multiple hamartomas of the retina	Fibroids gums
Cortical tuber	Hamartomas of visceral organs
Subependymal nodes	Achromatic portion of the retina
Giant cell astrocytoma	Hypopigmented spots "confetti" on the skin
Cardiac rhabdomyomas multiple and single	Multiple renal cysts **
lungs Lymphangioleiomyomatosis	
Multiple renal angiomyolipoma	

Notes: * - requires histological confirmation ** - enough radiological confirmation

Criteria for the diagnosis of TS unquestioned - one / two primary and two secondary features. Criteria for the possible diagnosis - one primary and one secondary sign, presumptive diagnosis - one primary or two (or more) of the secondary [11].

The difficulties of diagnosis of tuberose sclerosis (TS) are associated with a marked of clinical polymorphism. Patients with TS are observed by doctors nearly all specialties during the life, so only informed about the nature and characteristics of the course of these diseases, as well as the consistency of their diagnostic and therapeutic measures can ensure the correct choice of tactics of treatment of patients. The clinical course of the disease was isolated 4 forms: classical, psycho-neurological, skin, and liquor. The classical form of the disease is manifested by an adenoma of the sebaceous glands, epilepsy, mental retardation (Vogt`s triad). At the predominance of general and focal neurological symptoms caused by the enlargement of neuroglia of various divisions of the brain, we are talking about neuropsychiatric disease form, with primary skin lesions - dermatological form. When there are symptoms of spinal fluid hypertension - liquor one [4, 8].

Bourneville-Pringle's disease has a tendency to progression. Prognosis in most cases unfavorable and depends on the severity of internal organ involvement. Almost 30% of patients do not survive to 5 years, and patients aged 6 years and over 75% of cases - up to 20 years [5].

All patients with any type of TS, as well as their relatives, especially first-degree relatives, are subject of multidisciplinary dynamic observation and inspection throughout life. Only such an approach can provide adequate therapy and will allow for genetic counseling in families with identified patients with this severe multisystem disease [2].

Purpose of the research: to analyze the clinical features of tuberous sclerosis in children.

MATERIALS AND METHODS

The study included patients with Bourneville-Pringle's disease, who were examined and treated in the Republican hospital №1 - National Center of Medicine, Pediatric center neuropsychiatric department number №1 (head of department Basova E.V.) and №2 (head of department Androsova Z.P.), clinical advisory office (neurologist Nikolaeva G.E.), office of children's epileptologist of City Children's Hospital (neurologist Vyuchin A.V.).

For each patient it is filled developed formalized questionnaire survey, which includes the anamneses of life, disease, somatic and neurological status, the nature and frequency of attacks, data of paraclinical examinations (EEG, MRI of the brain), social data, therapy.

Diagnosis of Bourneville-Pringle's disease is put due to International diagnostic criteria recommended by the International Committee of Experts on the tuberous sclerosis. Magnetic-resonance tomography of the brain is carried out in the Department of radiation diagnostics of Pediatric centre National Center of Medicine.

RESULTS AND DISCUSSION

In the course of the study, for the period 2008 - 2013 years, 7 children were diagnosed with tuberous sclerosis. In all cases of the disease it was male patients. Surveyed children were ranged in age from 8 months to 10 years. At the time of the study 1 child was at the age of 8 months, 1 patient - 2 years, 2 children - 3 years, 1 patient - 5 years, 2 boys - 10 years. Nationality of patients are: 5 children - Yakuts, 1 child - Russian, 1 patient - Uzbek.

Three mothers (43%) of the surveyed children had burdened obstetric and gynecological history: pregnancy proceeded against the background of the constant threat of interruption, chronic nicotine intoxication, toxicity, anemia, chronic pyelonephritis. Heredity, according to parents, was not burdened.

The onset of disease before the age of 1 year was in 5 children, 2 years in 1 child, 3 years in 1 patient. In all cases, the disease began with a seizure attack. At the onset of disease West

syndrome was diagnosed in 3 children, with attacks in the form of infantile spasms, in other cases, the attacks bore the focal character. At the time of the study in 5 (71%) children seizures were in the nature of the focal with secondary generalization, with frequency up to 5 times a day, 2 (29%) children attacks had the focal character, with frequency up to 2 times a day.

The first physician who has treated patients, in all cases was the neurologist. In the neurological status of all children showed symptoms pyramid insufficient: quickened tendon and periosteal reflexes with limbs; increased muscle tone 1 child. In one patient noted common hyperkinesis. All children were diagnosed with symptomatic epilepsy. Delayed psycho-speech development was revealed in 3 children with onset under 1 year.

On examination of the skin in the lumbar region areas of modified skin type «shagreen», and the areas of depigmentation various areas of the body were detected in 3 (43%) of the surveyed (Fig.1), facial angiofibroma (adenoma Pringle) - in 1 patient. When conducting neuroimaging research methods brain subependymal nodes was noted in all the examined children (Fig.2), subependymal giant cell astrocytomas - 1 child. Fundus examination revealed the presence of retinal hamartoma in 1 child (14%). Held other clinical methods of examination (sonography of abdominal organs, Echocardiography, CT chest) possible to diagnose polycystic kidney disease in 2 children, multiple renal cysts in 1 child, multiple rhabdomyomas heart in 2 children, lymphangioleiomyomatosis lungs in 1 child.

The children received symptomatic therapy. Treatment of epilepsy was conducted with anticonvulsant valproate monotherapy in 5 children, valproate polytherapy + oxcarbazepine in 1 child, valproate+topiramate + in 1 patient.

CONCLUSIONS

The Bourneville-Pringle's disease despite the low incidence of it has a severe course, a high percentage of disability and poorly amenable to correction. Timely diagnosis allows determining the future management of patients, and providing genetic counseling to family members, which should help reduce the incidence of this disease. To improve the diagnosis of tuberous sclerosis all doctors need to pay attention when inspecting children on cutaneous manifestations of the disease. There is also a need for the introduction of molecular genetic analysis to confirm the diagnosis and to prenatal diagnosis of the disease Bourneville-Pringle in Yakutia.

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Figure 1. Cutaneous manifestations of the Bourneville-Pringle's disease

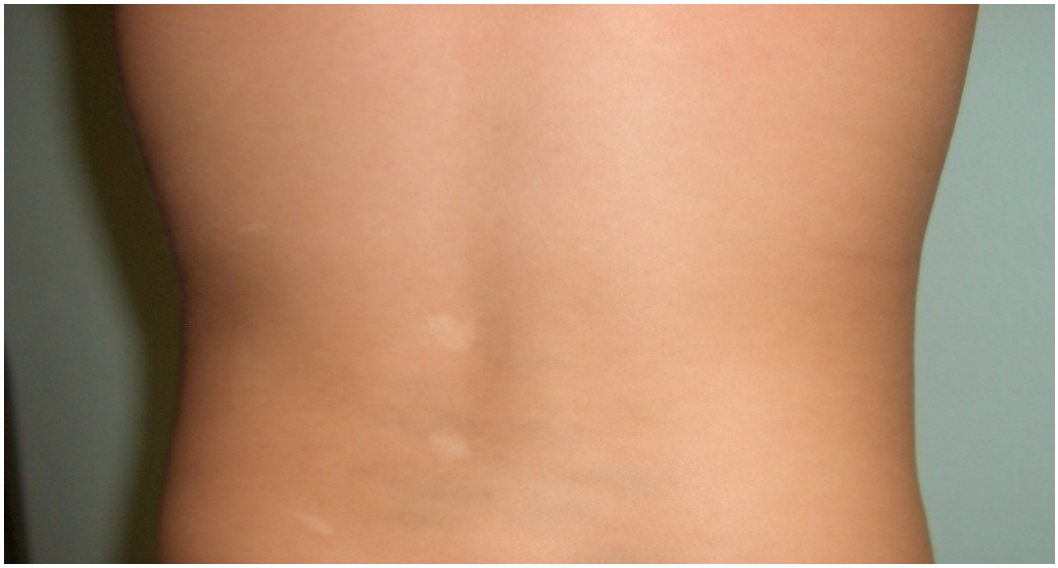
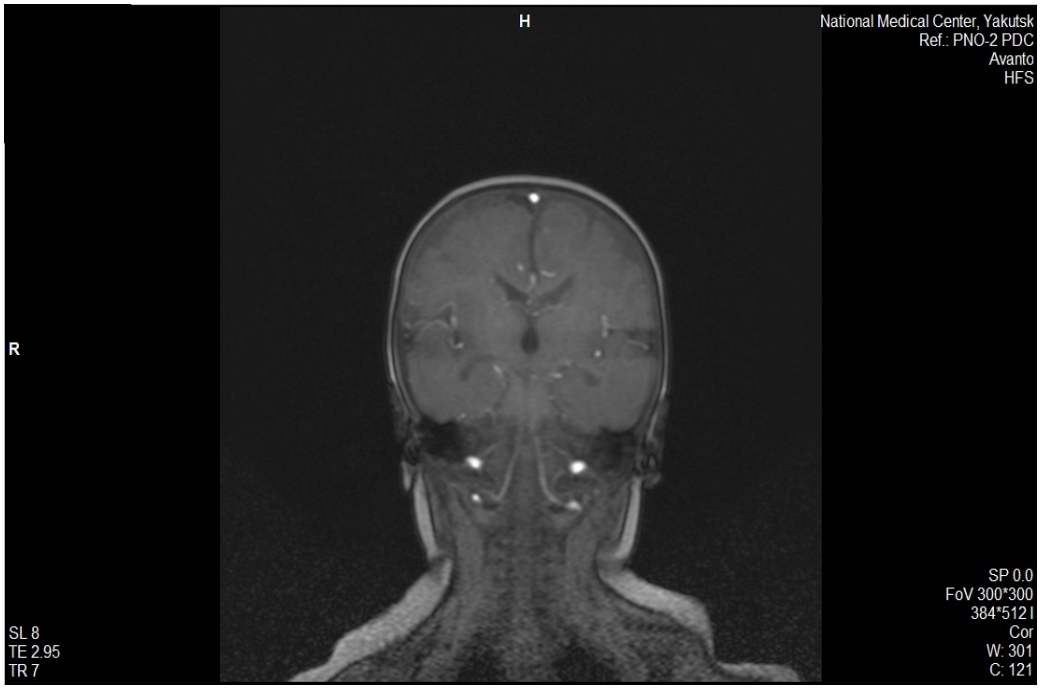
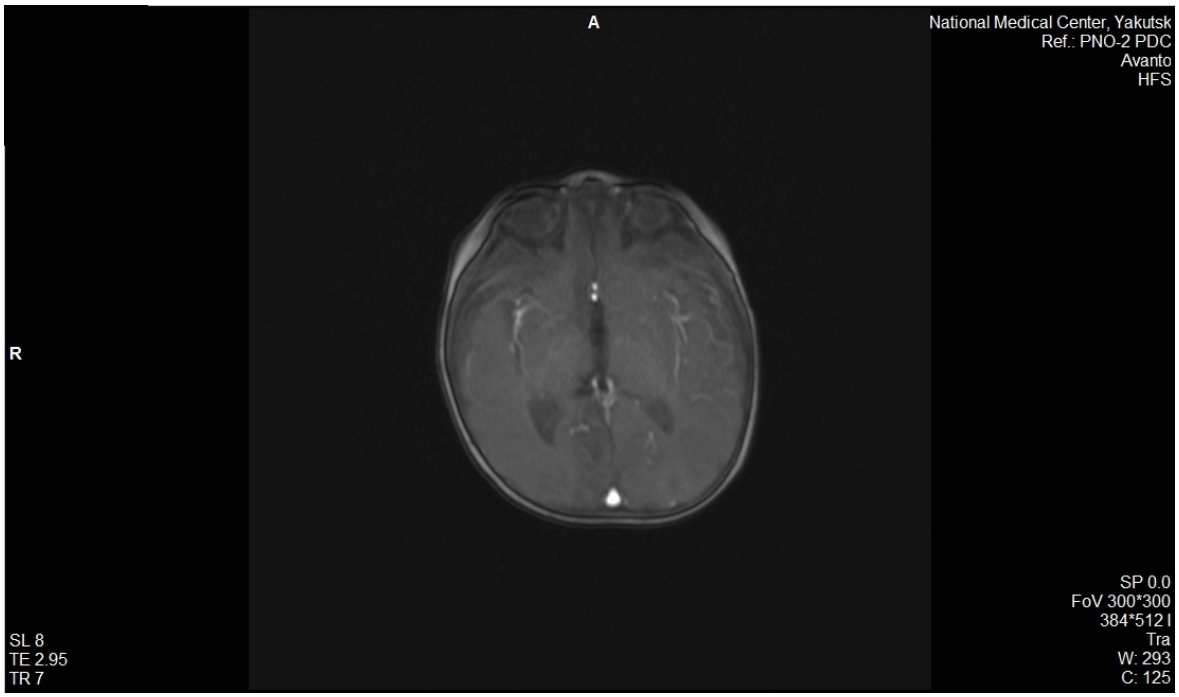


Figure 2. MRI data of patients with tuberous sclerosis.

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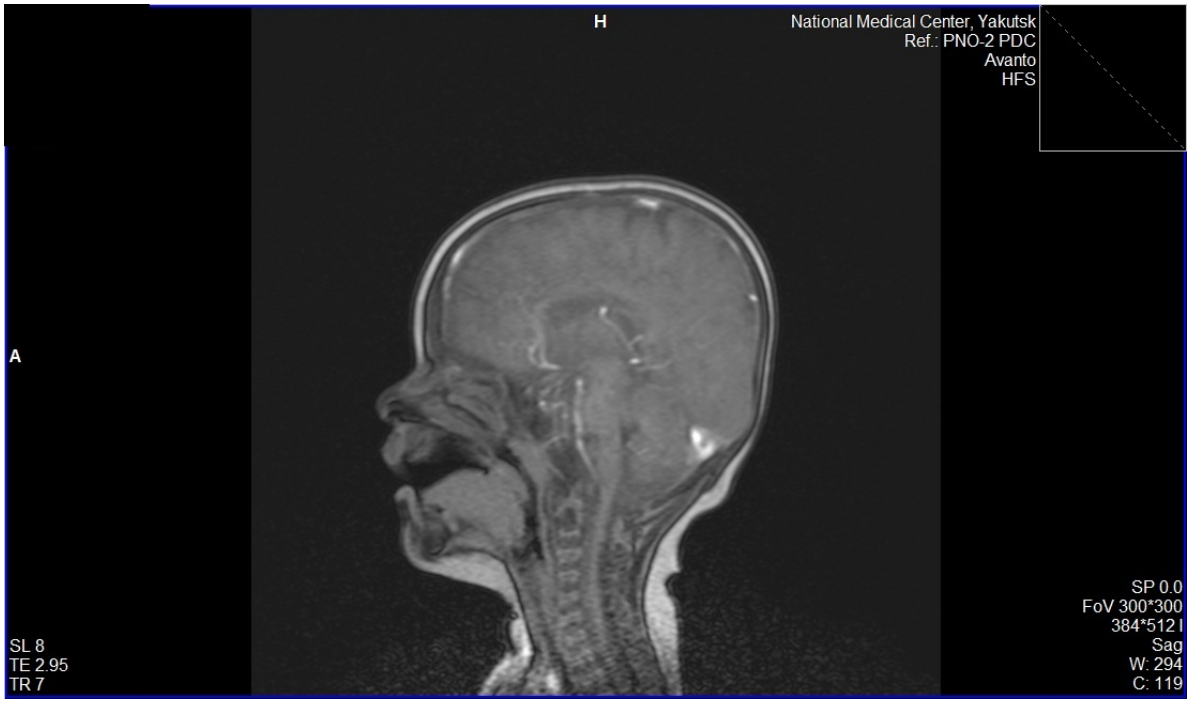
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Genetic Epidemiology of Hereditary Diseases among the Child Population in Eight districts of Tatarstan Republic

Zinchenko R.A., Vasilieva T.A., Elchinova G.I., Petrova N.V., Petrin A.N., Ginter E.K.

ABSTRACT

The results of the genetic epidemiological study of monogenic hereditary disorders (MHDs) among child population of eight districts of Tatarstan Republic are presented in the report. The total size of the investigated population is 268,894 individuals, from which the proportion of child population makes 21.44% (57648 children). The total population was examined by standard protocol of medical genetic research elaborated in laboratory of genetic epidemiology, Research Centre for Medical Genetics. About 3500 MHDs of OMIM could be identified by this protocol. Clinical investigations were performed by neurologists, ophthalmologists, orthopedic, otolaryngologists, dermatologists, pediatricians and clinical geneticists, focused on diagnostic of MHDs. The spectrum of MHDs detected in the eight districts RT comprises 256 diseases, including 135 autosomal dominant (AD), 97 autosomal recessive (AR), and 24 X-linked diseases. The MHDs diversity in the child population of RT comprised 158 diseases (61.72% the total number of registration disorders), including 84 (62.22%) AD, 54 (55.67%) AR, and 20 (83.33%) X-linked ones. The load of MHDs (AD, AR and X-linked) in rural and urban child population is calculated, the variety of common MHDs is described. The prevalence of MHDs among children from Tatarstan region occurs to be 1:103. Significant differentiation in the values of the MHDs load (AD, AR and X-linked disorders combined) between districts was detected. An attempt to explain the revealed differences is undertaken.

Keywords: the load and variety of hereditary diseases, genetic epidemiology, child population, prevalence rate, Tatarstan Republic.

INTRODUCTION

The Improving of the quality of medical care and the development of molecular genetics have led to relatively increasing of congenital and hereditary diseases proportion in the structure of morbidity, disability and mortality of the child population. According to this line the total

frequency of monogenic, chromosomal and genetic-related diseases is estimated at about 95-139 per 1000 people, of which monogenic hereditary diseases account for 5-17 per 1000 [4,7].

The main load of monogenic hereditary diseases (MHDs) falls on the child population; these hereditary diseases appear during the life of a particular neonate cohort. Up to 25% of MHDs manifests by the birth of a child, up to 70% of MHDs has already diagnosed by the 3rd year, debut and first diagnostic features of the disease has been defined for almost 90% of the MHDs by the end of puberty [4,7].

According to WHO, the approximate incidence of congenital MHDs is 10-15 per 1000 live births, 58% of which early dies, 31 % has cases of chronic conditions and disability, and only 11% is to be medically inpatient and outpatient treated. The precise data on loads of MHDs among children in the populations of the world and in Russia's regions are absent. Summary of the genetic loads is presented by data on chromosomal aberrations and monitoring of congenital malformations in lot of countries, while the MHDs burden remains mainly represented by the WHO data, as well as by the special registers of individual countries [2,7,8].

The purpose of the study. The aim of the study was to genetic epidemiological study of MHDs among the child population of the Republic of Tatarstan.

MATERIALS AND METHODS

In Russia the researches within genetic and epidemiological study of MHDs among children are conducted by the Research Center for Medical Genetics of the Russian Academy of Medical Sciences. Researchers are in progress according with the protocol of genetic-epidemiological studies. This protocol is developed in the laboratory of genetic epidemiology of the Research Center for Medical Genetics. The protocol includes three main research strategies: medical genetic study of populations (providing the capability to identify approximately ½ of currently known hereditary diseases), estimation of the genetic structure using nonbiological population statistical methods, and DNA polymorphisms analysis. The MHD diagnosing was performed by focused specialists in the expedition (they are genetic syndromologist, a pediatrician, a neurologist, an otolaryngologist, a dermatologist, an orthopedician, and an ophthalmologist from research and medical centers of Moscow) [3,5].

The material for analysis was collected in the course of genetic epidemiological study of the whole population of eight Tatarstan districts in 2009–2013 (Arsky, Atninsky, Kukmorsky, Buinsky, Drozhzhanovsky, Aktanishsky, Muslumovsky and Menzelinsky districts). After then, children (0-18 years) were selected the total sample, the load of MHDs was calculated and its variety estimated. The results obtained are compared with the data obtained earlier in some

regions of the European part of Russia (Chuvashia (RC), Udmurtia (RU), Bashkortostan (RB) and the Rostov region (RR) [1,6,9]. The total size of investigated population of Tatarstan comprised 268894 people, including 57648 (24.4%) children. The ethnic structure of the considered sample is presented by more than 80% Tatar population.

RESULTS AND DISCUSSION

While the medical genetic survey of eight districts we found in total 1597 patients from 1077 families with various clinical forms of MHDs. To estimate the hereditary load in the child populations, we considered separately patients with MHDs in the age interval from birth to 18 years inclusive. All in all we found 561 children (35.13% of the total number of patients) from 471 families with various clinical forms of MHDs, including 313 children from 258 families with AD diseases, 191 children from 167 families with AR diseases, and 58 children from 46 families with X-linked diseases.

On average, the total proportion of children with MHDs among all MHD patients was 35.19% (46.85%, 43.53%, 37.25% and 41.65% in RC, RU, RB, RR, respectively) [1,6,9]. The number of diseased children with AR and X-linked diseases prevails in each region and in the total sample. The mean proportions of children with AD, AR, and X-linked diseases in the five regions were 37.94%, 45.24%, and 50.44%, respectively. This situation is explained by the fact that part of the AD diseases manifests in older age. In addition, AR and X-linked pathology clinically is more severe, more often it is lethal either has a reduced fitness of patients for most diseases. Table 1 shows the load (and prevalence rates of MHDs in child populations in the eight districts studied and in some populations of Russia (RC, RU, RB,RR).

Table 1. The load (per 1000 children) and prevalence rates of MHDs child populations of the Republics of Tatarstan (RT), Chuvashia (RC), Udmurtia (RU), Bashkortostan (RB) and Rostov Region (RR).

Population (district)	Population size	Load of HDs (per 1000 children)			Prevalence rate
		AD	AR	X-linked	
Arsky	11029	5.35±0.69	2.36±0.46	1.45±0.51	1:119
Atninsky	2742	12.40±2.11	6.20±1.50	4.38±1.78	1:48
Kukmorsky	11431	4.11±0.60	3.06±0.52	1.92±0.58	1:123
Buinsky	9655	4.76±0.70	2.18±0.47	1.66±0.59	1:129
Drozhzhanovsky	5572	5.56±1.00	3.95±0.84	1.44±0.72	1:98
Aktanishsky	6770	5.76±0.92	2.81±0.64	2.07±0.78	1:104
Muslumovsky	4668	7.93±1.30	5.57±1.09	4.28±1.35	1:64
Menzelinsky	5781	3.46±0.77	4.32±0.86	1.38±0.69	1:118
Mean of RT	**57648**	**5.43±0.31**	**3.31±0.24**	**2.01±0.26**	**1:103**
Mean of RC	**57648**	**5.43±0.31**	**3.31±0.24**	**2,01±0.26**	**1:103**
Mean of RU	**67863**	**2.43±0.19**	**2.18±0.18**	**0.80±0.15**	**1:200**
Mean of RB	**60197**	**3.22±0.23**	**1.81±0.15**	**1.50±0.22**	**1:173**
Mean of RR	**64935**	**3.87±0.24**	**2.51±0.20**	**0.92±0.17**	**1:146**

We found significant differences in the AD genetic load between districts of RT (χ^2=38.26, D.f.=7) and for AR load (χ^2=23.98, D.f.=7). Differentiation between districts with X-linked pathology was not found (χ^2=7.25, D.f.=7). However, the basic medical genetic characteristics of most populations are formed as a result of complex interaction between various population dynamic factors. In addition to genetic drift, which is the main factor in microevolution, modern populations of Russia are also affected by natural selection and migrations [1–6]. With the purpose of definition of communication population structure and factors of microevolution in the formation of differences between districts in the level of MHDs load a correlation between the values of a random inbreeding F_{ST} (F_{ST} in Arsky – 0.00088, Atninsky – 0.00163, Kukmorsky – 0.00072, Buinsky – 0.00058, Drozhzhanovsky – 0.00062, Aktanishsky – 0.00081, Muslumovsky – 0.00075 and Menzelinsky – 0.00042) and load of AD and AR of MHDs in children was calculated. The linear correlation coefficients for AD and AR pathologies were r=0.91±0.17 and r=0.52±0.35. The revealed differences in load values between

districts can to some extent be explained by a lower level of migration, expressed by genetic subdivision and natural selection.

The spectrum of MHDs detected in the eight districts RT comprises 256 diseases, including 135 autosomal dominant (AD), 97 autosomal recessive (AR), and 24 X-linked diseases. The MHDs diversity in the child population of RT comprised 158 diseases (61.72% the total number of registration disorders), including 84 (62.22%) AD, 54 (55.67%) AR, and 20 (83.33%) X-linked ones.

There revealed 24 the most frequent nozological form (with a prevalence rate of 1 : 15000 or higher) among child population. With AD disorders they are the following: Ehlers–Danlos syndrome (prevalence rate 1:721), mental retardation (1:3391), ichthyosis (1:3843), palmoplantar keratoderma (1:4434), neurofibromatosis, type I (1:7206), scoliosis, idiopathic (1:9608), ptosis, hereditary congenital (1:11530), tuberous sclerosis (1:14412), Marfan syndrome (1:14412), Sturge–Weber syndrome (1:14412), curly hair with deafness syndrome (1:14412), polycystic kidney disease (1:14412), cataract hereditary (1:14412), retinoblastoma (1:14412), blepharophimosis with ptosis (1:14412), hypochondroplasia (1:14412).

The most frequent among AR diseases are: nonsyndromic neurosensory deafness (prevalence rate 1:1517), mental retardation (1:2306), oculocutaneous albinism (1:5241), amniotic band sequence (1:7206), congenital hypothyroidism (1:7206), orofaciodigital syndrome II (1:8235), phenylketonuria (1:9608), congenital glaucoma (1:14412).

The most frequent among X-linked disorders (with prevalence rate 1:15000 boys or higher) are: mental retardation (частота 1:1802), congenital nystagmus (1:5765), hemophilia, type A (1:9607), Parkinson disease 12 (1:14412), Duchenne PMD (1:14412), lymphoproliferative syndrome (1:14412).

The comparison of MHDs spectrum in the RT with other regions has shown the existence of pronounced regional features.

CONCLUSIONS

As follows from the present study, the total prevalence of MNDs among the child population of RT accounts for 1:103 children. Given that the methodology used by this research allows to diagnose about half of all known to date hereditary diseases, we may assume that the actual load of hereditary diseases in the child populations of Tatarstan Republic is approaching to 1.5%. Similar results were obtained during the examination of children in Udmurtia (1.2%), in Bashkortostan (1.4%), in Chuvashia (1%), in Rostov Region (1.3%). Most of the identified

diseases in children are significantly affect the duration and quality of their lives, what to consider when developing prevention of child morbidity, disability and mortality.

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Differential Diagnosis of Kennedy Spinal-Bulbar Amyotrophy and Familial Form of Motor Neuron Disease in the Republic Sakha (Yakutia)

T.K. Davydova, F.A. Platonov, T.E. Popova

ABSTRACT

In a result of clinical and epidemiological and genetic studies of the motor neuron disease in Yakutia, according to the register of the neurological department of Belarus № 2 for the period 1989-2013 1 MND family event was identified and 3 patients with spinal - bulbar amyotrophy Kennedy. The complexity of the differential diagnosis between these diseases has been very difficult on the clinical stage of diagnosis, because in all cases isolated lesion of peripheral motonevrona characteristic of both diseases was found.

Keywords: motor neuron disease (MND), amyotrophic lateral sclerosis (ALS), progressive muscular atrophy (PMA), spinal- bulbar amyotrophy Kennedy.

INTRODUCTION

Spinal- bulbar amyotrophy Kennedy is the main condition, which is necessary to differentiate the MND family event. Taking into account the issues of bioethics in the diagnosis of MND established diagnosis of spinal- bulbar amyotrophy for the patient becomes relatively favorable than the diagnosis of ALS.

Spinal- bulbar amyotrophy Kennedy or Kennedy 's disease (MIM 313200) - this is a rare neurodegenerative disease X-linked recessive mode of inheritance, which manifests itself in men in a relatively late age, usually after age 40. The disease was first described by Kennedy [et al.] in 1968. The clinical picture of the disease presented symptoms of peripheral motor neuron and is manifested by weakness and muscle atrophy in the proximal and distal extremities with fascicular twitching and bulbar impairment. In addition to these symptoms this disease is characterized by endocrine disorders, such as gynecomastia, infertility, testicular atrophy. Despite the late onset disease is characterized by slowly progressive course with very high life expectancy [12]. The incidence is - 1 in 40 000 men [13]. Kennedy disease is caused by damage of the androgen receptor (AR), located in the Xp11.2- 12 locus [6]. Mutation is the expansion of tandem repeats in the CAG- 1 exon gene AR. The normal number of copies of CAG is 10-36, while Kennedy amyotrophy patients have an increased number of tandem repeats - from 38 to 72. [11]. Considering Kennedy disease debut in later life and the electoral defeat of peripheral motor neuron, the disease is difficult to diagnose based only on clinical symptoms, because

conducting molecular diagnosis of the disease is possible in health care settings, with molecular genetics laboratory.

The differential diagnosis of spinal- bulbar Kennedy amyotrophy and ALS needs to be considered in the clinical picture of disease presence or absence of symptoms referable to the central motoneuron, loss of peripheral motor neuron disease lack of dependence on the sex of the patient is characteristic of ALS, a progressive disease course (fast and slow ALS disease in Kennedy), as well as rare hereditary abnormalities occurring in ALS. Unlike ALS, the presence of clinical symptoms of the electoral defeat of the peripheral motor neuron only, male sex, and endocrine disorders such as infertility, gynecomastia, testicular atrophy can be suspected spinal-bulbar amyotrophy Kennedy, even in the absence of family history. Although the particular difficulties can be found if the patient has a «false negative " family history, when a patient has no communication with relatives in both cases. In the diagnosis of Kennedy's disease may also help the relatively slow progression of the disease, in contrast to ALS.

For motor neuron disease (MND) are severe neurodegenerative disease of unknown etiology and pathogenesis unspecified , which is characterized by the selective loss of central and peripheral motor neurons and progressive course , invariably leads to death. According to the International Classification of Diseases - X (ICD - X, 2003), are a group of MND family motor neuron disease, amyotrophic lateral sclerosis (ALS), primary lateral sclerosis (PLS) , progressive bulbar palsy (PBP) and progressive muscular atrophy (PMA). The basis of this classification is the concept of the unity of the pathogenesis of bulbar, spinal central and peripheral motor neurons and CBE, FSN and ACA treated as isolated lesion of the central or peripheral motor neuron. The most common of this group in the world is amyotrophic lateral sclerosis (ALS), which accounts for 80 % of all other diseases, PMA -9 %, 8% of PRP - and PlS- 2% [5].

In clinical practice, due to the lack of a specific test and a variety of diseases that are accompanied by a syndrome of ALS diagnosis MND for neurologists is very challenging. The diagnosis of ALS is currently mainly clinical, as there is no specific marker and the data of laboratory and instrumental examination methods are complementary to clinical assessment of motor disorders that often creates difficulties in diagnosis, especially in the early stages of the disease. In favor of ALS in the early stages of the pathological process may indicate violations of respiratory function, dysarthria and dysphasia, which cannot be explained by other causes.

In 5-10% of all ALS patients are family cases [7, 9]. About monogenic inheritance of the disease among relatives a positive family history testifies. Family ALS cases can be transmitted as an autosomal dominant, autosomal recessive or X-linked pattern of inheritance. X - linked

dominant MND was found in a large American ancestry [15]. Different types of inheritance MND say about its genetic heterogeneity. To date, at least 12 chromosomal loci associated with the development of autosomal dominant, autosomal recessive, or X-linked forms of ALS as well as some ALS -like syndromes are identified. To half of these forms genes and their protein products are set [3]. Familial ALS, according to the clinical features and course of the disease, can be subdivided into two distinct subgroups: 1) "classic" late-onset ALS is inherited in an autosomal dominant manner and is associated with nine independent chromosomal loci 2) "juvenile» ALS with the manifestation of symptoms in adolescence) juvenile ALS is characterized by the onset of disease is most often on the 2nd decade of life, the variable ratio of expression of central and peripheral motor neurons, as well as a very slow progression, in some cases does not affect the natural life span [14]. Juvenile ALS can be inherited in an autosomal dominant and autosomal recessive manner.

Clinical manifestations and morphological changes in sporadic and familial forms generally have a similar picture, but in general for the familial forms of the disease, the clinical picture is characterized by several symptoms earlier debut [1].

Approximately 20% of patients with familial forms of ALS disease is caused by mutations in a gene located on chromosome 21q 21 and encoding cytosolic enzyme Cu / Zn superoxide dismutase (SOD1) [2]. This locus and the corresponding form of ALS were designated ALS1. According Andersen PM [1], underdiagnosis of familial ALS can artificially lower because of low penetrance gene SOD1, due to the lack of collected family history or " “false-negative” family history in the case of loss of communication between the different branches of the same family, the reluctance of family members to recognize hereditary nature of the disease, the death of family representatives, who were carriers of the gene from other causes of ALS at an earlier age, ALS underdiagnosed in earlier generations, the presence of illegitimate children in families.

Variability of forms of the disease within families was marked. Very rarely in patients within the same family one and the same form of ALS was described. The development of progressive muscular atrophy, classical ALS, progressive bulbar palsy, and primary lateral sclerosis, even within the same family is probable [10]. According to some studies intra-variability of ALS is even superior inter-family [8].

The next group of disease MND, which are encountered in doctor’s practice, is progressive muscular atrophy. First family event PMA was described in 1850 by F.Aran. He called this disease progressive muscular atrophy. In the described case, the patient, his sister and

two uncles of his mother died from the disease, which is characterized by atrophy, paresis of muscles of the upper and lower extremities. Around the same time, A. Duchenne described a similar syndrome, which was accompanied by progressive muscle weakness. Later, J.M. Charcot called PMA named it after both researchers Duchenne - Aran disease [11]. In 1880 W.Oster described progressive muscular atrophy in 13 patients in two generations of one family [1]. Absence of clinical PMA endocrine disorders, rapid progression of the disease, and the absence of hereditary factors suggest the possible PMA. But the decisive role belongs to direct DNA diagnosis, which in the case of spinal- bulbar amyotrophy Kennedy reveals increase trinucleotide CAG - repeats in the androgen receptor gene AR.

MATERIALS AND METHODS

Since 2006 all patients with MND entered in the register of the neurological department of Republican hospital № 2 with questioning and filling neurodevelopmental card followed by molecular genetic diagnosis to avoid Kennedy disease. Molecular genetic testing is carried out in the molecular genetics laboratory Genetic counseling (MGC) of the Republican hospital №1- National Center of Medicine (NCM). During the period from 1989 to 2013 130 patients with MND were included into Register.

RESULTS AND DISCUSSION

As a result of clinical and epidemiological studies according to the register of the neurological department of Republican hospital № 2 we revealed 113 or 86.9 % of patients with ALS; 13 patients with PMA (10 %); PLS 5 patients (3.8 %) and 2 patients with PRP (1.5%). 1 family event and 3 cases of MND spinal- bulbar amyotrophy Kennedy were revealed. According to the MGC NCM, Yakut three unrelated families in which it was revealed 7 patients with Kennedy disease, are under Neurogeneticist supervision [4].

At filling neurodevelopmental card in the test case of family MND autosomal dominant inheritance from the father in the three children from six (proband and his two older sisters) was established: All patients were diagnosed clinically MND in the neurology department of the Republican hospital № 2, except for father, who died at age 52 and at the time of addressing his son to medical facility, he was not alive. From the stories of relatives, he two years was bedridden because could not walk due to weakness in the legs, swallowed badly, suffered excessive salivation, his speech was slurred, at the end of his life he lost it. In youth he had pulmonary tuberculosis. There was no information about the other relatives from the father and mother sides. The family believed that their father died from lung cancer, because before his death he had dyspnea: slept in a semi-sitting position, could not cough up phlegm and spitting.

In the clinical picture of all patients with the family MND isolated lesion of peripheral motoneuron is noteworthy, which is manifested by areflexia, atony and bulbar disorders without sensitive and pelvic disorders, and early onset of the disease with a slow progression of the disease. In the clinical case in the proband the first symptoms appeared at age 32, when he noticed the weight loss of hands, their shaking, and body muscle twitching. Bulbar disorders joined only after 10 years. In total duration of disease was 14 years. In older of two sisters disease debut manifested itself in 40 years, when she began to complain of weakness in the legs: having trouble in climbing the stairs and into the bus. Gradually weakness in the hands joined, atrophy of hands, feet appeared, there were fascicular twitching and bulbar disorders. During the last 2 years of life, she was bedridden, unable to move independently, roll over in bed; she could not eat because of swallowing disorders. She died at the age of 48. Disease duration was 8 years. The younger sister, born in 1950, lives constantly in one of the republics of the CIS, and occasionally travels to relatives in Yakutia. She became ill at the age of 50 years. The disease began as well as his older sister with weakness in the legs, began to move slowly, climbing stairs with difficulty, could not hold a spoon in hand, cup and other items. There was atrophy of the hands and feet, slurring of speech appeared. Currently, the clinical picture of the disease in this patient appears with sluggish deep proximal tetraparesis, distal tetraplegia and moderate bulbar disorders without pelvic and sensory disorders. She moves in a wheelchair with the accompanying. Duration of her disease is today 14 years. All members of the described family are of Yakut ethnic group and are natives of Hangalassky area, which belongs to the Central region.

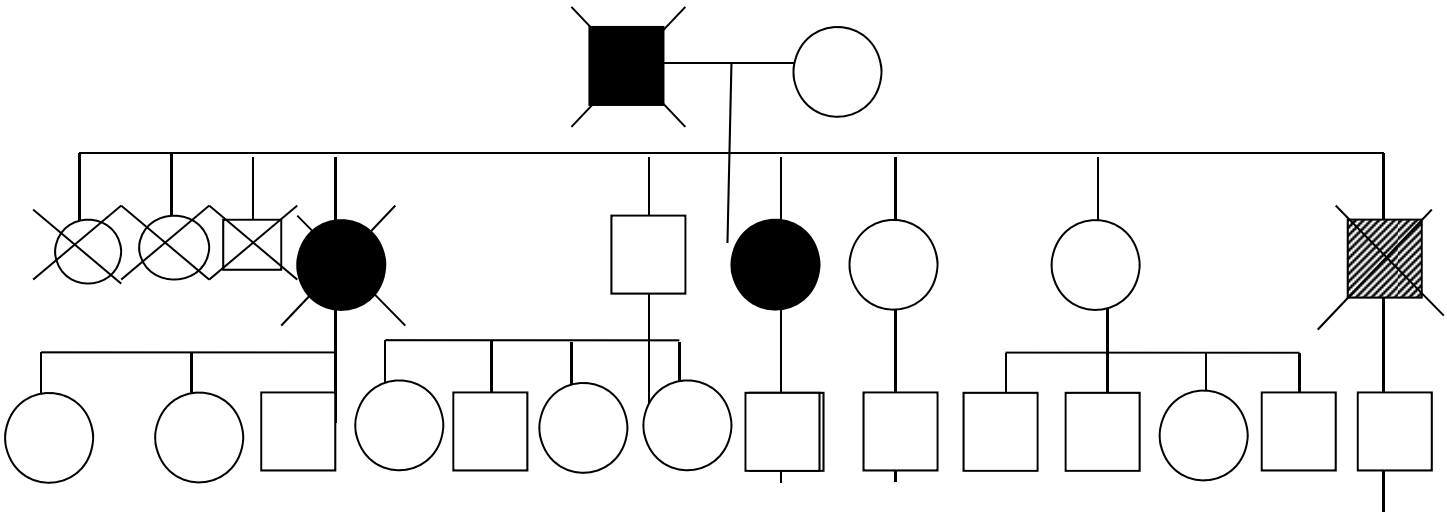

Fig. 1. Ancestry of a patient F., born in 1961, with a family MND

Fig. 1 shows that the disease is transmitted in an autosomal dominant pattern of inheritance from the sick father. From six adult children three children are diseased: two sisters and a younger son. Three older children died in infancy from a few months to 1.5 years. Characteristic of this family event is a relatively early onset of the disease, the lack of symptoms referable to the central motoneuron and relatively long duration of the disease with a slow progression that is not characteristic of ALS. These symptoms allow suggesting in the diseased members of this family a rare disease of MND groups - progressive muscular atrophy (PMA) or disease Duchenne - Aran.

Figure 2. Patient V., 72, with progressive muscular atrophy

In our practice in Yakutia a man, who was observed with ALS for 2 years in the neurology department of the Republican hospital № 2, was diagnosed with spinal- bulbar amyotrophy Kennedy in 2004. It was the first clinical diagnosis of SPA in Yakutia. The diagnosis of spinal - bulbar amyotrophy Kennedy was installed after the introduction into clinical practice of molecular genetic diagnostics at the National Center of Medicine in Yakutsk.

In total from the patients referred from different regions of the Republic to the neurological department of the Republican hospital number 2, suspected for MND, since 2004 according to the register 3 cases of spinal- bulbar amyotrophy Kennedy were identified. All patients underwent direct DNA diagnosis, which revealed the expansion of trinucleotide tandem repeats gene CAG- AR. The debut of the disease was observed from 44-56 years, with the development of total muscle fatigue in normal physical activities. 5-6 years later joined paresis mimic muscles with fasciculations of the facial muscles and bulbar disorders as choking, dysarthria, atrophy and fibrillation language flaccid tetraparesis with low reflexes. From endocrine disorders two patients were experiencing gynecomastia, infertility, one patient had early extinction of reproductive function.

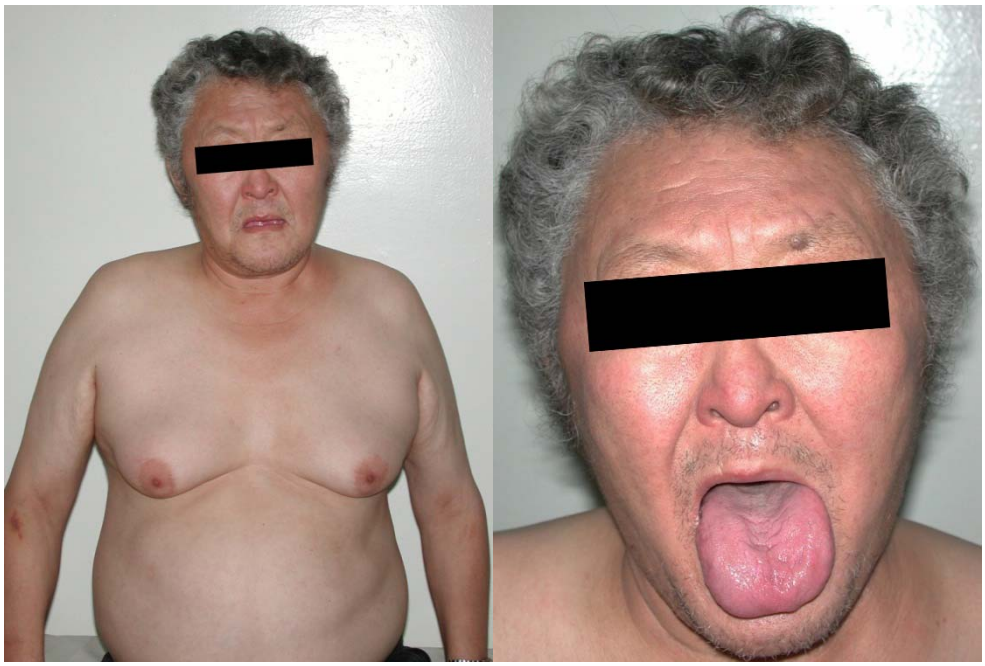

Figure 3. Photo of a patient with X-linked bulbar - spinal amyotrophy. Visible muscle wasting of the shoulder girdle, gynecomastia and tongue with signs of atrophy.

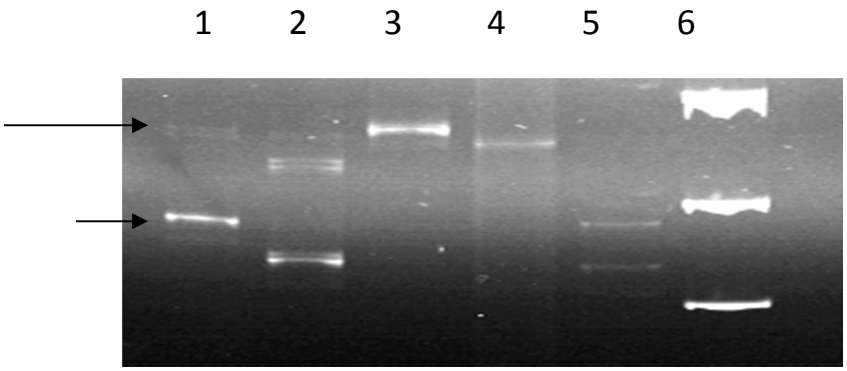

Figure 4. Electrophoregram of patient with Kennedy's disease

Direct DNA diagnosis of spinal - bulbar amyotrophy Kennedy. Electrophoregram 8% polyacrylamide gel: lane 6 - marker puc 19 / MspI, track 3.4 - patients with spinal-bulbar amyotrophy Kennedy, lane 2 - heterozygous carrier of the mutation (relative),

Lane 1, 5 - healthy. Long arrow marks the mutant allele (expansion CAG - repeat androgen receptor gene), a short arrow - the normal allele.

CONCLUSION

Thus diagnosis of familial forms of MND and Kennedy spinal- bulbar amyotrophy represented an important issue, because lack of specific clinical marker for the presence of MND and molecular genetic laboratory only in major medical centers and research institutes, create difficulties in the clinical diagnosis and molecular genetic studies. Study of molecular genetic basis of familial MND is a promising direction for understanding the mechanisms of disease development and requires further studies to identify the genotype of hereditary predisposition to the disease.

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Clinico-Epidemiological and Molecular Genetic Study of Lumbar-Limb forms of Progressive Muscular Dystrophy in the Republic of Dagestan

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ABSTRACT

For the first time in the Republic of Dagestan (RD) a comprehensive clinical - epidemiological and molecular - genetic research of limb girdle forms of progressive muscular dystrophies (LGPMD) was conducted. LG forms of PMD: 2A type, 2B type and distal type Miyoshi were identified. The prevalence of LGPMD in RD populations was determined.

LGPMD clinical polymorphism in patients living in the mountain, foothill and lowland regions of RD was studied and practical diagnostic algorithm for neurologists was made. Mutation which causes the development of LLPMD family forms was identified.

Keywords: progressive muscular dystrophy, limb girdle form, DNA diagnostics.

INTRODUCTION

Lap - extremity progressive muscular dystrophy (LLPMD) - a group of clinically and genetically heterogeneous polymorphous diseases characterized by a primary lesion of the pelvic and shoulder girdles , progressive course , the increase in activity of the enzyme creatine phosphokinase (CPK) in the blood plasma , primary muscle lesion in electroneuromyographic character study (EMG) [3]. Frequency of all LLPMD in different populations varies from 5 to 70 patients per 1 million population [1].

The Republic of Dagestan (RD) with a population of 2 million 946 thousand people, is characterized by a predominance of rural population living mainly in the highlands, where the preserved isolates with high levels of inbreeding.

MATERIALS AND METHODS

We selected and examined patients with LLPMD according magazines neurological hospitals , clinics , national urban and regional MSCE , medical unit , Republican Genetic counseling , magazines Advisory receiving the Department of neurology of the Dagestan State Medical Academy , as well as according to the register of inherited neuromuscular diseases " Neyroregistr Dagestan". The diagnosis based on clinical exhibited - genealogical research electroneuromyographic examination, biochemical analysis of the level of activity of CK in the blood plasma, a biopsy of the affected muscles and confirms the results of molecular genetic analysis.

RESULTS

The study identified 51 patients us in 32 families with AR mode of inheritance, living in 13 districts and 3 cities of the Republic of Dagestan, in a ratio of 1:1 for men and women. Prevalence PC action in different areas of the Republic of Dagestan ranges from 0.4 to 38.7 per 100,000 population. Based on clinical and genealogical, laboratory and instrumental data were three groups of patients: 1 - LLPMD 2A, 2 - LLPMD 2B and 3 - LLPMD Miyoshi distal type.

In our study, the most common AR pathology residents in mountainous areas, compared to foothill and lowland areas. Burdened correlates with the level of inbreeding: the higher the level of inbreeding, the higher overburdenness [4].

Conducted clinical and genealogical research allowed to allocate 2A, 2B and distal type Miyoshi.

2A LLPMD type was detected in 33 patients in 19 families, representing 64 % of all LLPMD. The ratio of male to female ratio was 1:1. Prevalence of 1.09 per 100 thousand of population. Age debut ranges from 5 to 24 years. The greatest number of patients with age onset of the disease in the first decade of life. In cities revealed 8 patients. It should be noted that the families of these patients are immigrants from high-altitude areas. Intra- analysis of patients from the same family revealed phenotypic polymorphism, which is expressed in the time of the first clinical signs of disease, the severity of the dynamics and movement disorders. Closely related to marriage burdened families was 63 %. Search for mutations in exons 4, 5, 10, 11, 12, 20, 21, 22 calpain gene, (the most frequent among the Western European population) [2] carried out in 15% of patients who did not give a positive result.

2B tip in our study is 20 % of the AR variants LLPMD in RD and identified 10 patients in 6 families, male to female ratio of 3:2. Age onset of the disease from 15 to 21 years. Prevalence in the population RD - 0.34 to 100 thousand people. Typical signs of the disease were debut with weakness in the proximal leg and right hand, with a primary lesion anterior muscle groups with psevdogipertrofie calf muscle in 5% of patients, moderate increase in CPK.

Win Miyoshi distal type from all forms of autosomal LL PMD was 16%. LLPMD Miyoshi distal type was detected in 8 patients in four families in the ratio of 1:2 for men and women, age 11-21 years old debut. The clinical picture prevailed atrophy of the posterior group of muscles distal parts of the legs and forearm muscles, Achilles tendon retraction , 11 patients with clinical variant type 2B and Miyoshi during molecular - genetic studies revealed a mutation in the gene DYSF, with replacement p.Val67Asp., mapped to locus of chromosome 2r13 .

In our epidemiological study in the Dagestan population identified mountain isolate population 2909 people, where accumulated genetic load LLPMD 2B and Miyoshi distal type and identified 15 patients in 8 families. The prevalence of this isolate was 5.1 per 1 thousand of people.

CONCLUSION

First held in RD clinical - genealogical and molecular genetic study of patients with LLPMD distal type 2B and distal type Miyoshi. The data obtained allow to carry out medical examinations active burden families and plan volume Genetic counseling in RD. Carried out molecular genetic studies in patients disferlinopatiyami allow residents to develop screening

research mountain isolates, identifying hereditary pathology at the early stages and carriage, conduct prenatal diagnosis.

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Genetic Aspects of Hemostasis in Children with Arterial Ischemic Stroke

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ABSTRACT

In acute and recovery periods it is obligatory to detect blood diseases and pathological conditions leading to recurrent cerebral thrombosis.

Aim of the study. To define genetic factors influencing thrombogenesis in children with AIS.

Materials and methods. At neurosurgical department of Children's City Clinical Hospital named after N.F. Filatov (St. Petersburg) we observed 33 children with AIS (20 male and 13 female) aged from 6 months to 17 years old. The diagnose was based on the developed focal neurological symptoms, that were present more than 24 hours, and changes, characteristic for AIS [18, 4], on the KT and MRT images. During history taking hemostasis disorders were detected in children and their relatives. 18 children had molecular genetic investigation of 10 genes thrombophilia markers.

Results and discussion.

In the history evidence of hemostasis disorders was found in 3 children with AIS and included: easy development and slow disappearance of bruises, inadequacy of hematomas to pattern and severity of injury, prolonged nasal, sclerotic and gum bleeding (also after traumas and operations). Parents of children with AIS hadn't suffered stroke, but their nearest relatives had different strokes – in 6 cases on the mother's side, and in 8 cases on the father's side. During history taking in some parents, mainly in mothers (15 cases), and in 2 fathers hemostasis disorders signs were detected, in the majority of cases – haemorrhagic.

At data assessment of thrombophilia genes' polymorphism (in 18 children) the occurrence frequency of mutant allele in the European population was considered. We determined the coefficient of ratio of gene mutation frequency in the study group (p%) to the mean figures in the general population (P%), and we may estimate the value of this polymorphism in the course of AIS in the study group. According to this ratio (p/P) prothrombin gene Prt (G20210A), MTHFR A1298C, fibrinogen FGB (G-455A), platelet receptor gene GP Ib and GP IIIa had the highest coefficient. The widespread cause of cerebral vessels' thrombosis, factor V Leiden mutation, was not detected.

Conclusion. The obtained results demonstrate that during history taking in children with AIS and their parents it is important to detect not only thrombosis but also haemorrhagic disorders. Children with confirmed AIS need further molecular genetic study of thrombophilic markers to detect etiological factors, as all the examined children had heterozygous and homozygous mutant genes.

Keywords: children, arterial ischaemic stroke, thrombophilia, gene markers.

INTRODUCTION

Recently researchers studying problems of children with stroke, especially arterial ischaemic stroke (AIS), pay attention to multiplicity of risk factors, directly or indirectly causing cerebrovascular diseases [8, 2, 13]. Besides congenital vascular defects, which make circulus willisii, and diseases manifesting with specific changes of vessel walls, including different connective tissue dysplasias, hemostasis disorders are described as one of the most frequent causes of thrombotic damages to cervico-cephalic vessels in children [21, 16]. In acute and recovery periods it is obligatory to detect blood diseases and pathological conditions leading to recurrent cerebral thrombosis in 7–20% of cases [15, 5, 11]. About 30% cases of AIS in children are considered idiopathic (cryptogenic) [10, 19], and it is the reason to continue search for etiological factors of this disease.

Aim of the study. To define genetic factors influencing thrombogenesis in children with AIS.

MATERIALS AND METHODS

At neurosurgical department of Children's City Clinical Hospital named after N.F. Filatov (St. Petersburg) we observed 33 children with AIS (20 male and 13 female) aged from 6 months to 17 years old. The most children were under 3 years old (21, or 63%), 1 child – from the age group 7–12 years. The boys prevailed (20 patients). Distribution by age and gender is presented in Table 1.

The diagnose was based on the developed focal neurological symptoms, that were present more than 24 hours, and changes, characteristic for AIS [18, 4], on the KT and MRT images.

Along with routine somatic and neurological assessment over time, personal background and medical history were examined, as well as risk factors of pregnancy, delivery and neonatal period. During history taking hemostasis disorders were detected in children and their families.

Detection of hemostasis disorders signs, both thrombotic and haemorrhagic, in children with AIS and their parents was fulfilled with questionnaire survey also. We searched in history for episodes of spontaneous haemorrhages of different localization under certain circumstances (for example, scleral haemorrhage, gum bleeding at teeth brushing), prolonged bleeding after operations (including tooth extraction). Also we detected nontraumatic subdermal hematomas, development of subdermal hematomas caused by minimal traumas, inadequacy of their size to pattern and severity of injury. Attention was paid to the duration disappearance duration of subdermal hematomas. All thrombosis episodes in relatives under 50 years old were registered. The mothers were questioned about prolonged profuse menstrual bleeding, excessive bleeding after abortion, and in postnatal period. Besides, we took into consideration slow wound healing in children with AIS and their parents.

Stroke pattern and extent were accessed by imaging. Computed tomography (CT) was performed in 30 children by Siemens Somatom Emotion scanner. Brain magnetic resonance imaging (MRI) findings were examined in 5 children by Toshiba excelart vantage 1.5T; in 12 children – by Hitachi «Aperto» 0.4T (T1, T2, DWI images). Seven children had phase contrast MR-angiography, 3D TOF MR-angiography was performed in 4 children.

18 children had molecular genetic investigation of 10 genes thrombophilia markers.

The results were processed with the standard programme Statistica 10.0 for Windows with criterion $\chi 2$ and Spearman rank correlation analysis.

RESULTS AND DISCUSSION

Two children had recurrent AIS, one in 6 months and the other one in 14 months after the first AIS. All other children hadn't had any thrombotic disorders before ischaemic stroke.

Clinical findings in children with AIS showed that motor disorders prevailed: hemiparesis – in 31 cases, ataxia – in 2 cases, visual impairments were the main symptoms of the disease. Minor craniocerebral trauma preceded stroke symptoms development in 23 children during different periods of time: from several minutes to 24 hours. By neuroimaging data we defined lacunar infarctions mainly in basal ganglia, thalamus and internal capsule (28 cases). In the rest 5 cases – in cortex, white matter, frontal, parietal and occipital lobes, cerebellum and brain stem. In 10 children during observation over time the secondary haemorrhage at ischaemic site was detected.

In the history evidence of hemostasis disorders in the form of haemorrhages was found in 3 children with AIS (Table 2).

Parents of children with AIS hadn't suffered stroke, but their nearest relatives had different strokes – in 6 cases on the mother's side, and in 8 cases on the father's side. During history taking we ascertained that 17 parents had haemorrhagic hemostasis disorders, the data is presented in Table 3.

Eighteen children had molecular genetic investigation of 10 genes thrombophilia markers (Table 4).

At data assessment of thrombophilia genes' polymorphism in children the occurrence frequency of mutant allele in the European population was considered. If we determine the coefficient of ratio of gene mutation frequency in the study group (p%) to the mean figures in the general population (P%), we may estimate the value of this polymorphism in the course of disease in the study group. According to this ratio (p/P) molecular genetic investigation data is presented in Table 5 in descending order.

Factor V Leiden mutation, rarely met in the European population, but in many publications standing first on the list among AIS development risk factors [6, 18], was not found in the observed children. While even more rare in this population genotypes G/g and G/A of G20210A prothrombin (1–4%) were detected in 50% of the observed patients. All the children were diagnosed with polymorphism of tissue plasminogen activator (tPA) Ins/Del gene. More than 60% of children had G/A and A/A genotypes of fibrinogen G-455A. Approximately half of the examined children had MTHFR A1298C and MTHFR C677T genes polymorphism, and 5 children were homozygous mutant allele. Homocysteine level was within normal ranges. Widespread in the European population plasminogen activator inhibitor (PAI-1) gene's mutation had mostly homozygous genotypes. The number of heterozygous genes of platelet receptors with pathological alleles 1b and A2 was also higher than general statistical data shows for European population.

DISCUSSION

Hemostasis changes play important role in ischaemic strokes pathogenesis in children. These changes have many clinical manifestations, which can be estimated only after close examination of case history, hemostasis factors dynamics and detection of symptoms group, influencing thrombogenesis.

In many recent studies there is data that AIS development factors in children are associated with blood coagulation system disorders and often are hereditary [14, 7]. That is why it is very important to detect thrombophilia signs in children, their parents and siblings. It is shown in many researches that hemorrhagic symptoms do not exclude thrombophilia

development, and in some cases are characteristic in the history of patients with thrombotic manifestations [1, 3, 20]. Such evidence demonstrates the necessity to detect in the history of children with AIS and their parents thrombosis episodes and hemorrhagic hemostasis disorders.

The obtained results (cases history) showed that only several observed children had signs of blood coagulation system disorders in the form of hemorrhages, as compared to the more frequently occurrence in their parents. This difference is likely related to the fact that parents during their life time suffered from effects of more exogenous and endogenous factors, influencing interrelations of hemostasis different components. We detected haemorrhagic signs in parents, and in other senior relatives, under 50 years old, – strokes in 8 cases. Thus, we can assume that complex, multicomponent hemostasis disorders are present, which in different situations make conditions for mainly haemorrhagic or thrombotic haemostasiopathias.

Polymorphism of genetic thrombophilic markers was noted in all the examined children with AIS, rather frequently we noted polymorphisms of the genes that have low incidence in the European population: prothrombin gene mutation G20210A, MTHFR A1298C, fibrinogen G-455A. It is well-known that namely these mutations play the most important pathogenetic role in thrombophilia development [9, 12].

CONCLUSION

The obtained results demonstrate that during history taking in children with AIS and their parents it is important to detect not only thrombosis but also haemorrhagic disorders often clinically apparent in these children. Children with confirmed AIS need further molecular genetic study of thrombophilic markers, as all the examined children had heterozygous and homozygous mutant genes. It shows the important role of genetic factors in AIS development in children, and reveals opportunities for causal and preventive treatment of thrombotic complications.

Table 1

Children's distribution by age and gender

Gender	6–12 months	1–3 years	3–7 years	7–12 years	12–18 years
Male	5	8	4	1	2
Female	4	4	3	0	2

Table 2

Clinical features of hemostasis disorders in children with AIS

Clinical features	Number of children
Easy bruises development, inadequacy to the trauma	3
Slow disappearance of bruises	3
Scleral haemorrhage	2
Epistaxis (gum bleeding)	1
Prolonged bleeding after tooth extraction	1
Prolonged bleeding after traumas, operations	1

Table 3

Clinical features of hemostasis disorders in children's relatives on mother's and father's sides

Clinical features	Mother	Father
Easy bruises development	15	1
Slow disappearance of bruises	4	1
Epistaxis (gum bleeding)	8	2
Scleral haemorrhage	6	0

Table 4

Blood analysis for thrombophilia markers' polymorphism (n = 18)

	Gene	Genotype	Number of the examined children (percent of the total number of children)	Incidence of mutant gene in European population
1.	Tissue plasminogen activator (tPA) gene	Ins/Del	18 (100%)	54%
2.	Prothrombin gene Prt (G20210A), genotype G/A	G/G (N)	9 (50%)	1–4%
		G/g	6 (33.3%)	
		G/A	3 (16.7%)	
3.	Factor V Leiden	Arg/Arg (N)	18 (100%)	2–7%
4.	Fibrinogen FGB (G-455A)	G/G (N)	7 (38.9%)	20%
		G/A	9 (50%)	
		A/A	2 (11.1%)	
5.	Integrin α-2 GPIa	C/C (N)	6 (33.3%)	35–44%
		C/T	12 (66.7%)	
6.	Plasminogen activator inhibitor gene (PAI-1)	5G/5G (N)	8 (44.4%)	26–50%
		4G/5G	5 (27.8%)	
		4G/4G	5 (27.8%)	
7.	Platelet receptor gene GP IIIa (HPA-1a/1b)	1a/1a (N)	11 (61.1%)	8–15%
		1a/1b	7 (38.9%)	
8.	Platelet receptor gene GP Ib (HPA-A1/A2)	A1/A1 (N)	10 (55.6%)	13–16%
		A1/A2	8 (44.4%)	
9.	MTHFR A1298C	A/A (N)	9 (50%)	10%
		A/C	6 (33.3%)	
		C/C	3 (16.7%)	
10.	MTHFR C677 T	C/C (N)	8 (44.4%)	32–40%
		C/T	8 (44.4%)	
		T/T	2 (11.1%)	

Table 5

Estimated value of thrombophilia genes' polymorphisms in children with AIS according to p/P ratio

Number	Gene	p/P
1.	Prothrombin Prt (G20210A), genotype G/A	12.5
2.	MTHFR A1298C	5
3.	Fibrinogen FGB (G-455A)	3.05
4.	Platelet receptor gene GP Ib (HPA-A1/A2)	2.78
5.	Platelet receptor gene GP IIIa (HPA-1a/1b)	2.59
6.	Tissue plasminogen activator (tPA) gene	1.85
7.	Integrin α-2 GPIa	1.51
8.	MTHFR C677 T	1.39
9.	Plasminogen activator inhibitor gene (PAI-1)	1.11
10.	Factor V Leiden	0

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Ethno-Territorial Groups of the Yakuts: Genetic Structure Characteristics

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ABSTRACT

This paper presents the characteristics of the gene pool of the three Yakuts ethno-territorial groups (Central, Vilui and Northern) through the lines of mitochondrial DNA and Y-chromosome. Data for study was collected in expeditions carried by Yakut Research Center of Complex Medical Problems, Siberian Branch of Russian Academy of Medical Sciences, during 2002-2004. The population sample included healthy unrelated individuals whose ethnicity was considered mainly until the third generation. The results mainly correspond with the historians' opinion of the close relationship between Vilyuy and Central Yakuts, and Northern Sakha with the immigrants from Central Yakutia. Special characteristics of different ethnic geographical groups of the Yakuts can be observed through Y-chromosome.

Keywords: mitochondrial DNA, Y-chromosome, ethno-territorial groups, Yakuts.

INTRODUCTION

A considerable number of studies on genetic history of the Yakuts were published over the past decade [1,8,10,12-15,17-19]. Geneticists gave close attention to the history of Yakut ethnic group due to special characteristics of the male population's gene pool which had been firstly mentioned by Tatiana Zerjal [16]. Most of the Sakha males (over 80%) are descendants of one ancestor with N1c Y-chromosome, which is quite uncommon for such a numerous ethnic groups [8,10,12,14,16,17]. Extremely low diversity of the Yakuts' male lineages is balanced by heterogeneity of female gene pool, although the ancestor effect is still observed - a fifth part of the Sakha women comes from one ancestor with D5a2a2 mitochondrial lineage [1,14,17-19]. According to our research diversification of N1c-subcluster which are specific for the Yakuts began about 1600 years ago, followed by the expansion about 900 years ago [14]. The first date

corresponds to the time of separation of the Yakut language from the ancient Turkic languages [5], and the second date coincides with supposed time of migration of the last and the most numerous group of Turkic ancestors of the Yakuts to the Middle Lena basin [4,6]. The Sakha people gene pool is mainly represented by genes of Asian origin (> 90%). The Caucasoid component is less than 10%: some part of these lineages was introduced by Russian-speaking population since the XVII century, the other part was brought from the Middle East through the steppe zone of Eurasia, Altai and South Siberia by the ancestors of Yakuts and Evenks [12,14]. According to genetic characteristics (mtDNA and autosomal loci testing) Yakuts are closest to Evenks [12,14,17], and then, according to the level of genetic proximity - to the peoples inhabiting southern areas – Buryats, Altaians, Khakassians, Tuvinians, Mongols [14]. Currently we can definitely say that the Yakuts is one of the most genetically studied ethnic group in the world, but sub-ethnic structure characteristics have not been sufficiently described yet.

The aim of the work is to define genetic characteristics of specific ethno-territorial groups of the Yakuts (Central, Northern and Vilyuy Yakuts).

Material and Methods: Data for study was collected in expeditions carried by Yakut Research Center of Complex Medical Problems, Siberian Branch of Russian Academy of Medical Sciences, during 2002-2004. The population sample included healthy unrelated individuals whose ethnicity was considered mainly until the third generation: Central Yakuts of Amginsky, Megino-Kangalassky, Namsky, Tattinsky, Ust-Aldansky, Khangalassky, Churapchinsky districts; Vilyuy Yakuts of Verkhnevilyuisky, Vilyuysky, Nyurbinsky, Suntarsky districts; Northern Yakuts of Verkhnekolymsky, Verkhoyansky, Zhigansky, Momsky, Srednekolymsky districts. MtDNA haplotypes were determined by sequencing of hypervariable segment I (HVSI) (16024-16390) and analysis of 95 sites of mtDNA coding region (n = 423). Y-chromosome haplotypes were identified by analysis of 28 diallel loci and 6 microsatellite loci of the nonrecombining region (n = 215). We used mtDNA haplogroups nomenclature according to http://www.phylotree.org/, and Y-chromosome haplogroups classification according to http://www.isogg.org/tree/. Calculations of genetic diversity (H) and genetic differentiation (Fst) were performed with ARLEQUIN software package, version 3.01. The analysis of genetic relationships between populations using principal component analysis was made through POPSTR program kindly provided by H.Harpending (Estonian Biocentre).

Results and Discussion

Table 1 shows the frequencies of mitochondrial DNA and Y-chromosome haplogroups in three ethno-territorial groups – Central, Northern and Vilyuy Yakuts. Statistically significant

differences were detected between Northern and Vilyuy Yakuts (in Y-chromosome N1c haplogroup), between Northern and the other two groups (in mtDNA D5a2a2 haplogroup). Statistically significant differences in other haplogroups frequencies between populations have not been identified. Figure 1 shows European and Asian lineages ratio. European component is mainly represented in Vilyuy Yakuts (16% of mtDNA lineages) and Central Yakuts (11% of Y-chromosome lineages). The differences between populations mainly represented by Y-chromosome (4.1%) and less by mtDNA (0.5%). There are significant differences in genetic diversity of the male gene pool of different groups (Table 2). Analysis of genetic relationship shows the close genetic relationship of Central and Vilyuy Yakuts and the remoteness of Northern Yakuts (Fig. 2).

The Central Yakuts represent the core of the Yakut ethnic group and refer their ancestors to the sons of the legendary forefathers Elley and Omogoy. Such Yakut groups as the Khangalastsys, the Megintsys, the Baturustsys, the Borogontsys, the Namcys descended from Elley and the Bayagantaytsys are descendants of Omogoy. Genetic characteristics of the Yakut ethnic group described in the introduction are fully applicable to Central Yakuts. 86% of male natives of the central districts have one ancestor with N1c-chromosome. It is interesting that in contrast to other populations of Yakutia (Vilyuy and Northern Yakuts, Evenks, Evens and Yukagirs) phylogenetically older N1c-lineages have not been detected in the gene pool of the Yakuts of Leno-Amginskoe interstream area [14]. This is also confirmed by DNA analysis of skeletal remains from ancient burials in Tattinsky, Churapchinsky and Khangalassky districts [15]. This fact points to isolation of the Middle Lena Yakuts for a long period of time. The frequency of N1b haplogroup which is peculiar to the peoples of Urals language family is 2.2%. The presence of rare C3d haplogroup (1.1%) indicates close genetic linkages between the Yakuts and the peoples of South Siberia and Mongolia: this haplogroup was found in Buryats, Teleuts, Tuvinians, Soyots, Mongols, Hamnigans [20], and notably that its frequency reaches 29% in the Buryats [2]. C3c Y-chromosome haplogroup which is peculiar to the Tungus ethnic groups was not detected in the examined sample. The independent research conducted upon another samples estimated no more than 2% frequency of this haplogroup in Central Yakuts [8, 17]. The Caucasoid component is represented by clusters I1, R1a1a7, R1b1b2 which are specific to Europeans, and R1a1 *- lineages which are also found among the peoples of Southern Siberia.

The Vilyuy Yakuts have low diversity of the male gene pool. At the same time 90% of Y-chromosome lineages coincide with the lineages of Central Yakuts [12], which is agreed upon by the opinion of historians that the origin of the Vilyuy Sakha is closely linked with the Yakuts of

central districts. According to the archives, the population of lower Vilyuy district was only a few hundred people in 1639, the number of immigrants from central districts increased only at the end of the XVII century [3, 7]. At the same time it should be noted that archaeologists found and studied settlements of cattle-breeders of kulun-atakhskaya culture of the XIV-XVI centuries on the territory of present Vilyuy district (unpublished data). Apparently the Yakuts had mastered not only the estuary part of the Vilyuy as it was previously considered, but much larger areas of the Vilyuy basin in its lower reaches by the arrival of the Russians in the 30s of the XVII century. According to G.V.Ksenofontov Vilyuy district was originally inhabited by "the Yakut reindeer herding people of mixed ethnic origin" who were later forced out to the north by the first Yakut cattle-breeders migrated from Verkhnelensky region [4]. According to the Vilyuy Yakuts legend “the Yakut indigenous population of Vilyuy area was formed not due to the russian refugees but due to refugees of the period of legendary Yakut king Tygyn" [4]. Y-chromosome C3c-lineage in the male gene pool of the Vilyuy people indicates the presence of Tungus component (3.4%). Indeed, historians have noted that the Vilyuy Yakuts included representatives of Sologon, Yugyuleet, Nyurbachaan clans who are descendants of such Tungus clans as Shelogon (Sologon), Fuglyad (Ugulyat) and Nyurmagat (Nyurbachan) of the XVII century [3,11]. The Vilyuy people Caucasoid component by Y-chromosome is only 3.4% (haplogroups I and R1a *), whereas it reaches 16% by mtDNA (haplogrous H, HV1, T, J, U, W).

The Northern Yakuts are characterized by high diversity of male gene pool lineages. It was determined that the Northern Yakuts have higher frequencies of haplogroups specific to the ethnic minorities of the North-East Eurasia (haplogroup G1b of mtDNA, haplogroups C3*, C3c of Y-chromosome). These data indicate a higher degree of miscegenation of Northern Yakuts with Evenks, Evens and Yukagirs. Frequency of N1c Y-chromosome haplogroup and D5a2a2 mtDNA peculiar to the Yakuts in general are lowered in the northern ethno-territorial group. The Northern Yakuts as well as Vilyuy Yakuts have phylogenetically older N1c-lineages in their gene pool [12,14]. The presence of N1b, C3d, R1a1 * haplogroups indicates a direct genetic linkages between Central and Northern Yakuts. According to historians, most of the northern Sakha descended from immigrants from Central Yakutia and they mainly include the same clans. The upper and the middle Jana was inhabited by the Yakuts long before the Russians’ arrival [3, 4, 7]. The territory of Verkhoyanskoe wintering was inhabited by the following Yakut clans in the 40s of the XVII century: the Duhaal (Yusaltsy), the Horo, the Bidy, the Kyure, the Enge, the Elget. Yasachnaya book dated 1640 mentioned the Betyuntsys and the book dated 1642 mentioned the Yakuts of Namskaya territory. The following years brought more newcomers

from various Yakut districts to Verkhoyanskoe wintering [7]. Representatives of Verkhoyansk clans (the Bidys, the Enge, the Horo, the Tumat) and the central Yakuts began to move to the territory of future Abyisky and Momsky districts since the XVIII century. Interaction between Yakuts and Evens and Yukagirs contributed to diversity of dialect and customs of these northern districts. Kolymsky district comprised 11 Yakut communities in 1810: Verhnekilymsky district included Myatyuzhsky and Baydunsky communities; Srednekolymsky district included Baydunsky, Borogonsky, two Kangalassky, two Myatyuzhsky and Eginsky communities; Nizhnekolymsky district included Myatyuzhsky community [9].

Thus, the results mainly correspond with the historians' opinion of the close relationship between Vilyuy and Central Yakuts, and Northern Sakha with the immigrants from Central Yakutia. Special characteristics of different ethnic geographical groups of the Yakuts can be observed through Y-chromosome.

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Table 1

Frequencies of mtDNA and Y-chromosomal haplogroups in Central, Vilyuy, Northern Yakuts

MtDNA				
Haplogroups	CY (n=164)	VY (n=111)	NY (n=148)	SUM (n=423)
A	2,4 (1,0-6,1)	3,6 (1,5-8,9)	0 (0-2,4)	1,9
B	0,6 (0,1-3,3)	0 (0-3,2)	2,7 (1,1-6,7)	1,2
C4	33,5 (26,8-41,1)	32,4(24,4-41,6)	32,4 (25,4-40,4)	32,8
C5	11,6 (7,6-17,4)	3,6 (1,5-8,9)	8,1 (4,7-13,6)	8,3
C7	0,6 (0,1-3,3)	0 (0-3,2)	0 (0-2,4)	0,2
D4	15,9 (11,1-	11,7 (7,0-19,0)	17,6 (12,3-	15,4

	22,2)		24,5)	
D5a2a2	17,1 (12,1-23,6)	18 (12,0-26,2)	6,1 (3,3-11,2)	13,5
D5b1	0 (0-2,2)	0 (0-3,2)	2,0 (0,7-5,8)	0,7
F	3,7 (1,7-7,7)	5,4 (2,5-11,3)	4,7 (2,3-9,4)	4,5
G1b	0 (0-2,2)	0,9 (0,2-4,9)	1,4 (0,4-4,8)	0,7
G2a	4,9 (2,5-9,3)	2,7 (1,0-7,6)	5,4 (2,8-10,3)	4,5
M7	0 (0-2,2)	0,9 (0,2-4,9)	1,4 (0,4-4,8)	0,7
M13	1,8 (0,7-5,2)	1,8 (0,6-6,3)	6,8 (3,7-12,0)	3,5
Y	1,2 (0,4-4,3)	2,7 (1,0-7,6)	0 (0-2,4)	1,2
Z	0 (0-2,2)	0 (0-3,2)	2,7 (1,1-6,7)	0,9
R1b	0 (0-2,2)	0 (0-3,2)	1,4 (0,4-4,8)	0,5
H	3,7 (1,7-7,7)	6,3 (3,1-12,5)	1,4 (0,4-4,8)	3,6
HV1	0 (0-2,2)	1,8 (0,6-6,3)	2,0 (0,7-5,8)	1,2
T	0,6 (0,1-3,3)	3,6 (1,5-8,9)	0 (0-2,4)	1,2
J	0,6 (0,1-3,3)	2,7 (1,0-7,6)	1,4 (0,4-4,8)	1,4
U	0,6 (0,1-3,3)	0,9 (0,2-4,9)	0,7 (0,2-3,7)	0,7
W	1,2 (0,4-4,3)	0,9 (0,2-4,9)	2,0 (0,7-5,8)	1,4
Y-хромосома				
Haplogroups	ЦЯ (n=92)	ВЯ (n=58)	СЯ (n=66)	Суммарно (n=216)
N1b	2,2 (0,7-7,6)	0 (0-6,1)	10,6 (5,3-20,3)	4,2
N1c	85,9 (77,3-91,5)	93,1 (83,5-97,2)	71,2 (59,3-80,7)	83,3
C3*	0 (0-3,9)	0 (0-6,1)	3,0 (0,9-10,4)	0,9
C3c	0 (0-3,9)	3,4 (1,1-11,7)	6,1 (2,5-14,6)	2,8
C3d	1,1 (0,3-5,8)	0 (0-6,1)	4,5 (1,7-12,5)	1,8
I1	2,2 (0,7-7,6)	1,7 (0,4-9,1)	0 (0-5,4)	1,4
R1a1*	4,3 (1,8-10,6)	1,7 (0,4-9,1)	4,5 (1,7-12,5)	3,7
R1a1a7	2,2 (0,7-7,6)	0 (0-6,1)	0 (0-5,4)	0,9
R1b1b2	2,2 (0,7-7,6)	0 (0-6,1)	0 (0-5,4)	0,9

Table 2

Indexes of genetic differentiation (Fst) and genetic diversity (H) of three populations of Yakuts by mtDNA and Y-chromosomal haplogroups

Population	Fst	H
mtDNA		
CY (n=164)	0.0052	0.82±0.02
VY (n=111)		0.84±0.02
NY (n=148)		0.85±0.02
Y chromosome		
CY (n=92)	0.0408	0.26±0.06
VY (n=58)		0.13±0.06
NY (n=66)		0.48±0.07

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Genetic Aspects of Metabolic Syndrome of Yakut Ethnic Groups

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ABSTRACT

In this article, we have studied polymorphisms in the genes encoding components of carbohydrate metabolism and fibrinolysis system in Yakuts. Genotypes SNPs rs9939609 gene FTO, rs1137101 gene *LEPR*, rs1799889 and rs6046 gene *SERPINE1 F7* gene were identified in the study group patients with metabolic syndrome (n = 100) and healthy (n = 100). We have revealed that the MS group «+» prevailed polymorphic genotype 4G/4G gene *SERPINE1*, associated with obesity (p=0,009). The risk of developing MS in the Yakut population became involved with genotype 4G/4G gene *SERPINE1* (OR = 3,568; CI 95%: 1,534-8,299). In case of polymorphic variants of other genes we studied the risk of MS has not been identified (OR < 1). There was a statistically significant association of gene polymorphism Gln223Arg *LEPR* level of total cholesterol (p = 0,038), triglycerides (p = 0,033) and SC (p = 0,030) in Yakut ethnic group. Association analysis of polymorphism -675 5G/4G gene *SERPINE1* with MS components in the sample revealed significant differences in anthropometric parameters: BMI (p = 0,016), WC (p = 0,001), the ratio of WC to HC (p=0,019).

Keywords: metabolic syndrome, polymorphism, *FTO, LEPR, SERPINE1, F7.*

INTRODUCTION

Currently, WHO experts consider metabolic syndrome (MS) as "pandemic of the XXI century". According to the recommendations of experts All-Russian Scientific Society of Cardiologist metabolic syndrome is characterized by an increase in visceral fat mass, decreased sensitivity of peripheral tissues to insulin and hyperinsulinemia, which cause the development of carbohydrate, lipid, purine metabolism and arterial hypertension (AH). MS is also a prothrombotic state because of endothelial dysfunction, the presence of hypercoagulable imbalance between coagulation factors and substances that regulate fibrinolysis. Violations by the blood coagulation system in the metabolic syndrome characterized by increased levels of fibrinogen and fibrinolysis inhibitor content level - factor VII (F7) , and plasminogen activator inhibitor type 1 (PAI 1) [17].

The study of molecular and genetic factors in the development of metabolic syndrome, the search for susceptibility genes and analysis of their association with various components polymorphisms syndrome are given more attention in research of recent years [9]. In meta-

analyzes of the European population has ties gene associated with fat mass (FTO) with overweight and obesity [4,11]. There are works of the controversial nature related to the Asian population. In Asian populations M. Horikoshi (2007) and H. Li (2008) showed no association of FTO with obesity [26,25], whereas J. Chen (2009), W. Tong (2010) concluded that there is a connection FTO with the metabolic syndrome [13,22]. There are also conflicting results in case of leptin receptor gene polymorphism (LEPR) and obesity. T. Furusawa (2010) et al. revealed that carriers of allele 223 Q (223Arg) had a significantly higher body weight (p = 0.0009) and BMI (p=0,0022) [24]. However, other studies failed to find a positive and significant relationship between obesity and the gene polymorphisms studied *LEPR* [6,16].

-675 5G/4G polymorphism of the gene encoding PAI 1 - *SERPINE1* (rs1799889), is regarded as a risk factor for cardiovascular disease [15]. According to some studies, the 4G-allele carriers are more prone to the development of obesity and MS, but in other works of a similar dependence was identified [5].

Several studies have been shown to increase the concentration of factor VII in patients with type 2 diabetes and its relationship with insulin resistance indices and triglyceride levels [10,14]. The level of factor VII in the blood is determined to a large extent genetic component [21]. Thus, gene polymorphism F7 - rs6046 (Arg353Gln) in exon 9 of the gene is associated with low blood levels of F7. Changes in gene F7 in most cases have a protective effect. Genotype A/A causes F7 decrease enzyme activity by 72%, compared to wild type (genotype G/G) [8].

Studies aimed at identifying genetic polymorphisms associated with metabolic syndrome are continuing. There is a large number of genes with established function and an even greater number of genes - candidates playing defined role in the formation of the main manifestations of MS. Currently, there are few works on studying the association of metabolic syndrome with polymorphic genes in the Yakut ethnic group. But at the same time there are no studies on the association with MS genes encoding components of carbohydrate metabolism and fibrinolysis system in Yakuts, namely gene *FTO* (T/A), *LEPR* (Gln223Arg), *SERPINE 1* (-675 5G/4G), *F7* (Arg353Gln). This paved the way for the study of polymorphisms of these genes in Yakuts with metabolic syndrome.

MATERIALS AND METHODS

The study is included 200 people Yakut with no relationships between them. All patients resided in the Far North - in the Republic of Sakha (Yakutia). According to the clinical, laboratory and instrumental survey, according to the recommendations of All-Russian Scientific Society of Cardiologist in 2006, patients were divided into two groups: those with metabolic syndrome and

healthy people. The main group consisted of 100 patients diagnosed with metabolic syndrome, a hyperbolic (MS «+»). The age of patients ranged from 18 to 70 years, the average age of the group MS «+» 47,63±1,25. Comparison group consisted of healthy volunteers almost corresponding to the group of patients with MS «+» by age, gender, ethnicity, without MS (MS «-»).

All participants signed informed consent. All persons at study performed a comprehensive clinical examination, questioning by a specially developed map of the subject, with the explanation of socio-demographic characteristics, anamnestic data, heredity, physical activity, smoking data, the presence of menopausal women. They have made complaints in the card, such as physical examination, anthropometry: measurement of height, weight, waist circumference (WC), hip circumference (HC), the ratio of WC/ HC, the calculation of body mass index (BMI), blood pressure (BP) and frequency heart rate (HR) and the results of biochemical analysis: glucose, total cholesterol (TC), low density lipoprotein cholesterol (LDL) , high density lipoprotein cholesterol (HDL), triglycerides (TG), atherogenic ratio calculation (ARC) in formula: ARC = (TC - HDL)/HDL, the definition of a single nucleotide polymorphism (SNP) rs9939609 gene *FTO*, rs1137101 gene *LEPR*, rs1799889 and rs6046 gene *SERPINE1* gene *F7*.

DNA was extracted from venous blood leukocytes phenol-chloroform method [3]. Determination of gene polymorphism was performed by polymerase chain reaction (PCR) using the reagent kit for amplification «SNP-Express» production firm «Lytech» thermocycler at ABI 9700. The amplification products were analyzed by electrophoresis in 2% agarose gel stained with ethidium bromide.

We have used a modified criterion $\chi 2$ (p) to check compliance with the empirical frequency distribution of genotypes theoretically expected equilibrium distribution of Hardy-Weinberg, defined using the RxC algorithm. This algorithm allows us to estimate the statistical significance of deviations from the expected frequency distribution when the number of observations for a significant number of classes is less than 5, and the application of the standard criterion $\chi 2$ incompetent. Obtained during the study data were processed using the software package SPSS. Figures in the study groups were described using mean values (M) and standard error (m). Comparison of genotype frequencies in groups of patients and healthy individuals using the chi-square Pearson. Threshold of significance for all statistical tests used accepted meaning of $p < 0,05$. Relative risk for a particular genotype was calculated as the odds ratio (odds ratio - OR) according to the formula: OR = (a x d) / (b x c), where a - genotype frequency in the sample of patients, b - genotype frequency in the control sample, with - the sum of frequencies

other genotypes in a sample of patients; d - the remaining sum of the frequencies of genotypes in the control sample. If one of the indicators is 0, adopted an amendment to the continuity of – 0,5. If OR = 1 - no association, OR> 1 - a positive association with the disease genotype and OR < 1 - negative association.

The project was implemented within the framework of the research work «Metabolic syndrome and chronic non-communicable diseases among residents of Yakutia» (Registration number YSU 11 - 01M.2009.) Department of Internal Medicine and General Practice (Family Medicine) Faculty of Postgraduate Medical Education Medical Institute NEFU. Study was approved by the local ethics committee YSC CMP SB RAMS (Prot № 24 dated June 29, 2010). This work was partially funded by a grant individual NEFU rector for students and young scientists by 2013.

RESULTS AND DISCUSSION

Distribution of polymorphisms studied theoretically corresponds to the equilibrium expected Hardy-Weinberg equilibrium ($p > 0,05$).

The results of the frequency distribution of alleles and genotypes of polymorphic markers of genes are shown in Table. 1. According to the results, when we had compared with a group of MS group healthy frequency distribution we observed differences in genotype frequencies of -675 5G/4G gene *SERPINE1*. As this can be seen from the data presented in the most common group of MS «+» was heterozygous genotype 5G/4G (49%) and gene *SERPINE1* marked predominance of genotype 4G/4G (37%) of the genotype 5G/5G (14%). In the comparison group, we also revealed the prevalence of heterozygous genotype 5G/4G (53%), but there is predominance of genotype 5G/5G (27%) of the genotype 4G/4G (20%). A comparative analysis revealed a statistically significant difference data distribution of genotypes ($p=0,009$). Our findings are consistent with earlier studies, according to which version of 4G/4G polymorphism -675 5G/4G gene *SERPINE1* correlates with central obesity and the relation of options 5G/4G polymorphism with average levels of PAI 1 in blood in the presence of obesity [18,20]. Comparative analysis of the frequency distribution of genotypes of the remaining genes in the test group of patients with MS «+» and the control group showed no significant differences.

The present study has revealed the lowest incidence of carriage "unfavorable" A allele of the *FTO* gene and 223Arg allele of gene *LEPR*, that gives us assume that these alleles are less significant in patients with MS Yakut ethnic population.

The risk of developing MS in the Yakut population became involved with genotype 4G/4G gene *SERPINE1* (OR = 3,568; CI 95%: 1,534-8,299). Similar data were obtained V.H.

Khavinson et al. (2010) on the Russian population. The study showed that the genotype 4G/4G gene SERPINE1, associated with the slowdown of fibrinolysis, systolic hypertension, the risk of acute coronary syndrome, increasing the concentration of glucose and cholesterol in the blood, can be attributed to genetic risk factors of metabolic cardio - vascular syndrome [1].

Speaking about the polymorphic variants of other genes we studied the risk of MS has not been identified (OR < 1). Perhaps in the surveyed Yakut ethnic group gene polymorphisms *FTO, LEPR* and *F7* do not contribute significantly to the development of MS.

For each studied polymorphic variant of the gene was analyzed associations with MS components - blood pressure levels with measures of carbohydrate and lipid profile and anthropometric data.

Passing in review of the contribution of T/A polymorphism of the *FTO* gene in the variability of MS components it was shown that, the polymorphic marker is not associated with any of the studied components of MS. However, a positive trend due to this polymorphic marker with HDL (p = 0,054). Our results are consistent with M.A. Garbuzova (2010), who found no association of rs8050136 and rs9939609 marker gene FTO with anthropometric and metabolic parameters MS: BMI, WC, HC, insulin resistance index, immunoreactive insulin, total cholesterol, HDL cholesterol, LDL cholesterol, TG , TG/HDL, blood glucose levels fasting and after an oral glucose tolerance test (p> 0.05) in the Russian population [2].

There was a statistically significant association of gene polymorphism Gln223Arg *LEPR* level of total cholesterol (p=0,038), triglycerides (p=0,033) and ARC (p=0,030) in Yakut ethnic group. Also, the study G.M. van der Vleuten (2006) found that the carrier 223Gln leptin receptor allele (homozygotes and heterozygotes 223Gln allele) was associated with combined hyperlipidemia, reduced sensitivity to insulin and obesity [23].

Association analysis of polymorphism -675 5G/4G gene *SERPINE1* with MS components in the sample revealed significant differences in anthropometric parameters: BMI (p = 0,016), WC (p = 0,001), the WC/HC (p = 0,019). Regarding the parameters showed a trend toward connection with the polymorphic marker (p = 0,069). This association was showcased the study Zaid H. Al-Hamodi et al. (2012), where the inhabitants of Malaysia polymorphism -675 5G/4G gene *SERPINE1* significantly associated with BMI [19].

The present study revealed no statistically significant association of polymorphism Arg 353 Gln *F7* gene with MS components in the Yakut ethnic group. A number of studies supports our findings. Thus, the study A.P. Reiner et al. (2007) showed that the minor Arg/Arg genotype was associated with a lower BMI, cholesterol and reduced risk of coronary heart disease, but this

association disappeared after adjustment for BMI and HDL [6]. Also not found an association between the level of blood triglycerides and Arg 353 Gln polymorphism F7 studies J.S. Pankow et al. (1998) and A. Lane et al. (1992) [27,12].

CONCLUSION

All things considered in the MC group «+» gene polymorphism 4G/4G genotype *SERPINE1,* associated with obesity played the main part (p=0,009). The risk of developing MS in the Yakut population became involved with genotype 4G/4G gene *SERPINE1* (OR = 3,568; CI 95%: 1,534-8,299). Speaking about the polymorphic variants of other genes we studied that the risk of MS has not been identified (OR<1). There was a statistically significant association of gene polymorphism Gln223Arg *LEPR* level of total cholesterol (p=0,038), triglycerides (p = 0,033) and ARC (p=0,030) and -675 5G/4G gene polymorphism with anthropometric *SERPINE1* BMI (p = 0,016), WC (p=0,001), WC/HC (p = 0.019) in the Yakut ethnic group.

Table 1

Comparative analysis of the distribution of allele and genotype frequencies of polymorphic markers of candidate genes of the metabolic syndrome in the Yakut population

Gene	Allele and genotype	Frequency of alleles (shares) and genotypes (%)		p	OR	
		MC «+» n = 100	MC «-» n = 100		value	CI 95%
SERPINE1 –675 5G/4G (rs1799889)	allele *5G*	0,39	0,54	-	-	-
	allele *4G*	0,62	0,47		-	-
	genotype *5G/5G*	14	27	0,009	0,280	0,121 – 0,652
	genotype *5G /4G*	49	53	-	-	-
	genotype *4G/4G*	37	20	0,009	3,568	1,534 – 8,299
LEPR Gln223Arg (rs1137101)	allele *Gln*	0,87	0,91	-	-	-
	allele *Arg*	0,13	0,9		-	-
	genotype *Gln/ Gln*	86	91	0,277	0,977	0,947- 1,009
	genotype *Gln/ Arg*	12	9		-	-
	genotype *Arg/ Arg*	2	-		-	-
FTO T/A (rs9939609)	allele *T*	0,88	0,89	-	-	-
	allele *A*	0,13	0,11		-	-
	genotype *T/T*	87	89	0,587	0,989	0,967 - 1,011
	genotype *T/A*	12	11		-	-
	genotype *A/A*	1	-		-	-
F7 Arg 353 Gln	allele *Arg*	0,57	0,61	-	-	-
	allele *Gln*	0,43	0,39		-	-

(rs6046)	genotype *Arg/ Arg*	55	52		1,135	0,452 – 2,849
	genotype *Arg/ Gln*	33	38	0,734	-	-
	genotype *Gln/ Gln*	12	10		0,881	0,351 – 2,214

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Nutrigenetics Bases in the North

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ABSTRACT

In studying of influence of food on a genome of the person is engaged nutrigenetics science which is extremely demanded, investigating how different nutrients are capable to modify human genes and as it in turn influences health of the person.

The scientific research institute of health of SVFU for decades carries out studying of nature of food of the population, living in an extreme and severe zone. Results the researches conducted in various medico-economic zones of the republic showed that there is a distinction in the daily caloric content of a diet among respondents depending on a floor and an ethnic origin.

Low consumption of the main micronutrients connected with insufficient consumption of the main food is established.

Despite the occurred changes, food of the population is unbalanced on all main components, including on essentsialny for health – to mineral substances and vitamins and doesn't correspond to recommended Russian physiological requirements of adult population.

Conditions of environment and increase in incidence of people of all age caused the necessity of creation of functional food, i.e. products with the additional functions, useful nutritious and physiological characteristics.

Using in production of foodstuff of new generation unique Yakut herbs and wild-growing berries, it is possible to improve adaptation and immune opportunities of the person.

At population catering services in the conditions of the North it is necessary to consider and food habits of nationalities living here.

Keywords: nutrigenetics, nature food population, food optimization, food in the north, biotechnology, functional products.

In studying of influence of food on a genome of the person nutrigenetics is engaged - the science which has arisen in the USA about ten years ago. Despite relative "youth" of the geneticist of food it is extremely demanded, first of all, of course, abroad. Nutrigenetics investigates how different nutrients are capable to modify human genes and as it in turn influences health [3, 9].

The knowledge of interaction between environment and our genes laid the foundation to a new era in dietology and medicine. Practically it means that we can force to work by means of food the genes for us so that to remain healthy much more long (healthy nutrition, according to the last scientific researches, is capable to prolong life for a period of up to 14 years) [8].

Optimization of food is daily use of products which influence activity of the revealed polymorphic genes, help with prevention of diseases of individual group of risk and with achievement of a goal, for example, with weight correction. After all volumes of our body is a result of a combination of such factors, as interaction of genes, metabolic processes, a way of life, an environment, the general culture and socially economic status of the person [1].

It is established that more than 100 gene combinations influence weight changes. Some alternative combinations of genes play a key role in energy consumption, appetite control, a lipid exchange, emergence of a syndrome of an insulin resistance. Each individual genetic variation has rather small influence on excessive weight and not necessarily causes obesity, but "awards" a susceptibility to obesity and changes balance of control of weight if the person chooses the wrong way of life. That is has impact on, whether the person to gain weight is inclined and to keep it, how fast its organism reacts to physical activity, and also to which exercises and what type of a diet it reacts.

Such approach is called as "individual prevention" or preventive individual medicine and helps to protect effectively each person from various multifactorial violations connected with influence of environment (oncological diseases, cardiovascular risk, the return reaction to medicines, allergies, etc.), and also it is essential to slow down processes of aging [9].

To limit animal fats in a diet, to diversify it with sea fish and vegetables, there is no need to do the expensive genetic analysis. But there are nuances. For example, the person owing to genetics acquires polynonsaturated fats, than mononnonsaturated better. Or weaker perceives sweet taste – then it will sweeten more strongly tea and to sugar strawberry that, certainly, it isn't useful [8].

Ability to digestion of vitamins too genetically happens different: one people have a need for vitamins A, D, E, groups B are higher, than at others. If it is accompanied also genetically by hypersensibility to bitter taste when for anything won't take people in a mouth, for example, broccoli, it is necessary to pick up an individual complex of vitamins [3].

According to bioclimatic division into districts the region of the Republic of Sakha (Yakutia) belongs to an extreme and severe zone. The scientific research institute of health of SVFU for decades carries out studying of nature of food of the population, living in an extreme

and severe zone. Early researches were conducted in 2001 in 6 areas and 2 cities. Results researches of food the population conducted in 6 areas of the republic showed that there is a distinction in the daily caloric content of a diet among respondents depending on a floor and an ethnic origin [4, 6].

So, the average daily power value of a diet of men made 2308, women have 1801,3 kcal ($p < 0,05$). Caloric content of a diet of indigenous people was statistically significantly higher (1787,1 and 2129,2 kcal, respectively, $p < 0,05$)than at not indigenous people. The highest caloric content of a diet was noted in Verkhoyansk, Suntarsk uluses and in Yakutsk. The lowest – in Neryungri. Comparing the power value of a diet 2001 and 2012 it is possible to note that by 2012 the power value of a diet decreased by 11%, and averaged 1885,7 kcal. The greatest decrease in power value happened in an industrial zone (1797 kcal), the smallest in the Arctic zone (2020 kcal).

According to "Norms of physiological requirements for energy and feedstuffs for various groups of the population of the Russian Federation" the average daily physiological need for proteins averages 75 g. By results of research, in 2001 the population of the republic consumed 63 g of protein per day, in 2012 - 67 g per day that also doesn't correspond to physiological requirements. In comparison with 2001 consumption of fats with 71 g per day decreased to 67 g (norm of 83 g per day) and carbohydrates from 289 g per day to 242 g per day (norm of 365 g per day) [4, 6].

Low consumption of the main microfeed stuffs is undoubtedly connected with insufficient consumption of the main food. The carried-out comparative characteristic of food of the population in 2001 and 2012 showed that food sets of inhabitants of RS (Ya) were characterized by decrease in quantity of products of an animal origin, fruit and increase in a quota grain and potatoes in 2001.

The comparative characteristic of consumption of the main products on bioclimatic zones showed that in all climatic zones there were changes consumption of products. So, in the Arctic zone, consumption of fish by 3 times with (from 9,2 g increased to 25 g per day), milk by 4 times (from 161 g to 647 g), eggs by 6 times (from 2,9 g to 12,6 g), bread twice (from 108 g to 252 g). Considerably consumption of fruit (from 2 g increased to 63 g). By 4 times consumption of vegetable oil (51,1 g and 13,4) and potatoes (453 g and 86 g) decreased. Consumption of vegetables remains same low (69 g and 79,6 g according to).

Thus, the power value of a diet for a 10-year interval decreased by 2012 by 11%, and averaged 1885,7 kcal. Thus the greatest decrease in power value happened in an industrial zone

(1797 kcal), the smallest in the Arctic zone (2020 kcal). Average daily consumption of proteins, fats and carbohydrates for a 10-year interval significantly didn't change, remaining is much lower than recommended physiological norms. Considerable deficiency of potassium, magnesium, calcium, iron, B1, B2, C, PP vitamins, Retinolum remains.

Despite the occurred changes, food of the population is unbalanced on all main components, including on essentially for health – to mineral substances and vitamins and doesn't correspond to recommended Russian physiological requirements of adult population.

Metabolism studying at the children living in northern regions of the country, both local, and the alien population, showed that specific climatic conditions (long severe winter, sharp temperature drops of air and atmospheric pressure, magnetic influences) cause increase of requirement of a children's organism in some feedstuffs (protein, fat, group B vitamins, C) and energy.

For children of regions of the North the increase in caloric content of a diet at 10% in comparison with norm for a midland of the country and increase in the contents in protein diets for 8 - 10% and fat for 5 - 10% is recommended[6, 7].

It must be kept in mind that the specified increase in protein, fat and diet caloric content approximately and depends on conditions of accommodation in each certain region. The more severe climate and is sharper weather change, the increase is higher. Seasonality matters also, so in a cold season the need for proteins, fats and caloric content will be higher, than in summertime [6, 2].

In this regard for the region of the Far North, with long winter, higher norms of consumption are recommended.

Feature of catering services of the population of northern regions of RS (Ya), especially Far North, difficulties in providing with their natural products in a winter and spring season and at the beginning of summer are.

The high need of the person for some biologically active agents at adaptation to North conditions, and also can lead wide use of dry, frozen and unenriched tinned products to insufficient receipt with food of some vitamins (C, P, B, etc.).

It is necessary to encourage development of the enterprises with subsidiary farm for population supply by fresh vegetables, and also to use products of a local source of raw materials more widely [7].

The flora of Far North and the Areas of Siberia gives the chance to use a large number of wild-growing cultures in food - in the forest-tundra and a tundra zone there are trade stocks of

black and red currant, a dogrose, cloudberries, blueberry, a honeysuckle, cowberry, and the region of the Far North - a kislichnik of a two-column, Arctic sorrel [5]. It is expedient to include these products in diets of children not only in an aestivo-autumnal season, but also to make preparations them for the winter and spring.

To number of products of a local source of raw materials of the North, containing a large number water - and fat-soluble vitamins, enough of mineral salts and microcells, belong a venison, river and lake breeds of fishes, meat of sea animals.

At population catering services in the conditions of the North it is necessary to consider and food habits of nationalities living here.

Conditions of environment and increase in incidence of people of all age caused the necessity of creation of functional food, i.e. products with the additional functions, useful nutritious and physiological characteristics [1].

Main goal of our scientific team is development of innovative biotechnologies of specialized food of a functional purpose from local raw materials taking into account medicobiological features of their health and the actual food, really helping the North population in preservation of health is "future food", completely corresponding to idea of transition to preventive biocorrection and the medicine which main objectives are protection of the genetic device of cages, prevention of emergence of diseases and delay of processes of aging.

Using in production of foodstuff of new generation unique Yakut herbs and berries, it is possible to improve adaptation and immune opportunities of the person.

Also development of the new technologies providing rational complex processing of raw materials is necessary for ensuring competitiveness of production of the food industry and public catering. It is connected with use of secondary material resources. Application of new technologies of deep processing of raw materials will allow to create quality safe domestic food [7].

The knowledge of an individual genetic profile allows: to make an optimum power supply circuit for the prevention of genetic risks;

• to choose food additives according to individual requirements;

• to choose an optimum diet for weight correction.

Nutrigenetic recommendations have nothing in common with the general councils. They represent development of individual food behavior and lifestyle according to knowledge that healthy food is a food which corresponds to a specific genetic profile [8]. Recommendations

about the food, based on genetic tests will never order a rigid diet. They only give new understanding of that is optimum food for each specific person.

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Genetic Predisposition of Chronic Obstructive Pulmonary Disease in Extremely Cold Climate

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ABSTRACT

This article reflects the genetic aspects of chronic obstructive pulmonary disease. Modern molecular biology concept comes from an imbalance in the system proteolysis - antiproteoliz , alpha 1- antitrypsin is a major inhibitor of serine proteases, which include trypsin, chymotrypsin, neutrophil elastase, tissue kallikrin, Factor X "a" and plasminogen. The gene PI (proteinase inhibitor) located on the long arm of chromosome 14 (14q31- 32), and the gene product is glycoprotein alpha$_1$ - antitryprisin. Two types of cells expressing the gene PI - macrophages and hepatocytes, the function of hepatocyte, associate tissue specificity of the inhibitor.

Keywords: chronic obstructive pulmonary disease, alpha$_1$ - antitrypsin.

Chronic obstructive pulmonary disease (COPD) develops when failure alpha$_1$ - antitrypsin based on etiopathogenesis lie mutations in genes CF (cystic fibrosis) and PI (protease inhibitor) [6].

There are several local defense units of bronchopulmonary system: mucociliary apparatus, ciliated cells and rheological properties of mucus humoral - immunoglobulins, lysozyme, lactoferrin, antiprotease, complement components, interferon, a cellular link, including alveolar macrophages (AM), neutrophils and lymphocytes; bronhoassociated lymphoid tissue (BALT) [1]. Based on the analysis of molecular and cellular changes in the respiratory tract of patients with COPD can be divided into four main stages of the disease:

1. Stage of aseptic inflammation. It is caused by excessive production of ROS and nitric oxide in the respiratory organs. Its main clinical manifestation is a productive cough. Significant biomarkers of this stage of COB are: increase in blood chemiluminescence of leukocytes and bronchoalveolar lavage of smokers and patients with increased concentration of nitric oxide in exhaled air in non-smoking patients.

2. Stage of obstructive changes. Its reason - the relative lack of otsantitripsin arising due to an imbalance of proteolytic enzymes and their inhibitors in lung tissue. Criterion is the degree of decrease in expiratory flow rate and elevated levels in the urine of patients' oksiprilin. It is also found increased content of hydrogen peroxide in the exhaled air.

3. Stage of reducing germicidal protection. It arises as a result of the blockade of oxygen-dependent bactericidal system of alveolar macrophages and neutrophils on the background of the development of atrophic changes in the bronchial mucosa. Method of estimating the degree of suppression of lung antibacterial protection needs to be improved. For this purpose it may be used to determine the degree of neutrophil myeloperoxidase oppressing blood or bronchoalveolar lavage, or the degree of suppression of production of ROS by neutrophils and macrophages. Clinical sign of this stage is the appearance of purulent sputum.

4. Stage of severe respiratory failure. The final stage of the development of chronic obstructive bronchitis caused by two reasons: a decrease in total respiratory alveolar surface due to emphysema and exhale collaboration of bronchioles due to atrophy of the elastic fibers and smooth muscle of their walls. Leading sign - hypoxemia. It is characterized as tense, but little effective work of external respiration [2].

In the 60s gene alpha$_1$- antitrypsin (α_1 -AT) was discovered and till this day it is continued to search for new genes. Laurell and Ericsson found that patients with low levels of alpha$_1$ - globulin serum revealed a high incidence of pulmonary emphysema, i.e., chronic obstructive pulmonary disease. Genetic polymorphism of α_1 -AT is not confined to a mutation leading to a decrease in the level of α_1 -AT in the serum. Currently, described a series of mutations, accompanied by a decrease in functional activity of the inhibitor. However, such cases are rare, and they can be linked only a small percentage of individuals predisposed to the development of COPD [5]. Also has been described principally new mutation in the gene α_1 -AT in 3 ' flanking area which has no relation to the amino acid sequence and therefore does not lead to amino acid substitutions in the molecule α_1 -AT, does not alter its physic -chemical properties. Theoretically, such a mutation should not have any clinical consequences. Nevertheless, a clear connection has been found this mutation (even in the heterozygous state) with susceptibility to COPD and bronchoecstasies. This mutation was discovered by two independent groups of researchers and it was not so rare - 15-20% of patients with emphysema and bronchoecstasies (in the control group of healthy individuals - not more than 5 %). Almost the same frequency was detected E.I. Samilchuk et al. (1997) in the Russian population [3]. This is most likely due to the fact that a mutation in the 3' -flanking region of the regulation of gene expression gives α_1 -AT. It is known that α_1 -AT relates to proteins known as "acute phase ", and its concentration in serum is increased in inflammatory processes 2-3. Such an increase in the concentration of α_1 -AT has a large biological sense, since to prevent tissue damage by proteolytic enzymes in the field of acute inflammation. Lack of acute phase increase in the level α_1 -AT in viral and

bacterial respiratory infections may contribute to tissue damage in neutrophil elastase and other proteolytic enzymes. At present the first results, which reveal the specific molecular mechanisms by the 3'- mutation which disrupts acute phase response. In the 90s drew attention to the gene encoding the alpha 1- antichymotrypsin (Samilchuk E.I., 1997) [3]. This inhibitor is also included in the group of serine; its gene is located in chromosome 14, in the same plot as its related gene PI.

Currently there are 75 known alleles of PI gene. They are divided into four groups: normal, they are characterized by physiological concentrations of serum alpha-1 - antitrypsin; scarce - the concentration of inhibitor reduced by at least 65 % of the norm, the "zero» – in a serum not detected and finally in serum normal level of inhibitor is registered, but its activity against elastase is reduced. PI- allele nomenclature is based on the electrophoretic mobility of glycoprotein - alpha1 - antitrypsin, option "A" is closest to the anode, the most popular variant "M" and a cathode - labeled «Z». The main shares of the gene pool (over 95 %) are three subtypes of the normal allele "M": M1, M2, and M3. Human pathology, of associated gene PI, falls on deficit and zero alleles. The main clinical manifestations of deficiency of alpha1 - antitrypsin are emphysema and juvenile cirrhosis. Delineated genetic emphysema cases , sometimes it is referred to as Essential , falls on a young age ; this form is often associated with cirrhosis [5].

However, there are described cases when in the elderly type ZZ was detected at moderate forms of emphysema. Revised data for the epidemiological study of genetic predisposition to emphysema suggest that in patients with chronic obstructive pulmonary disease, it is about 2 to 5%. The main pathogenetic mechanism that underlies the pathological process of low - inhibitory activity in lung neutrophil elastase structures, which leads to proteolytic degradation of respiratory tissue and primarily elastic fibers [3, 5].

Yakutia is the pole of cold, and clinical COPD picture proceeds more difficult than in other regions of the Russian Federation and the world in general. As a result of simultaneous clinical and instrumental studies in 2856 and residents of Yakutsk village Churapcha, proportion of COPD in the city was 14.1 % in rural areas - 20.5%. One of the main reasons for this is a significant prevalence of tobacco smoking among urban and rural residents especially Yakutia. The incidence of COPD among males over 60 years higher than that of women, it is almost 2 times in rural areas are 7.1 and 3.3 %, and in the ratio of men and women were about equal in city. According to our data, the frequency of colds was in 54.5% in city, and in rural areas - 43.9

%, which also explains the severe climatic conditions and is one of the risk factors for COPD. In this study, we noted that the disease is hereditary [4].

In the near future as a result of the research will be first identified genes for COPD in the Yakut population , will generate new data on chronic and COPD , the ways of transmission and prevention methods have been developed . The results can be used to improve the diagnosis, prevention and treatment of chronic human diseases associated with respiratory tract infection in extremely cold climates. A large number of gene mutations described COPD may cause a variety of clinical manifestations. Among those older than 40 years, the most frequent form with a primary lesion of the bronchopulmonary system, this determines the course and prognosis of the disease.

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Genetic Testing for Hereditary Diseases with Autosomal -Recessive Type of Inheritance in the Republic of Sakha (Yakutia)

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ABSTRACT

Prevalence of some autosomal - recessive diseases in the Republic of Sakha (Yakutia) for genetic testing has been estimated. Among the Yakuts no widespread monogenic diseases such as phenilketonuria and mucoviscidos have been noted. However, there was accumulation of such rare syndromes as 3M and methhemoglobinemia type 1 with major mutation. To decrease a rate of the monogenic diseases in the republic genetic testing system of autosomal-recessive diseases has been elaborated.

Keywords: autosomal-recessive diseases at population of the world, Yakutia, DNA research, phenilketonuria, mucoviscidosis, methhemoglobinemia type 1, 3M syndrome.

INTRODUCTION

Monogenic hereditary diseases are conditionally subdivided on rare, i.e. less than one case per 10 000 newborns, and frequent – more than one case per 10 000 births. Diseases with prevalence of 1 per 6 000 newborns and more can be referred to very frequent monogenic states. As a rule, such diseases have autosomal-recessive type of inheritance. Prevalence of heterozygotic carriage of such diseases in population was noted at 1 per 35-40 the people. For the majority of territories of Russia there are four very frequent monogenic diseases: phenilketonuria, mucoviscidosis, spinal amyotrophy, neurosensor relative deafness. Approximately every tenth person of the population at least is considered as a carrier of one of them. And each person is a heterozygotic carrier of 3-10 mutant genes of autosomal-recessive diseases. In the developed countries of Europe and the USA the screening of all population at reproductive age on carriage of these diseases is carried out (Table).

In the Republic of Sakha (Yakutia) methhemoglobinemia, 3M syndrome, nanizm with optic nerves subatrophy and pelgerovsky anomaly of leukocytes, neurosensor deafness type 1A are frequent monogenic diseases with autosomal-recessive type of inheritance at Yakuts. At Slavic and other nationalities more than 46% of the population of the republic often suffers from phenylketonuria and mucoviscidosis.

Hereditary methemoglobinemy (MGE) caused by deficiency of methemoglobin reductase (Methhemoglobinemia, NADH-Cytocromeb5 Reductase Deficiency, Diaphorase Deficiency, DIA1, MIM 250800) is the autosomal-recessive disease, followed by cyanosis of integuments, mental retardation of various severity degree, a higher level of methhemoglobin and insufficiency of methemoglobin reductase in blood. Vives-Corrons with coauthors [abst. 2] allocated 2 forms of deficiency of methemoglobin reductase. At MGE type 1 there are only symptoms of MGE, fermental defect is limited by erythrocyte soluble cytochrome-b5-reductase. At MGE type 2 mental retardation is manifested, and fermental defect is generalized and affects both soluble, and a microsomal cytochrome-b5-reductase in erythrocytes and leukocytes. Shirabe [abst. 2] noted that missens -mutations at MGE type 2 are located close from the catalytic center of enzyme, it causing high decrease in its catalytic activity while mutations at MGE type 1 affect marginal part of enzyme which influences only on its stability [11].

The first research of hereditary enzymopenic methhemoglobinemia (HEM) was conducted by Zakharova F.A. in Yakutia in 1982. Among the surveyed 120 people (patients and relatives) there were 48 homozygotes, 45 heterozygotic (i.e. HEM patients) (23 of them revealed for the first time) and 12 healthy members of the families living in Yakut Autonomous Soviet Socialist Republic, and also 7 homozygous and 6 heterozygotic ones from other regions of the USSR (Moscow, RSFSR, Ukraine and Uzbekistan). Homo-and heterozygotic HEM patients were identified on the basis of determination of methemoglobin reductase activity (MGR) and MetHb content in erythrocytes, as well as data of family and clinical inspection. Heterozygotic patients had no clinical symptoms of the disease though their MHR activity was reduced on 40-50% in comparison with the norm. The main clinical manifestation of homozygote patients was cyanosis of integuments. The expressiveness of symptoms depended on the MetHb level of "valent hybrids" in blood. The general state of the surveyed was satisfactory that didn't correspond to the expressed cyanosis at some of them. In blood analyses the initial MetHb level fluctuated from 10 to 44%. No MHR activity was noted almost at all (0) [4].

Banshchikova E.S. studied 50 children with HEM who were treated in hematologic department of RH No. 1-NCM of Yakutsk [1]. All children were the Yakuts (Sakha); the greatest number of children lived in the central part of Yakutia and the Viluysky group. At all children HEM type 1 was diagnosed. For the senior age group of children complaints to fast fatigue (31.25%), headache (28.1%) were characteristic. Complaints to heartaches had direct dependence on methemoglobin level in blood, heartbeat, dyspnea (37.5%). A large number of degenerate forms of erythrocytes were detected that could testify to accelerated ageing of

erythrocytes. Among the children with HEM, a higher rate of lipids peroxide oxidation was noted with considerable weakness of antioxidant systems that can promote the prevalence of organ and fabrics hypoxia. The HEM patients underwent the treatment by means of ascorbic acid according to their age. Due to the carried-out therapy the state of children improved: the weakness and heartaches disappeared, the cyanosis decreased. The hemogram didn't change significantly, the level of methemoglobin raised up to 10-12%, but further the data didn't change and the indicators of peroxide oxidation rose moderately. Such moderate increase of peroxide oxidation atHEM patients while treated with ascorbic acid is possibly connected to the increased concentration of ascorbic acid that can cause the pro-oxidant effect and lead to undesirable effects. The children suffering from HEM are subject to dispensary supervision through the whole period of the childhood then transferred to adult network [1].

The identification of methhemoglobinemia mutation at Yakuts was carried out in laboratories of MSSC of Russian Academy of Medical Sciences (Moscow). Samples of genomic DNA of 16 patients and 8 healthy relatives from 16 Yakut families with HEM type 1 were applied for the survey. The diagnosis was verified in the Scientific Institute of Medical Genetics RAMS (Tomsk). Also samples of blood of 9 patients from 8 families suffering from methhemoglobinemia having been sent for DNA diagnostics to the laboratory of MSSC RAMS during the period from 2006 to 2012 were used. For detecting CYB5R3 gene malformation a method of direct automatic sequencing was conducted in order to study all coding area and locations of exonic-intron links of this gene of one genomic DNA proband sample, homozygous on haplotype D22S276 – D22S1178 – D22S418 – D22S1179: 4 – 4 – 1 – 5 . As the result of that survey in exone 9 a change of c.806 C>T mutation in homozygous state was detected for the first time.

The prevalence of heterozygous carriage of c.806C>T mutation among the Yakuts amounted to 55:1000. Using a Hardy-Weinberg's equation as well as basing on experimental data 2pq = 0,055 the prevalence of mutant allele in the population was determined. The calculating prevalence of the disease is equal to 1:1250 in Yakutia. The prevalence of heterozygous carriage of c.806C>T mutation was analyzed in samples of two geographically close Yakut nationalities – the Koryaks (64 chromosomes) and the Chukchi (98 chromosomes). In these samples the mutation wasn’t detected. Considering the geographical and historical neighborhood, and data about prevalence of methhemoglobinemia type 1 among the Eskimos of Alaska, a DNA of representatives of the Canadian Eskimos (78 chromosomes) was investigated. In this sample c.806>T mutation wasn’t found as well [2, 10].

Since 2006 research associates of the department of molecular genetics of YSC CMP SB RAMS optimized a PCR-method in diagnosing HEM and transferred to the laboratory of practical medicine of MGC RH №1-NCM. In total the research of Pro269Leu mutation was carried out at 522 people by the PCR method, where the homozygous state (patients with a clinical picture) was noted at 56 people while the heterozygous one being 59 relatives (parents and sibs)and no mutation being found among the rest 407 people [12].

3-M syndrome (MIM 273750) is a rare autosomal-recessive disease called by initial letters of three authors' surnames (Miller, McKusick, Malvaux), for the first time they described the syndrome in 1975. The disease is characterized by facial dismorphy, pre-and a post-natal hypoplasia of extremities and bodies with normal sizes of a head and normal intelligence, and radiological changes in bones. CUL7 gene (Cullin) causing 3-M syndrome is mapped and identified in 2005 by a group of scientists of several countries of the world. At present patients with 3-M syndrome from 29 families of various nationalities have 25 mutations in CUL7 gene. The prevalence rate of the disease in the world is unknown as only 50 clinical cases and only one case of ultrasonic diagnostics of 3-M syndrome at a fetus has been described for the last 30 years.

In the Yakut population the 3-M syndrome prevalence is high and makes about 1 case per 8600 Yakuts. Molecular and genetic research of absolute haploid genome screening, genetic mapping of a gene, identification of the 3-M syndrome mutation were carried out during 2004 to 2006 together with Japanese colleagues in the Scientific Institute of brain of the University Niigata (Japan) (the head of department of molecular neuroscience Osama Onoder). This project was approved by ethical committee of the University Niigata. We described 43 Yakut patients with the confirmed molecular and genetic diagnosis 3-M syndrome. All patients had the same 458insT nonsense mutation in 25-coding exone of Cullin 7(CUL7) gene. The only mutation and the revealed haplotype testify to effect of the founder. Among the populations of Evenks, Yukaghirs, Evens, Buryats of Buryatia, Russians of the Tomsk region no heterozygous carriers of 4582insT mutation in *CUL7* gene were revealed [8]. The method of DNA diagnostics of 3-M syndrome in the Yakut population (the patent for invention RF№2315310) was devised. Growth inhibition mechanisms at 3-M syndrome are unknown. A fetus placenta with 3-M syndrome was characterized by dissociated maturation of chorionic villae with a large number of cynsytial kidneys. At 41.9% of the Yakut patients at birth asphyxia and a respiratory distress syndrome were noted, 25,6% newborns needed neonatal emergency care because of respiration disturbance. The histologic researches of lungs manifested cartilaginous tissue hypoplasia in

bronchial medium and large caliber with villous space narrowing [13]. The Patent for the invention RF №2315310 "A method of 3-M syndrome diagnostics in the Yakut population" (date of registration of 20.01.2008) has been issued. The research associates of the department of molecular genetics YCS CMP SB RAMS developed and introduced PCR-diagnostics of 3-M syndrome in applied medicine. By appointment of a doctor geneticist in the laboratory of MGC RH№1-NCM the 3M-syndrome DNA diagnostics at nanous patients is carried out. In total 1668 people were investigated by PCR method from 2006 to 2013, 4582insTy mutation being found at 37 patients in the homozygous state (with a clinical picture), at 135 relatives (parents and sibs) in the heterozygous one, no mutation registered at 1496 people. When pregnant women suspected on 3-M syndrome in fetus ultrasonography and both spouses detected as 458insT mutation carriers, they are registered in MGC, as genetic risk of a fetus with 3-M syndrome is estimated at 25%. If a family is cooperative, the prenatal diagnostics is carried out. In a case when the fetus is homozygous, with the consent of a family fetus elimination is performed. By 2013 within the MGC RH№1-NCM the prenatal DNA diagnostics on carriage of 3M-syndrome mutation had been performed at 40 pregnant women, 11 cases of them with homozygous carriage diagnosed, 7 heterozygous carriers of 3M-syndrome revealed. In 8 cases when a fetus was homozygous on mutation, with the consent of parents it was eliminated. In 3 cases parents decided to prolong pregnancy. In 7 cases when the fetus was heterozygous, all pregnancies were preserve and continued [12].

The hereditary diseases with autosomal-recessive type of inheritance, frequent in other regions of the Russian Federation (phenylketonuria, mucoviscidosis), are included there in the list of mass neonatal screening. Phenylketonuria (PKU) is a group of the diseases, being characterized by metabolic imbalance of irreplaceable amino acid xenylamine, coming through a human body with protein food. The developed clinical picture of the disease includes mental retardation, behavioral disorder, pigmentation defect, convulsive syndrome and dermatitis. The prevalence rate considerably varies depending on the population: 1:4370 in Turkey (Ozalp Y. et al. [abst. on 2]), 1:80500 in Japan (ShigematsuY.et al. [abst. on 2]). The prevalence PKU is 1:7 697 in the Russian Federation, 1: 8 376 in Krasnodar Kray.

In MGC RY№1-NCM, according to the Republican genetic register 12 patients with phenylketonuria from 11 families with healthy parents of Slavic and other nationalities are registered. Among the Yakuts no patients with phenylketonuria has been revealed. Of 11 families 6 ones live in Yakutsk, 5 families with 6 patients in other settlements of the republic. One of the parents in all families is a native of other regions of the Russian Federation. In 2012

direct diagnostics for identifying 8 frequent mutations (*IVS10-11G>A, R261Q, R252W, R408W, IVS12+1G>A, R158Q, P281L, IVS4+5S>T)* in *PAH*gene – xenylamine hydroxylase (PKU) was introduced. DNA testing was carried out at 53 patients, as the result 18 heterozygous carriers, 3 patients with *R408W, R158Q, R408W/R408W*mutations in homozygous state were revealed.

Mucoviscidosis (cystous fibrosis; MV) is a frequent monogenic autosomal-recessive disease, being characterized by defeat of excretory glands and vital systems both having severe acute disease and prediction. The prevalence of MV varies in different European populations from 1:600 till 1:12000 (on the average 1:5000) newborns. Mucoviscidosis in the Russian Federation is 1:11 585, in Krasnodar Kray is 1:11 425 [9].

In the genetic register of MGC RH№1-NCM 6 patients with mucoviscidosis are included. In 2011 the direct DNA diagnostics of probands and parents for identification of 16 frequent mutations *Del 21kb, L138ins,3944delTG, delF508, DelI507, 1677delTA, 2143delT, 2184insA, 394delTT, 3821delT, 604insA, 3849+10kbC>T, N1303K, R334W, G542X, W1282X)* in *CFTR* gene with mucoviscidosis (MV) has started. Of 246 DNA investigated by multiplex PCR and visualized by 10% polyacrylamide gel *F508del/N* mutation in heterozygous state was detected in 11 cases, , *del21Kb/W128X* in 1, *F508del*/*F508del* in homozygous state at 3 patients.

The aim of the study is to identify heterozygotic gene carriers of frequent hereditary diseases with autosomal-recessive type of inheritance among the population in the Republic of Sakha (Yakutia).

MATERIALS AND RESEARCH METHODS

For the DNA diagnostics of hereditary enzymopenic methhemoglobinemia type 1 venous blood is used as a material of the research, as for DNA diagnostics of 3-M syndrome venous blood and fetus chorion are applied. Patients' blood collection has been carried out by filling in a questionnaire and signing an informed consent. The DNA has been extracted from leukocytes of peripheral blood by a standard method with proteinase K and subsequent phenol - chloroform extraction. Molecular and genetic diagnostics of Pro269Leu mutation in *DIA1* gene was carried out by the PCR method with original oligoprimers by means of electrophoresis in 2% agarose gel. In norm fragments of 340 and 87 base pairs have to be formed, in case of the homozygous state fragments with length 245, 95 and 87b.p. are formed. In case of the heterozygous carriage of mutation (a healthy carrier) the lengths of fragments will be 340, 245, 95 and 87 b.p.

The molecular and genetic diagnostics of 4582 insT mutation in *CUL7* gene was carried out by the PCR method with original oligoprimer by means of electrophoresis in 3% agarose gel. In norm fragments 125, 115 b.p. have to be formed, in case of the homozygous state (patient)

instead of fragments 125 and 115 a fragment with length of 240b.p. is formed. In case of the heterozygous carriage (a healthy carrier) lengths of fragments will be 240, 125, 115p.n.

As a material for the research the venous blood of 282 students at the age of 21-26 years has been tested. All students from a random sample of the Medical institute NEFU of M. K. Ammosov by Yakut nationality, were engaged in the voluntary testing, they having been informed before about the aim and tasks of this research and filled in an informed consent.

To study phenylketonuria and mucoviscidosis outcomes of neonatal screening have been taken. The neonatal screening in the republic for 2006-2013 covers 99.7%.

RESULTS AND DISCUSSION

The questionnaire results and the analysis of genealogical students have shown that no close relatives with HEM and 3-M syndrome have been revealed. Other hereditary and hereditarily liable diseases have been revealed at relatives of the first and second familial relationship: autosomal-dominant type of inheritance, cardiovascular pathology, diabetes, tuberculosis of lungs, bronchial asthma, hypertension, etc. Testing for the heterozygous carriage of methemoglobinemy was held by the PCR method while studying Pro269Leu mutation. From 282 students 13 people were diagnosed with heterozygous mutation on HEM. The prevalence of the HEM in heterozygous carriers of the Yakut population at reproductive age is 46: 1000 people, or 1 HEM heterozygous carrier: 22 people.

When studying the second hereditary disease of 3-M syndrome at students the heterozygous carriage was found out at 9 people. The prevalence of carriage of 3-M syndrome at population Sakha at reproductive age is rated at 31.3 per 1000, or 1 heterozygotic carrier of 3M syndrome on 32 people.

According to statistical data there are about 8 thousand marriages in a year all over the republic, i.e. 16 thousand men and women marry. Of them 50%, i.e. 8 thousand men and women are representatives of the Yakut nationality, who should be examined by the genetic testing for revealing heterozygous carriers of autosomal-recessive pathology [3].

For 8 thousand people of the Yakut nationality 386 people are supposed to be heterozygous carriers with HEM, and 250 heterozygotes being with 3-M syndrome. At marriage occurring by chance it is rather possible to be a heterozygous couple with identical disease as among the Yakuts there are demographic distinctive features of marriage structure, this issue having been investigated in the republic (Picture).

When studying causes of the accumulation of monogenic diseases in the republic, we have noted that remoteness of settlements with small population from each other, bad transport

network, a high level of birth rate, assortative marriage by nationalities raise conditions for the marriage of heterozygous carriers with identical recessive diseases [6,7].

The data of neonatal screening for 2006-2013 have been investigated for performance of the frequent hereditary diseases, including phenylketonuria and mucoviscidosis which are already conducted at marriage in some countries.

Of 124019 newborns who were born in the republic from 2006 to 2013, 5 patients with phenylketonuria were revealed, no Sakha patient were among them. The prevalence of phenylketonuria in the republic is 1:24803 [12]. The ethnic origin of parents in forms isn't noted. According to demographic sources [5], in the republic about 46% of the population (i.e. 57 048 newborns) concern to the Slavic and other nationalities and the prevalence of PKU among them is comparable to other regions of the Russian Federation. Thus, the PKU prevalence among newborns with the disease (the Slavs and others, except theSakha) is 1:11 404.

For 2006-2013 112164 newborns were screened for mucoviscidosis, 4 patients are revealed, all children being of the Slavic nationality, no patients of Sakha noted. The prevalence of mucoviscidosis in the republic is 1:28041 among all newborns, approximately 1:14020 among the Slavs and others [13].

The prevalence of phenylketonuria and mucoviscidosis in the republic is lower than 1: 10000, and due to a higher degree of genetic heterogeneity at phenylketonuria and mucoviscidosis it is difficult to conduct mass preventive research on heterozygous carriage of these diseases among couples. Parents of sick children with PKU and mucoviscidosis are studied for the detection of heterozygous carriage, in more complicated cases they are sent to federal centers for the purpose of prenatal diagnostics.

Taking into account that no phenylketonuria and mucoviscidosis are noted among the Yakuts, they being frequent hereditary diseases among other nationalities of the Russian Federation, it is necessary to conduct researches on heterozygous carriage in the nearer future.

CONCLUSION

The heterozygous carriage of 2 frequent hereditary diseases in the population of reproductive age has been studied. The prevalence of HEM and 3M syndrome is comparable at students of Sakha with the prevalence of population studies in the Republic of Sakha (Yakutia), carried out earlier by other researchers.

It has been revealed that:

– The accumulation of hereditarymethemoglobinemy type 1 and 3M syndrome in the republic is related to major mutations that makes easier to conduct molecular diagnostics at homo and heterozygous carriers;

- The development of the effective diagnostic testing system of frequent monogenic diseases in the Republic of Sakha (Yakutia) for definition homo-and heterozygous carriers, including HEM type 1 and 3M syndrome is recommended;

– Phenylketonuria and mucoviscidosis in populations of Europe, the USA and the Russian Federation are frequent hereditary pathology and are recommended for the genetic testing for preventive measures. The prevalence of phenylketonuria and mucoviscidosis at newborns in the Republic of Sakha (Yakutia) is lower than in other regions of the Russian Federation and in Europe.

-In accordance with the preliminary molecular researches of parents and probands with phenylketonuria the high degree of genetic polymorphism of *PAH* gene mutation is observed that complicates the planning of prenatal preventive diagnostics of this serious illness;

– The prevalence of mucoviscidosis the republic among the Slavic and other nationalities according to the preliminary data is lower than in other regions of the Russian Federation and Europe; the genetic heterogeneity is observed;

Thus, the introduction of the diagnostics of monogenic diseases into applied medicine in the Republic of Sakha (Yakutia) is carried out by research associates of YSC CMP SD RAMS, further laboratory geneticists of MGC in association with doctors geneticists carry out DNA diagnostics of patients, members of families, prenatal diagnostics at families with high genetic risk on the budgetary basis. The wide application of DNA diagnostics at frequent monogenic diseases has great social and economic value for Yakutia as the remote region of the Russian Federation. If an effective test system of heterozygous and homozygous carriage of frequent monogenic diseases for the diagnostics of marrying people is elaborated thoroughly, it will be possible to lower the rate of hereditary diseases in the republic.

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table

Prevalence of heterozygous carriage of monogenic diseases in world populations

Population	Disease	Prevalence rate
Afro-Americans	Sickle cell anemia	1:10
	Mucoviscidosis	1:65
	Beta-Talassemia	1:75
Jewish andAshkenazi	Goshe disease	1:15
	Mucoviscidosis	1:26-29
	Tay Sacksdisease	1:30
	Disautonomy	1:32
	Cana-Van disease	1:40
Asia	Alpha-Talassemia	1:20
	Beta-Talassemia	1:50
America, Europe	Mucoviscidosis	1:25-29
French-Canadian	Tay Sacksdisease	1:30
Spaniards	Mucoviscidosis	1:46
	Beta-Talassemia	1:30-50
Mediterranean	Beta-Talassemia	1:25
	Mucoviscidosis	1:29
	Sickle cell anemia	1:40
Yakuts	SOPHsyndrome	1:100
	3-M syndrome	1:33
	Hereditary enzymopenic methemoglobinemia	1:25
	APcongenital deafnessIA	1:20

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Diagnosis of Genome Pathology in Children with Mental Retardation and Autism by SNP-Oligonucleotide Molecular Karyotyping

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ABSTRACT

Since mental retardation and autism are the commonest brain disorders in children, uncovering their genetic basis is an actual direction in current biomedicine. Application of whole genome scan for studying unbalanced DNA copy number variations allows not only the detection of chromosomal and genomic rearrangements leading to these diseases, but also offer an opportunity to describe pathogenic processes resulting in abnormal functioning of the central nervous system. To assess SNP-oligonucleotide molecular karyotyping (molecular cytogenetic method having the highest resolution for detection of such genomic variations) in diagnostic context, we have analyzed the genome of 100 children suffering from mental retardation and/or autism. Eight numerical and structural chromosome abnormalities (size: >5 Mb) and 20 submicroscopic genomic rearrangements (size: 0.5-3 Mb) were detected. Nine cases exhibited gene mutations manifesting as exonic deletions and duplications associated with the phenotype. As a result, it was concluded that this molecular cytogenetic technique has diagnostic yield no less than 37%. Additionally, further analysis by an original bioinformatics technologies allowed uncovering pathogenic processes associated with the aforementioned mental disturbances in another 55 cases. Thus, combination of SNP-oligonucleotide molecular karyotyping and bioinformatics analysis has high efficiency for detection of genomic pathology and identification of molecular mechanisms for mental retardation and autism.

Keywords: molecular cytogenetics, whole genome scan, SNP-oligonucleotide molecular karyotyping, mental retardation, autism

INTRODUCTION

Chromosomal abnormalities and genomic rearrangements are the commonest genetic defects associated with mental retardation and autism. During the last decade, an increase of communications dedicated to contribution of submicroscopic chromosomal abnormalities and copy number variations (CNV) to brain diseases has been noted. This is likely to be linked to active introduction of whole genome scan techniques by molecular karyotyping with an unprecedentedly high resolution (1 kbp and higher) [1-4]. Summarizing the data on genomic variations was used for recommendations concerning detection of unbalanced genomic rearrangements, which suggest using whole genome scan by SNP-oligonucleotide molecular karyotyping as the first tier diagnostic technique [8]. In children with mental retardation and/or autism, such types of genomic rearrangements can be detected in 10-50% cases depending on cohort peculiarities and application of additional bioinformatics techniques for assessment of the pathogenic value. The latter becomes even more actual inasmuch as CNV can be either pathogenic or benign [5, 10]. Here, diagnostic potential of SNP-oligonucleotide molecular karyotyping in combination with original bioinformatics technology [2, 6, 7] for identification of genome pathology in children with mental retardation and autism was addressed.

MATERIALS AND METHODS

SNP-oligonucleotide molecular karyotyping was used to analyze unbalanced genomic variations in 100 children with mental retardation, autism and/or congenital malformations according to previous protocols [2, 6]. Affymetrix platform for SNP-oligonucleotide molecular karyotyping, consisting of about 2.7 million probes and scanning the genome at a resolution of 1 kbp or higher, was used. Moreover, all the genomic variations were addressed by an original bioinformatics technology for assessing the phenotypic outcome as described previously [6, 7].

RESULTS AND DISCUSSION

Each individual demonstrated from 150 to 480 unbalanced genomic changes (CNV manifesting as deletions and duplications; sized from 1 kbp to 1.5 Mb). Bioinformatics analysis has led the way to differ between pathogenic and benign deletions/duplications. Large chromosome abnormalities were detected in eight cases: deletions of 1p32.1p31.1 (12.075 Mb),

6q11.1q14.1 (18.779 Mb), 7q32.3q35 (15.86 Mb), 8p23.3p23.1 (11.152 Mb); duplication of 8p23.1p11.22 (26.642 Mb) concomitant with deletion of 8p23.3p23.1 (6.782 Mb); supernumerary rearranged chromosomes 17 and X, as well as mosaic trisomy of chromosome X. We also detected submicroscopic genomic rearrangements affecting the following chromosomal loci: 1p36.33 (deletion), 1p36.23 (deletion), 2q23.3 (deletion), 2q24.2 (deletion), 5p13.3 (duplication), 5p13.2 (duplication), 5q14.3 (deletion), 5q15 (deletion), 6p11.2 (deletion), 9q21.13 (deletion), 11p14.3 (duplication), 12p13.31 (duplication), 15q11.2 (deletion), 15q13.1 (deletion), 16p11.2 (deletion), 17p13.3 (duplication), Xp22.12 (deletion), Xq21.1 (duplication), Yq11.223 (duplication) и Yq11.23 (deletion/duplication). The size of submicroscopic genomic rearrangements varied from 0.5 to 3 Mb. Interestingly, 4 cases of autism exhibited somatic mosaicism for structural chromosome abnormalities confirming previous studies of somatic genome variations in autism spectrum disorders [11]. Nine cases demonstrated gene mutations: exonic duplications in *KANSL1, EP300, PHEX, AFF2 (FMR2)*, *FMR1, RB1* and exonic deletions in *WT1, ATXN3*, *AKT3*. Summarizing these data, we concluded that diagnostic yield of SNP-oligonucleotide molecular karyotyping in combination with original bioinformatics technology is no less than 37%.

In 55 cases, CNV affecting genes highly expressed in the pre- and postnatal brain as well as involved in pathways of transcriptional regulation, cell cycle regulation, DNA reparation and replication, axonal guidance and neurogenesis. Therefore, combination of SNP-oligonucleotide molecular karyotyping and bioinformatics has provided for uncovering of molecular mechanism for brain malfunction in children with mental retardation and autism, as well.

CONCLUSION

The experience of applying SNP-oligonucleotide molecular karyotyping in combination with bioinformatics analysis, described herein, indicates that this approach towards detection of genetic causes for mental retardation and autism possesses high diagnostic yield. Similar results are able to offer opportunities for further studies of molecular processes leading to such kind of mental impairment in order to develop effective therapeutic interventions. Opening new prospects in personalized (genomic) medicine, the aforementioned technologies can significantly increase life quality in patients with mental retardation and autism due to a wide spectrum of genomic pathology.

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1p36 Microdeletion Syndrome: Diagnostic Problems and the Use of Molecular Cytogenetic Technologies for the Solution

Vorsanova S.G., Iourov I.Y., Demidova I.A., Kolotiy A.D., Liehr T., Yurov Y. B.

ABSTRACT

Partial 1p36 monosomy is one of the most frequent microdeletion syndromes. However, the diagnosis appears to be hindered due to the exceptional clinical diversity. Here, we present a comparative analysis of cytogenetic and molecular cytogenetic methods employed for testing of this chromosomal aberration in children with mental retardation and congenital malformations. Application of cytogenetic techniques allowed us to reveal terminal 1p36 deletions in 0.1% of cases (2 of 1874). Molecular cytogenetic analysis performed by conventional comparative genomic hybridization (CGH) uncovered 1p36 deletions in 1.3% cases (2 of 150). Using high-resolution microarray CGH (array CGH) we found microdeletions in 2.4 % (3 of 125) cases. Detected chromosomal rearrangements were confirmed by a fluorescent in situ hybridization. Array CGH allowed us to characterize the loss of genetic material in microdeletions with 1-10 kbp resolution. It was concluded that molecular diagnosis of 1p36 microdeletion syndrome requires the application of such innovative molecular cytogenetic technologies as array CGH. Our study enabled to estimate for the first time the frequency of 1p36 microdeletion syndrome among children with mental retardation and congenital malformations in the Russian Federation, which appeared to be about 2%.

Keywords: 1p36 deletion, microdeletions, molecular cytogenetics, whole genome scan, comparative genomic hybridization.

INTRODUCTION

1p36 deletion syndrome is characterized by severe intellectual disability, developmental delay, microcephaly, facial dismorphisms (prominent forehead , deeply set eyes, midface retrusion, depressed nasal bridge, asymmetric ears, cleft lip and palate) and congenital heart disease. This chromosomal pathology is considered as one of the most common causes of mental retardation and congenital malformations associated with microdeletions with estimated incidence about 1:5000 in general population, and 0.5-0.7 % — among children with mental retardation [1-4, 6].

Despite the fact that 1p36 deletion syndrome represents one of the most common genetic diseases associated with an unbalanced structural genomic rearrangement, its molecular diagnosis is complicated. This problem is apparently associated with exceptional clinical diversity and size variability of DNA sequences affected by deletions [5]. The aim of this work is to compare diagnostic techniques used for 1p36 deletion detection, which are based on either conventional cytogenetic or molecular cytogenetic innovative methods towards detection of unbalanced chromosomal and genomic rearrangements.

MATERIALS AND METHODS

We analyzed samples of peripheral blood lymphocytes obtained from 1874 children with mental retardation and congenital malformations using conventional cytogenetic methods of G- and C-banding. One hundred fifty cases were studied by applying conventional comparative genomic hybridization (CGH) in according to previously described protocol [7]. Additionally, 125 cases of mental retardation and congenital malformations were investigated using a genome-scanning technology with a resolution from one to several thousand bp or array CGH [8]. Patients presented with loss of genetic material in the aforementioned region of chromosome 1 were confirmed by fluorescence in situ hybridization (FISH).

RESULTS AND DISCUSSION

Cytogenetic analysis revealed terminal deletions of the short arm of chromosome 1 in 2 cases out of 1874 (0.1%). Although both cases were confirmed by FISH, deletion size and genes involved in chromosomal rearrangements were not identified. Conventional CGH revealed deletions in 1p36 region in children with severe mental retardation and congenital malformations in 2 cases (1.3%). These aberrations were microdeletions within 1p36.1p36.3 and 1p36.13p36.21 chromosome regions. Deletion sizes were estimated as 12+/- 2Mb in the first case and 7+/- 1Mb in the second one. Nevertheless, the use of conventional CGH did not allow us to determine the gene imbalance caused by these microdeletions in the short arm of chromosome 1. High-resolution genome scan by array CGH (with 1 kbp resolution and more) revealed the presence of deletions in 1p36 region in three cases out of 125 (2.4%). Deletions sizes were determined as 4.44, 7.09 and 8.15 Mb. The whole-genome scan technology has made possible to characterize the genomic rearrangements up to 1 kbp and uncover genes which became homozygous due to the deletion.

These data suggest that the use of the high-resolution array CGH is the most effective method for detecting recurrent chromosomal rearrangements in children with mental retardation and congenital malformations. It is noteworthy that, despite of retrospective clinical

ascertainment showed the presence of 1p36 deletion syndrome phenotype, all patients were not clinically diagnosed until molecular testing was performed. It is also important to note that cytogenetic analysis revealed only terminal deletions, which size was more than 25 Mb. Conventional CGH revealed deletions in 1p36 but did not allow accurate description of DNA sequence losses. Whole genome scan or array CGH proved to be the most effective in terms of molecular diagnosis. This correlates with the previously obtained data on microdeletion syndromes investigated by a genome-wide scan [1, 2, 6]. In addition, our data demonstrates that the occurrence of 1p36 deletion syndrome among children with mental retardation and congenital malformations is not less than 2% in the Russian Federation.

CONCLUSION

Comparative analysis of cytogenetic and molecular cytogenetic methods used in genome analysis showed that for highly effective diagnosis of one of the most common syndrome associated with microdeletions, the use of high-resolution array CGH is essential, because it as increases the efficiency of molecular diagnostics as allows to define DNA sequences and genes involved in the rearrangement. Considering current achievements in molecular therapy, the information obtained can serve as a basis for the development of science-based tactics for treatment of this disease. It should also be noted that similar studies of 1p36 deletion syndrome in the Russian Federation has not been carried out. This is probably due to the fact that the methods of high-resolution molecular cytogenetic diagnostics were only recently introduced into genetic diagnosis. This study allowed us to solve this problem and showed that the occurrence of 1p36 deletion syndrome among children with mental retardation and congenital malformations in the Russian Federation is about 2%.

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A Case of Familial Translocation between Chromosomes 2 and 18

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A.L. Sukhomyasova

ABSTRACT

Authors describe case of translocation trisomy in chromosome 18. Proband's chromosomal aberration was a result of maternal reciprocal translocation t (2; 18) (q13; q23). The proband A., 28 years old, was directed to the genetic consultation at 12 weeks of pregnancy about detected by ultrasonography chromosomal markers in the fetus. Pregnant burdened reproductive history: the first pregnancy - miscarriage at an early date, second pregnancy is underdeveloped. This third pregnancy was uneventful. Genealogical history of monogenic hereditary diseases and congenital malformations is not burdened. The proband pregnancy was terminated with the consent of the family for the period of 12 weeks for medical reasons due identified chromosomal disorders in the fetus - translocation trisomy 18 (Edwards syndrome) with a poor prognosis.

Empirical risk of giving birth to a sick child in families with reciprocal translocations is about 33%, the theoretical risk is 50% [5].

Keywords: balanced translocation, chromosome 18, Edwards syndrome, karyotyping.

INTRODUCTION

Chromosomal pathology is one of the leading places in the structure of human hereditary pathology. The different types of chromosomal and genomic mutations are described. Genomic mutations include aneuploidies and ploidy changes of the structurally unchanged chromosomes. A trisomy is a type of aneuploidies (an abnormal number of chromosomes). This is due to incorrect differences of certain chromosomes during meiosis in gametogenesis from a parent. The most famous trisomies are Down syndrome (trisomy 21), Edwards syndrome (trisomy 18) and Patau syndrome (trisomy 13).

Edwards syndrome is a trisomy of 18 chromosome. The frequency of occurrence of this syndrome in the population - one in about 7,000 births [5]. Complete or full trisomy 18 is the most common form. In addition, there is also a mosaic form. An alternative, but rare, cause of Edwards syndrome is «unbalanced translocation»[4]. This can occur because one of the baby's parents carriers what is known as a "balanced translocation".

The aim of the study is to conduct a cytogenetic study of the family, which is the carrier of balanced translocation between chromosomes 2 and 18.

MATERIALS AND METHODS

All samples for cytogenetic studies were received by «nego» method of the chorionic villi and «indirect» method of peripheral blood lymphocytes cultured within 72 hours, in accordance with standard procedure.

A standard cytogenetic analysis of metaphase chromosomes using differential coloring GTG on the microscope Olympus BX43F equipped with a digital camera, with auto karyotyping CytoLabView is made.

RESULTS AND DISCUSSION

The proband A., 28 years old, directed to the genetic consultation at 12 weeks of pregnancy about detected by ultrasonography chromosomal markers in the fetus. Pregnant burdened reproductive history: the first pregnancy - miscarriage at an early date, second pregnancy is underdeveloped. This third pregnancy was uneventful. Genealogical history of monogenic hereditary diseases and congenital malformations is not burdened Fig.(1).

Phenotype: asthenic physique, poor posture, scoliotic deformity, head of the usual form, a symmetrical face, hypocaloric, uneven tooth alignment. From the side of other organs and systems without violations

Ultrasound examination of the fetus done at 12 weeks of pregnancy: identification of chromosomal markers - a nuchal translucency measurement of 6 mm, aplasia of the nasal bone, abnormal yolk sac, pathological spectrum of blood flow in a venous duct, it is not excluded congenital heart disease.

Maternal serum screening: the β –subunit of hCG gonadotropin (beta-hCG)- 2,67 mlU/L /0,051 MoM (low), pregnancy – associated plasma protein A (PAPP-A) - 3,120 mlU/L /1,051MoM (normal).

The calculation of individual risk for trisomy 21,18,13 using the program «Astraya»: high risk for trisomy 21 (down syndrome) - 1:18, trisomy 18 (Edwards syndrome)- 1:4, trisomy 13 (patau's syndrome) - 1:88.

Given the presence of chromosomal markers identified for the ultrasound examination of the fetus and the high risk of combined screening, invasive prenatal diagnosis - transabdominal chorionic biopsy was done. Karyotyping was carried out in the proband, her husband.

Cytogenetic studies: cytogenetic analysis of the proband's husband showed normal karyotype 46, XY. Cytogenetic analysis of the proband revealed 46, XX, t (2; 18) (q13; q23), Fig. (2).

When fetal karyotyping were identified derivative two chromosomes that are the result of maternal reciprocal translocation t (2; 18) (q13; q23) and three copies of chromosome 18 through derivative of chromosome 18, inherited from the mother. Thus, fetal karyotype was identified as 47, XY, t (2; 18) (q13; q23) mat, +18 Fig. (3).

Proband's family was also examined for chromosome analysis. A t(2;18)(q13;q23) was found in the proband's mother, sister and niece.

Considering the mechanism of formation «translocation» trisomy Edwards syndrome, you may notice that when heterozygous carrier of reciprocal translocations in prophase of meiosis chromosomes form not bivalent and quadrivalent - complex of four chromosomes [1]. This complex spatial structure violates easy chromosomes in the anaphase to the poles cells may diverge as two chromosomes (segregation 2:2), and three and one (segregation 3:1).

In our case Fig.(4) a gamete got 3 chromosome: two chromosomes involved in reciprocal translocations and one chromosome 18. When combined such gametes with normal gamete, occurs trisomy 18 - Edwards syndrome.

The proband pregnancy was terminated with the consent of the family for the period of 12 weeks for medical reasons due identified chromosomal disorders in the fetus - translocation trisomy 18 (Edwards syndrome) with a poor prognosis.

Empirical risk of giving birth to a sick child in families with reciprocal translocations is about 33%, the theoretical risk is 50% [5].

CONCLUSION

Thus, the identified family translocation is essential for genetic counseling from the point of view of assessing the genetic forecast and opportunities of prenatal diagnosis. Timely prenatal diagnosis of fetal allowed to properly diagnose, identify a balanced translocation from the mother and to assess the risk of recurrent cases of the disease in the offspring.

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The Clinical Case of Crohn's Disease in Child of 3 Years and 7 Months

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ABSTRACT

The paper presents a clinical case of Crohn's disease, diagnosed in 2014 in a child of 3 years and 7 months. This pathology is rare among children (in the Republic Sakha (Yakutia) - 2 cases). Due to the variability of the clinical picture of the disease it is difficult to diagnose.

Keywords: system of histocompatibility, clinical remission, gastroenterology, inflammation, gastrointestinal tract.

INTRODUCTION

Crohn's disease is a chronic nonspecific progressive transmural granulomatous inflammation of the digestive tract. Most often affected the terminal small intestine, so there are synonyms of this disease, as «terminal ileit», «granulomatous ileit» etc. In the pathological process may involve any of the digestive tract from the root of the tongue to the anus. Frequency of lesions of the intestine and decreases in the following order: terminal colitis, ileocolic, anorectal form and other funds are also focal, multifocal and diffuse form[1,2,7,12,15,24]. For Crohn's undulating, with exacerbations and remissions. Crohn's disease is detected in children of all age groups. The peak incidence occurring at 13-20 years. Among the cases the ratio of boys to girls 1:1.1 [3-5, 19,23,25]. The etiology and pathogenesis of the disease is unknown. Discuss the role of infection (mycobacteria, viruses, toxins, food, certain medications, considered as the starting moment for the development of acute inflammation. Much emphasis immunological, dysbiotic, genetic factors [7,10,11,16,22]. The relation between the system HLA and Crohn's disease, which often reveal the loci of DR1 and DRw5. Clinical picture of the disease differs the big variety. Beginning of the disease is usually gradual, over many years with occasional outbreaks. The main clinical symptom in children's persistent diarrhea (up to 10 times a day). The volume and frequency of stools depend on the level of destruction of the small intestine - the higher it is, the more often a chair and, accordingly, the heavier the disease. The defeat of the small intestine accompanied with malabsorption syndrome. The chair periodically appears in the blood.

Complications of Crohn's disease are most often associated with the formation of fistulas and abscesses of different localization, ulcer perforation, peritonitis. Possible intestinal obstruction, acute toxic dilation of the colon. In the General analysis of blood reveal anemia (loss of red blood cells, hemoglobin, hematocrit), reticulocytosis, leucocytosis. When the biochemical

analysis of blood find hypoproteinemia, hypoalbuminemia, hypokalemia, reduction of the content of microelements, increased alkaline phosphatase, A2-globulin and C-reactive protein[1,2,7,12,15]. The severity of biochemical changes correlates with disease severity. Endoscopic picture of Crohn's disease is characterized by a great polymorphism and depends on the stage and extent of the inflammatory process. Endoscopically are three phases of the disease: infiltration, ulcers-cracks, scarring.

In phase infiltration (the process is localized in the submucosa of the) mucosa has a type of «quilt» Matt, vascular picture is not visible. Later, erosion type aft with separate superficial ulceration and fibrinous overlays. In phase ulcers-crack identify the individual or multiple deep longitudinal ulcerous defects affecting and muscular layer of the intestinal wall. The intersection of cracks gives the mucous membrane type «cobblestones» [8, 12, 17, 18, 20, 23]. Due to the significant swelling under the mucous membrane, and also defeats the deep layers of the intestinal wall of the bowel lumen narrows. In phase scarring detecting sites irreversible stenoses of the intestines. Characteristic radiological signs (research is usually done with double contrast study) - segmentation lesions, wavy and uneven contours of the intestine. In the colon determine the bumps and sores on the top edge of the segment with preservation of Australia on the bottom. In stage ulcers-crack - type «cobblestones». Diagnosis is based on clinical and anamnestic data and the results of laboratory, tool, morphological studies [13, 21, 26]. Differential diagnosis of Crohn's disease is carried out with a sharp and prolonged intestinal infections of bacterial and viral etiology, diseases caused by protozoa, worms, malabsorption syndrome, tumors, ulcerative colitis and other The most effective medicines believe drugs 5-aminosalicylic acid (mesalazine), sulfasalazin [24, 25]. At the same time should be taking folic acid multivitamin with microelements in the dose of age. In the acute phase of illness and severe complications (anemia, cachexia, damage of joints, erythema and others) prescribed glucocorticoids (hydrocortisone, prednisolone, dexamethasone), less immunosuppressants (azathioprine, cyclosporine).

The prognosis for recovery is unfavorable, for life - depends on the severity of the illness, the character of its course, the presence of complications. It is possible to achieve long-term clinical remission.

Thus, Crohn's disease is a severe disease with variable flow. Currently in the Republic of Sakha (Yakutia) has two children with the diagnosis of Crohn's disease. This clinical diagnosis was a girl aged 3 years and 7 months in 2014.

Objective: To present a clinical case of Crohn's disease in girls 3 years and 7 months

MATERIALS AND METHODS

The analysis of two case histories Children clinical hospital № 2 and National centre of medicine№ 1 Pediatric centre was done.

RESULTS

The Girl - child from the first pregnancy occurring without features. Childbirth for 38 weeks, operative. Breastfeeding to 2.5 months. Psychomotor and physical development is age appropriate. The child is sick from 10.11.2013. Feces is blood-streaked. The divisional pediatrician prescribed «smectite», the diagnosis is not installed. 13.11.2013 deterioration, bowel movement is regular with blood and child was hospitalized to the regional hospital with clinical diagnosis: enterocolitis unclear etiology. The girl received treatment: Ceftriaxone, Mezim Forte, Linex, Arbidol, ampicillin, loperamide. The child's condition deteriorated. Parents arrived to Yakutsk to hospitalize at children's clinical hospital №2. Child was diagnosed with acute gastroenterocolitis, moderate severity. Toxicosis, exicosis 1 degree. The child had a feces bacteriological analysis, sowing negative. In the General analysis of the blood was a high rate of sedimentation rate up to 42 mm/h, decrease in hemoglobin to 107 g/l, platelets to 414. In feces coprogram: erythrocytes 37-43-45 in sight. The girl received treatment: infusion therapy for two days, amikacin, smecta, Gidromet, Bifidumbacterin, furazolidone. After treatment, the child was discharged home. After a week there has been a sharp deterioration: a temperature of 39 degrees, diarrhea with blood stains 4-5 times a day. The child is sent to Yakutsk to the National centre of medicine № 1 Pediatric centre, where the reception was held with the surgeon and sigmoidoscopy. In the study Protocol is the following: in the bowel lumen scarlet blood, mucous intestines swollen and hyperemic, with hemorrhagic lesions 0.2 sm. After consulting the child was sent to Children's clinical hospital №2. Girl was diagnosed with Adenovirus enterocolitis moderate severity. Mixed infection. Acute intestinal infection of unknown etiology with gymocolit moderate severity and treatment: sylfperazon, trichopol, suprax, bromhexine. In the blood is the ESR to 60 mm/h, hemoglobin g/l, thrombocytosis to 438. The child examined by a gastroenterologist. Conclusion gastroenterologist: nonspecific ulcerative colitis. On 4.02.14 the child's state is seen as a serious, due to gymocolit. February 5, 2014 she was transferred to the gastroenterology Department of the National centre of medicine № 1 Pediatric centre, where she stayed for 5 days and got the following treatment: baktisubtil, salofalk, microclysters with hydrocortisone, Kreon, smectite and infusion therapy. There is an improvement of the patient and 10.02.2014 child is transferred by agreement in the National centre of health children of

Russia in gastroenterology Department for further diagnosis. In the National centre of health children of Russia diagnosis of Crohn's disease was confirmed.

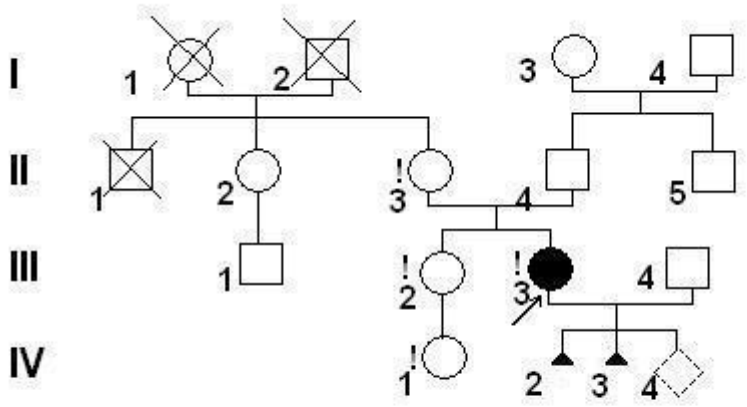

Fig.1. Pedigree

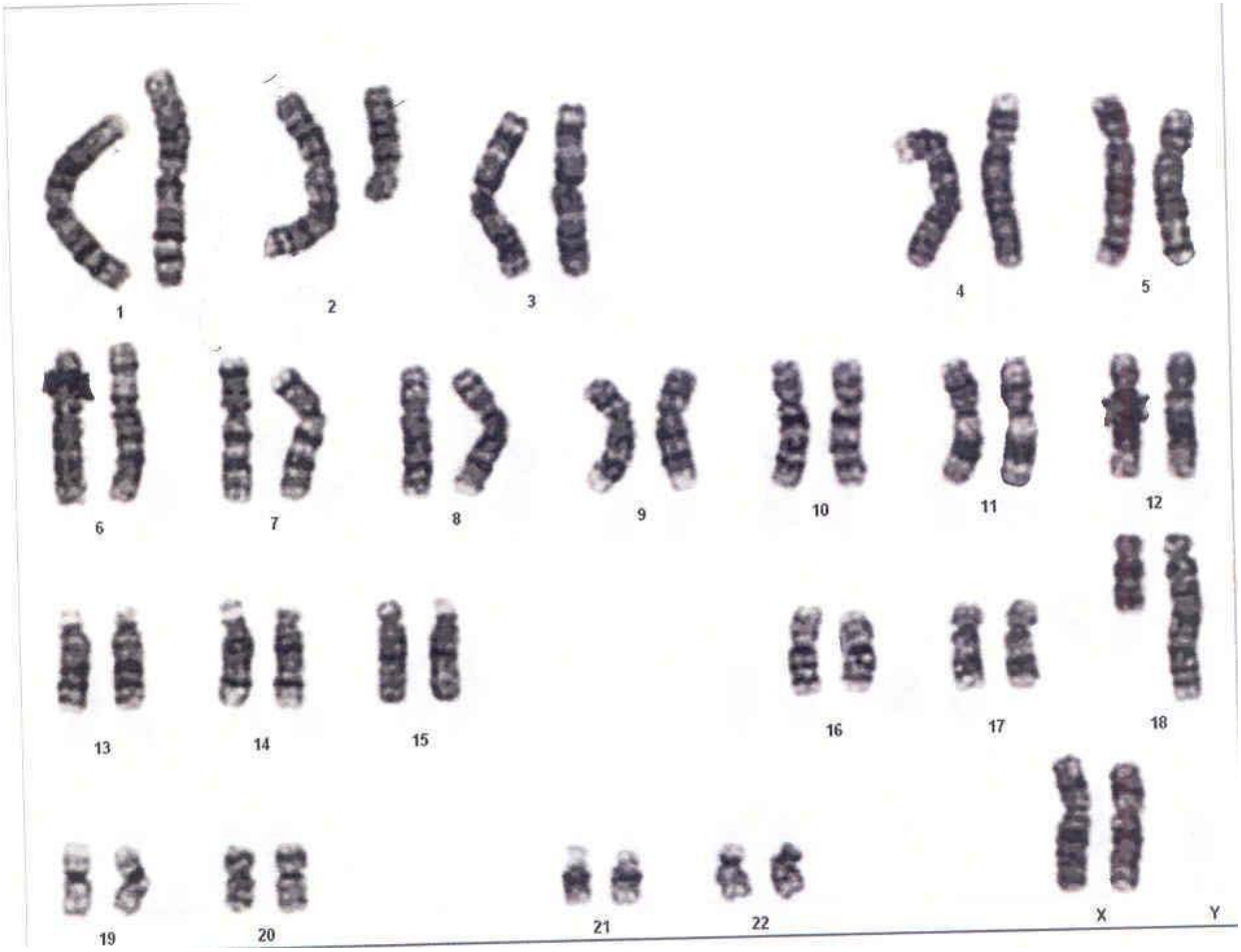

Fig.2. The proband's karyotype: 46,XX,t(2;18)(q13;q23) – balanced translocation between chromosomes 2 and 18

Fig.3. The fetal karyotype: 47,XY, t(2;18)(q13;q23)mat,+18.

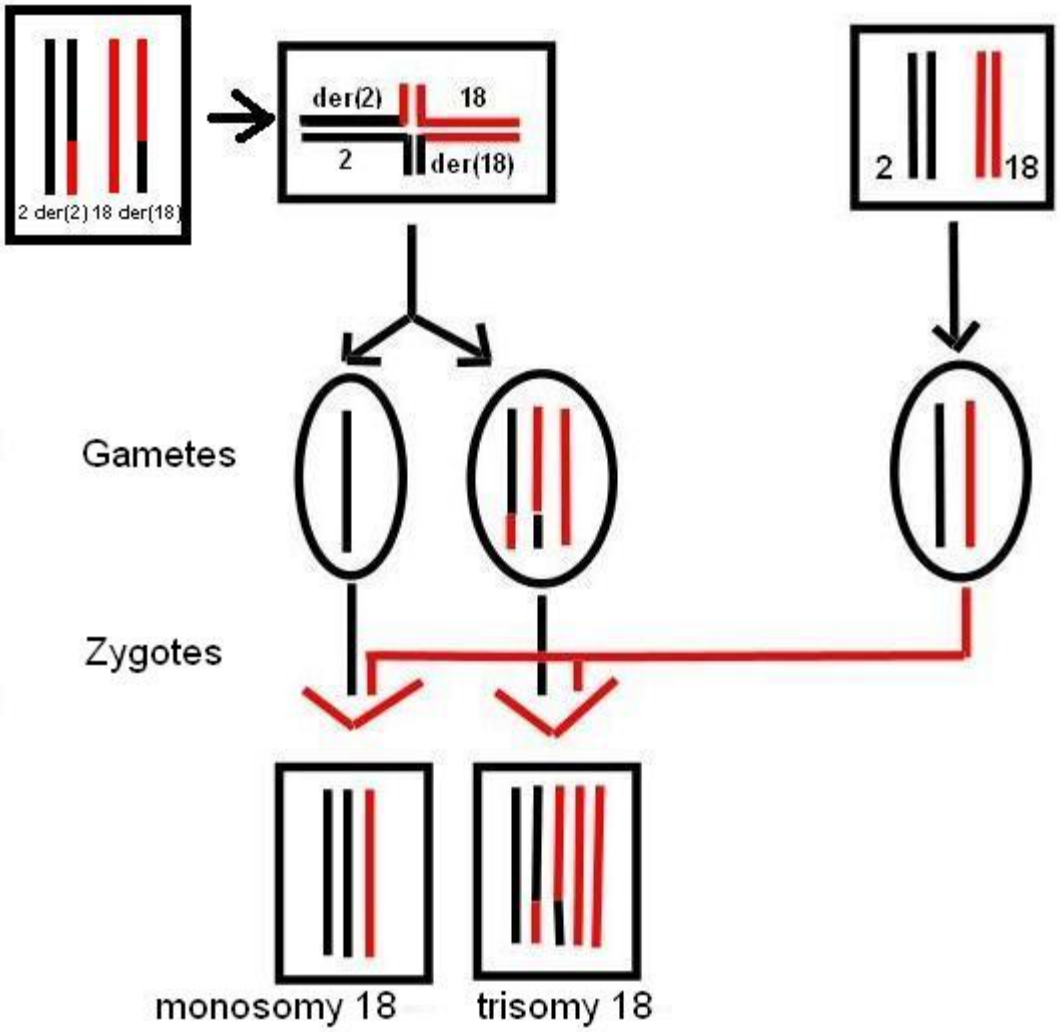

Fig.4. Carriage of reciprocal translocation. Formation of quadrivalent. Chromosome disjunction (3:1 segregation).

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Potential and Perspectives of a-CGH Method in Clinical Practice

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ABSTRACT

The identification of chromosomal abnormalities is an important component of clinical genetics. Currently, the methods of classical cytogenetics cannot meet the increasing demands of clinical medicine. The development of fluorescence *in situ* hybridization techniques (FISH, CGH) has made possible the identification of submicroscopic chromosome aberrations. Progress of the technology has led to the emergence of new high-tech method - comparative genomic hybridization on microarrays (array-CGH, a-CGH). This method is used in many areas of clinical genetics, reproductive medicine and oncology. Besides practical applications, a-CGH is used in fundamental medicine, particularly to study the causes of congenital malformations and undifferentiated intellectual disability, as well as fundamental oncology.

Keywords: a-CGH, cytogenetic diagnostics, microarrays.

Submicroscopic chromosomal deletions and duplications (less to 10 Mb) constitute up to 15% of all mutations underlying human hereditary diseases [6]. Comparative genomic hybridization on microarrays is the most appropriate method to detect such abnormalities. Resolution of modern microarrays reaches several tens of kb, while up to 1 million unique genomic loci is scanned. The main advantage of the method is extremely high resolution, allows for the identification submicroscopic chromosomal aberrations as well as copy number variations of large blocks of DNA repeats (Copy Number Variation, CNV). CNV accounts for roughly 12% of human genomic DNA. Copy number variation can be inherited from parents or arise *de novo* in any part of the genome, its size can be relatively small – a few hundred thousand base pairs. More than 41% CNV overlap with known genes, indicating their possible role in the regulation of the expression through the effect of gene dose or position [1]. In the future, the analysis of the genes on the chromosome regions affected by submicroscopic chromosomal abnormalities or CNV will help to identify genes with imbalance leading to the development of pathology. Noticeable shift of focus in cytogenetic studies from chromosome to gene, as well as the expected introduction of high-throughput genome sequencing allows the appearance of a new direction in biology and medicine – cytogenomics [2].

Array CGH method is based on the principles of the conventional comparative genomic hybridization (c-CGH). Differentially labeled control DNA (usually labeled with a red fluorochrome) and target DNA (labeled with green fluorochrome) hybridize together on small fragments of human DNA deposited in a specific order to the microchip. Differences between gain and loss or a balanced status are based on the ratio of green to red fluorescence for each DNA fragment. Further, by specific processing programs all DNA segments are positioned on a specific chromosomal region, wherein the hybridization profile generated reflecting the amount of DNA material in each region of the genome.

Microarrays differ in their resolution. Microarrays that use large segments of DNA (100-200 kb) built-in artificial bacterial chromosome (BAC - bacterial artificial chromosome) are applied for low resolution a-CGH. In average, they have a resolution of 1 Mb (i.e. register gain or loss of genetic material quantity of 1 Mb, which roughly corresponds to 1/10 of the chromosomal band). High-resolution microarrays have a resolution of 50-100 kb. They are consisted of the oligonucleotide sequences of about 60 nucleotides in length, relatively homogenously covering the whole genome. For example, available commercial microarrays provide an average coverage of the genome with a resolution up to 0.02 Mb that is 1000 times more informative than karyotyping [3].

Array CGH method allows to identify all unbalanced chromosomal rearrangements, including aneuploidy, unbalanced translocations and microstructural abnormalities in a single analysis. Disadvantages of the method include the inability to detect balanced structural abnormalities (reciprocal translocations, inversions and Robertsonian translocations) and polyploidy [8].

Reproductive medicine is a key area where CGH microarray technology is demanded. Usage of assisted reproductive technology (ART) is accompanied by a high frequency of chromosomal abnormalities in embryo cells. After the application of ART, the frequency of abnormal embryos with chromosomal abnormality in women younger than 35 years is 60%, and for women over 41 – 80% [9]. Preimplantation genetic diagnosis (PGD) can detect abnormal embryos, greatly increasing the chance of successful implantation of the blastocyst and successful delivery. Currently, for purposes of PGD FISH and a-CGH are used. FISH commonly allows identification of aneuploidy for five chromosomes (13, 18, 21, X and Y) only with clinical significance at once, whereas the use of a-CGH allows genome-wide assessment of aneuploidy and unbalanced structural chromosome aberrations.

Listed advantages of microarrays in comparison with FISH remain in prenatal diagnosis. Array CGH in prenatal diagnosis of chromosomal abnormalities is used primarily on the testimony, for example, if the parents are carriers of balanced translocation. In addition, a-CGH is used to identify the causes of spontaneous abortion. So, we investigated 13 miscarriages with normal karyotype determined by conventional cytogenetics. From 3 to 20 CNV were detected in each case. In 4 embryos only benign variants were observed, while 9 abortions had potentially pathogenic CNV.

Microarray technology is most prevalent in areas of clinical genetics such as search for genetic causes of intellectual disability and congenital malformations [4]. This trend is due to the fact that many undifferentiated forms of these pathologies result from various CNV and submicroscopic chromosomal rearrangements. CNV underlie the 14-18% of undifferentiated intellectual disability [5]. Currently, 211 microdeletion and 79 microduplication syndromes are described that cover 267 genomic loci [7]. Research in this field was also conducted by our team. Samples from 79 children with intellectual disability and congenital malformations were analyzed using Agilent 44 K and 60 K arrays. Array CGH analysis did not identify any unbalanced chromosomal aberrations in 35 of the patients (44%). Copy number variations that were observed in the remaining 44 patients were first classified using the Database of Genomic Variants. Twenty-two children carried only benign CNV. A total of 26 pathogenic or likely pathogenic CNV were detected in the other 22 affected children (28%) [4].

Microarray technologies are an integral part of modern cytogenetics. Demand for this method is due to the possibility of simultaneous analysis of the whole genome with an extremely high resolution. Given the significant advances in this area in the last decade, as well as due to ever-lower cost, a-CGH technology in the near future can be widely used in medical practice. Effective implementation of this technology into clinical practice requires not only technical equipment and qualified personnel, but also the maximum awareness of clinicians.

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Prenatal Diagnostics in the Republic Sakha (Yakutia)

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ABSTRACT

The purpose of research. To analyze of the effectiveness of prenatal diagnostics in the Republic of Sakha (Yakutia) and the effect of combined prenatal screening for chromosomal abnormalities.

Materials and methods. In work the analysis of invasive prenatal diagnostics in the medical genetic consultation Perinatal Centre, Republican Hospital №1 - NCM for 2005-2013, the results of combined prenatal screening in the RS (Y) for 2012 and 2013 are presented. In the laboratory of the prenatal diagnosis of the medical genetic consultation Perinatal Centre 2012 and 2013 by the combined of screening program 16397 pregnant women were examined.

Results. In the system of actions of prenatal diagnostics one of the most important places belongs to the invasive methods for obtaining the fetal material. In most cases, invasive prenatal diagnosis is carried for excluding chromosomal and of monogenic diseases of the fetus. Groups for invasive prenatal diagnosis are formed on the results of the combined prenatal screening, the presence of the echographic markers in the fetus, as well as on the results medical genetic counseling.

Keywords: prenatal diagnostics, invasive diagnostics, congenital malformations of the fetus, combined screening, chromosomal abnormalities.

INTRODUCTION

Prenatal diagnostics of congenital and hereditary diseases - is a complex branch of medicine that uses ultrasound examination invasive diagnostics (chorion biopsy, amnio-and cordocentesis, muscle and skin biopsies of the fetus), and laboratory methods (cytogenetic, biochemical and molecular genetic). Prenatal diagnostics is essential in medico genetic consultation, because it enables to move from the probable an unambiguous prediction of the child's health in families with genetic complications. Currently, diagnostics almost of all chromosomal syndromes and hereditary diseases is possible, the biochemical defect in which is set significantly (1).

Reduction of perinatal morbidity and mortality is one of the main tasks in the system of maternity and childhood protection, in which structure congenital and hereditary diseases occupy

a leading position. With hereditary defects that have, as a rule, severe course, are born to 2.5% of newborns.

One of the most common forms of congenital and hereditary diseases are chromosomal abnormalities whose frequency reaches among newborns the 7-8:1000. Rude chromosomal defects are having a poor prognosis for life and health, registered in 2-3 cases per 1000 live births.

Up to 70-80% chromosomal defects arises in the young and in no way burdened families as a result of new mutations. This eliminates the possibility of effective prevention of chromosomal disorders and requires improvement approaches to their early prenatal diagnosis. Among pregnant older (35 and older) age and of genetic risk group (weighed down by family, obstetric and somatic anamnesis), the incidence of chromosomal abnormalities in fetuses above general population and can reach 4-5% (2).

In the system of actions of prenatal diagnostics one of the most important place belongs to the invasive methods for obtaining the fetal material. In most cases, invasive prenatal diagnosis is carried for excluding chromosomal and of monogenic diseases of the fetus. Groups for invasive prenatal diagnosis are formed on the results of the combined prenatal screening, the presence of the echographic markers in the fetus, as well as on the results medical genetic counseling.

An effective method of detection prenatal echographic markers of chromosomal abnormalities in the fetus is the ultrasound diagnostics. The probability of detecting chromosome abnormalities in the fetus significantly increased in the presence of several echographic markers. The combination of the echographic markers of chromosomal abnormalities with other risk factors (especially age factor) is an absolute indication to prenatal definition of fetal karyotype in connection with the highest risk of detection of chromosomal abnormalities.

The purpose of research. To analyze the effectiveness of prenatal diagnostics in the Republic of Sakha (Yakutia) and the effect of combined prenatal screening for chromosomal abnormalities.

MATERIALS AND METHODS

In work the analysis of invasive prenatal diagnostics in the medical genetic consultation Perinatal Centre, Republican Hospital №1 for 2005-2013, and the results of combined prenatal screening in the RS (Y) for 2012 and 2013. Material for research were the results of the invasive diagnostics in 1484 pregnant women and the results of two years of combined screening with the analyzer KRYPTOR and software "Astraya" at 16397 of pregnant.

In the medical genetic consultation Perinatal Centre, Republican Hospital №1 within the national project in december 2011 delivered biochemical analyzer KRYPTOR [BRAHMS, Germany], performing the determination of levels of PAPP-A and free β - hCG in the blood serum of pregnant. All operations are fully automated. Specialized software "Astrayya" (obstetric database) allows to calculate the the combined risk fetal abnormalities of trisomy 21 (Down syndrome), trisomy 18 (Edwards syndrome), trisomy 13 (Patau syndrome)taking into account the biochemical parameters determined in test double the first trimester and ultrasound findings made in the timeline 11-13,6 weeks of pregnancy. Such a test is called combined with of nuchal translucency double test first trimester of pregnancy or triple test first trimester. Risk calculation results obtained by a combined double test is much more accurate than calculations based on only the risk of biochemical parameters, or based only on ultrasound.

The software "Astraya" is based on algorithms developed by Fetal Medicine Foundation (FMF) and the Foundation for fetal medicine (London). The program takes into consideration the skills of specialists of ultrasound diagnosis on the basis certificate FMF, allows you to audit all indicators as well as maintains a database for research.. The qualifications of specialists ultrasound theoretically exclude the false from entering pregnant in high-risk group, which leads to the need for in this case unfounded, economically costly, having 1-2% of complications of invasive diagnostic procedures.

RESULTS AND DISCUSSION

Number of the invasive researches in the Republic of Sakha (Yakutia) is increasing every year in connection with increase of early coverage of pregnant prenatal diagnosis with the introduction of the combined prenatal screening, in which a high-risk group includes the pregnant with changing of maternal serum of biochemical parameters without ultrasound markers, increasing the number of the aged pregnant (tabl.1).

Tabl.1

Indications for the invasive cytogenetic diagnostics in the MGC for 2005-2013, abs. number (%)

	2005	2006	2007	2008	2009	2010	2011	2012	2013
Number of pregnant who underwent invasive diagnosis	49	118	111	123	141	180	257	235	270
Yakutsk	24 (49)	48 (41)	56 (51)	64 (52)	80 (57)	83 (46)	145 (56)	111 (47,2)	125 (46,3)
Districts	25 (51)	70 (59)	55 (49)	59 (48)	61 (43)	97 (54)	112 (43)	124 (52,8)	145 (53,7)
Age 35 years age	35 (71)	81 (69)	82 (74)	78 (63)	106 (75)	131 (73)	178 (69)	147 (65)	163 (60)
over 35 years	14 (29)	37 (31)	31 (26)	45 (37)	35 (25)	24 (13)	79 (31)	79 (35)	111 (41)
Of these, over 39 years	11 (79)	28 (76)	19 (61)	28 (62)	16 (46)	25 (14)	53 (21)	43(54)	64(57)
Number of invasive research	49	118	111	144	155	184	257	226	274

Table 2 presents the indications for invasive methods of prenatal diagnosis. The main indications for invasive prenatal diagnosis in the last years are a combination of biochemical and ultrasound markers.

Table 2

Indications for the invasive cytogenetic diagnostics in the RS (Y), abs. number (%)

	2009	2010	2011	2012	2013
Indications:					
Combined (ultrasound, age)	30 (21%)	27 (15%)	48 (20%)	18 (8%)	16 (6%)
Biochemical markers and ultrasound	5 (4%)	4 (2,2%)	2 (0,8%)	63 (29%)	53 (20%)
The echographic markers	55 (40%)	102 (57%)	101 (42%)	48 (22%)	45 (17%)
Age over 35 years	5 (4%)	31 (17%)	24 (10%)	12 (6%)	9 (3,3%)
The carrier status of balanced rearrangements	3 (2%)	5 (3%)	3 (1,2%)	2 (0,9%)	4 (1,5%)
Biochemical markers	2 (1,5%)	6 (3,4%)	8 (3,3%)	14 (6,4%)	83 (31%)
congenital malformations of the fetus	30 (22%)	13(7%)	46 (19%)	46 (21%)	40 (15%)
Others	7 (5%)	7 (4%)	7 (2,9%)	14 (6%)	16 (6%)
i. Pathologies are revealed by indications:					
ii. The echographic markers	8 (9%)	9	10 (59%)	2 (10%)	11(30%)
iii. Age over 35 years and biochemical markers	0	0	0	0	4 (11%)
iv. Combined (ultrasound, age)	8 (27%)	1	5 (29)	1 (5%)	6 (16%)
v. Biochemical markers and ultrasound vi. (in women are under 35 years)	1 (20%)	0	2 (11,8%)	9 (45%)	5 (14%)
vii. The carrier status of balanced rearrangements	1 (33%)	1	1 (5,8%)	1 (5%)	2 (5%)
viii. Biochemical markers and ultrasound (in women	-	-	-	8 (40%)	9 (24%)

age over 35 years)					

b. Table 3.

c. Effectiveness of invasive diagnosis in detecting chromosomal abnormalities, %

i.	2005	2006	2007	2008	2009	2010	2011	2012	2013
Pathologies is revealed in the fetus	6 (12%)	12 (10%)	9 (8%)	14 (11%)	18 (13%)	10 (5,5%)	17 (6,6%)	22 (9,7%)	43 (15,9%)
ii. Yakutsk	3 (50%)	5 (42%)	3 (33%)	8 (57%)	11 (61%)	9 (90%)	11 (65%)	13 (59,1%)	31 (60,8%)
iii. Districts	3 (50%)	7 (58%)	6 (67%)	6 (43%)	7 (39%)	1 (10%)	6 (35%)	9 (40,9%)	20 (39,2%)
iv. In women are under 35 years	5 (83%)	10 (83%)	6 (67%)	9 (64%)	11 (61%)	9 (90%)	10 (59%)	11 (50%)	24 (47,1%)
v. In women age over 35 years	1 (17%)	2 (17%)	3 (33%)	5 (36%)	7 (39%)	1 (10%)	7 (41%)	11 (50%)	27 (52,9%)

From 2005 to 2013 the medical genetic consultation Perinatal Centre are diagnosed chromosomal abnormalities in the fetus 151, and since 1999, since the organization of invasive cytogenetic diagnostics in the RS (Y) 182 cases. Specific weight of the frequent genetic pathology - Down syndrome in the structure of the pathology in the prenatal period was 71% (21 cases) in 2013 (in 2012 - 44% (11), in 2011 - 47% (8), 2010 - 10%, in 2009 - 61%, 2008 - 40%). The high level of syndrome Edwards diagnosis.

Effectiveness of prevention congenital malformations of the fetus, in 2013 increased significantly in chromosomal aberrations (79.6%), including Down's syndrome (71%), moderately increased in musculoskeletal pathology, multiple congenital malformations of the fetus, congenital heart diseases. At this stage abortion decreased by congenital malformations of the fetus, which is successfully carried out at surgical correction of the fetus in the conditions of the Perinatal Center.

In the Republic invasive prenatal diagnosis was carried out since 1999, for the last 5 years diagnosis with invasive methods was performed in 1121 pregnant women.

Table 4

The main indicators of prenatal diagnosis from 2009 to 2013

Indicators	2009	2010	2011	2012	2013	Всего
All invasive procedures, including:	164	184	258	241	274	1121
-amniocentesis	2	3	5	5	2	17 (1,5%)
Horion biopsy	42	38	89	99	121	389 (34,7%)
placenta biopsy	101	114	104	88	120	527 (47,1%)
cordocentesis	19	29	60	48	31	187 (16,7%)
Investigations of fetal material	174	187	258	240	290	1149
Including cytogenetic	155	168	234	226	274	1057
Including molecular genetic	19	19	25	27	16	106
Deviations	23	13	23	29	47	135
Including chromosomal aberrations	18	10	17	22	43	110
Including monogene pathologies	5	3	6	7	4	25
Sent to interrupt pregnancy	101	109	100	101	97	508
- with a chromosomal abnormality	18	10	17	22	43	110
- monogenic disease	4	3	6	7	4	24
with unviable congenital malformations	66	45	64	67	50	292

The large-scale organization of prenatal screening in the Republic of Sakha (Yakutia) was launched in late 2011. As part of the priority national project "Health", "Prenatal diagnosis of the child's of disorders development" launched a massive combined screening pregnant women in timeline 11-13 weeks 6 days. According to the order MZ of Sakha (Yakutia) № 01-8/4-196a of 01.03.2011 "On conducting prenatal diagnostics of disturbance of a child's development in the Republic of Sakha (Yakutia)" prenatal screening is required for all pregnant women living in the Republic refer for medical observation in health facilities.

In the laboratory, of prenatal diagnostics the medical genetic consultation for 2012-2013 were examined for programme combined screening 16397 pregnant (6726 pregnant women in 2012 and 9671 pregnant women in 2013). From them 8582 (52.3%) of the sent areas and the 7815 (47.6%) - from Yakutsk. 2985 (18.2%) of pregnant women over the age of 35 years and 582 (3.5%) women over the age of 39 years.

In the combination of prenatal screening in a high risk group (cut-off 1:100) included 411 (2, 5%) pregnant (the value of high-risk groups for qualitative of indicator screening is 1.3% - 2%). From them 257 (51.3%) of women aged 35 years.

In the audit participated 668 (4%) pregnant women with increased nuchal translucency greater than 95 percentile (with effective screening should be 2-5% of the measurements nuchal translucency than 95 percentile and 45 - 50% more than the median of measurements nuchal translucency.

Changes of biochemical parameters of maternal serum was noted in 1131 (6.9%) pregnant women (Table 5).

Table 5.

Indicators of biochemical markers in pregnant women

	5th percentile	median	95th percentile	
Free beta- hCG (MoM)	0,288	0,839	2,362	3,6% above 95 th
PAPP-A (MoM)	0,381	1,075	2,916	7,2% below 5th

The effectiveness of screening is determined by its sensitivity (detection rate) and a specificity (level false - positive and false - negative results). Since the that the level of false - positive results affects the amount needed invasive procedures, this indicator is not less important than detection to evaluate the efficacy of screening.

Invasive diagnostics on the results of the combined screening for two years carried 200 pregnant. Should be noted that with the introduction of the combined prenatal screening has increased the number of invasive procedures in the I trimester of pregnancy (horionbiopsy accordingly), decreased the number of cordocentesis. Detected 48 (24%) of chromosomal abnormalities including Down's syndrome - 21, Edwards syndrome - 12, Patau syndrome - 2, Turner syndrome - 3, the other chromosomal abnormalities - 10. From them in women aged 35 years and older found 22 (46%) cases of chromosomal aberrations, including Down's syndrome - 11 (50%). At revealing chromosomal abnormalities in 32 (67%) of pregnant women was an increase in nuchal translucency greater than 95 percentile, the change of biochemical parameters of maternal serum in 20 (42%) pregnant.

The detectability of chromosomal aberrations in the Republic amounted to a combined screening - 24% (efficiency should not be less than 15%). False-negative results in 8 (0.05%) pregnant women, of whom 4 because of the age, congenital malformations of the fetus, the echographic markers of fetal chromosomal aberrations in II trimester conducted prenatal karyotyping, detected chromosomal abnormalities (2 - Down syndrome, 1 - Edwards syndrome, 1 - marker chromosome).

Qualitative conducting combined of screening associated to the specificity of our republic: a big problem in the Arctic region is in connection with the remoteness of from the capital and localities of the regional centers, a sophisticated transport scheme, inadequate personnel base.

The data received as a result of the combined prenatal screening, justify the need not only to accumulate its own results to determine the normal level of markers in the blood of pregnant

women the examined population, but also corrections MoM from a regional perspective, as in the software "Astraya" considered ethnicity pregnant from which determines the final calculation of the risk. This obliges compiling that each laboratory the table of measurements on a certain week of pregnancy to calculate the eigenvalues of the median. Regional of the median value should serve as the basis for calculating individual risk having a child with a chromosomal abnormality.

Education and external audit for programme FMF allows unification ultrasound methodology and allows considered reliable results fetometry performed data specialists. This determines finally accuracy of the calculation of the individual risk of congenital malformations in the baby.

Effectiveness of prevention of fetal malformations in 2013 increased significantly in chromosomal aberrations (79.6%), including Down's syndrome (71%).

CONCLUSION

1. Actual task is the further study of the prognostic value of fetal nuchal translucency thickness as a prenatal echographic marker of congenital and hereditary diseases and to develop an algorithm of complex examination of fetuses with the advanced nuchal translucency in various stages of pregnancy.

2. Work out regional indicators for measurement of the fetus at risk for the formation of invasive cytogenetic diagnostics.

3. The use of molecular prenatal screening for aneuploidy diagnostics to 5 chromosomes (X, Y, 21, 13, 18) by PCR.

4. The introduction of new methods of molecular genetics the fetal karyotype analysis to decide on the prognosis for the fetus and management of pregnancy.

5. To improve the quality parameters of combined prenatal screening: coverage of screening at least 80%; to improve the quality parameters measurement of ultrasonic indicators: to do this continuous learning of doctors of prenatal diagnostics, the maximum certification of physicians by FMF (international certificates for ultrasonic diagnostics) for more accurate prenatal screening of international standards diagnostic, equipping in Yakutsk and regional centers regions of the republic ultrasonic apparatuses high and expert class; to develop regional median value of biochemical markers for accurate calculation of individual risk of congenital disorders in the child.

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Neonatal Screening for Cystic Fibrosis in Republic Sakha (Yakutia)

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ABSTRACT

Neonatal screening for cystic fibrosis involves early presymptomatic diagnosis of the disease in newborns. The aim of the study was to determine the effectiveness of neonatal screening for cystic fibrosis in the Republic Sakha (Yakutia). The results of neonatal screening, sweat samples and molecular genetic testing of DNA samples from 126 newborns hypertripsinogenemia (level of immunoreactive trypsin in the first test – IRT>70 ng/ml) and 120 patients to the most common gene mutation *CFTR*. Mutations were found in 17 individuals (11 - heterozygotes, 6 - homozygotes).

Keywords: cystic fibrosis, neonatal screening, sweat test, gene, *CFTR*, DNA-diagnostics

INTRODUCTION

Neonatal screening for cystic fibrosis (CF; OMIM no.219700), is one of the most effective methods, which provides early detection of the disease and prompt initiation of treatment to prevent or slow down development of severe manifestations of the disease leading to disability and/or early death of a child. Cystic fibrosis has now become a national priority program in the Russian Federation. Sakha Republic (Yakutia) in 2006 held mass screening of newborns for CF as part of the national "Health" priority project, along with phenylketonuria, galactosemia, congenital hypothyroidism and congenital adrenal syndrome were all included in the list of hereditary diseases subject to mandatory newborn screening.

Increased levels of immunoreactive trypsin (IRT) in blood plasma of patients with CF were discovered in the 1970s; and this was a beginning of mass newborn screening for this disease. Screening protocol for CF Russia includes 4 stages: determination of IRT in newborn dried blood spot, IRT repeat (retest), sweat test and DNA-diagnostics, and only three are required [2].

Cystic fibrosis (CF) is the most common hereditary multiorgan pathology characterized by pronounced genetic heterogeneity and clinical polymorphism. This monogenic disease is caused by mutations of the *CFTR* gene (cystic fibrosis transmembrane conductance regulator), characterized by lesions of the exocrine glands vital organs and usually requiring a severe

rehabilitation course and prognosis [1]. Currently more than 1500 described mutations and 250 polymorphisms in the *CFTR* gene (*CFTR* mutation database, http://www.genet.sickkids.on.ca/cftr/). *CFTR* gene was isolated in 1989. It contains 27 exons, spans 250000 nucleotide pairs and is located in the middle of the long arm of chromosome 7. A multicenter study involving local scientists (N.I.Kapranov, E.K.Ginter, V.S.Baranov) conducted in 1999-2000, has covered 17 countries in Central and Eastern Europe, including Russia. As a result of these studies a list of 33 common mutations specific to those countries was suggested. Among them, the most frequent mutation is delF508; second in frequency - del21kb (*CFTR* dele 2.3). Frequency of the next 6 mutations (N1303K, G542X, W1282X, 3849+10 kbC> T, 2143delT, 2184insA) exceeded 1% [3]. The frequency of cystic fibrosis varies in different populations of the world very widely (for example, in Europe from 1:1800 births in Ireland to 1:26000 in Finland). Estimates of the frequency of cystic fibrosis in different populations of the Russian Federation are also quite different (from 1:4900 to 1:12000 live births) [4].

MATERIALS AND METHODS

Material for the study is based on the data of a survey on the CF 112 019 newborns in the period 2006 - 2013. Determination of immunoreactive trypsin (IRT) level in dried blood spots was performed by immunofluorescence method with a time resolution using reagents DELFIA Neonatal IRT (Perkin Elmer/Wallac, Finland). The study was conducted in newborn blood biochemical laboratory Genetic counseling Perinatal Center Republican Hospital №-1 - National Medical Center. Positive result of screening (IRT>70 ng/ml) served for re-determination of IRT in blood samples. High-level re- IRT (>40 ng/ml) served for a sweat test. Sweat test was carried out by titration Gibson - Cooke Sweat and using aNanoduct (Wescor, USA) analyzer (system to stimulate perspiration and sweat analysis). Measured "equivalent" concentration of sodium chloride in the sweat fluid. Accepted as the norm results 0-60 mmol/l, figures 60-80 mmol/l were considered borderline, 80 mmol/l or higher were considered positive for cystic fibrosis. As part of neonatal screening in the molecular genetics laboratory in 2011 initiated molecular genetic testing for cystic fibrosis *CFTR* gene using the test system CF-11 (mutations del21kb, L138ins, delI507, delF508, 394delTT, 604insA, 1677delTA, 2143delT, 2184insA, 3821delT, 3944delTG) and CF5-L (G542X, W1282X, N1303K, 3849+10kbC>T, R334W), developed by the "Center of molecular Genetics", Moscow. Isolation of DNA for molecular genetic studies performed from venous whole blood and blood spots on filter paper, using a reagent kit «DNA-Prep» when

dealing with blood stains and «DNA-Blood» on liquid blood, according to the method recommended by the manufacturer.

Since December 2013 a diagnosis of CF by reverse hybridization on the microarray with a set of "Cystic fibrosis - biochips" ("Alcor Bio", St. Petersburg) was introduced Detection of hybridized microarray performed on PerkinElmer ScanRi microarray scanner (PerkinElmer, USA). The method allows to detect 25 most frequently occurring in the territory of the Russian Federation of mutations in *CFTR* at the same time: F508del, Delex2-3, 2143delT, G542X, G551D, 2184insA, W1282X, N1303K, 3732delA, 1717-1G> A, 1677delTA, 2188AA-G, S1196X, 3821delT, R553X, 1078delT, I507del, 2789 +5G>A, R1162X, 3849+10kbC>T, G85E, 621+1G>T, R347P, R347H, R334W. The current method is being optimized.

RESULTS AND DISCUSSION

Mass screening for cystic fibrosis was launched in August 2006. During this time, were examined 112019 births (screening coverage - 99.5 %). The number of newborns with initially elevated levels of IRT - 1474 (1.3%), held on 21 - retest the 28th day of life - 1050 (71.2 %). Improving IRT at retest - 52 (5 %).

Gibson-Cooke sweat test method was held since 1989 in biochemical laboratory;the sweat test titration performed about 200 patients annually. The Nanoduct analyzer was used since 2008 and has been used in 239 cases (we tested both children with a positive retestand children with a single positive test, which for some reason has not been retested).

Four children with CF were revealed according to the results of the sweat test. One child from Yakutsk, 3 - from rural areas: Tomponsky, Mirnynsky and Aldanskyregions. Meconium ileus has occurred in one case. We identified patients with children at the first level of IRT survey averaged 184.5 ng/ml. During the retest, patients IRT level ranged in average 160.8 ng/ml. Thus in this form of screening, there was a direct correlation between degree of increase of a biochemical marker and a share of diagnosed patients.

Molecular genetic study in 2011 was conducted with 246 patients. Homozygous for the mutation delF508 found in 4 patients, heterozygous state in 11, and compound del21kb/W1282X del21kb/2184insA - 2 cases. One child died with IRT > 200 ng/mL, sweat test is not carried out. The diagnosis of cystic fibrosis was confirmed by molecular genetic studies have identified mutations in the homozygous state delF508.

The results of molecular genetic testing for the period 2011-2013

Year	Number of the investigated	Genotype	Number of identified
2011	24	delF508/-	3
		delF508/delF508	2
		del21kb/W1282X	1
2012	168	delF508/-	6
		delF508/delF508	1
		del21kb/2184insA	1
2013	54	delF508/-	2
		delF508/delF508	1

As a result of neonatal screening, patients affected by CF in most of the cases are diagnosed before the onset of clinical manifestations (except for individuals who have CF manifests meconium ileus). A link between *CFTR*-genotype and phenotype in CF is not direct. Clinical manifestations of CF are a consequence of interaction of three factors: *CFTR*-mutations modifying factors in the *CFTR* gene and/or other genes and environmental influences [5]. Classification of *CFTR*-mutations based on the nature of the molecular defect and its impact on the function of chloride channels. Class I, II, and III mutations, such as W1282X, delF508, G542X, significantly reduce or completely destroy activity of *CFTR*-channel and associated with classic CF phenotype: lung disease, elevated levels of chloride in sweat, pancreatic insufficiency and impaired fertility in men. When mutations IV, V and VI classes, such as R334W and R117H, partly chloride channel activity is still present, although reduced chloride conductance. These mutations are associated with preservation of pancreatic function and late-onset disease. However, it should be borne in mind that nature and severity of lesions varies widely even in patients with the same genotype [6].

CONCLUSION

Neonatal screening is the only method of early diagnosis of cystic fibrosis that allows early treatment and improves the quality and life expectancy of patients with cystic fibrosis. Neonatal screening is onlyeffective if the coverage is not less than 98 % of newborns;the test forms are timely delivered to the laboratory and supply of reagents and consumables is uninterrupted. Use of confirmatory techniques such as sweat test for Gibson-Cooke, sweat

analyzer type Nanoduct and molecular genetic methods are requiredfor a complete verification of the diagnosis.

Given the complexity and sensitivity of the test sample, the laboratory performing the test must meet certain requirements: it must conduct more than 150 tests per year. The method has high requirements for qualification of performing personnel and quality of applied reagents. At the same time, the titration method does not require special equipment and consumables. This classic method is the "gold standard" for diagnosis of cystic fibrosis. For the first months of life, which are difficult or impossible to obtain sufficient sample of sweat on the filter paper for a reliable analysis, the Nanoduct analyzer is a choice method. The analyzer also provides an opportunity for a sweat test outside the laboratory.

Today, the most relevant molecular genetic methods for the diagnosis of cystic fibrosis are multiplex PCR and hybridization on microarray that simultaneously detect a number of mutations in *CFTR*. Molecular genetic methods allow us to confirm the diagnosis of cystic fibrosis in borderline sweat test results above. Value of molecular genetic diagnosis is also in getting more information for research. The disadvantage of molecular genetic techniques is the high cost and inability to identify unintended mutations used method. Whereas conducting a sweat sample test can reveal near-negative, as well as a small percentage of false-negative case results, this requires conducting DNA diagnosis of cystic fibrosis.

Introduction of molecular genetic analysis of the *CFTR* gene identified in newborn screening patients has revealed the type of mutation delF508, del21kb, W1282X and 2184insA in the population of the Sakha Republic (Yakutia).

Diagnosis of the disease through neonatal screening allows early treatment and rehabilitation activities that should lead ultimately to improve the quality and duration of life of these children. Genetic analysis to the manifestation of clinical manifestations needed for preventive measures and for the prognosis of the disease. Research findings are important for planning and implementation of programs for early diagnosis, treatment and rehabilitation of children with cystic fibrosis.

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The Clinical Case of Crohn's Disease in Child of 3 Years and 7 Months

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ABSTRACT

The paper presents a clinical case of Crohn's disease, diagnosed in 2014 in a child of 3 years and 7 months. This pathology is rare among children (in the Republic Sakha (Yakutia) - 2 cases). Due to the variability of the clinical picture of the disease it is difficult to diagnose.

Keywords: system of histocompatibility, clinical remission, gastroenterology, inflammation, gastrointestinal tract.

INTRODUCTION

Crohn's disease is a chronic nonspecific progressive transmural granulomatous inflammation of the digestive tract. Most often affected the terminal small intestine, so there are synonyms of this disease, as «terminal ileit», «granulomatous ileit» etc. In the pathological process may involve any of the digestive tract from the root of the tongue to the anus. Frequency of lesions of the intestine and decreases in the following order: terminal colitis, ileocolic, anorectal form and other funds are also focal, multifocal and diffuse form[1,2,7,12,15,24]. For Crohn's undulating, with exacerbations and remissions. Crohn's disease is detected in children of all age groups. The peak incidence occurring at 13-20 years. Among the cases the ratio of boys to girls 1:1.1 [3-5, 19,23,25]. The etiology and pathogenesis of the disease is unknown. Discuss the role of infection (mycobacteria, viruses, toxins, food, certain medications, considered as the starting moment for the development of acute inflammation. Much emphasis immunological, dysbiotic, genetic factors [7,10,11,16,22]. The relation between the system HLA and Crohn's disease, which often reveal the loci of DR1 and DRw5. Clinical picture of the disease differs the big variety. Beginning of the disease is usually gradual, over many years with occasional outbreaks. The main clinical symptom in children's persistent diarrhea (up to 10 times a day). The volume and frequency of stools depend on the level of destruction of the small intestine - the higher it is, the more often a chair and, accordingly, the heavier the disease. The defeat of the small intestine accompanied with malabsorption syndrome. The chair periodically appears in the blood.

Complications of Crohn's disease are most often associated with the formation of fistulas and abscesses of different localization, ulcer perforation, peritonitis. Possible intestinal obstruction, acute toxic dilation of the colon. In the General analysis of blood reveal anemia (loss of red

blood cells, hemoglobin, hematocrit), reticulocytosis, leucocytosis. When the biochemical analysis of blood find hypoproteinemia, hypoalbuminemia, hypokalemia, reduction of the content of microelements, increased alkaline phosphatase, A2-globulin and C-reactive protein[1,2,7,12,15]. The severity of biochemical changes correlates with disease severity. Endoscopic picture of Crohn's disease is characterized by a great polymorphism and depends on the stage and extent of the inflammatory process. Endoscopically are three phases of the disease: infiltration, ulcers-cracks, scarring.

In phase infiltration (the process is localized in the submucosa of the) mucosa has a type of «quilt» Matt, vascular picture is not visible. Later, erosion type aft with separate superficial ulceration and fibrinous overlays. In phase ulcers-crack identify the individual or multiple deep longitudinal ulcerous defects affecting and muscular layer of the intestinal wall. The intersection of cracks gives the mucous membrane type «cobblestones» [8, 12, 17, 18, 20, 23]. Due to the significant swelling under the mucous membrane, and also defeats the deep layers of the intestinal wall of the bowel lumen narrows. In phase scarring detecting sites irreversible stenoses of the intestines. Characteristic radiological signs (research is usually done with double contrast study) - segmentation lesions, wavy and uneven contours of the intestine. In the colon determine the bumps and sores on the top edge of the segment with preservation of Australia on the bottom. In stage ulcers-crack - type «cobblestones». Diagnosis is based on clinical and anamnestic data and the results of laboratory, tool, morphological studies [13, 21, 26]. Differential diagnosis of Crohn's disease is carried out with a sharp and prolonged intestinal infections of bacterial and viral etiology, diseases caused by protozoa, worms, malabsorption syndrome, tumors, ulcerative colitis and other The most effective medicines believe drugs 5-aminosalicylic acid (mesalazine), sulfasalazin [24, 25]. At the same time should be taking folic acid multivitamin with microelements in the dose of age. In the acute phase of illness and severe complications (anemia, cachexia, damage of joints, erythema and others) prescribed glucocorticoids (hydrocortisone, prednisolone, dexamethasone), less immunosuppressants (azathioprine, cyclosporine).

The prognosis for recovery is unfavorable, for life - depends on the severity of the illness, the character of its course, the presence of complications. It is possible to achieve long-term clinical remission.

Thus, Crohn's disease is a severe disease with variable flow. Currently in the Republic of Sakha (Yakutia) has two children with the diagnosis of Crohn's disease. This clinical diagnosis was a girl aged 3 years and 7 months in 2014.

Objective: To present a clinical case of Crohn's disease in girls 3 years and 7 months

MATERIALS AND METHODS

The analysis of two case histories Children clinical hospital № 2 and National centre of medicine№ 1 Pediatric centre was done.

RESULTS

The Girl - child from the first pregnancy occurring without features. Childbirth for 38 weeks, operative. Breastfeeding to 2.5 months. Psychomotor and physical development is age appropriate. The child is sick from 10.11.2013. Feces is blood-streaked. The divisional pediatrician prescribed «smectite», the diagnosis is not installed. 13.11.2013 deterioration, bowel movement is regular with blood and child was hospitalized to the regional hospital with clinical diagnosis: enterocolitis unclear etiology. The girl received treatment: Ceftriaxone, Mezim Forte, Linex, Arbidol, ampicillin, loperamide. The child's condition deteriorated. Parents arrived to Yakutsk to hospitalize at children's clinical hospital №2. Child was diagnosed with acute gastroenterocolitis, moderate severity. Toxicosis, exicosis 1 degree. The child had a feces bacteriological analysis, sowing negative. In the General analysis of the blood was a high rate of sedimentation rate up to 42 mm/h, decrease in hemoglobin to 107 g/l, platelets to 414. In feces coprogram: erythrocytes 37-43-45 in sight. The girl received treatment: infusion therapy for two days, amikacin, smecta, Gidromet, Bifidumbacterin, furazolidone. After treatment, the child was discharged home. After a week there has been a sharp deterioration: a temperature of 39 degrees, diarrhea with blood stains 4-5 times a day. The child is sent to Yakutsk to the National centre of medicine № 1 Pediatric centre, where the reception was held with the surgeon and sigmoidoscopy. In the study Protocol is the following: in the bowel lumen scarlet blood, mucous intestines swollen and hyperemic, with hemorrhagic lesions 0.2 sm. After consulting the child was sent to Children's clinical hospital №2. Girl was diagnosed with Adenovirus enterocolitis moderate severity. Mixed infection. Acute intestinal infection of unknown etiology with gymocolit moderate severity and treatment: sylfperazon, trichopol, suprax, bromhexine. In the blood is the ESR to 60 mm/h, hemoglobin g/l, thrombocytosis to 438. The child examined by a gastroenterologist. Conclusion gastroenterologist: nonspecific ulcerative colitis. On 4.02.14 the child's state is seen as a serious, due to gymocolit. February 5, 2014 she was transferred to the gastroenterology Department of the National centre of medicine № 1 Pediatric centre, where she stayed for 5 days and got the following treatment: baktisubtil, salofalk, microclysters with hydrocortisone, Kreon, smectite and infusion therapy. There is an improvement of the patient and 10.02.2014 child is transferred by agreement in the National centre of health children of

Russia in gastroenterology Department for further diagnosis. In the National centre of health children of Russia diagnosis of Crohn's disease was confirmed.

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Methylation Status of the Cell Cycle Control-Associated Genes in the Carotid Artery Tissue of Atherosclerotic patients

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ABSTRACT

Objective: We tested the hypothesis that the aberrant methylation of the promoters and first exons of *CDKN2A* (*p16INK4a*, *p14ARF*), *CDKN2B* (*p15INK4b*) and *RB1* genes was associated with carotid atherosclerosis.

Methods and results: The DNA methylation status of these cell cycle control-associated genes was analysed in 120 samples of carotid arteries using two different techniques: methylation-sensitive polymerase chain reaction (MS-PCR) and methylation-specific PCR (MSP-PCR). DNA methylation was not detected in advanced atherosclerotic plaques or nearby macroscopically intact tissues of the vascular wall from the same patients.

Conclusion: The methylation status of *CDKN2A*, *CDKN2B* and *RB1* genes does not appear to be a marker of human carotid atherosclerosis.

Keywords: DNA methylation, atherosclerosis, *CDKN2A*, *CDKN2B, RB1*.

INTRODUCTION

Atherosclerosis and associated ischaemic events remain a major cause of morbidity and mortality worldwide, including Russia. In the last years, insights into the molecular mechanisms of disease pathogenesis have progressed considerably.

The 9p21 locus is currently considered the most robust genetic marker of atherosclerotic vascular disease [10]. Disease-associated SNPs are located in proximity to *CDKN2A* (coding for the cyclin-dependent kinase inhibitor p16INK4a and its alternative reading frame transcript variant p14ARF in humans and p19ARF in mice) and *CDKN2B* (coding for the CDK inhibitor p15INK4b). These genes are involved in regulating the cell cycle and apoptosis. Deletions and aberrant DNA methylation of the *INK4b-ARF-INK4a* locus are frequent events in tumours. Recent studies have demonstrated that the loss of cell cycle control-associated proteins, such as p19ARF and Rb in gene-targeted animal models, are related to atherosclerotic lesion progression coinciding with changes in cellular composition [4, 7].

It is becoming clear that the inappropriate epigenetic regulation of the 9p21 locus via a large antisense ncRNA named ANRIL also contributes to atherosclerosis [10]. It is possible that this mechanism occurs not in isolation but in close relation to other epigenetic modifications, such as DNA methylation. Aberrant methylation in promoters leads to the transcriptional inactivation of genes involved in the cell cycle regulatory p15INK4b-p16INK4a-cyclin D/CDK4-RB1-mediated pathway (RB1 pathway) in human malignancies. To our knowledge, the methylation status of these genes has never been investigated in atherosclerosis.

In this study, the methylation status of the promoters and first exons of *CDKN2A* (*p16INK4a*, *p14ARF*), *CDKN2B* (*p15INK4b*) and *RB1* genes was evaluated in carotid artery samples of patients with atherosclerosis.

MATERIALS AND METHODS

The study group included 60 asymptomatic/symptomatic patients (men, age 62.3±6.7 years, mean±S.E.) diagnosed with >70% carotid artery stenosis (NASCET criteria) referred to the Research Institute for Complex Problems of Cardiovascular Diseases for the surgical treatment of severe carotid artery stenosis. Twenty-one patients (35%) presented with an ischaemic cerebral event history before surgery. All cases were diagnosed as having coronary heart disease and hypertension. A total of 26 patients (43.3%) had peripheral artery disease. Fifty-three men (88.3%) had hyperlipidaemia. Diabetes mellitus was found in 50% of patients. Cardiovascular risk factors and disease history were recorded at the time of surgery. This study was approved by the local ethics committee, and written informed consent was obtained from all patients.

Vascular samples were collected from advanced atherosclerotic plaques and nearby macroscopically intact tissues from the same patients. Immediately after endarterectomy, the samples were examined by a pathologist, carefully cleaned of calcifications, fatty deposits, and thrombotic material, and washed with a sterile physiological saline solution. All samples, which consisted of intima and the inner media, were fixed in liquid nitrogen and stored at -80°C until used for molecular analysis.

Genomic DNA was purified using standard proteinase K digestion and phenol/chloroform extraction methods.

The methylation status of the promoters of *CDKN2A* (*p16INK4a*, *p14ARF*) and *RB1* genes was determined with methylation-sensitive polymerase chain reaction (MS-PCR), as previously described [1]. Genomic DNA samples were digested with *Hpa*II (Fermentas, Lithuania) before PCR. A 351-bp fragment of the *CDKN2A* (*p16INK4a*) promoter containing 4

*Hpa*II sites, a 283-bp fragment of the *CDKN2A* (*p14ARF*) promoter containing 6 *Hpa*II sites, and a 239-bp fragment of the *RB1* promoter containing 4 *Hpa*II sites were amplified using PCR. Fragments of *EXT2* exon 8 (253 bp) and microsatellite D9S145 (144 bp) were used as internal PCR controls.

In addition to MS-PCR, another approach, methylation-specific PCR (MSP-PCR), was used to analyse the methylation status of exon 1 of *CDKN2A* (*p16INK4a*, *p14ARF*) and *CDKN2B* (*p15INK4b*). The sodium bisulfite conversion of DNA was performed using an EZ DNA Methylation Kit (Zymo Research, United States). Bisulfite-modified DNA was amplified with PCR using two primer sets specific for methylated sequences and two primer sets specific for unmethylated sequences, as described by Herman et al. (1996) [8] and Amatya et al. (2004) [2]. In total, twenty-three CpG dinucleotides from exon 1 of *CDKN2A* (*p16INK4a*, *p14ARF*) and *CDKN2B* (*p15INK4b*) were investigated using MSP-PCR.

RESULTS AND DISCUSSION

The methylation of *CDKN2A*, *CDKN2B* and *RB1* was analysed in 120 carotid artery samples of atherosclerotic patients using two different techniques: MS-PCR and MSP-PCR. No PCR band was observed in the *Hpa*II-digested DNA samples from atherosclerotic plaques (APs) or the macroscopically intact tissue (IT) of carotid arteries (fig. 1). In the experiments using MSP-PCR, all arteries exhibited only unmethylated alleles (fig. 2).

Atherosclerosis is a common disease in which cell proliferation plays an important role. This biological process underlies lesion evolution at all stages, from establishment to plaque complications [6]. The unique roles of *CDKN2A* (*p16INK4a*, *p14ARF*), *CDKN2B* (*p15INK4b*) and *RB1* in cell proliferation suggest a possible role of these genes in atherogenesis.

Numerous studies have underscored the importance of DNA methylation changes in atherosclerosis [3, 13]. However, only a few studies to date have reported DNA methylation changes in vascular tissues from patients with atherosclerosis using a candidate gene approach [9,11,12,14,15] and microarray-based genome-wide analysis [5].

The principal finding of the current study was that the promoters and/or first exons of *CDKN2A* (*p16INK4a*, *p14ARF*), *CDKN2B* (*p15INK4b*) and *RB1* genes were unmethylated in the atherosclerotic plaques and nearby macroscopically intact tissues of the carotid arteries from the same patients.

DNA methylation within gene promoters is suggested to have the highest functional relevance to gene expression control. Our findings are in agreement with those of other authors

who showed that *CDKN2A* (*p16INK4a*, *p14ARF*) and *CDKN2B* (*p15INK4b*) were expressed in the smooth muscle cells of coronary atherosclerotic plaques and normal human arteries [10].

There are several limitations of the current study. The DNA methylation profile may be affected by differences in the cell type composition in various arterial beds, which was not characterised in the present study. Laser microdissection with an appropriate quantitation method (e.g., pyrosequencing) would provide cell-specific information on DNA methylation.

CONCLUSION

Our study of patients with advanced carotid atherosclerosis provides evidence that the methylation status of *CDKN2A*, *CDKN2B* and *RB1* does not appear to be an important determinant of human carotid atherosclerosis.

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VKORC1 and *CYP2C9* Genes Polymorphisms, Affecting the Sensitivity of Anticoagulant Therapy in Patients with Acute Cerebral Circulation Impairment

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ABSTRACT

Preliminary data of the frequency of genetic variants *VKORC1* and *CYP2C9*, affecting sensitivity to anticoagulant therapy, in Asian indigenous and Caucasian patients with acute ischemic stroke in Yakutia is presented in this article. The frequencies of increased warfarin sensitivity alleles *CYP2C9*2* (rs1799853), *CYP2C9*3* (rs1057910) and C1173T *VKORC1* (rs9934438) polymorphism's variants were determined in the group of 15 patients. *CYP2C9* genotyping revealed 3 heterozygous for *CYP2C9**1/*2. The allelic variant *CYP2C9*3* have not been identified in this group. *VKORC1* genotyping revealed 4 heterozygous for 1173CT *VKORC1*. There were 22.2% carrier with allele *CYP2C9*2* and 22.2% with T allele of C1173T *VKORC1* polymorphism among Asian indigenous patients. Genotyping of *VKORC1* and *CYP2C9*, including *CYP2C9*2* allele, is recommended for personified prevention of the cardioembolic stroke in Yakutia.

Keywords: *VKORC1* gene polymorphism, *CYP2C9* gene polymorphism, cardioembolic stroke, warfarin.

INTRODUCTION

The oral anticoagulants have used for the primary and secondary stroke prevention in the certain clinical situations. Warfarin, the most commonly prescribed anticoagulant, significantly reduces the risk of cardioembolic ischemic stroke and transient ischemic attacks if it received the adequate dose [2, 7]. In patients with atrial fibrillation the anticoagulant's efficiency for stroke

prevention is much higher than the one of the aspirin therapy (risk reduction 64 % and 22 %, respectively) [8]. The warfarin effect depends from many factors such the individual characteristics (e.g., body size, gender, race), diet, medication, liver and kidneys diseases, as well as other factors.

It has been established that the sensitivity to warfarin dependents on genetic factors, e.g. , genotype isoforms of CYP2C9, which codes for the cytochrome P450, and VCORC1, which codes one subunit of vitamin K epoxide reductase complex [9]. *VKORC1* gene mutations associated with the sensitivity to warfarin [10]. C1173T *VKORC1* polymorphism associated with increased sensitivity to warfarin. Carriers of the mutant 1173T allele require lower doses of warfarin compared to carriers of allele 1173C *VKORC1*, while the average daily dose is reduced by 43 % for homozygous 1173TT *VKORC1* and by 22 % for heterozygous 1173CT *VKORC1* [1]. It was found differences in genetic polymorphisms frequency that cause different sensitivity to warfarin. For example, African Americans are relative stability and Asians are relative sensitivity to warfarin [4]. The low dose haplotype of *VKORC1* leads to the rapid achievement of target INR, on the other hand, also leads to INR be more than 4 quickly and is associated with the hemorrhagic complications risk [6]. Currently *CYP2C9* genotypes are recognized the most clinically significant factors for the diagnosis of sensitivity to warfarin. Alleles *CYP2C9*2* and *CYP2C9*3* are associated with a slow metabolism of the warfarin [13]. The frequency of the "slow" *CYP2C9* alleles varies widely in different ethnic groups [3, 11]. *CYP2C9* gene polymorphisms also affect to the time INR achieve > 4 [6].

Racial differences in the frequency of "defect" alleles which affect warfarin metabolism require consideration in therapy. Thus, Asian patients must have lower average dose of warfarin, while patients of the black race - higher average dose for therapeutic INR levels [12].

Aims: To study the frequency of *VKORC1* and *CYP2C9* allele polymorphisms, affecting the metabolism of warfarin, in acute ischemic stroke Asian indigenous and Caucasian patients in Yakutia.

MATERIAL AND METHODS

The study group includes 15 patients with acute ischemic attacks and stroke, which were hospitalized in the Regional Vascular Center (Yakutsk) in 2013 and received warfarin therapy for secondary stroke prevention. The diagnosis was confirmed by the data of neurological examination, medical history, neuroimaging. Computed tomography (CT) of the brain was performed on 64-slice CT scanner multispiral Siemens SOMATOM Definition AS, the data were

interpreted by radiologist. Ultrasound of the heart and brachiocephalic vessels carried on the unit AcusonS 2000 (SiemensAG). The blood coagulation (international normalized ratio (INR), activated partial thromboplastin time (aPTT), PTI (prothrombin index)), complete blood count, electrocardiogram were performed. All patients provided informed consent. Genetic study was conducted in "Genomic Medicine" laboratory of Medical Clinic of NEFU named after M.K. Ammosov. DNA extraction was performed using a set ExtraGene (Germany) and phenol-chloroform method. Genetic typing of the *CYP2C9*2* (rs1799853), *CYP2C9*3* (rs1057910) and *VKORC1* C1173T (rs9934438) held with PCR kits "Литех" (Moscow).

RESULTS AND DISCUSSION

There were 7 male patients (46.7 %) in the study group (n = 15). The average age was 65 years (min – 41, max – 81). There were 9 patients (60 %) of the indigenous Asian ethnicity, 5 patients (33.3 %) of Caucasian race, and others – 1 (6.7 %). 13 ischemic stroke cases (86.7 %) and 2 transient ischemic attack cases (13.3 %) were diagnosed. The following comorbid diseases were diagnosed: atrial fibrillation – 11 cases (73.3 %), heart defects – 4 (26.7%), including operated heart valves – 2 (13.3%), chronic rheumatic disease – 5 (33.3%), ischemic heart disease (IHD) – 9 (60%), acute myocardial infarction – 1 (6.7%), hypertension – 13 (86.7 %), diabetes mellitus – 3 (20 %), dilatation of the heart – 9 (60 %), osteoarthritis – 1 (6,7 %). Patients had no contraindications to anticoagulation therapy, including hematologic diseases, peptic ulcer disease, liver or kidney failure, hemorrhagic stroke in history, severe degree of hypertension at the time of the survey.

The genotyping of *CYP2C9* identified homozygous of allele *CYP2C9*1* in most cases (n = 12, 80%). Heterozygous carriers of *CYP2C9*2* detected in 3 cases (20%), all of them had heterozygotes with *CYP2C9*1/*2*. The allelic variant *CYP2C9*3* have not been identified in this group.

Genotyping C1173T *VKORC1* found 11 cases of homozygous with allele C (73.3 %). Heterozygotes of this polymorphism C/T were found in 4 cases (26.7 %). Homozygous carriers of the T allele *VKORC1* were not found in this group. The genotypes frequencies of *CYP2C9* and *VKORC1* in the target group are presented in Table 1.

Table 1

CYP2C9 and *VKORC1* genotypes frequencies depending on ethnicity and gender

Ethnicity	Gender	Genotype			
		*CYP2C9 *1/*1*	*CYP2C9 *1/*2*	*VKORC1 1173 C/C*	*VKORC1 1173 C/T*
indigenous of Asian race (n=9) (abs.)	male	3	1	3	1
	female	4	1	4	1
Caucasian race (n=5) (abs.)	male	3	0	3	0
	female	1	1	0	2
others (n=1) (abs.)	female	1	0	1	0
Total (abs, %)	15 (100)	12 (80)	3 (20)	11 (73,3)	4 (26,7)

Thus, the proportion of carriers of increased warfarin sensitivity alleles was 40 % (n = 6), including 4 heterozygous for T allele C1173T polymorphism *VKORC1* (26.7 %) and 3 carrier of *CYP2C9*2* genotype (20 %), while 1 patient was both "defect" alleles carrier. Among the carriers of "defect" alleles there were 4 Asian indigenous patients (genotype CYP2C9 *1/*2 (n = 2 , 22.2 % of this ethnic group) and genotype C/T of polymorphism C1173T *VKORC1* gene (n = 2 , 22.2 %); and there were 2 Caucasian patients, one of which was with two "defect" allele (genotype *CYP2C9* *1/*2 (n = 2 , 40% of this ethnicity) and genotype C/T of C1173T polymorphism *VKORC1* gene (n = 1, 20%)).

It was found that there are ethnic differences in the frequencies of *CYP2C9* gene variants. Thus, according to [5], in the Korean population allelic variant *CYP2C9*3* is less common compared to the European one. Allelic variant *CYP2C9*2* is absent in East Asian populations, including Koreans, or it is present in a small proportion of cases and is more rare than allelic variant *CYP2C9*3*. Accordingly, it was concluded that routine *CYP2C9*2* genotyping no need in this population [5]. Our data, on the contrary, confirm the need for allele *CYP2C9*2* typing for the study of individual sensitivity to warfarin among Asian indigenous in Yakutia.

We present two clinical reports of acute ischemic stroke cases with the mapping *CYP2C9* and *VKORC1* genotyping data on the dynamics of blood coagulation due the warfarin therapy. The demographic and laboratory data, including concomitant diseases, addictions, medications, initial warfarin dose are presented.

Clinical report 1. Patient R., 68 years old, Russian, male gender, diagnosis: Ischemic stroke in the left posterior cerebral artery (cardioembolic variant). Patient also had ischemic stroke in 2012; IHD, miocardial infarction in hystory, exertional angina FC II; permanent atrial fibrillation, tachysystolic variant; heart defect, mitral valve and tricuspidal valve failure 1 degree; secondary dilatation of both atria of the heart; chronic heart failure II A; hypertension stage III; hypertrophy of the left ventricle; multifocal atherosclerosis; osteoarthritis of ankle joints.

Height 175 cm, weight 95 kg. No smoker. Medications: amiodarone, antifungals, sulfonamides at the time of the study – no; statins – yes (atoris 20 mg drug); nonsteroidal anti-inflammatory drugs (NSAIDs) – yes (75 mg per day). Initial INR – 1.14; Platelets – 242 10^9/ L; PTI – 82.1 %; aPTT – 29.7; initial warfarin dose – 2.5 mg. Genotype: combination *CYP2C9*1/*1* allele and C/C of C1173T polymorphism *VKORC1* gene.

INR value has not reached the "therapeutic range" on the initial dose of warfarin 2.5 mg per day (INR less than 2.0). The anticoagulant low-dose was appointed in connection with the need for NSAIDs due to the joints disease. On the 8th day INR value remained low and amounted to 1.21, therefore the dose correction was performed.

Clinical report 2. Patient M., 72 years old, indigenous ethnicity, female gender, diagnosis: Transient ischemic attack in vertebral-basilar system. Patient also had IHD, exertional angina FC II; permanent atrial fibrillation, normosistolic variant; нypertension stage III; hypertensive heart disease III; secondary dilatation of the heart; hypertrophy of the left ventricle; multifocal atherosclerosis.

Height 164 cm, weight 67 kg. No smoker. Medications: amiodarone, antifungals, sulfonamides at the time of the study - no; statins – yes (atoris 20 mg). Initial INR – 1.13; Platelets – 159X10^9/ L; PTI – 81.1 %; aPTT – 25; initial warfarin dose – 2.5 mg. Genotype: combination *CYP2C9*1/*2* and C/C of C1173T polymorphism *VKORC1* gene.

The initial warfarin dose was 2.5 mg per day. The INR value achieved the "therapeutic range" to 7th day (INR 2.26). Subsequently, the warfarin dose 2.5 mg daily was prescribed after discharge from the hospital under the supervision of laboratory INR. During 6 months the patient's INR value was in the range from 2.21 to 2.76 with 2.5 mg warfarin daily.

These case reports demonstrate the various blood coagulation dynamics in response to an identical dose during the first few days of warfarin therapy. Patient M. (case report 2) had genotype *CYP2C9*1/*2* with functionally "defect" allele and thus had «slow» warfarin metabolism, and so there was a more rapid achievement of INR «therapeutic range». Warfarin «slow» metabolism causes the hypocoagulation state for a long time. Carriage of this genotype

suggests a tendency to haemorrhagic complications [6], so recommendation of warfarin low dose need to patients. In case report 2 the therapeutic effect (INR value of 2.0 to 3.0) could be achieved at a low dose warfarin (2.5 mg) during a long time (6 months). Subsequently this patient should be monitored INR carefully to avoid haemorrhagic complications.

Patient R. (case 1) had not "defect" alleles of studied polymorphisms (genotype *CYP2C9 *1/*1*; 1173CC *VKORC1*). During the first days of warfarin therapy the INR value remained low at 2.5 mg and did not reach the "therapeutic threshold". The carriage of this genotype is associated with a "fast" warfarin metabolism and thus to a more rapid elimination of the drug, respectively, the patient needs to the higher dose.

Recently the genetic testing is recommended for optimization of the selection of the warfarin start dose. For example, according to [14], algorithm including genetic typing of genes *CYP2C9* and *VKORC1*, improves the prognosis of the optimal dose in comparison with algorithms based on clinical and demographic factors solely.

CONCLUSION

Preliminary data of the frequency of genetic variants *VKORC1* and *CYP2C9*, affecting sensitivity to anticoagulant therapy, in acute ischemic stroke indigenous Asian and Caucasian patients in Yakutia is presented in this article. The proportion of carriers of *VKORC1* and *CYP2C9* genotypes, determining high sensitivity to warfarin, accounted 40 % in the observed group. There were 22.2% carrier with allele *CYP2C9*2* and 22.2% with T allele of C1173T *VKORC1* polymorphism among Asian indigenous patients.

Blood coagulation control taking into data of *CYP2C9* and *VKORC1* polymorphisms genotyping contributes to the safety and effectiveness of warfarin therapy in secondary prevention of cardioembolic stroke. It is necessary to note the presence of confounding factors affecting metabolism, including the medication, comorbid diseases, gender, ethnicity. Genotyping of *VKORC1* and *CYP2C9*, including *CYP2C9*2* allele, is recommended for personified prevention of cardioembolic stroke in Yakutia.

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*LPL*rs320 Lipoprotein Lipase Gene Polymorphism: Comparative Characteristics in Different Populations

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ABSTRACT

Gene *LPL*, encoding enzyme lipoprotein lipase, is the one of the most important genes associated with lipid disorders and the risk of metabolic syndrome. Earlier *LPL* rs320 gene polymorphism has been reported to be significantly associated with type 2 diabetes in the Yakut population. The **aim** of this study was to compare *LPL* rs320 SNP (G/T) allele frequency in different world populations, including the Yakuts and analyze blood glucose level, systolic blood pressure and body mass index in patients with type 2 diabetes and healthy individuals of Yakut ethnic group, depending on the genotype rs320 LPL. So the paper presents a comparison of allele frequencies SNP (G / T) gene *LPL* rs320, which contributes to the development of a predisposition to type 2 diabetes mellitus in the Yakut population, with distribution of allelic variants of this SNP in other world populations. Also the authors studied the blood glucose level, systolic blood pressure and body mass index, depending on the variant genotype rs320 *LPL* in patients with type 2 diabetes and healthy individuals of the Yakut ethnic group. The obtained during the study data reveal that in the Yakut population allele G variant rs320 *LPL* is most prevalent, with which a reduced risk of dyslipidemia and metabolic syndrome under the conditions of traditional nutrition type in the North with a high content of proteins and lipids is associated.

Keywords: gene *LPL* rs320, lipoprotein lipase, metabolic syndrome, type 2 diabetes mellitus, genetic predisposition.

INTRODUCTION

Lifestyle of modern man has undergone dramatic changes over the last decade. It causes high prevalence of diseases associated with metabolic disorders such as metabolic syndrome and type 2 diabetes mellitus (T2DM). In understanding the molecular mechanisms that lead to disruption of metabolic processes that evolved millennia of human adaptation to the environment, genetic studies play an important role. Yakutia is distinguished by its extreme climatic conditions, peculiar traditional lifestyle and diet of the indigenous population. Study of gene polymorphism in comparison with that of other populations living in different conditions will contribute to a better understanding of changes in the metabolism of modern human.

One of the most important genes associated with lipid disorders and the risk of metabolic syndrome, is the gene *LPL*, encoding a key fat metabolism enzyme - lipoprotein lipase. Previously, we found that genetic polymorphism *LPL* rs320 is a major contributor to susceptibility to type 2 diabetes in the Yakut population [1]. *LPL* gene is located on 8 chromosome - 8p22. Nowadays, more than 100 mutations were identified, many of which were associated with a reduction in the enzymatic activity of *LPL* [11]. Gene polymorphism is associated with dyslipidemia: high triglycerides, low HDL, and high levels of blood pressure (BP) [5, 7, 8, 10]. The connection between gene and insulin resistance and type 2 diabetes is marked [13].

The paper presents the comparative frequency of allelic variants of the gene *LPL* rs320 in different populations, including the Yakut one. The data on the effect of genotypes on blood glucose level, systolic blood pressure and body mass index in the Yakut population are reported.

MATERIALS AND METHODS

The study included 344 people - the indigenous inhabitants of the Central Yakutia, 194 were patients with T2DM and 150 individuals who were not in relationship with patients and did not suffer from T2DM. Body mass index (BMI) of patients was calculated using the formula BMI = body weight (kg) / height (m^2). Blood pressure was determined by the double measuring with mercury sphygmomanometer on the right arm in a sitting position. Measurement of capillary blood glucose was performed using glucometer ACCU-CHEK, ADVANTAGE, and SOFTCLIX (trademarks of a Member of the Roche Group).

DNA of patients was isolated from white blood cells by the method of Wizard ® Genomic (Promega, Madisan, WI). Genotyping was performed at the Institute of Neurological Disorders and Strokes of Health Institutes, USA (Bethesda) by the procedure described in detail in a previous publication [1].

All study participants gave written voluntary informed consent to participate in the study. The study protocol is approved by the local Committee on Biomedical Ethics YSC CMP SB RAMS (protocol number 5, June 21, 2006).

In our study, quantitative measures of blood glucose, blood pressure and BMI did not have a normal distribution, and were described as median and interquartile range (Me (25-75 %)). Comparison of allele frequency of *LPL* rs320 in different populations was carried out by comparing the 95% confidence interval (95% CI). *LPL* gene variants association with glucose and blood pressure levels was detected using the nonparametric Kruskal -Wallis criteria.

RESULTS AND DISCUSSION

In European populations, the minor allele of *LPL* SNP (G / T) rs320 is G allele; its frequency is estimated from 24 to 32 % [3, 9, 12]. With this gene allele high enzyme activity is associated. The prevalence data of genotypes and alleles of this gene in Asian, Indian and Arab populations do not differ from European ones (Table 1) [2, 4, 6, 12, 13]. The highest frequency of G allele among healthy individuals is revealed in Saudi Arabia. [4]

Table 1

Minor allele frequency *LPL* rs320 in different world populations

Population	Minor allele frequency (G), %	Number of patients	Reference	95% CI minor allele frequency, %
China	19.7	654	[13]	17.6-21.9
Canada	24	334	[9]	21-27
India	24.6	1015	[2]	
China	25	56	[6]	
Spain	32	1029	[3]	30-34
Saudi Arabia	37.6	65	[4]	29.5-46.6
European race (19 studies)	29.1	3540	[12]	
Asian race (4 studies)	28.9	479	[12]	

For comparison of the frequency of the minor allele polymorphism *LPL* rs320 we calculated 95% CI of the minor allele share in some populations. The frequency of the minor allele G in the Chinese population in one of studies is 19.7%, in the Spanish Mediterranean population 32, Saudi Arabia 37. 6 %. In our studies of the Yakut population frequency of this allele in patients with type 2 diabetes is 21 %, while in the control group 54% (Table 2). Comparison of the 95 % CI in healthy individuals shows that the Yakut population differs in allelic gene frequency *LPL* (rs 320) from other world populations.

Table 2

Allele and genotype frequency *LPL* gene rs320 in type 2 diabetes mellitus patients and healthy individuals in Yakut population

	Allele				Genotype					
	G		T		GG		GT		TT	
	%	95%CI	%	95%CI	n	%	n	%	n	%
Healthy	53.8	49.6-57.9	46.2	42.0-50.4	117	42.4	63	22.8	96	34.8
T2DM patients	21.1	17.3-25.3	78.9	74.6-82.7	4	1.9	78	38.2	122	59.8

According to the literature, pathological allele polymorphism of *LPL* rs320 gene is T allele, with which high triglyceride level and low HDL are associated (2, 3, 5, 8).

To assess the impact of *LPL* gene variants in the Yakut population to the level of blood glucose, systolic blood pressure (SBP) and BMI analysis of these indicators depending on the genotype of SNP (G / T) rs320. The results show statistically significant differences in individuals with different genotypes in the level of glucose, SBP and BMI (Table 3). However, consideration of the indicators within the group of patients with type 2 diabetes and healthy subjects did not reveal significant differences (Tables 4, 5). Differences in the level of glucose throughout the sample due to the fact that initially selected for the study were patients diagnosed with type 2 diabetes , i.e. high levels of blood glucose. Nevertheless, in the control group as a trend due TT genotype with higher levels of glucose, although not statistically significant (Table 5).

BMI is also associated with genotype *LPL* rs320 in a joint sample of patients and healthy subjects (Table 3). The highest BMI was found in TT genotype in patients with type 2 diabetes (Table 4).

Table 3

Glucose level, SBP and BMI according to *LPL* rs320 genotypes in sample including type 2 diabetes patients and healthy individuals of Yakut population

LPL rs320 genotype	Glucose, mmol/l		SBP, mmHg.		BMI, kg/m^2	
	Me (25-75%)	n	Me (25-75%)	n	Me (25-75%)	n
GG	4.95 (4.55-5.6)	52	140 (130-150)	50	25.13 (23.1-26.5)	48
GT	9.3 (5.3-13.3)	85	147.5 (135-160)	96	26.37 (25.0-29.3)	84
TT	8.4 (5.6-14)	129	150 (140-170)	127	27.08 (24.5-30.2)	96
p	**0.0000**		**0.0005**		**0.0014**	

Table 4

Glucose level, SBP and BMI according to *LPL* rs320 genotype in type 2 diabetes patients of Yakut population

LPL rs320 genotype	Glucose, mmol/l		SBP, mmHg.		BMI, kg/m^2	
	Me (25-75%)	n	Me (25-75%)	n	Me (25-75%)	n
GG	15.5 (10.1-18.5)	4	150 (135-170)	4	25.2 (23.7-30.0)	3
GT	12.2 (9.1-14.8)	56	150 (130-165)	96	26.7 (25.7-29.9)	53
TT	12.1 (7.4-15.0)	95	160 (140-170)	109	27.8 (25.5-32.0)	75
p	0.48		0.41		0.49	

Table 5

Glucose level, SBP and BMI according to *LPL* rs320 genotypes in healthy individuals of Yakut population

LPL rs320 genotype	Glucose, mmol/l		SBP, mmHg.		BMI, kg/m^2	
	Me (25-75%)	n	Me (25-75%)	n	Me (25-75%)	n
GG	4.8 (4.4-5.4)	48	140 (130-150)	46	25.1 (23.0-26.4)	45
GT	5.0 (4.2-5.4)	29	140 (140-150)	29	25.6 (22.9-27.4)	31
TT	5.2 (4.8-5.7)	34	140 (130-150)	18	23.7 (22.9-26.1)	21
p	0.07		0.37		0.47	

CONCLUSION

The data testify that in the Yakut population with the highest incidence G allele variant rs320 *LPL* (54%) occurs, with which a reduced risk of dyslipidemia under the conditions of traditional nutrition type in the North with a high content of proteins and lipids is associated.

With the T allele rs320 *LPL* gene the development of predisposition to overweight is connected, that leads to the development of metabolic syndrome and type 2 diabetes mellitus. For a deeper understanding of the impact on the metabolic processes of the gene and its role in the pathogenesis of type 2 diabetes further study of a representative sample of healthy individuals is required.

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Molecular Genetic Studies of Hereditary Predisposition to Alcoholism

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ABSTRACT

The article presents a literature review on the results of molecular genetic studies of predisposition to alcoholism. Major molecular genetic studies of alcoholism conducted for identifying associations polymorphisms neurotransmitter system and ethanol - metabolizing enzymes in populations of different ethnic groups. Identification of gene polymorphisms association with the risk of developing alcoholism will promote preventive addictology and technologies of personalized therapy.

Keywords: alcoholism, genetic predisposition, serotonin, dopamine, ethanol - metabolizing enzymes.

Heredity has a big role in the development of alcoholism. On the incidence of alcoholism among children of alcohol-dependent people attention was paid in the XIX century [36]. Since the second half of XX century modern genetic researches began. Thus, J.Seixas et al. (1985) reported that children of the alcohol-dependent people develop alcoholism in 4 times more likely than children whose parents do not suffer from alcohol dependence, even if they are raised in different families, and the risk of disease in the sons of alcoholics is higher than in daughters. It was confirmed in further studies.

Back in 2000 a group of researchers [5] has been studied elevated basal level of gene activity Tryptophan in alcohol preferring mice C57BI. In C57B1 mice basal activity of this enzyme was elevated, which presumably was the probable cause of the reduced level of tryptophan in the blood and serotonin in the brain associated with a predisposition to the alcoholism.

Ten years of research (Anstee QM et al., 2013) in a mouse model, it was found that mutation of two types of base pair substitutions in Gabrb1 - the gene encoding the beta- subunit of one of the two GABA- receptor responsible for the body's response to major inhibitory neurotransmitter CNS gamma- aminobutyric acid - lead to permanent preference experimental animals ethanol water.

Even in the XX century has been proven that psychoactive substances stimulate the «reinforcement system" of the brain, central to which is occupied dopaminergic and serotonergic systems [1].

Recent advances in molecular genetic studies of substance dependence, the most common of which is alcohol, showed that the basis of the formation of the disease state is the individual features of activity of neurotransmitter systems and their compensatory potential for prolonged effects of these substances on the body. An important role in dopaminergic neurotransmission plays dopamine transporter DAT1 (encoded by the gene *SLC6A3*), limiting the activity of dopaminergic neurotransmission by reuptake of the neurotransmitter into the presynaptic terminals.

The results of molecular genetic studies VNTR polymorphism in the gene *SLC6A3* revealed that allele 9 repeat units, primarily in the homozygous state, is associated with an early form of alcoholism (the beginning of 35 years) and with the development of acute alcoholic psychosis [8, 24, 34].

In the literature there is a single study of polymorphic marker 2319G> A gene *SLC6A3* in alcoholism, which reports on its involvement in the development of alcohol dependence [23]. Other authors [14, 17] established the role of the polymorphic locus VNTR DRD4 gene in the development of alcohol and drug addiction and the formation of personality traits in healthy individuals. At the same time, a comparative analysis of the distribution of allele frequencies VNTR locus of the *DRD4* gene found similarities in the group Yakut and Evenks with East Asians (Chinese, Japanese), while all ethnic groups studied statistically significantly different from the people of Africa [33]. The study identified markers of increased risk of alcohol and drug abuse only in individuals Tatar and Russian ethnicity genotype *DRD4**S/*S and/or allele of DRD4*S. The analysis of gene polymorphism of the dopaminergic system in patients with alcoholism Yakut and Chukchi ethnicity [4] analyzed the association of *SLC6A3* gene haplotypes with alcoholism in the population of the Yakut Republic of Sakha (Yakutia) and the Tatars of the Republic of Bashkortostan [10]. Various results can be explained by the difference in the geographical origin of the individuals involved in the study and ethnic heterogeneity of samples.

On the territory of the Republic of Sakha (Yakutia), a group of researchers [2, 13, 18] studied polymorphism 1342 A/G in exon 9 of the dopamine transporter DAT1 (SLC6A3) in samples Yakuts, dependent on alcohol and healthy individuals. The study has been found close to the statistically significant association of polymorphism A/G (p= 0,015) with alcoholism in the Yakut population. Genetic variability of the studied loci not linked with alcoholic psychosis [18]. Statistically significant differences between the control groups Yakut and Evenks were found in the frequency distribution of genotypes and alleles of polymorphic loci 25G> A *DRD3* gene and 5 -HTTLPR of the 5 – *HTT* gene [17].

A.O.Kibitov [16] in 2013 established the molecular genetic profile of the dopamine neurotransmitter system in addicted patients. Other authors [6] studied polymorphism RS1611115 (-1021C / T) gene dopamine -β- hydroxylase (DBH) in patients with alcoholic psychosis and healthy donors from Kemerovo region.

The next authors [12] tested the hypothesis of an association of polymorphic variants rs9373085, 743964, rs1743966, rs1057293 *SGK1* gene with alcoholism, comparison of the frequencies of genotypes and alleles in groups of alcoholics and controls revealed no significant differences.

Thus, it is possible to suppose that polymorphic variants of dopaminergic systems genes may lead to the development of alcoholism, modulating alcohol action on brain "reinforcement system". For more thorough and accurate assessment of the role of these genes will require further molecular genetic studies of polymorphic variants in different populations of the world, as well as the study of the functional significance of the protein products in the pathogenesis of alcohol dependence.

Further review of the literature allows us to establish that the molecular genetic studies in the field of Addiction, revealed the correlation between polymorphisms of genes regulating serotonin exchange with the risk of formation of alcoholic psychosis often conducted among the Slavic population, which examined the role of hormonal parameters, genetic markers and psychometric parameters in the regulation of aggressive behaviors in patients with alcoholism [3].

The serotonin 5- hydroxytryptamine, 5 -HT is one of important hormones, which has the role of neurotransmitter of the CNS. Chemical structure of serotonin relates to biogenic amines, tryptamine class. Physiological functions of serotonin are extremely diverse. Serotonin controls so many functions in the body. The metabolism of serotonin is involved alcohol dehydrogenase; serotonin may participate in the formation of endogenous opiates, reacts with acetaldehyde (a decay product of ethanol). Serotonin itself is formed from the amino acid tryptophan. However, this reaction only occurs in natural light. Lack of ultraviolet in the winter season and the reason for the widespread seasonal depression (R.Sandyk, 1999), including the indigenous population of the Arctic North, where the end of November start polar night. In this regard, it is assumed that the reduction in the level of serotonin in the brain is one of the factors in the formation of depressions under the Arctic North, which may be the direct cause of substance abuse. Analysis of published data indicates failure association study of serotonin receptor gene polymorphism with the development of alcoholism in Alaska Arctic North.

Aggression in alcohol-dependent individuals is now widely studied, but yet the question why in response to alcohol intake in some individuals there is the desire to commit aggressive acts, while others have not such a reaction has not resolved, the role of biological factors in the mechanisms of criminal aggression patients with alcoholism is not defined. For this purpose, Tomsk scientists [3,7] carried a comprehensive study of the role of hormonal indicators of genetic markers and psychometric parameters in the regulation of aggressive behavior in 450 people, including 193 patients with alcoholism. The study of genetic markers revealed associations with their psychometric characteristics. So convicted patients with alcoholism wrongful conduct, media S/S genotype of 5HTTLPR polymorphism of the serotonin transporter gene, characterized by low levels of depression questionnaire Beck compared with genotype L/L. This result corresponds to the data in the literature and has a biological explanation. S allele reduces the promoter strength and as a consequence , the level of expression of the transporter in the brain, acting essentially as an antidepressant - selective serotonin reuptake inhibitors, which may lead to disruption in signaling synaptic 5 -HT in people with genotype S / S, and S / L as compared with native genotype L / L [21].

Ethanol-oxidizing systems have great importance in the genesis of alcoholism. Many Mongoloids have their identity [15, 35]. Studies in Eskimos and Indians of Alaska, Evenksof Yakutia, showed a high frequency of "abnormal" AlDG1 isoenzymes and (alcohol dehydrogenase) ADH. According to researchers, this leads to a greater possibility of developing alcoholism.

In ethanol metabolism two enzymes play an important role - alcoholdehydrogenase (ADH) and aldehydedehydrogenase (ALDH). The cytochrome system takes a main position, and a relatively small role takes a catalase. Currently 6 *ADH* classes are revealed [38]. *ADH* classification was based on differences in electrophoretic mobility. These differences were significant ones in the respective structures and functions of enzymes. Seven *ADH* genes are located on chromosome 4 (4q21-q25), within a portion size of 380 kB. Independent studies revealed the presence of clutch of this site with alcoholism [22, 27]. *ADH* enzymes of classes I, II and IV influence significantly on the metabolism of ethanol. Class I enzymes are encoded by three genes: *ADH1A, ADH1B* and *ADH1C*, highly expressed in human liver.

ADH oxidizes ethanol to acetaldehyde. As a biologically active molecule, acetaldehyde induces a range of pathological processes, damaging a number of enzymes, supramolecular structures, membranes, etc. Acetaldegidrogenazes, *ALDH1* and *ALDH2* have a major role in the metabolism of acetaldehyde. *ALDH2* gene is mapped to chromosome 12 (12q24.2), contains a

polymorphism G1510A, which results in an aminoacid substitution Glu487Lys. *ALDH2 * Lys* allele is found only in Asian populations. Enzyme, resulting in a heterozygous individual has only 20% of normal enzyme activity. To explain this, a model of partial dominance allele *ALDH2 * Lys* was proposed towards to *ALDH2* Glu* [21]. Genotype *ALDH2 *Lys/*Lys* is associated with the occurrence of the flush-syndrome (discomfort, facial flushing, nausea, vomiting) after the intake of ethanol, due to elevated levels of acetaldehyde. Therefore, this genotype is considered to be a protective against the development of alcohol dependence [31].

In research of H. Shoshana et al. [32], conducted in 2001 by students of the University of California in San Diego (USA), they revealed that in the population of Asians change in the level of the enzyme alcohol dehydrogenase gene ADH2 was correlated with alcohol consumption.

ADH1B gene contains two actively studied functional polymorphisms: *Arg47His* and *Arg369Cys*, which are the result of transitions *143A> G* and *1108T>C*, respectively. The enzyme encoded by *ADH1B *A*, has improved activity provides a more rapid accumulation of acetaldehyde, which has a toxic effect on many tissues of the body [31]. *ADH1B *A* allele and the corresponding atypical *ADH* are rarer among alcohol-dependent patients than among healthy individuals. Among alcoholics allele carriers use smaller doses of ethanol than the individuals in whom it is absent. Thus, the allele may be considered as protective against alcohol abuse. Frequency of allele *ADH1B * A* in different populations varies from values of more than 70% of indigenous peoples of Southeast Asia to 7.5 % and below in the peoples of Europe. Among indigenous peoples from Africa and America this allele is virtually absent. Several researchers suggest that the frequency of allele *ADH1B*A* increased by the positive selection [25].

In addition, a polymorphic marker *143A> G* (rs1229984, *Arg47His*) *ADH1B* gene has an interest in the study of alcoholic etiology of carcinogenesis [26, 29].

In research of M.V. Osier et al. [30] the role of alcoholdehydrogenase gene (ADH) in alcoholism has shown, namely that gene polymorphism *ADH1B Arg47His*, associated with the *ADH1B * 47His*, carries a protective effect.

Other researchers [2, 13, 18] studied the distribution of allele and genotype frequencies of polymorphisms of two genes ethanol - metabolizing enzymes *ADH1B * Arg47His* and *CYP2E1 Pst I (G/C)*, length polymorphism of tandem repeats (VNTR) in the 3'-noncoding region (3 '-UTR) in samples from three Yakut populations in comparison with the group of patients with alcoholism Yakuts. In this case, in the Yakuts no connection of ethanol-metabolizing enzymes polymorphism with susceptibility to alcoholism was found.

Established markers of increased risk of alcoholic psychosis, which accounted for polymorphisms in the genes of enzymes of alcohol metabolism: ADH1C 272 R> G, ALDH2 357 A>G, CYP2E1 7632 T> A Russian population in the Belgorod region of Central Black Earth region of the Russian Federation [9].

Thus, analysis of published data on the polymorphism of candidate genes for alcoholism in humans indicates the existence of population differences in the frequency distribution of genotypes and alleles of these genes, and the genetic background of alcohol dependence. Use of polymorphic loci as genetic markers of genes whose expression products may at neurotransmitters and enzymes involved in the metabolism of alcohol, especially determine reactions to alcohol, as well as provoke potentially pathogenic changes in the light of ethnicity, will provide comprehensive information on the molecular genetic mechanisms of formation of alcohol dependence. It must be emphasized that the study of candidate genes for alcohol dependence was conducted on samples from Western Europe, North America and some Asian populations, while in Russia, according to an analysis of literature, such studies have relatively small amount [9, 14, 17, 19, 20].

Conducted clinical and genetic analysis of ethanol - metabolizing enzymes polymorphism with susceptibility to alcoholism and alcoholic psychosis persons of indigenous nationalities (Yakut) revealed no statistically significant association. Low frequency of alleles having a protective effect (8.0 and 12.0%), possibly contributes to the high prevalence of alcoholism among the Yakut [16].

It was revealed that markers of increased risk for alcoholism in Yakut is allele *ADH1B * 143G*, a marker of low risk - *MAOA *H*T* haplotype, whereas in the Evenks marker of reduced risk of chronic alcoholism is a heterozygous genotype *HTR1B * 861C/G*, as well as increased risk of alcoholism markers in the combined sample of the Sakha (Yakutia) is *ADH1B * 143G* allele and genotype *ADH1B * 143G/G*, low-risk markers - genotype *DRD4*L/ L* and the haplotype *MAOA*H*T* [17].

Analysis of intergenic interactions among the Yakut from Republic of Sakha (Yakutia) allowed us to establish a statistically significant ($p<0.05$) model the interactions of genes affecting the formation of alcohol dependence: 1) *ADH1C* rs698, *PDY*N 68 no VNTR; 2) *ADH 1B* rs2966701, *PDYN* 68 n.o. VNTR, *OPRM1* rs3823010 [4].

Pharmacogenetic investigations (Chamorro A.J. et al., 2012) established the role of G allele polymorphism *A118G OPRM1* at naltrexone treatment of patients with alcohol dependence [37].

Despite a proven role of genetic factors, it is extremely difficult to establish its contribution to the formation of alcoholism in the entire population of patients with alcohol dependence. This is due to the fact, that always selected patient populations are examined in which the disease occurs more rapidly and proceeds more unfavorably. The largest number of alcohol-dependent persons never seeks medical help due to the rather good adaptation and relatively benign course of the disease. It is possible that these patients groups differ significantly in environmental, ethnocultural and genetic factors on the formation of alcoholism. In this regard, further molecular genetic investigations in populations of indigenous peoples of the Arctic North are very important problem of the modern addictology. Also, revealing the association of gene polymorphisms with the risk of alcoholism will promote preventive addictology and technologies of personalized therapy.

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Analysis of Aggression in Male and Female Populations in Two Coherent Ukrainian Generations

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ABSTRACT

Human aggression is a species characteristics and subject of interest for anthropologists, geneticists, criminologists, psychologists, psychiatrists and other specialists. This paper studies some types of aggression in Ukrainian population in two coherent generations. The researches involved 2305 people from Ukraine (including 741 men and 1501 women) aged 14 to 72 from Kharkiv and Kharkiv region mostly. It was found that distribution of aggression is mostly normal. The signs of sexual dimorphism and cohort effect related to the disparities between generations were revealed. For example physical aggression is more common for men of both generations. There are signs of verbal aggression and negativism among older generation. On the whole in terms of the majority of aggression types the differences between generations are traceable among women only.

Keywords: aggression; Ukraine; population distribution; sexual dimorphism; generations.

INTRODUCTION

The number of works devoted to biology of aggression in both animals and human is enormous [7, 8, 12]. It has been recognized that a human in terms of his biological natures is an aggressive creature. This aggression has been evolving during evolution of Homo *sapiens* as of a biological species. It is believed that inherent for a human are both pro-social behavior with its utmost trait of altruism and antisocial behavior with its utmost form as aggression. Aggression of a human is a species characteristics which means that a human possesses physical, cognitive and emotional systems capable for inflicting intentional harm to others. In animals and in humans aggression is an inborn response to potential threat or provocation. This conclusion is based on the researches of outstanding ethologists, anthropologists and psychologists: C. Laurentz [3], E. Wilson [15], S. Freud [15], R. Baron, D. Richardson [1] etc.

Apart from aggression which is typical to all the members of population antisocial behavior generally includes psychological disorders in particular psychopathy, antisocial personality disorders diagnosable in 5-10 % of population as well as offensive patterns of behavior demonstrated by 20-30 % of population. The variants of antisocial behavior

interpretation by psychologists, psychiatrists and criminologists are highly inter-correlated [14]. For this reason the behavioral genetics often most researches both antisocial behavior as it is and its individual components like aggression. Knowledge of the nature of aggression, genetic control of its physical and biochemical processes we can find a means of positive aggression control.

The aim of this research is to study population-related distribution of certain aggression types among the population of Ukraine in two successive generations.

THE MATERIAL AND METHODS OF RESEARCH

The research covered 2305 people of Ukraine (741 men and 1501 women) aged 14 to 72 from Kharkiv City and Kharkiv region mostly who gave informed consent for questionnaire survey. The information was collected in compliance with ethical standards of communication. The questionnaire gave social and demographic information. The probands were 741 men and 1501 women. The researched population comprised 74 married couples, 105 couples of siblings and 352 parent-child couples, 1174 peoples were researched with no relative. The groups were formed depending on the task of research. One group included the persons aged under 35 with the youngest one being 14 years old. The second group included the people who are more than 35 with the oldest being 72 years of age. The average age of the examined from younger generation was 19.3±0.1 years old (s = 3.8), modal age was 17 years old and medial age made 17 years old. Among the older generation respondents the average age was 43.8±0.3 years (s = 7.2), the modal age made 40 years and medial age was 42 years. The difference between the average ages of younger and older generations of respondents is 24.5 years which corresponds to the time segment equal to one generation in terms of genetics.

Different types of aggression were assessed under Buss-Durkey Inventory [4].

Verification of data for compliance with the law of normal distribution in big groups (n>30) was made by the method of Kolmogorov-Smirnov. The parameters of symmetry and excess with subsequent verification of zero hypothesis about their equaling to zero were calculated. Comparison of two groups arithmetic average was accomplished by Student method. The conclusion on statistical hypotheses was made at $p \leq 0.05$ level [2, 5].

The database was formed with the help of Microsoft Excel software. The calculations were made in Microsoft Excel и Biostat software.

FINDINGS AND DISCUSSION

Study of population distribution in terms of behavioral features is not only a means of behavior polymorphism assessment but rather an essential preliminary stage of genetic analysis, determination of population incidence, risks, etc. For population analysis the biologically interpreted behavioral features were selected which are significant in terms of medicine, education and social life and important for the professional performance. For all qualitative features which were studies under classic methodologies in two successive generations and which are described in this section the complete statistics was calculated which includes 13 indices: $\bar{x}$, *Me, Mo, min, max,* Q_{25}, Q_{75}, *s,* $s_{\bar{x}}$, *As,* s_{As}, *Ex,* s_{Ex}. The paper contains data on the significance of differences between representatives of different generations and different sexes as well as the results of correlation analysis between the ages within one generation and behavioral features.

In a number of researches completed in students sampling no sexual differences in the level of physical aggression were revealed [10]. As for the physical aggression Ukrainian population is not an exception from the general rule (Table 1). Thus younger generation men tend to be more aggressive than women ($\bar{x}$ = 5.0 and $\bar{x}$ = 3.9, p<0.001), the same being true for the researched men and women of older age ($\bar{x}$ = 4.6 and $\bar{x}$ = 3.5, p<0.001).

Some scholars explain aggression from the evolutionary point of view. According to this view the people having common genes tend to show less aggression towards each other [9, 11, 13].

For verbal aggression and negativism (Table 1) the gender differences were revealed in older generation while in younger generation they were absent. Some obtained data can be put down to the following factors of mixed biological and social nature. Thus the studies accomplished by the Western scholars reveal no sexual dimorphism [10]. Nevertheless the population researched in this study show a higher verbal aggression in men as opposed to women

($\bar{x}$ = 6.3 and $\bar{x}$ = 5.5, $p<0.01$). Gender differences specific for younger generation only were determined in 3 types of aggression only: resentment, sense of guilt and displaced aggression (Table 1).

The Western scientists believe that women more often tend to experience displaced aggression and interpersonal aggression in particular social expulsion [10]. The findings of our research confirm that the same regularity is also traced among the representatives of younger generation (Table1). Thus in women displaced aggression is higher ($\bar{x}$ = 5.0) than in men ($\bar{x}$ = 4.6, $p<0.01$). It is worth mentioning about a higher level of the sense of guilt in younger women as opposed to men ($\bar{x}$ = 5.7 and $\bar{x}$ = 5.1, $p<0.001$). As the sense of guilt is self-aggression which means aggression directed by the human to himself or herself than a higher level of this behavioral features in women sends them to the group of suicide behavior risk. Although the data available from foreign resources say that men are 3 to 4 times more likely to die as a result of suicide than women the attempts to kill oneself are more common among women but not men. A probable explanation of this is that men more often use the tools of suicide which lead to immediate death. Another cause is that women tend to self-injury more often in an attempt to attract attention or ask for help [6].

d. Table 1

i. Distribution of different aggression types among population under Buss-Durkey Inventory

Features of behavior	Statistical indices													
	N	$\bar{x}$	Me	Mo	min	max	Q_{25}	Q_{75}	s	$s_{\bar{x}}$	As	s_{As}	Ex	s_{Ex}
Younger generation, ♂														
Physical aggression	155	5.0	5.0	6.0	1.0	9.0	4.0	6.0	1.8	0.1	-0.17	0.19	-0.47	0.39
Displaced aggression	155	4.6	5.0	4.0	2.0	8.0	3.0	6.0	1.5	0.1	-0.01	0.19	-0.86	0.39
Irritation	155	5.0	5.0	6.0	0.0	10.0	3.0	6.0	2.3	0.2	-0.07	0.19	-0.35	0.39
Negativism	155	2.9	3.0	3.0	0.0	8.0	2.0	4.0	1.4	0.1	0.06	0.19	-0.06	0.39
Resentment	155	3.9	4.0	5.0	0.0	8.0	2.0	5.0	1.8	0.1	0.12	0.19	-0.69	0.39
Suspicion	155	5.8	6.0	7.0	1.0	12.0	4.0	7.0	2.2	0.2	0.06	0.19	-0.31	0.39
Verbal aggression	155	6.3	6.0	7.0	1.0	11.0	5.0	8.0	2.3	0.2	-0.12	0.19	-0.37	0.39
Sense of guilt	155	5.1	5.0	6.0	0.0	9.0	4.0	7.0	2.2	0.2	-0.38	0.19	-0.37	0.39
Younger generation, ♀														
Physical aggression	402	3.9	4.0	4.0	0.0	8.0	2.0	5.0	1.9	0.1	0.27	0.12	-0.77*	0.24
Displaced aggression	402	5.0	5.0	5.0	1.0	9.0	4.0	6.0	1.4	0.1	-0.14	0.12	-0.42	0.24
Irritation	402	5.6	5.5	5.0	0.0	11.0	4.0	7.0	2.2	0.1	0.01	0.12	-0.35	0.24
Negativism	402	3.1	3.0	4.0	0.0	5.0	2.0	4.0	1.4	0.1	-0.30	0.12	-0.83*	0.24
Resentment	402	4.3	4.0	4.0	0.0	8.0	3.0	6.0	1.8	0.1	-0.07	0.12	-0.59	0.24
Suspicion	402	6.0	6.0	6.0	1.0	11.0	4.0	8.0	2.1	0.1	0.00	0.12	-0.53	0.24
Verbal aggression	402	6.5	6.5	5.0	0.0	11.0	5.0	8.0	2.3	0.1	-0.18	0.12	-0.57	0.24
Sense of guilt	402	5.7	6.0	6.0	0.0	9.0	5.0	7.0	1.9	0.1	-0.45*	0.12	0.02	0.24
Features of behavior	Statistical indices													
	N	$\bar{x}$	Me	Mo	min	max	Q_{25}	Q_{75}	s	$s_{\bar{x}}$	As	s_{As}	Ex	s_{Ex}
Older generation, ♂														
Physical aggression	68	4.6	4.0	4.0	1.0	8.0	3.0	6.0	1.9	0.2	-0.01	0.29	-0.72	0.57
Displaced aggression	68	4.6	4.5	4.0	1.0	8.0	4.0	5.5	1.5	0.2	0.10	0.29	0.07	0.57
Irritation	68	5.3	5.0	6.0	0.0	11.0	3.0	7.0	2.6	0.3	-0.01	0.29	-0.61	0.57
Negativism	68	2.8	3.0	4.0	0.0	5.0	2.0	4.0	1.4	0.2	-0.15	0.29	-0.88	0.57
Resentment	68	4.4	4.0	4.0	1.0	8.0	3.0	6.0	1.9	0.2	0.03	0.29	-1.04	0.57

Suspicion	68	5.8	6.0	7.0	1.0	9.0	5.0	7.0	2.0	0.2	-0.69	0.29	-0.16	0.57
Verbal aggression	68	6.3	7.0	7.0	1.0	11.0	5.0	8.0	2.3	0.3	-0.61	0.29	-0.01	0.57
Sense of guilt	68	6.0	6.0	5.0	1.0	9.0	5.0	7.5	2.0	0.2	-0.29	0.29	-0.56	0.57
Older generation, ♀														
Physical aggression	180	3.5	3.0	2.0	0.0	8.0	2.0	5.0	1.7	0.1	0.35	0.18	-0.57	0.36
Displaced aggression	180	4.4	4.0	4.0	1.0	8.0	4.0	5.0	1.4	0.1	-0.00	0.18	-0.30	0.36
Irritation	180	4.6	5.0	5.0	0.0	10.0	3.0	6.0	2.1	0.2	0.05	0.18	0.03	0.36
Negativism	180	2.4	2.0	2.0	0.0	5.0	1.0	3.0	1.3	0.1	0.26	0.18	-0.70	0.36
Resentment	180	4.4	4.0	5.0	1.0	8.0	3.0	6.0	1.8	0.1	0.09	0.18	-0.75	0.36
Suspicion	180	5.8	6.0	5.0	2.0	11.0	4.0	7.0	2.2	0.2	0.23	0.18	-0.70	0.36
Verbal aggression	180	5.5	5.0	6.0	0.0	11.0	4.0	7.0	2.2	0.2	0.01	0.18	-0.49	0.36
Sense of guilt	180	5.8	6.0	6.0	1.0	10.0	4.0	7.0	1.8	0.1	-0.16	0.18	-0.35	0.36

*e. Note. * – $p<0.05$.*

In one types of aggression (the sense of guilt, Table 1) the differences were fixed between men of different generations. Thus younger men's sense of guilt was slighter as opposed to the men of older generation ($\bar{x}$ = 5.1 and $\bar{x}$ = 6.0, $p<0.01$). Hyper self-aggression can be a suicide risk factor not only in younger women as it has been noted above but in the men of older age group as well.

It is worth noting that in the majority of aggression the difference between representatives of different genders are characteristics for women only. For example younger generation women show higher levels of the following types of aggression as opposed to women of older generation: physical aggression ($\bar{x}$ = 3.9 and $\bar{x}$ = 3.5, $p<0.01$), displaced aggression ($\bar{x}$ = 5.0 and $\bar{x}$ = 4.4, $p<0.001$), verbal aggression ($\bar{x}$ = 6.5 and $\bar{x}$ = 5.5, $p<0.001$), irritation ($\bar{x}$ = 5.6 and $\bar{x}$ = 4.6, $p<0.001$), negativism ($\bar{x}$ = 3.1, and $\bar{x}$ = 2.4, $p<0.001$).

CONCLUSIONS

The analysis of aggression types in terms of different population has shown that distribution of the most of these types correspond to Gauss' Law. Defining the character of behavioral features distribution will make it possible to select the proper methods for assessing heritability coefficients in the subsequent genetic analysis. The value of gender differences was

in average 10% of the range of features deviation. More significant differences between representatives of different generations was fixed among women.

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Pharmacogenetics and its Role Understanding by Future Pharmacists: Gender Specificity

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ABSTRACT

Pharmacogenetics is one of the areas of genetics, the ground of modern personalized medicine and an educational discipline (or its part) for specialists training in different fields of pharmacy. With the aim of awareness studying in the area of pharmacogenetics, the students questioning, specializing in pharmacy, has been carried out for the first time in Ukraine. It has been stated that, more than70% of questioned got the information about pharmacogenetics in University for the first time. However, only more than one-third of respondents (37.7% males and 43.9% females) correctly understand the idea of this discipline. About half of questioned students thought that, pharmacocorrection of hereditary diseases is impossible, while 16.7 % of males and 13.4 % of females did not have a definite opinion as to this question. Thus, the awareness increasing of future pharmacists, as a link between a physician and a patient, about genetic aspects of pharmococorrection is an important problem that requires an immediate reaction.

Keywords: pharmacogenetics, personalized medicine, Ukraine.

INTRODUCTION

Pharmacogenetics is a field of genetics, which studies the peculiarities of body's reaction to medical preparations, depending on genetic characteristics of an organism. It is well known that medical preparations may not have proper effect on 30-60 % of patients according to a number of reasons, including genetic polymorphism of pharmacodynamics and pharmacokinetic human systems. Taking this fact into consideration, in modern medicine it is very important to use individual approaches to treatment, based on genetic testing, and define the treatment regimen on the base of organism genetic characteristics [1, 2]. This approach to treatment is a part of personalized medicine. According to the literature data, just patient genetic peculiarities are determined to 50 % of all atypical pharmacological responses. Recently, the investigations of personalized medicine are especially intensively conducted in former Soviet Union and beyond [4, 7], single studies are known in Ukraine [3]. A pharmacist plays an important role in

pharmacogenetics introduction in to health care practice. He/she is a link between a physician and a patient [5, 6].

The aim of the present work is an analysis of students of National University of Pharmacy (NUPh) awareness as to pharmacogenetics.

MATERIALS AND METHODS

Field investigations have been used in this work. Questioning of NUPh students (Kharkov, Ukraine) have been carried out. The questionnaire consisted of two parts: socio-demographic and basic. In socio-demographic part of questionnaire there was information about sex, age, address, educational level, respondents' profession and presence in his/ her family persons, working in the field of health care system. The basic part of questionnaire consisted of questions, directed on genetic factor role understanding during pharmocorrection. Only some questions of basic part of a questionnaire have been used for current analysis.

The material analysis has been carried out on the base of questioning of 637 NUPh students (1-4 year of studying), specializing in pharmacy. According to specific character of studying, contingent selection has been shifted to female side. Among the questioned students, there were 557 females (87.4%) and 80 males (12.6%). The age of questioned students is from 17 to 23 years old. In different age groups females and males were presented in such way: 17 years old – 7.5% and 11.5%, 18 years old – 31.2% and 25.0%, 19 years old – 28.7% and 25.3%, 20 years old – 18.7% and 27.5%, 21 years old – 10% and 10.2%, 22 years old – 2.5% and 0,5%, 23 years old – 1.2% and 0% respectively. 98.8% of questioned males and 99.5% of females lived in Ukraine (the rest respondents lived in Russia and Kazakhstan or stayed in Ukraine temporarily).

Among males, 46.4% lived in large cities with population of more than 1 million people, 34.5% – in big towns (population 250-500 thousand) and in towns (population 100-250 thousand), and 19.1% – in small towns (up to 50 thousand) and in rural areas. Among females questioned, 41.7% lived in large cities, 38.5% in big towns and 19.8% – in small towns and rural areas.

38.1% of questioned males and 42.4% of questioned females have family members, who work in the field of medicine and pharmacy.

Information gathering has been conducting, taking into consideration the ethical requirements during working with a person. All the participants of investigation gave informed consent to anonymous questioning.

The connection between qualitative characteristics has been evaluated, using the criterion χ^2. Conclusion as to statistic hypotheses has been performed at a significance level $p \leq 0,05$.

Data basis has been formed on the program Microsoft Excel. Calculations have been carried out in Microsoft Excel and Statistica 6 software.

RESULTS AND DISCUSSION

According to basic socio-demographic characteristics of males and females selection were put together, that is why any significant differences could be stipulated only by sexual factor.

As the basis for many atypical human reactions as to medical preparations there is a genetic factor; it is reasonable to include the question about possibility understanding of hereditary conditions (diseases) pharmacocorrection. The results of investigation showed that the majority of males questioned believed that it is impossible to correct hereditary diseases by medical preparations (51.2%). Females, who thought that hereditary diseases cannot be corrected by pharmaceutical preparations, divided approximately equally – 41.4% and 45.2 % (Fig.1). Any significant differences between females and males answer peculiarities to this question haven't been detected ($\chi^2 = 2.70$, $\nu = 2$, $p = 0.26$).

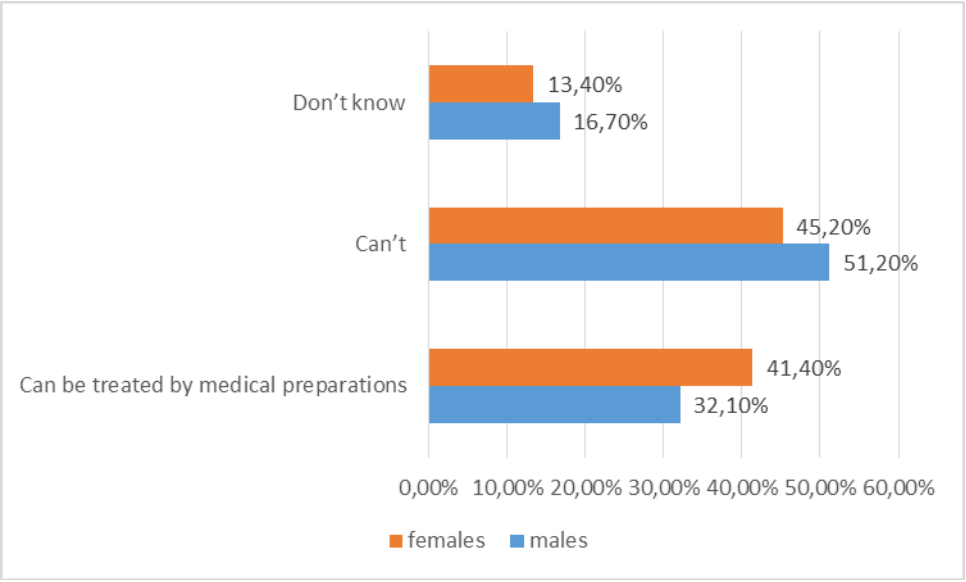

Fig.1. **Analysis of pharmacocorrection possibility of hereditary diseases among males and females.**

Analyzing answers to the question about idea of pharmacogenetics, it has been shown , that this trend was understood differently by males and females (Table 1), and obtained differences are statistically important ($\chi^2 = 13.84$, $\nu = 6$, $p = 0.03$). Thus, 11.8% of questioned males and 6.4% females didn't hear anything about pharmacogenetics. 14.1% of males and of females

25.2% have heard about it, but couldn't get to know exactly what pharmacogenetics studied. Right answer about the idea of pharmacogenetics was given by one – third of questioned males (37.7%) and females (43.9%).

Table 1

The distribution of answers to the question "What does pharmacogenetics study?" among respondents of different sex

Sex	What does pharmacogenetics study?						
	Don't know	Have heard, but can't say exactly	Hereditary diseases	The influence of medical preparations on human	Gene impact on medical preparations	The possibility of gene mutations appearance as a result of medical preparations taking	Organism response on medical preparations due to its genetic peculiarities
Male	11.8%	14.1%	17.6%	3.5%	4.7%	10.6%	37.7%
Female	6.4%	25.2%	9.7%	4.5%	3.6%	6.7%	43.9%

Note. χ^2=13.84, ν =6, p=0,03.

It has been stated, that females – future pharmacists were more progressive as to awareness of pharmacogenetics ideas. Consequently, just females are more informed about genetic peculiarities of organism and its reaction to medical preparations, and they probably will advise chemist's visitors to conduct these tests in future. Besides, females, as a subject of a pharmaceutical market, potentially more often can be consumers of this production themselves (pharmacogenetics tests), and less likely will have side effects due to incorrect treatment. In connection with this, male population can get into potential risk group of increased frequency of atypical reactions.

During studying the information sources about pharmacogenetics, it has been shown that most students received information about this trend in the University.Moreover, any significant differences between males and females has not been detected ($\chi 2$ = 2.23, ν = 5 , p = 0.82). In particular, the curriculum for the discipline "Biology with genetics fundamentals" for the 1st year students of "Pharmacy" specialty in NUPh pays some attention to pharmacogenetics, when considering topics related to population genetics and hereditary diseases. According to the

questionnaire data, 70.7% of males and 72.9% of female respondents heard about pharmacogenetics superficially in University. In 13.4% of males and 10.5% of females information about this trend was not set aside (Table 2).

Table 2

The distribution of answers to the question "Where did you get information about pharmocogenetics?" among males and females

Sex	The source of information about pharmogenetics				
	Don't have any information	Have heard in University (superficially)	Studied in University (as a discipline)	Got to know from friends	Got to know from media
Male	13.4%	70.7%	3.7%	3.7%	8.5%
Female	10.5%	72.9%	5.6%	4.9%	6.1%

Note. $\chi^2 = 2.23$, $\nu = 5$, $p = 0.82$.

The conducted analysis showed that, even in the sphere of future pharmacists, the awareness about pharmacogenetics and its role in personalized medicine is not satisfactory. Thus, it is necessary to pay more attention to pharmacogenetics aspects during preparing competent specialists in the field of pharmacy, who are up to date. Effective development of corresponding infrastructure at pharmacogenetics testing introduction among the population of Ukraine is also necessary.

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CONCLUSIONS

1. The analysis of future pharmacists 'awareness in the field of pharmacogenetics, which pointed out problem aspects of this trend understanding and their sexual specific, have been carried out for the first time in Ukraine.
2. It has been stated that, more than 70% of questioned got the information about pharmacogenetics in University for the first time. However, only more than one-third of respondents (37.7% males and 43.9% females) correctly understand the idea of this

discipline. About half of questioned students thought that, pharmacocorrection of hereditary diseases was impossible.

3. It has been shown that, on the whole females were more informed about pharmacogenetics than males. So they can become more active persons of pharmaceutical market in future.

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Morphology of Leukocytes as an Indicator of Yakutia Athletes' Adaptability to Intense Exercises

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ABSTRACT

Hematologic researches of leukogram and nonspecific adaptive reaction of an organism of highly skilled fighters and boxers of Yakutia revealed a loading hypoxia in 30% and organism disadaptation in more than 10% of athletes.

Material for the study was conducted among athletes - single wrestlers of the State high sports skills school, School of Olympic Reserve of the Republic Sakha (Yakutia) and Churapchinsky State Institute of Physical Culture and Sports. 169 athletes Yakuts in age from 18 to 28 years old, involved in acyclic sports were surveyed: 112 freestyle wrestlers and 57 boxers. The control group consisted of 30 students of the North- Eastern Federal University named after M.K. Ammosov, aged 19 to 25 years, engaged in physical training program for general university.

Keywords: athletes, leukogram, loading hypoxia, nonspecific adaptive organism reactions.

INTRODUCTION

Composition of white blood indicates the status of the immune system [12, 14], and is an indirect measure of stress reaction in athletes in assessing the extent of the training load [2, 3]. According to some authors, the composition of white blood cells in norm in athletes is quite stable and varies little over time [13, 14]. However, according to V.I. Boldina (4), one-year dynamics of hematological parameters in conditions of regular muscular loads reflects positive adaptive changes in the blood system of athletes.

Nonspecific adaptation (or anti-stress) response (NAR) is the main body's response to the action of any stimulus [2]. In the basis of NAR differentiation are different levels of neuro- endocrine-immune homeostasis. NAR is defined by the intensity of the processes of accumulation of energy and plastic resources in the body and their expenditure in adapting to complex stimuli, external and internal environment, as well as the reactivity of host defenses, including phagocytosis, immunoreactivity, and the formation of the inflammatory response etc. Ultimately NAR characterizes the degree of intensity of metabolism in the adaptation process and the body's resistance to the action of the stimulus for a short time and in the future. It is known that going for

great sport is accompanied by intense exercise loads and leads to a breach of the general condition and the frequent development of stress that causes a decrease in immuno-biological reactivity and occurrence of disease [5, 9].

Adaptive response in athletes often does not correspond to the characteristics of health. These blood tests given in the literature [2, 3], speak for the predominance of intense training and activation reactions or stress, as well as reactivation. Particularly tense are reactions before - and competitive periods [8]. Therefore, a study of the morphological composition of white blood and NAR in elite athletes in the conditions of Yakutia, where climatogeographic factors lead to increased stress on the human body, is important.

The purpose of the study. To assess leukogram peripheral blood and nonspecific adaptive response (NAR) in elite athletes Yakutia.

MATERIALS AND METHODS

Material for the study was conducted among athletes - single wrestlers of the State high sports skills school, School of Olympic Reserve of the Republic Sakha (Yakutia) and Churapchinsky State Institute of Physical Culture and Sports. 169 athletes Yakuts in age from 18 to 28 years old, involved in acyclic sports were surveyed: 112 freestyle wrestlers and 57 boxers. The control group consisted of 30 students of the North- Eastern Federal University named after M.K. Ammosov, aged 19 to 25 years, engaged in physical training program for general university.

Peripheral blood corpuscles per unit volume (1mkl) levels were measured on a hematology analyzer with integrated semi dilutor - NA-5710 (made in USA) using reagents firm JTBAKER (Netherlands).

In vitro the following parameters were determined in peripheral blood: WBC (White Blood Cell, WBC).

Nonspecific adaptive response (NAR) of athletes- single wrestlers was determined by leucocyte procedure Garkavi L.H. (2006).

RESULTS AND DISCUSSION

Average white blood indices of athletes - single wrestlers are within acceptable normal values (Table 1). Comparative analysis of data athletes- single wrestlers and the control group showed statistically significant difference in the total number of leukocytes, lymphocytes and monocytes. Thus, in the control group, these figures were lower than those of athletes - single wrestlers, while in the athletes - single wrestlers number of monocytes varied within the upper limit of normal. Significant differences were found in relative and absolute terms, nuclear neutrophils and monocytes in absolute terms, wrestlers and boxers. In the boxers these figures were higher than in

the fighters (Table 1). Lowering of the total number of white blood cells (leukopenia) was detected in 4.46% of wrestlers and 3.5% of boxers, and an increase (moderate leukocytosis) - at 4.46% of wrestlers and 5.26% of boxers (Table 2). Differential leukocyte analysis revealed relative segmentonuclear neutropenia in 16.07% of wrestlers and 24.56% of boxers and absolute segmentonuclear neutropenia in 10.52% of boxers. Relative lymphocytosis was observed in 23.21% of wrestlers and 28.07% of boxers, and absolute lymphocytosis in 6.25 and 7.02% respectively (Table 2). The study results do not contradict the published data. According to Zh.I. Karpova [et al.] (6), changes in the number and properties of leukocytes and lymphocytes are one of the first responses of an athlete organism to excessive exercise. At fatigue the number of leukocytes increases, in this case the so-called "myogenic leukocytosis" with phase change is revealed [10]. Above outlined leukocyte changes are observed also in our sportsmen. Thus, the first lymphocytic phase with distinct rise in the number of lymphocytes (40-50%) at a relatively small increase in the total number of cells (up to 9000-1000) and with relative neutropenia were observed in the two wrestlers. Neutrophilic leukocytosis with phase (up to 10,000), with an absolute neutrophilia and relative lymphopenia was detected in 3 fighters and 1 boxer, and with relative eosinophilia – in 1 boxer. Muscle leukocytosis (up to 11000) relative and absolute monocytosis was detected in 1 wrestler. However, the relative and absolute eosinophilia, without increasing the total number of leukocytes, was ascertained in 20% of wrestlers and 14% of boxers. Relative and absolute monocytosis was detected in 28% of wrestlers and 43% of boxers, and the relative and absolute lymphocytosis was observed in 27% of wrestlers and 35% of boxers.

It is known that during intense physical exertion of submaximal power (wrestlers and boxers) many metabolites, unoxidized decay products are formed - low molecular weight acids (lactic, pyruvic, etc). Accumulation of acids in muscle cells alters the properties of their internal contents, obstructing the course of the process of muscle contraction. Under such conditions, the cell tends to get rid of acid, giving them to blood. Penetration of large amounts of acids in blood leads to change in important biological constants - the acid - alkalinity (pH) of blood. Reduced blood (pH) changes the properties of blood proteins and is a threat to their destruction. At the same time the rate of formation of acids during submaximal power is so high that the buffer system of the blood do not have time to neutralize acidification, which subsequently leads to hypoxic conditions.

It was experimentally proved that any effects of hypoxic stress in the early stages of character and increasing tissue hypoxia is the activation of mononuclear phagocytes of the bone marrow, monocytosis and therefore is non-specific reaction to any stress effect [11]. Increasing the load on the system - increasing rate of oxygen consumption and CO2 production, which is observed in the

amplification function of the organ or tissue, especially at muscle activity also affects the system as a whole, for all its units. Hypoxic conditions, arising by increasing the load on the system, i.e., with a significant increase in oxygen consumption, are allocated in a separate type - load hypoxia [7].

According to our data the relative monocytosis is ascertained at 5.25% of wrestlers and 10.52% of boxers, and the absolute monocytosis - in 25.89 and 33. 33% of sportsmen (Table 1).

Nonspecific adaptive response of the body (NAR) is the basic response of the organism to the action of any stimulus. Depending on the nature of the stimulus to the body such a specific adaptive response is formed (e.g., to the action of cold - increased heat release, dilution of cell membranes, skin reactions, etc.). However, this specific reaction only models, directs track nonspecific response of the body. NAR provides at the adaptive process energy resources, plastic materials for the synthesis of the necessary enzymes and other cellular structures, the level of activity of protective systems and regulation of the entire metabolism. NAR character depends on the ratio of the intensities of the stimulus and the response of an organism. At the basis of NAR differentiation are different levels of neuro-endocrine-immune homeostasis [2].

NAR is defined by the intensity of the processes of accumulation of energy and plastic resources in the body and their expenditure in adapting to complex stimuli, external and internal environment, as well as the reactivity of host defenses, including phagocytosis, immunoreactivity, formation of inflammatory reactions, etc. Ultimately NAR characterizes the degree of intensity of metabolism in the adaptation process and the body's resistance to the action of the stimulus for a short time and in the future.

It is known that modern sport involves significant physical and emotional stresses that cause stress on the athletes' body. Therefore, in the long-term adaptation of an athlete is formed, both general and specific mechanisms of adaptation. Under these extreme conditions, for the body's athletes natural defense, more acceptable is calm NAR and increased activation.

Results of the nonspecific adaptive response (NAR) study are shown in Table 3. At the impact of potent factors to the organism or stress reaction (characterized by severe lymphopenia - less than 20%), or the reactivation reaction develop.

As seen from Table 3, in the reaction characterized as calm and increased activation were over 60% wrestlers and boxers, and also 90% of non-athletes, with NAR levels being on average reactivity. Calm and increased activation NAR of the average reactivity body is characterized by the following features: in the blood test there are small deviations from the normal of one or two elements of leukocyte formula. Blood coagulation is normal at a quiet activation and at elevated - moderately reduced.

According to the literature from the CNS side at a quiet activation moderate physical excitation is observed, and more pronounced - at higher excitation. Emotional state at a quiet activation is good, while at increased activation - excellent. At calm activation efficiency of the organism is good on a length, but a little worse for speed. At elevated activation, conversely, operability is great for speed, but inferior in durability. Endocrine glands in calm activation is active secretion of glucocorticoids (anti inflammatory-hormones) is within the lower half of the limit of normal and at elevated - secretion of glucocorticoids is on 10-30% above normal. The immune system is good. High resistance of the organism. Plastic and energy metabolism are well balanced, anabolic processes (synthesis) predominate. Both the activation reactions (especially increased) stimulate and enhance the activity of the body's defense. At these reactions is the fastest and adequate protective forces restructuring in response to the damaging effects [1, 2].

According to our data the reaction “high reactivity stress” was detected in 4.46 % of wrestlers and 5.26 % of boxers, and reactivation was observed in 10.71% and 12.28 respectively. This reaction of the organism to the NAR is characterized as follows: in the CNS inhibition prevails, emotional state is satisfactory , the average activity, operability is slightly reduced for speed , there is a sleep disorder, apathy, reduced appetite, secretion of glucocorticoids is moderately increased, the activity of cellular immunity is slightly below normal, humoral - within the normal range, the body's resistance is slightly reduced, metabolism - energy and plastic - slightly violated, catabolism prevails(but not dramatically) (breakdown). This type of reactivity is observed in violation of general health and premorbid conditions.

At reactivation coagulation is slightly reduced. From the CNS marked agitation is observed. Greatly enhanced sensitivity. Unstable emotional state. The mood is good, but there is anxiety and stress with symptoms of irritability and aggressiveness. Disturbed sleep - difficult to fall asleep and waking up in the night. Great appetite. Especially increased activity of cellular immunity. Moderately lowered body resistance. Plastic and energy metabolism is very active, accelerated anabolism (synthesis) and catabolism (breakdown), but anabolism prevails. This reaction is not a disease, but there are health and sleep disorders. Biological sense of reactivation is in attempt of the body to keep the activation in response to an impossible burden without the "reset" in stress. Reactivation is better than stress, but it's dangerous by «breakdown» into it, besides reactivation is nonspecific basis of certain diseases.

Thus, in highly skilled Yakutia athletes relative segmented neutropenia was detected in 16.1% of wrestlers and 24.6% boxers, and absolute segmented neutropenia - in 10.5 % of boxers. Relative and absolute eosinophilia was ascertained in 20% of wrestlers and 14 % of boxers. Identified in

28% of wrestlers and 43% of boxers relative and absolute monocytosis and relative and absolute lymphocytosis in 27% of wrestlers and 35% of boxers, and increasing of all agranulocytes (monocytosis + lymphocytosis) without increasing the total number of leukocytes, occurring in 11% of wrestlers and 14 % of boxers show nonspecific adaptive response of the organism due to the occurrence of stress hypoxia.

Nonspecific adaptive reaction of the organism in athletes is characterized by stress response in 4.46% of wrestlers and 5.26% of boxers and the reactivation in 10.71% of wrestlers and 12.28% of boxers, which is organism disadaptation to physical stress.

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Table 1

Morphological composition of the blood of athletes

(Significance of the differences represented non-parametric Wilcoxon-Mann-Whitney)

	WBC· 10^9/l	Basophils (%)	Eosinophils (%)	Stab neutrophils (%)	Segmented neutrophils (%)	Lymphocytes (%)	Monocytes (%)
Wrestlers n = 112	6,2±0,1^{x} p=0,01	0,1±0,02	2,7±0,1	1,1±0,3* p=0,005	51,6±0,6	36,0±0,6	8,5±0,2^{x} p=0,005
	Absolute figures	3,9±1,02	167,4±11,5	65,3±27,9* p=0,005	3230,7±82,8	2167,7±49,6^{x} p=0,04	518,8±14,9*^{x} *p=0,05 ^{x}p=0,000
Boxers n=57	6,4±0,1^{a} p=0,008	0,1±0,02	2,8±0,2	2,7±0,5* p=0,005	50,8±0,8	36,0±0,8	8,9±0,3^{a} p=0,001
	Absolute figures	6,1±1,4	188,4±15,3	193,6±37,3* p=0,005	3303,6± 110,573	2293,8±66,3^{a} p=0,03	563,9±19,9*^{a} *p=0,05 ^{a}p=0,000
Control group n=30	5,27±0,59^{xa} ^{x}p=0,01 ^{a}p=0,008	0,0±0,0	2,4±0,9	1,2±0,5	55,9±2,9	34,8±2,7	5,7±0,6^{xa} ^{x}p=0,005 ^{a}p=0,001
	Absolute figures	0,0±0,0	124,5±36,4	59,9±23,3	2950,0±360,6	1847,5±273,0^{xa} ^{x}p=0,04 ^{a}p=0,03	294,4±39,8^{xa} ^{x}p=0,000 ^{a}p=0,000

Note: * - The wrestlers and boxers, ^{x}- wrestlers and control group, ^{a}- boxers and control group

Table 2

Leukocytes Deviations depending on the sport *

Blood counts	Indicators reduction		Indicators increase	
	Wrestlers n=112	Boxers n=57	Wrestlers n=112	Boxers n=57
WBC	4,46	3,5	4,46	5,26
Eozinotsitoz relative	-	-	8,92	7,02
Eozinotsitoz absolute	-	-	11,6	8,77
Segmented neutropenia relative	16,07	24,56	-	-
Segmented absolute neutropenia	-	10,52	-	-
Segmented relative neutrophilia	-	-	3,57	1,75
Relative lymphopenia	4,46	1,75	-	-
Lymphopenia absolute	6,25	5,26	-	-
Relative lymphocytosis	-	-	23,21	28,07
Absolute lymphocytosis	-	-	6,25	7,02
Monocytosis relative	-	-	5,25	10,52
Monocytosis absolute	-	-	**25,89**	**33,33**

* Percentages (%) of the number of examinees

Table 3

Indices of nonspecific adaptive reaction of an organism (NAR) in athletes (%) *

	Wrestlers	Boxers	Control group
Stress	**4,46**	**5,26**	0
Training	16,07	14,03	6,67
The activation of tranquil	20,53	29,82	**46,67**
Increased the activation	**46,43**	**38,59**	**43,33**
Reactivation	**10,71**	**12,28**	3

* - Percentages (%) of the number of examinees

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Levels of Low Molecular Antioxidants in the Indigenous and Non- Indigenous Population of Yakutia

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ABSTRACT

Two groups of the population were under study: the first group included indigenous people, adapted to the high latitudes, the second – the non- indigenous, not adapted to local conditions. In the non- indigenous compared with the indigenous people low molecular antioxidant levels were significantly higher and combined with significantly high levels of lipid metabolism. About strengthening the energy metabolism in the Yakutia non- indigenous population, related to adaptation to northern conditions, significantly high levels of glucose, creatinine, and high creatine kinase compared with the indigenous people, showed. Correlation analysis revealed a statistically significant direct relationship between levels of uric acid and the northern experience in the non- indigenous people.

Keywords: albumin, urea, uric acid, lipids, adaptation, North.

INTRODUCTION

Accommodation in high latitudes associated with higher energy costs is associated with adaptation, in which inevitably the intensity of cellular respiration, enzyme activity and the quantity of reactive oxygen species - initiators and followers of lipid peroxidation chains increase. The normal concentration of active oxygen species and lipid peroxidation products are small, as their excess inactivated AO system. With high speed and course of active oxygen species amplification of lipid peroxidation accumulate toxic substances that do not have time to be inactivated by the AO system, which leads to cell death or dysfunction of the development and pathological conditions.

Antioxidant system of the organism is represented as well as macromolecular antioxidants and antioxidants (superoxide dismutase, glutathione peroxidase, glutathione reductase, catalase) as the low molecular weight antioxidants.

The aim of our research was to study levels of low molecular weight antioxidants in the serum of indigenous and non-indigenous inhabitants of Yakutia.

MATERIAL AND METHODS

1262 residents of Yakutia in age from 18 to 72 years were surveyed. Indigenous people of Yakutia was 631 people (mean age 45,02 ± 3,54 years), the alien population - 631 (mean age 43,67 ± 2,61 years). Women was 778, men - 484. Yakutia Residents were divided into two groups: the first group included persons adapted to the North, to which the attributed indigenous people - Yakuts, Evens, Evenki, the second - person unadapted to northern conditions, newly arrived residents (Russian). The studies were conducted in the winter. Biochemical studies were conducted on automatic biochemical analyzer «Cobas Mira Plus» utilizing commercial reagents «Biocon» (Germany).

Statistical processing of data was performed using statistical software application package SPSS for Windows 17.0. Standard methods of variation statistics calculation of mean values, standard errors , 95% confidence interval. Data in tables are presented as M ± m, where M - average , m - error of the mean. The significance of differences between mean values was assessed using Student's t test and Kolmogorov-Smirnov. Probability of the null hypothesis accepted at $p < 0,05$. Correlation analysis was performed by the method of Pearson and Spearman.

Results and discussions. Comparative analysis in serum of blood concentrations low-molecular antioxidants of Yakutia residents showed their dependence of ethnicity. As seen from Table 1, the levels low molecular weight antioxidants,which include albumin, urea, uric acid, varying in within normal values were higher in statistically significantly alien inhabitants.

Albumin, the major protein of blood is one of the endogenous antioxidants. Antiradical and antiperoxidant properties are due albumin the presence of thiol groups which represent important

the extracellular antioxidants.

Antioxidant effect of urea associated with the stabilization of of membranes and modification of enzymes, thereby reducing the number of iron centers of lipid peroxidation.

Uric acid is able to inhibit the O_{2}-, OH-radicals, $ONOO^{-}$, heme oxidants and amino groups at the expense of bind metal ions of variable valence, forming stable complexes. Due to the high content of uric acid in blood serum it accounts for from 35 to 65% protection against oxidation of lipoproteins, 10 - 15% the inhibition hydroxyl radical and singlet-oxygen.

A statistically significant increase in serum low-molecular antioxidants in alien inhabitants of Yakutia was beset with a significantly higher total cholesterol, atherogenic cholesterol fractions and A statistically significant increase in serum low-molecular antioxidants

in alien inhabitants of Yakutia was beset with a significantly higher total cholesterol, atherogenic cholesterol fractions and low value antiatherogenic cholesterol fractions than with indigenous peoples (Table 2).Exceeding the normal factor values atherogenicity alien inhabitants indicates an increase in circulation the modified LDL which exposed peroxidation of lipids.

To strengthen energy metabolism alien inhabitants related with adaptation to northern conditions indicate statistically significant high activity of creatine kinase (115,04 ± 3,09 U / L) compared with the indigenous people (103,46 ± 4,59, p = 0.001) . In addition, the alien population of Yakutia than with indigenous peoples was high serum creatinine (90,99 ± 0,98 mmol / l) compared with indigenous peoples (74,47 ± 1,26 mmol/l, p = 0.000).

With the intensification energy metabolism could explain the increased levels of albumin in alien inhabitants, as one of the important functions of albumin is its part in the transport of fatty acids. Follows also be noted that glucose levels in serum alien inhabitants have been significantly higher (4,96 ± 0,04 mmol/l, p = 0.000) compared to natives (4,62 ± 0,04 mmol/l).

Increase in blood low molecular weight antioxidants is an indicator of suppression antioxidants of enzymatic unit the radical protection of cells, as under conditions of oxidative stress defense enzyme has a less effective action compared to protective effect low molecular weight antioxidants.

However, the excessive education free radical oxidation may be depleted a pool of and nonenzymatic antioxidants, which, acting as free radical scavenger, are transformed into inactive dimer and other forms. In Yakutia, observed that decrease of low molecular weight antioxidants is due to exogenous substances - vitamins with antioxidant activity - ascorbic acid, vitamin E and A, that is most noticeable among the rural population.

Described in Tables 3 are statistically significant correlations with the levels of low molecular weight antioxidants lipid metabolism in non-adapted inhabitants of Yakutia, and detected a statistically significant direct relationship between levels of uric acid in the northern experience indicates that the biochemical parameters of blood serum reflect the adaptive metabolic processes.

The conclusion. Statistically significant high levels of low molecular weight antioxidants increase of atherogenic cholesterol fractions and reduced antiatherogenic cholesterol fractions, and also the activity creatine kinase, adjoint with increase in blood creatinine and glucose in Yakutia alien inhabitants as compared with indigenous peoples indicate that they had showing signs depletion of the reserve capacity of the organism.

Since in the northern conditions endogenous antioxidants can not cope with the increasing concentrations of lipid peroxidation products, it is necessary to ensure the delivery of antioxidant vitamins from outside.

Table 1

The levels low molecular weight of antioxidants, depending on ethnicity

Biochemical parameters	Native population (n=631)	Non-native population (n=631)	Veracity (p)
Albumin, g / l	47,44±0,22	50,26±0,17	0,000
Urea, mmol / l	4,76±0,07	5,21±0,07	0,000
Uric acid, μmol / l	281,84±3,93	319,09±4,23	0,000

Table 2

Serum lipid profiledepending on the ethnicity

Biochemical parameters	Native population (n=631)	Non-native population (n=631)	Veracity (p)
Cholesterol, mmol / l	5,54±0,04	6,19±0,05	0,000
Triglycerides, mmol / l	1,07±0,02	1,27±0,04	0,000
HDL-C, mmol / l	1,59±0,02	1,48±0,02	0,009
LDL-C, mmol / l	3,46±0,04	4,08±0,05	0,009
VLDL-C, mmol / l	0,49±0,01	0,56±0,01	0,009
Coeff. atherogenicity	2,81±0,05	3,48±0,07	0,009

Table 3

Correlations between biochemical parameters and the low molecular weight with antioxidants in alien inhabitants

Biochemical parameters	Albumin		Uric acid		Urea	
	Coeff. correlation (r)	Veracity (p)	Coeff. correlation (r)	Veracity (p)	Coeff. correlation (r)	Veracity (p)
Northern length of service	-	-	0,208	0,001	-	-
Triglycerides, mmol / l	-	-	0,392	0,000	0,171	0,000
Cholesterol, mmol / l	-	-	-	-	0,270	0,000
HDL-C, mmol / l	0,091	0,05	-0,254	0,000	-	-
LDL-C, mmol / l	-	-	-	-	-0,137	0,001
VLDL-C, mmol / l	-	-	0,362	0,000	-	-
Coeff. atherogenicity	-	-	0,329	0,000	-	-
Creatine kinase, U/l	0,147	0,002	-	-	-	-

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Prevalence of Abdominal Obesity in the Aboriginal and non- Aboriginal population of Yakutsk Aged 60 and Older

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ABSTRACT

Aim: to study the prevalence of abdominal obesity among the elderly and senile (including long-lived people) population of the city of Yakutsk, and to determine waist circumference cutoff values associated with the components of the metabolic syndrome (MS) in the Yakut and non-aboriginal population aged ≥60 years.

Material and methods: The study of the prevalence of abdominal obesity (AO) was done based on representative sample comprising 485 citizens (210 males and 275 females), aged 60 or over.

Results: Mean values of waist circumference (WC) in Yakuts were smaller (90.5 sm.), than in the non-aboriginal population (94.1 sm.). Prevalence of AO in the total study population was 42.9% (95% CI 38.5–47.4) using NCEP ATP III (2001) criteria, or 65.2% (95% CI 60.8–69.3) using RSC (2009) criteria. All WC cutoff values used were associated with less prevalence of AO in the aboriginal population, compared to non-aboriginal population: 32.9% in Yakuts and 52.4% in non-aboriginal patients using WC cutoff value of ≥102/88 sm., and 58.2% and 71.8%, respectively, using WC cutoff value of ≥94/80. Mean WC cutoff points associated with the 5 components of the metabolic syndrome (arterial hypertension (AH), hypertriglyceridemia (hTG), low HDL cholesterol (hypo-HDL-C), fasting hyperglycemia (5.6 mmol/L)) were 83.0 sm. in aboriginal males, 92.6 sm. in aboriginal females, 97.1 sm. in non-aboriginal males, and 93.1 sm. in non-aboriginal females.

Keywords: epidemiology, metabolic syndrome, abdominal obesity, waist circumference, ethnicity.

INTRODUCTION

Numerous cohort studies have shown that obesity plays a role as an important predictor of all death causes, and cardiovascular death [9]. In Novosibirsk, large-scale epidemiological studies have been conducted since mid-80s of the XX century. Data on high prevalence of risk factors for cardiovascular diseases among the population aged 25-64 had been reported from 1984 to 1995, as part of the WHO project MONICA (Principal Investigator in Novosibirsk center: Y.P. Nikitin). The risk factors included overweight and obesity [3]. National Health and

Nutrition Examination Surveys (NHANES) conducted in 2001-2002 have revealed ethnic-dependent variation in the incidence of obesity in Americans. This correlation was true for females, but not for males. The highest incidence of overweight was found in African American females (68.6%), with a slightly lower incidence in Caucasian (56%) and Mexican females (54.5%) [13]. In Europe, the situation with obesity has been more favorable. For example, in Great Britain 37% of males and 24% of females had overweight; obesity was present in 17 and 19.5%, respectfully. Finland has the lowest registered incidence of obesity among the countries of European Community (19% in males and 18% in females) [15]. Furthermore, based on the findings from some epidemiological studies, not just the presence of obesity, but the type of body fat distribution as well, influences overweight-related complications [4]. The predominating role of abdominal obesity can be explained by its stronger "pathogenicity". Moreover, obesity acts not only as an independent aggressive factor, but is often associated with the other components of metabolic syndrome [1,4-6]. The prevalence of AO in the population of Novosibirsk aged 45-69 was 43% using the criteria of NCEP ATP III (2001), and 65% using the criteria of IDF (2005), IDF, AHA/NHLBI (2009), and RSC (2009) [7]. Significant ethnic variations (between European, Asian, American and other populations) are known to exist in the prevalence of metabolic syndrome, and in particular, its basic component, abdominal obesity. Ethnic and regional differences of AO have been increasingly studied during the last years [10-12,14,16]. The prevalence of MS and AO in the Asian part of the Russian Federation, where the population is ethnically very heterogeneous, is poorly studied to date, especially among the elderly and senile population. Hence epidemiological study of the prevalence of MS and its components (specifically, AO) in the elderly and senile population in Yakutia is an important basic & applied research task.

AIM: to study the prevalence of abdominal obesity among the elderly and senile (including long-lived people) population of the city of Yakutsk, and to determine waist circumference cutoff values associated with the components of the metabolic syndrome (MS) in the Yakut and non-aboriginal population aged ≥60 years.

MATERIAL AND METHODS: This paper presents data from the research project "Epidemiology and risk factors for some of the chronic non-infectious diseases in the elderly and senile (including long-lived people) in Yakutsk" (Principal Investigator: O.V. Tatarinova, Scientific Supervisor: Y.P. Nikitin, RAMS academician). This study was conducted under methodological guidance of the Institute of Therapy SB RAMS, Novosibirsk (Supervisor: Y.P.

Nikitin, member of the Russian Academy of Medical Sciences) (government contract no. 274). Cross-sectional population study design was used.

We studied the population of Yakutsk aged 60 and over. On January 1, 2005, there were 18 320 people aged 60 and over in Yakutsk. For the purposes of population study, a representative sample was formed, using Yakutsk electoral lists and computer random number generation. The sample comprised 970 individuals, who made up 5.3% of the total population of the city of Yakutsk, excluding the city suburbs. Next we verified the lists, confirming the home addresses, the places of residence and their correspondence to residence permits, and rechecking the data on the deceased persons. After verification, the total number of subjects in the sample was 775 individuals (response level was 79.9%).

The size of the sample for the study of the prevalence of MS made 491 person, and was calculated using M/Blend formula (2000), with 95% CI ± 4% around 30% estimated prevalence (according to data from publications). 485 persons were examined. All patients participating in the project gave informed consents for examinations. The study was approved by the Ethical Committee of the Yakutsk Scientific Center SB RAMS (record no. 26, 30 March, 2011). All patients were grouped by sex, age (60-69; 70-79; 80-89; 90 and over) and were divided to 2 groups by their ethnicity: aboriginal (Yakuts, n=237; 48.8%) and non-aboriginal (Caucasians: Russians, Ukrainians, Byelorussians, Poles, and Germans, n=248; 51.1%).

Examination included: assessment of social and demographic data, clinical assessment of health status, measurement of blood pressure, anthropometry, fasting biochemical tests (blood glucose levels, triglycerides (TG) levels, high-density lipoprotein cholesterols (HDL-C), and low-density lipoprotein cholesterols (LDL-C)). Standard screening methods were used, in compliance with the guidelines accepted in epidemiological studies [Rose G. et al., 1982; Oganov R.G., 1990].

Fasting blood was collected by vein puncture using vacutainers. After centrifugation, the serum samples had been stored for 1-3 months in low-temperature chamber at –70^{o}C until the day of test. Biochemical blood tests were performed in the Biochemistry Laboratory of the Institute of Therapy SB RAMS (Head of the laboratory: Professor YU.I. Ragino). Prevalence of MS was analyzed using the guidelines of NCEP ATP III (2001), RSC (2009), IDF (2005), AHA/NHLBI (2005), AACE (2003), and JIS (2009) [6,7].

Statistical analysis was performed using SPSS (ver. 11.5) software. Two-sample methods (Mann-Whitney U-test, paired Student t-test) were employed. ROC-analysis was performed. Sample normality was tested using Kolmogorov-Smirnov test. In case of incomparability of the

data the values were standardized for one or two characteristics. The results were considered significant if $p<0.05$.

RESULTS AND DISCUSSION

In the elderly (≥60 years) population of Yakutsk, mean WC values were similar in the first 2 age groups (94.4 and 94.3 sm.), but after 80 years of age, this value decreased to 88.0 sm. Distribution of WC values in aboriginal and non-aboriginal populations was slightly deviating from normal distribution (Kolmogorov-Smirnov test, $p<0.05$). Mean WC values for aboriginal population were: 90.2±12.7 sm. (Mean±SD), Mo=80.0 sm., Me=90.5 sm. Mean WC values for non-aboriginal population were: 94.3±13.8 sm. (Mean±SD), Mo=95.2 sm., Me=95.2 sm. 10% and 90% WC cutoff values were 73.0 sm. and 106 sm. for aboriginal patients, and 76.0 sm. and 110.1 sm. for non-aboriginal patients. Age-standardized mean WC values were significantly lower in aboriginal, than in non-aboriginal patients ($p_{A<NA=}0.003$) (Table 1). Interestingly, in aboriginal patients WC values did not change much between age groups, but in Caucasians WC values decreased markedly after 80 years of age.

Male aboriginal patients aged ≥60 had lower mean WC values, compared to non-aboriginal male population (91.0±11.3 sm. vs. 96.4±12.7 sm., $p_{A<NA}=0.01$), the same was true for WC values in women (89.3±14.1 sm. vs. 93.2±14.3 sm., $p_{A<NA}=0.025$, respectively).

Using WC cutoff value of ≥102/88 sm. (NCEP ATP III, 2001), abdominal obesity (AO_1) was present in 32.9% of the aboriginal patients, which was 1.5 times lower, than in non-aboriginal patients of the same age (52.4%, $p_{A<NA}=0.0001$) (Table). In the age groups of 60-69 and 70-79 these differences were more vividly pronounced, than in other age groups. In female population, the prevalence of AO_1 was 2 twice higher, then in male population ($p_{M<F}=0.0001$). In males, prevalence of AO_1 was twice lower in aboriginal, than in non-aboriginal population; likewise in females, AO_1 was 1.3 times less prevalent in aboriginal, than non-aboriginal females.

The same analysis of the prevalence of AO was done, using more strict WC cutoff value of >94/80 sm. (IDF and RSC). Using this definition, the prevalence of abdominal obesity (AO_2) reached 71.8% in non-aboriginal population (Table). In aboriginal patients, the prevalence is still high, but reliably lower, than in non-aboriginal patients (58.2%, $p_{A<NA}=0.002$). Prevalence of AO_2 decreased reliably with aging, both in aboriginal, and non-aboriginal populations, and in women more, than in men.

We used also JIS criteria of abdominal obesity (AO_3): WC >94 sm. for Caucasian males; WC >90 sm. for Asian males; WC >80 sm. for females (Table 2). As far as WC cutoffs for non-aboriginal males and females remained the same as in the above definitions of AO, all comparisons were done only with aboriginal males. Prevalence of AO_3 in the aboriginal cohort was 66.2%, which was close to prevalence in non-aboriginal population (71.8%, $p_{A-NA}=0.189$).

Analysis by age groups shows the absence of ethnic differences in the prevalence of AO_3 in males, although ethnic difference was present, when other WC cutoff values were used (in the total population and in the age group of 60-69) (Table).

The prevalence of AO in the population of Yakutsk aged ≥60 was high (ranging from 42.9% to 69.1%), using various definitions of MS. Prevalence of AO in the aboriginal population was lower, than in non-aboriginal population (in the age groups of 60-69, 70-79). AO was more incident in females, compared to males, independent of the ethnicity factor. In non-aboriginal patients, there was a decrease in the prevalence of AO after 80 years of age. Using WC cutoff values for Caucasian people (WC>94/80 sm.), the prevalence of AO was higher in non-aboriginal, than in aboriginal population. Using the WC of >94/80 sm. in combination with ethnicity-adjusted WC cutoff value for Asian males (WC≥90 sm.), the difference in the prevalence of AO between aboriginal and non-aboriginal populations was insignificant. Meanwhile, gender differences in the prevalence of AO remained the same, both in aboriginal, and non-aboriginal elderly and senile populations, with higher prevalence rates in females aged <80 years.

Concluding from the results of ROC-analysis, the most WC-value-sensitive factors were: AH defined as AP >130/85 mmHg in aboriginal and non-aboriginal males, and lipid levels (hypo-HDL-C and hyper-LDL-C in aboriginals; hypo-HDL-C in non-aboriginal) (Fig. 2). Mean WC cutoff values associated with the detection of all the 5 MS components (AH, hTG, hypo-HDL-C, fasting hyperglycemia (5.6 mmol/L)) in the population aged ≥60 were: 83.0 sm. for aboriginal males, 92.6 sm. for aboriginal females, 97.1 sm. for non-aboriginal males, and 93.1 sm. for non-aboriginal females.

CONCLUSIONS:

1. Mean WC values are lower in the aboriginal aged ≥60 years (90.5 sm.), than in non-aboriginal population (94.1 sm.).
2. Prevalence of AO in the total population aged ≥60 was 42.9% (95% CI 38.5–47.4), using NCEP ATP III (2001) criteria, and 65.2% (95% CI 60.8–69.3), using RSC (2009) criteria.
3. AO frequency for all the WC studied criterion in the indigenous aged60-80 years is lower relative to non-indigenous residents over the age of 80 years – in the indigenous substantially unchanged, while in the non-indigenous - is reduced and ethnic differences are smoothed.

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Table

Prevalence of abdominal obesity using WC cutoff values of ≥102/88 sm. * and ≥94/80 sm. ** in the population of Yakutsk aged ≥60 years (both male and female)

Age groups (years)	Aboriginal			Non-aboriginal			p к-нк	Total population		
	N	n	%	N	n	%		N	n	%
	WC ≥102/88§*									
(I) 60-69	61	23	37.7	89	51	57.3	0.020	150	74	49.3
(II) 70-79	85	29	34.1	82	50	61.0	0.001	167	79	47.3
(III) 80-89	61	16	26.2	56	21	37.5#§	0.193	117	37	31.6 #§
(IV) ≥90	30	10	33.3	21	8	38.1	0.728	51	18	35.3
60 – ≥90	**237**	**78**	**32.9**	**248**	**130**	**52.4**	**0.0001**	**485**	**208**	**42.9**
	WC ≥94/80§§									
(I) 60-69	61	35	57.4	89	69	77.5	0.010	150	104	69.3
(II) 70-79	85	56	65.9	82	65	79.3◊	0.055	167	121	72.5◊
(III) 80-89	61	29	47.5§	56	33	58.9#§	0.220	117	62	53.0#§
(IV) ≥90	30	18	60.0	21	11	52.4#	0.591	51	29	56.9
60 – ≥90	**237**	**138**	**58.2**	**248**	**178**	**71.8**	**0.002**	**485**	**316**	**65.2**
	WC ≥94(90)/80§§§									
(I) 60-69	61	40	65.6	89	69	77.5	0.109	150	109	72.7
(II) 70-79	85	62	72.9	82	65	79.3◊	0.340	167	127	76.0
(III) 80-89	61	36	59.0	56	33	58.9#§	0.992	117	69	59.0
(IV) ≥90	30	19	63.3	21	11	52.4#	0.438	51	30	58.8
60 – ≥90	**237**	**157**	**66.2**	**248**	**178**	**71.8**	**0.189**	**485**	**335**	**69.1**

Notes (title of the table):

* definitions of NCEP ATP III, AACE, and AHA;

** definitions of IDF, ВНОК;

***definitions of JIS.

Notes (values in the table):

 # p<0.05 compared to (I) age group

§ p<0.05 compared to previous age group

◊ p <0.05 compared to (IV) age group

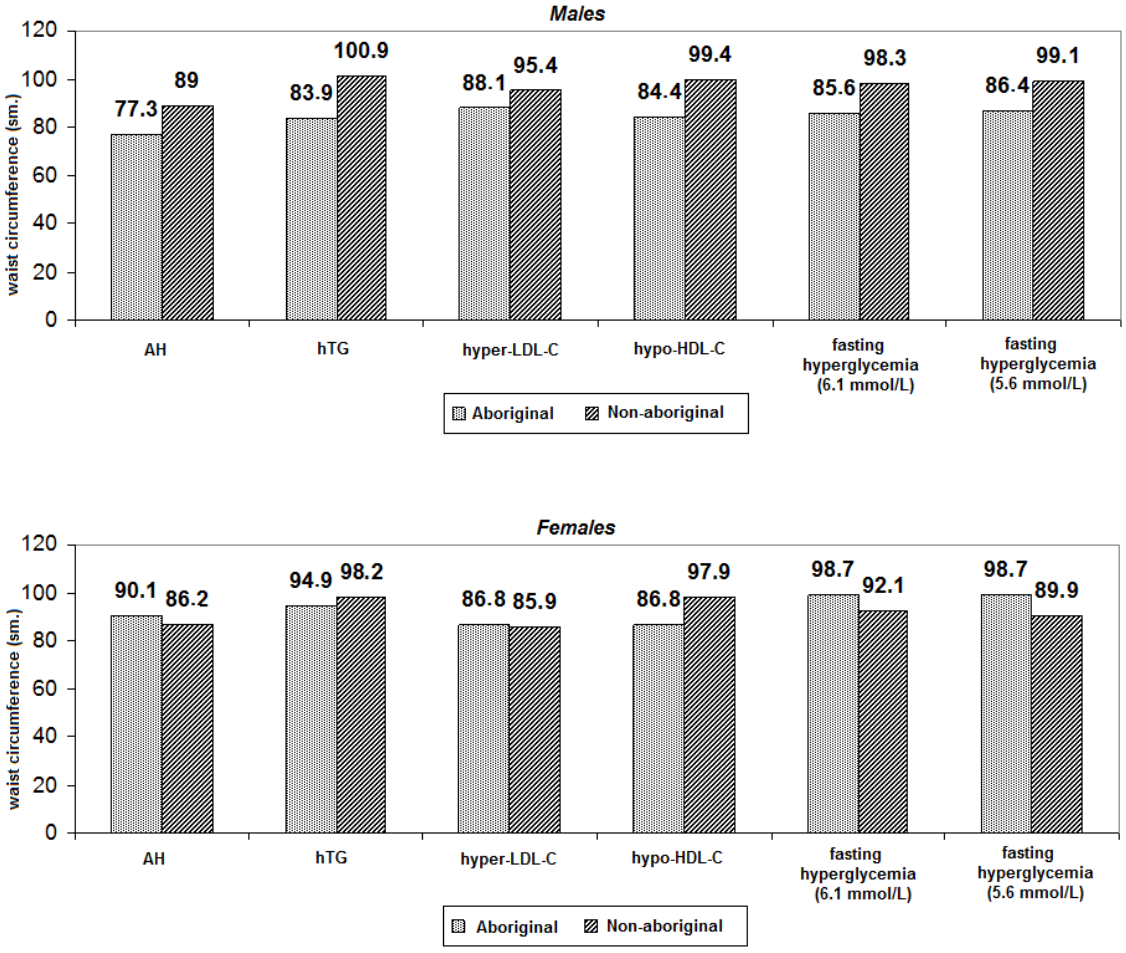

Fig. Waist circumference cutoff values, used for diagnostics in patients with metabolic risk factors, among the population aged ≥60 in Yakutsk.

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The Prevalence of HPV Infection in Patients with Cervical Cancer and Dysplasia in Yakutia

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ABSTRACT

The research was conducted among 164 women from the Sakha Republic (Yakutia) (of average age of 44.6+/- 0.82) diagnosed with histologically verified IIIrd degree dysplasia (CIN III) and uterine neck cancer. Analysis of HPV-contamination of high oncogenous risk among women with uterine neck cancer pathologies showed 78.10 % of HPV DNA detected from the total of 164 female patients. Genotyping assay of HPV-positive samples showed that Type 16 HPV is the most common one (82.81 %).

Keywords: human papilloma virus, cervical cancer, screening.

INTRODUCTION

According to published research data cervical cancer (CC) is the second most frequent and the third most frequent cause of death among the oncologic pathologies of female population. CC is the most frequent cause of death and dominates among women of reproductive age diagnosed with genital forms of cancer.

Numerous works published in 1980-2000s show the relation between human papilloma virus (HPV), dysplasia and epidermoid cervical cancer. Hybridization showed that 80 to 100 % of CC contains HPV DNA [9, 12]. A rough correlation between CC frequency and HPV detectability among the patients was found. In the countries with high frequency of CC the detectability of HPV-infection was shown to be found within 10-20 %, while the in the countries with low frequency that of 5-10 % [20]. The study of papilloma viruses in cervix tumors conducted in 22 regions of the world showed the presence of known types of HPV in 93 % of cases [19]. Various types of HPV were detected in 99.7 % of biopsy samples obtained from CC patients across the world; both in cases of dermoid cancer and adenocarcinoma [16, 21, 22].

According to the research conducted in N.N. Blokhin Russian Oncological Research Center (Moscow) the high cancer-inducing HPV in CC and CIN II/III (n=525) medications could be found in 99.6 % of cases [5]. According to other research data, the high risk HPV with II/III degree of CIN was detected among female patients from Moscow in 99.4 % [2]; from Saint Petersburg – in 92 % [6]; from Yekaterinburg – in 77.5 % [1]; and from Tyva Republic – in 81.8 % of cases [7].

All the human papilloviruses are divided in two groups. HPV types are numbered in order of their identification [14]. Viruses of high cancerous risk (types 16, 18, 31, 35, 39, 45, 51, 52, 56, 58, 59, 68) are more frequent with frank dysplasia, preinvasive and invasive cancer. Types 16 and -18 HPV together take more than 70 % of UNC cases, while types 16, 18, 45 and 31 are the cause of nearly 80% of all the CC cases. The same four types are responsible for more than 90 % of uterine neck adenocarcinoma development [10, 15]. Based on the variation of DNA sequence of *E6* oncogene the following variations of HPV-16 were detected: European, Asian, African 1 and 2, North-American and Asian-American [13]. Cases of CC most frequently show HPV type 16 and 18. However, contamination of these viruses does not always cause cancer of the given localization [4, 8, 17].

Different countries of the world show different patterns in HPV type frequency. The most frequent serotypes in Europe and USA are 6, 11, 31, 33 and 35; in Asian countries – 52 and 58; in Philippines – 45; in Latin America – 31 and 45 [11, 18].

Epidemiological data on contamination and prevalence rate of the virus in the regions of Siberia and Far East are very few. However, it is here where the highest rate of CC morbidity and death rate is noted.

Therefore, any preventive measures against CC must be preceded by careful study of the specifics of HPV prevalence in various regions.

Aim of the research is to study the relative frequency, definition of viral load and typing of HPV with Real-Time PCR method among women with precancerous pathology and CC in the Sakha Republic (Yakutia).

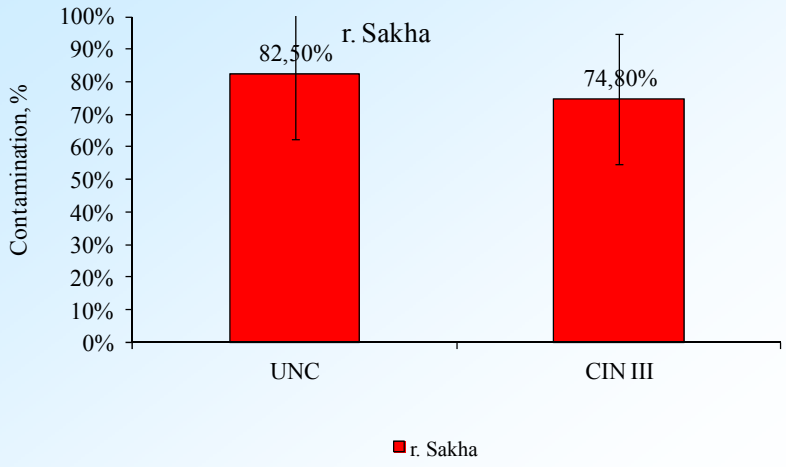
MATERIALS AND METHODS

The research was conducted among 164 women from the Sakha Republic (Yakutia) (of average age of 44.6+/- 0.82) diagnosed with histologically verified IIIrd degree dysplasia (CIN III) and cervical cancer. Genotyping and viral load definition of high cancerous risk (HCR) HPV (types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59) was conducted with multiplex-PCR by “AmpliSensR” (Russia) diagnostic sets.

OUTCOMES AND THEIR DISCUSSION

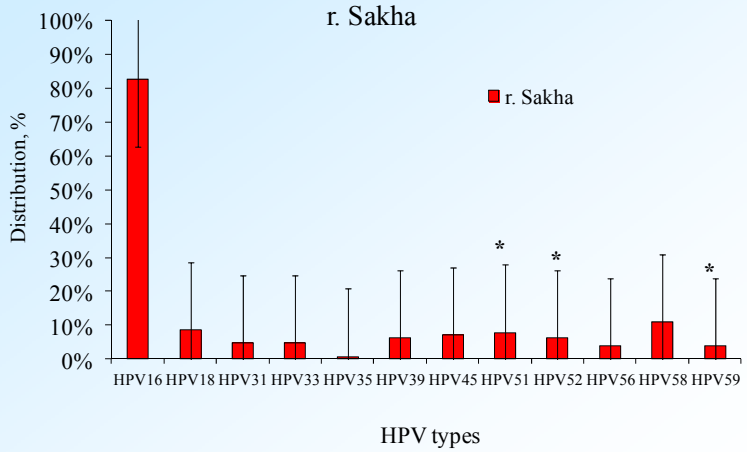
Analysis of HCR HPV contamination among women with CIN III and CC in the region under study showed that out of 164 patients HPV DNA was detected with 74.8 and 82.5 % respectively.

General contamination rate among the population of the Sakha Republic (Yakutia) – 78.1 %



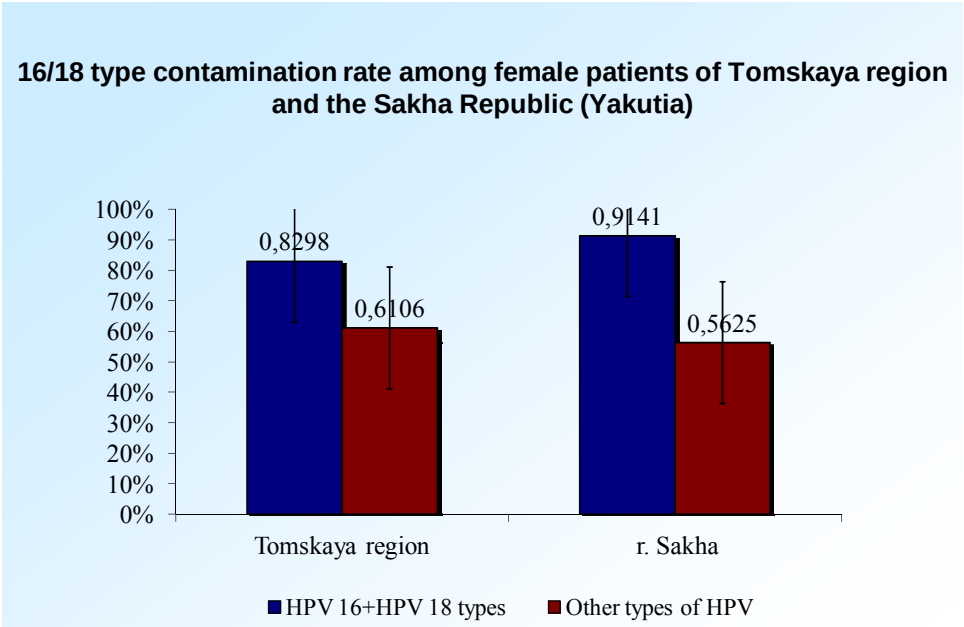
Genotyping of HPV-positive samples showed that type 16 HPV leads in the frequency rate. HPV of types 58, 18, 51, 39 and 45 are less frequent among the female population of the Republic. Other types are rarely found.

Distribution of viral genotype among HPV (+) female patients of the Sakha Republic (Yakutia)

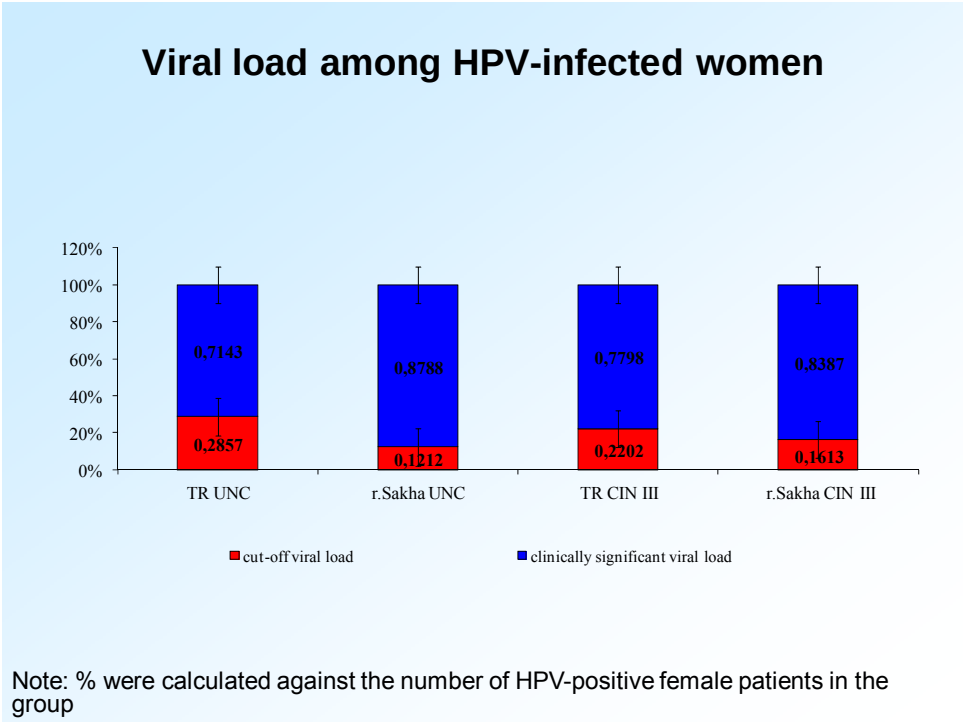


Note: % were calculated against the number of HPV-positive female patients in the group

Types 16 and 18 HPV contamination, which, according to published data is detected in 70% of CC totals in 91.4 % in the Sakha Republic and 82.9 % in Tomskaya region. Other types of HPV total in 56,2 %.



Note: % were calculated against the number of HPV-positive female patients in the group

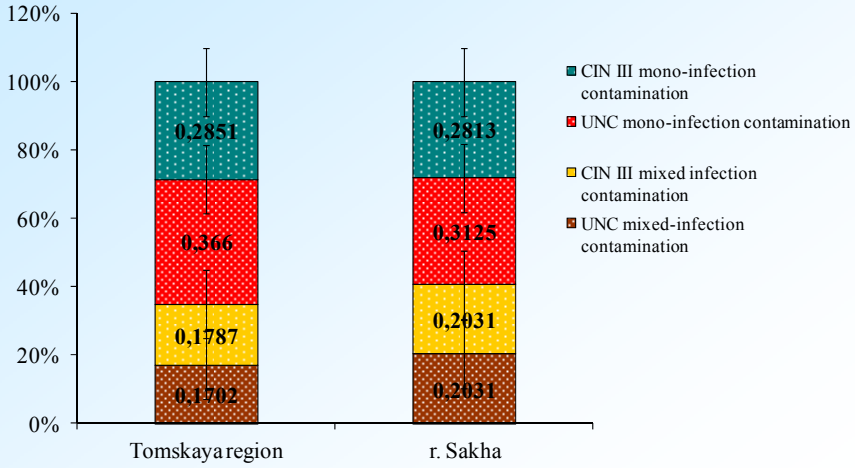


Note: % were calculated against the number of HPV-positive female patients in the group

Definition of DNA virus concentration (viral load) in samples showed that clinically significant index (> 3.1 gx of 105 cells) among CC -diagnosed female patients in the Sakha Republic made

87.88 %, CIN III – 83.87 % which exceeds similar index among female patients of Tomskaya region.

Frequency rate of mixed- and mono-infection contamination among HPV-positive female patients of Tomskaya region and the Sakha Republic diagnosed with uterine neck cancer



One must take notice of the tendency of mono-infection prevalence: this type of contamination is detected in 59.38 % of cases in the Sakha Republic (Yakutia).

CONCLUSION

Conducted research resulted in obtaining the data on the specifics of HPV contamination rate, viral load rate and high oncogenous risk HPV distribution among female patients of the Sakha Republic (Yakutia) diagnosed with CIN III and CC.

The data obtained once again emphasize the necessity of CC early detection programs, such as screening which includes (apart from colposcopical and cytologic examination) HPV DNA testing. It will allow detecting risk groups and monitoring genital papilloma virus infection patients, as well as conducting preventive and medical vaccination. Such measures are aimed at lowering contamination and death rate caused by uterine neck cancer.

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Evaluating the Effectiveness of Vitamin Drink «Valetek -SP Aktiv" in Yakutia Freestyle Wrestlers

Konstantinova L.I., Mironova G.E., Torgovkin V.G., Krivoshapkina Z.N.

The influence of vitamin and mineral complex "Valetek-SP Aktiv" on an organism of wrestlers of Yakutia was investigated. Drinking of vitamin and mineral complex within 20-days increases vitamins levels in blood serum and removes the vitamin A, E, C and B_1 deficiency in sportsman's organism and has a favorable effect on lipid metabolism.

Keywords: vitamin sufficiency, blood serum, vitamin and mineral complex "Valetek-SP Active", biochemical status, freestyle wrestlers.

RELEVANCE

In the Far North are fairly widespread states related to vitamin deficiency or insufficiency [1,3,7,9]. Among the population of Yakutia most frequently observed hypovitaminosis A, E and C, especially pronounced in winter [2,5].

The need for vitamins increases with systematic physical activities. Especially the role of vitamins increases during training and competition cycles. Lack of vitamins and minerals can cause a decrease in physical performance of athletes and to break the recovery processes. In this regard, the use of biologically active supplements in the world of sports is becoming more common. In order to increase the adaptive capacity of athletes for increasing physical activities most commonly used biologically active additives (BAA). BAA is habitual component of sports nutrition worldwide.

Aim of the study was the estimation of influence of vitamin-mineral complex "Valetek-SP Active" for supply of vitamins A, E, C and B1 an organism wrestlers.

MATERIAL AND METHODS

The study involved 39 healthy young men native nationality, aged 18 to 24 years, professional athletes. Among them were 1 category athletes, candidates for master of sports, sports master, and master of sports of international class. All participants are wrestlers and students of the Institute of Physical Culture and Sports (IFKiS) M.K. Ammosov North-Eastern Federal University and GU "High school sports" (SVSM) Yakutsk.

Investigation Group were athletes who had taken vitamin and mineral drink "Valetek-SP Active", numbering 21 people. The control group included 18 athletes who did not receive additional sources of vitamins and minerals. Comparison groups were composed of athletes with

similar levels of physical activity and physical fitness. The study was conducted during the winter (November-December).

For one portion of the vitamin-mineral 10 g of the dry drink mix was dissolved in 250 ml of bottled water at room temperature. Ready to drink "Valetek-SP Active" sportsmen took 1 times a day, after the evening workout for 20 days in the presence of researchers.

Material for the study served as serum and heparinized blood taken from the cubital vein in the morning on an empty stomach in a state of relative muscular rest.

Biochemical studies have been performed 3 times: 1st time - before start taking drink; 2nd time - 11 days and receive the third time - after the 20-day course.

Ascorbic acid (vitamin C) was determined by titration using 2.6 dinitrofenildifenolyat ; determination of retinol (vitamin A) and α- tocopherol (vitamin E) was performed by fluorimetric method analyzer bioliquids " Fluorat -02- ABLF " firm " Lumex " configured for retinol at excitation wave 335 nm and an emission wavelength of 460 nm , for α - tocopherol - with an excitation wavelength of 292 nm and an emission wavelength of 320 nm; thiamine (vitamin B1) was determined by the photometric method analyzer bioliquids " Fluorat -02- ABLF " firm " Lumex ".

Blood biochemical parameters were determined by biochemical analyzer «Labio 200" using reagents «Biocon» (Germany).

Body mass index was calculated as Ketle: BMI = M (kg) / H*H (M2).

Statistical processing was performed using the software package SPSS 19,0. For quantitative indicators were calculated the mean and standard error, denoted as M±m. Estimation of the importance differences of average in the comparison groups performed using the Mann-Whitney, Kruskal-Wallis. Mann-Whitney test was used for paired comparisons independent groups and nonparametric ANOVA Kruskal-Wallis was used to compare the mean values of three or more groups. The dependent samples when measuring quantitative indices were in the same individual at different times, the comparison of mean values was performed using the non-parametric Friedman ANOVA. For all the criteria used for the threshold level of significance accepted value of $p < 0.05$.

RESULTS AND DISCUSSION

All athletes surveyed belonged to one age category, without disabilities in physical development. Table 1 shows the anthropometric data of the surveyed athletes.

Body weight of athletes who drink ranged from 56.3 to 86.7 kg. In 7 (33%) athletes overweight was observed. In the control group, body weight ranged from 50 to 80.5 kg. Excess body weight was observed in only 1 athlete.

Height athletes who drink ranged from 1.60 to 1.84 m, while the control group of athletes from 1.60 to 1.81 m (Table 1).

Evaluation of vitamin sufficiency of athletes showed that hypovitaminosis A was noted in 22% of those surveyed wrestlers. Hypovitaminosis E before start taking drink was observed in 90% of athletes who drink and 86% of the athletes in the control group. Deficiency of vitamin C and vitamin drink reception was observed in 24% of athletes to drink, and in the control group - 39%. Hypovitaminosis of vitamin B1 was detected in only 1 athlete, taking a drink of the number before the study and the control group was not observed.

Daily intake of vitamin drink athletes within 20 days contributed to increase the effective provision of the organism antioxidant vitamins A, E, C and B1 (Table 2).

According to our data, the athletes taking the drink, in 10 days the content of vitamin A levels increased by 17% and after 20 days - 30% compared from baseline (Table 2). A statistically significant difference in the concentration of vitamin A blood fighters before and after a 20 day course of treatment with vitamin drink. At the same time, in the control group, the average concentration of vitamin A in the blood remained unchanged.

Daily intake of vitamin drink "Valetek-SP Active" significantly increased levels of vitamin E in the blood of athletes both on the 10th and on the 20th day of the study, compared with the initial level: on 10 days - 33%, and 20 days after receiving - by 52% ($p \leq 0.05$). These changes in the average concentration of vitamin E are statistically significant. It should be noted that the results of the initial assessment of 90% of athletes who drink are deficient in vitamin E. As a result, the 10-day receiving vitamin drink hypovitaminosis E was reduced to 42% and by the end of the study vitamin E deficiency was observed in only 38% of the wrestlers. In the control group of athletes levels of vitamin E did not change.

The initial level of vitamin C content in both study groups was normal. At the same time, before you start taking vitamin drink, vitamin C has been installed in 24% of athletes to drink, and in the control group hypovitaminosis C was observed in 39% of the wrestlers. On the 10th day of the drug average ascorbic acid content in the blood athletes who drink increased by 33% and by the end of the 20-day course of treatment with vitamin drink - 57%, compared with the

initial level. In the control group noted minor variations in average concentration of vitamin C in the blood.

The following vitamin average which rose as a result of a 20-day course of treatment with vitamin-mineral complex "Valetek-SP Active" is B1. By the end of the 20 day course of administration of the drug concentration in blood of vitamin B1 athletes who drink increased by 20% compared with the initial level. At the same time, the average concentration of vitamin B1 in the control group blood fighters virtually unchanged (Table 2).

Thus, the course of a vitamin drink "Valetek-SP Active" significantly increases the levels of vitamins in the blood of athletes. By the end of the course taking the drug in athletes who drink original hypovitaminosis vitamin E preserved in only 38% of the wrestlers. The concentration of the other investigated vitamins increased to normal values. In the control group insufficient supply of vitamins remained.

Cause of sub-optimal provision of athletes vitamins can be first - insufficient supply them with food, and secondly - increased utilization rate during intense physical exertion in the North, third - lack of pharmacological correction of hypovitaminosis.

One of the approaches to improve the vitamin status of athletes is consistently included in their diet enriched with vitamins food or dietary supplement. An important problem in the use of such products is correct, evidence-based selection [4,6,8].

Used by us vitamin-mineral complex "Valetek-SP Active" is designed specifically for athletes. Components included in the said drink have a tonic, immunomodulatory effects, neutralize free radicals, prevent muscle spasms, muscle contraction and provide nerve conduction, and contribute to the rapid replenishment of energy conservation status, reinforce the processes of oxidation, reduced salivation. This vitamin complex is recommended as increasing performance and endurance, loss of energy and replenish vitamins, recovery of water-salt balance and strengthen the immune system.

The following is a vitamin and mineral composition of the instant natural beverage "Valetek-SP Active", containing a fairly complete set of vitamins and minerals in doses up about 80% of the physiological needs of athletes.

We conducted a 20-day course of vitamin and mineral complex "Valetek-SP Active" a positive effect on vitamin status of athletes. The data presented in Table 2 indicate the statistical significance of mean values of vitamins in the body athletes taking supplements, only between the 1st and 3rd blood sampling. At the same time, significant differences there are between the two groups in the mean values of ascorbic acid and tocopherol.

The dispersion analysis showed a statistically significant increase in the concentration of vitamins A, E, C and B1 in the blood serum under the influence of dietary supplements athletes already on the 11th day admission ($\chi 2$ = 0,000*). In addition, the Mann-Whitney test shows a significant difference between the two groups by the end of observation (A - 0,049*, E - 0,004*, C - 0,000*, B1 - 0,047*).

Biochemical parameters of studied athletes' blood serum are presented in Table 4.

Our data on the biochemical parameters revealed that the initial average level of lactate dehydrogenase (LDH) is higher than normal values in athletes who drink 2,08 times, while in the control group - 1,87 times. By the end of the observation LDH decreased in both groups compared with baseline values. Increased activity of LDH in the blood serum of athletes, probably due to a high rate of utilization of oxygen and hypoxia in the working muscles, as high demand muscle energy substrates under conditions of oxygen deficiency is met by anaerobic oxidation of glucose.

The average concentration of creatine kinase (CK NAC) in the first day of the study in both groups exceeded the norm: in athletes who drink - 1,75 times in the control group - 2,16 times. 10 days after receiving a drink in athletes who drink higher than normal level of 1,32 times, while the control group was within normal limits. At the end of the study, athletes who drink average 1,61 times higher than the normal value, and in the control group - 1,41 times. In athletes who drink an average level of CK MB insignificantly exceeded the normal value in the first day at 1,16 times, whereas in the control group - 1,52 times ($p < 0,05$), and end of the study in a group of athletes taking a drink - 1,08 times, while in the control group - 1,16 times higher than the normal value.

Alkaline phosphatase (ALP) activity in athletes who drink before you start taking the drink was somewhat higher than normal values. After 10 days, the average level of ALP was observed on the upper limit of normal, and after 20 days of receiving the beverage increased by 10% compared to baseline. In the control group the mean values of ALP activity throughout the study were much higher than the norm and values were higher than in athletes taking a drink. A statistical significance is observed after the 2nd blood sampling in both groups.

After a 10-day ingestion of drink "Valetek-SP Active", we noted a tendency to reduce serum triglycerides athletes who drink 23%, and 20 days after ingestion of drink triglyceride levels returned to baseline. In the control group of athletes observed same regularity. Some reduction in triglyceride levels after 10 days receiving vitamin drink, probably due to the mode

of training. At this time, the athletes competed, which leads to large energy costs and enhances lipid catabolism.

After 20-day ingestion of drink occurred lowering aspartate (AST) ($p < 0,05$), which is a very good result. As is known, increased AST in the blood is indicative of an inadequate exercise and development of fatigue. Decrease in AST activity was observed in both groups.

The results of biochemical studies suggest that have not shown significant differences between athletes who drink and the control group.

The average value of total cholesterol throughout the study in both groups varied within limits. Meanwhile, we have seen a trend towards lower cholesterol levels after 20 days of receiving the drink "Valetek-SP-Active" in the group of athletes to drink.

When analyzing the lipid metabolism have been identified wrestlers atherogenity low coefficient (Ka) of the athletes who drink after a 20-day receiving vitamin drink, caused an increase in HDL cholesterol compared to baseline values. Whereas in the control group atherogenic factor in each study increased and HDL cholesterol dropped. LDL cholesterol and VLDL in both groups was within the normal range, but a group of athletes who took vitamin drink, there is a tendency to decrease.

Discussing the findings of biochemical studies, it is necessary to note the same pattern of changes in enzyme activity in both groups. However, the marked reduction of Ka, due to increase HDL cholesterol indicates the beneficial effect of vitamin drink for athletes' body.

Improvement of lipid metabolism, while reducing transaminase activity and increase the concentration of vitamins A, E and C, shows that vitamin drink "Valetek-SP Active" has hepatoprotective effect, increases the redox potential of the body by increasing the level of low molecular weight antioxidant system.

The fact that after a 20-day course of vitamin prevention, the part of the athletes remained hypovitaminosis E, probably related to the short-term course or receiving a relatively low dose of vitamin E in this preparation. It is known that low doses in all cases can eliminate hypovitaminosis in the short term, especially when the initial failure of a vitamin. Probably for sportsmen training in the North require higher doses of vitamin E, has a membrane and antioxidant properties.

Thus, the inclusion in the diet of freestyle wrestlers vitamin drink containing about 80% of the recommended daily intake of vitamins associated with a significant improvement in security of athletes' vitamins A, E, C, B1, and improves lipid metabolism. Results of the study show the

effectiveness of the use of vitamin drink "Valetek-SP Active" in the practice of wrestling as an additional source of vitamins and minerals for the wrestlers' organism.

CONCLUSIONS

1. The 20-days reception of drink "Valetek-SP Active" improves the security of athletes' vitamins. So, after 20 days of receiving the beverage content of vitamin A (before receiving - 46,25±2,52 mg/dL after administration - 66,30±3,34 g/dl) and vitamin B1 (to reception - 6,56±0,18 ng/ml after administration - 8,24±0,22 ng/ml) in serum athletes increased by 20%, vitamin C (until receiving 1,01±0,09 mg/dL after administration of 1±0,06 76 mg/dL) - 43% of vitamin E (prior to receiving 0,52±0,06 mg/dL after administration - 1,08±0,11 mg/dL) - 52%.

2. 20-day course of vitamin and mineral drink "Valetek-SP Active" has a favorable effect on lipid metabolism of freestyle wrestlers: a significant decrease in total cholesterol levels (U=0,036* after a 10-day course), increased cholesterol HDL (U=0,046* after 10 days of treatment; U=0,047* after 20 days of treatment).

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Table 1

Anthropometric characteristics of the study athletes

	Age, years	Height, m	body weight, kg	BMI
	M±m	M±m	M±m	M±m
Athletes who had taken a drink n=21	21,29±0,97	1,69±0,01	69,14±2,18	24,06±0,66
Control group n=18	19,89±0,37	1,70±0,01	66,26±1,90	22,85±0,44

Table 2

Dynamics of average concentrations of vitamins in the body of athletes depending on the of duration of reception of vitamin drink "Valetek-SP Active"

	Athletes who had taken a drink (n = 21)			Control group (n = 18)		
	M±m			M±m		
	First study day	11th day of exploring	the 21st day of the study	First study day	11th day of exploring	the 21st day of the study
Vitamin A, 30-80 ug/dl	46,25±2,52	55,71±2,39	66,30±3,34*	48,10±2,99	50,23±3,19	51,30±3,51
Vitamin E, 0,8-1,5 mg/dl	0,52±0,06	0,78±0,11	1,08±0,11*x	0,55±0,03	0,56±0,03	0,57±0,03
Vitamin C, 0,7-1,5 mg/dl	1,01±0,09	1,51±0,07	1,76±0,06*x	1,01±0,12	1,02±0,17	1,04±0,20
Vitamin B1, 5-20 ng/ml	6,56±0,18	7,23±0,14	8,24±0,22*	7,26±0,41	7,39±0,26	7,44±0,41

Note: *) p <0,05 compared with the value of the 1st day of the study; x) p <0,05 compared with control group

Table 3

The content of vitamins and minerals in one serving of beverage

Composition	Contents in 1 portion
A	0,39 mg
E	7,5 mg
D3	5,35 mcg
C	64,0 mg
B_1	0,95 mg
B_2	1,1 mg
B_6	1,2 mg
B_{12}	1,6 mcg
PP	9,65 mg
K_1	48,5 mcg
Pantothenic acid	2,9 mg
Folic acid	0,38 mg
Biotin	16,0 mcg
Calcium	140 mg
Mg	100 mg
Amber acid	50 mg
Carbohydrates	6,7 g
Caloric content	29 kkal

Table 4

Dynamics of biochemical parameters of blood serum of athletes

	Athletes who took a drink (n=21)			Control group (n=18)		
	M±m			M±m		
	First day of the study	11th day of the study	21st day of the study	First day of the study	11th day of the study	21st day of the study
Lactate dehydrogenase (LDH), 225-450 U/L	519,86±30,64	464,69±41,09	427,24±18,13	468,11±23,12	424,78±23,72	382,50±20,59
Creatine kinase (CK NAC), <190 U/L	332,78±51,41	250,50±39,84	305,71±43,52	410,76±57,17	193,36±26,31	267,70±63,81
Creatine kinase (CK MB), <25 U/L	28,86±2,76	25,13±2,69	27,14±2,07	37,89±4,72*	27,00±3,40	29,30±2,34
Alkaline phosphatase, until 258 U/L	294,19±19,64	258,93±15,81	285,86±23,85	350,83±30,90	345,44±28,39*	319,40±34,30
Triglycerides, 0,5-1,5 mmol/l	0,85±0,10	0,69±0,06	0,85±0,14	0,75±0,07	0,60±0,07	0,74±0,07
Total cholesterol, 3,6-6,5 mmol/l	4,06±0,13	4,10±0,18	3,73±0,24	3,64±0,12	3,51±0,13	3,45±0,12
Gamma-GT, 11-50 U/L	25,05±2,02	25,54±2,33	24,81±1,93	23,22±1,19	22,30±1,54	21,10±1,55
Alanine aminotransferase (ALT), until 30 IU	19,48±1,95	19,63±2,96	18,43±1,80	17,17±2,26	13,36±1,69	12,90±1,29*
Aspartate (AST), until 40 IU	30,05±2,28	24,93±2,78	20,38±2,39*	28,44±1,94	16,45±0,96	15,20±2,03*
Glucose, 3,3-5,5 mmol/l	4,37±0,19	4,34±0,11	4,36±0,06	4,13±0,18	4,24±0,14	4,16±0,06

Urea, 2,5-8,3 mmol/l	4,64±0,27	4,96±0,32	4,80±0,20	4,51±0,23	4,46±0,34	4,72±0,30
Creatinine, 53-97 mcmol/l	92,24±1,66	94,15±2,36	94,24±1,53	90,33±2,75	93,30±2,81	95,00±3,76
Total protein, 65-85 g/l	74,70±0,68	73,57±1,05	72,91±0,81	72,12±0,72	72,64±0,93	71,70±1,07
Albumin, 34-48 g/l	45,98±0,33	44,86±0,66	44,03±0,40	44,69±0,35	40,56±4,33	44,21±0,59
HDL cholesterol, 0,78-2,2 mmol/l	1,34±0,09	1,46±0,11	1,43±0,09	1,25±0,06	1,19±0,07	1,13±0,09
LDL cholesterol, 1,68-4,53 mmol/l	2,23±0,12	2,22±0,17	2,10±0,11	2,04±0,11	2,04±0,10	1,97±0,09
VLDL, 0,8-1,5 mmol/l	0,41±0,05	0,31±0.03	0,39±0,07	0,35±0,04	0,28±0,03	0,34±0,03
Coefficient atherogenity (Ka), <3	2,11±0,18	1,98±0,18	1,89±0,14	2,02±0,19	2,01±0,16	2,14±0,23

Note: *) The level of significance of differences p <0,05 as compared with baseline content

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Hygienic and Epidemiological Aspects of the Organization for the Prevention of Infection of Patients, Employees and Visitors of TB Institutions

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ABSTRACT

Because of the continued tense situation on TB morbidity and high mortality problem of strengthening the prevention of this infection is important. In the last 7 years in Khabarovsk Krai antituberculosis institutions a program of the International Society of the Red Cross and WHO Crescent against TB has being realized. One of the conditions of this program is to create a set of epidemiological, clinical and hygienic measures aimed at preventing the spread of infectious disease, comprehensive infection control programs. Infection Control Program includes three levels of control: administrative, engineering and personal. The authors describe ways to implement infection control at all levels and express the view that the use of such approaches in general health care institutions, especially outpatient clinics.

Keywords: infection control, tuberculosis.

INTRODUCTION

Taking into the current epidemic situation on tuberculosis, which is characterized by the global spread of tuberculosis (TB) and extensively drug-resistant tuberculosis, reduce of the effectiveness of treatment and increased mortality from this infection, the problem of strengthening the prevention of the studied disease, including the sanitary-epidemic measures is rather topical [1,3].

In the Khabarovsk region on the basis of antitubercular establishments the TB program of the International Society of Red Cross and Red Crescent is realized more than 7 years. One of the conditions of implementation of this program is creation on the basis of epidemiological, clinical and hygienic measures, used in the international practice, aimed at preventing the spread of an infectious disease, a comprehensive infection control program. Experts who conducted inspection infection control programs, which allowed continuing the implementation of the international program on the territory of Khabarovsk region.

The epidemiology of tuberculosis in modern conditions includes the following:

- high incidence of tuberculosis;

- the spread of drug-resistant strains of Mycobacterium tuberculosis, including the appearance of pathogens with multidrug-resistant TB, extensively drug-resistant and resistant to disinfectants;
- the spread of HIV infection, which is accompanied by the increase in the number of persons much more prone to infection as the ILO and the reactivation of latent TB infection with fast and malignant development of active disease.
- nosocomial TB outbreaks. An increased incidence of TB medical personnel.

In connection with the peculiarities of the transmission of TB infection, it is essential that the nosocomial transmission of TB from patient to another patient, employee of a medical institution for the visitor. Recent studies performed in different countries have shown that health care workers treatment-and-prophylactic establishments, including anti-TB profile, contact with patients that release into the environment of the office, are at increased risk of infection and developing TB disease. In the absence of effective preventive measures significant factor in the spread of tuberculosis, including its drug-resistant forms, is the transmission of the pathogen not only in TB facilities, and in medical institutions of different profile, as well as in prisons, orphanages and residential living homeless [2,5].
TB prophylaxis includes many components that have different degrees of importance and require decisions at various levels. These include: early detection and proper treatment of TB, examination of contacts and timely detection among them are infected with M. tuberculosis, the correct tactics of isolation and placement of patients in a hospital, depending on their degree of epidemiological danger - bacillers, the presence of drug resistance, HIV status, and other, fulfilling all the requirements of sanitary and antiepidemic regime, including measures to reduce contamination of the air, the requirements of respiratory protection and personal hygiene etc.

The World Health Organization (WHO) in the prevention of nosocomial TB [2], speaking about the measures to control the spread of infection, divides them into three directions:

- administrative control - a set of administrative measures aimed at prevention of spread of infection from contaminated areas in clean areas and including including planning of premises, the organization of work, personnel training methods, providing the reduction of the risk of infection and other;
- engineering control of the engineering measures providing safety of the premises and the environment) - a complex of engineering (design and technical) measures aimed at reducing the concentration of infectious aerosols in the air - forced ventilation, the use of effective devices air disinfection by filtering, radiation and other;

- personal (individual) control activities in relation to risk groups - susceptible contingents of patients and medical workers and directed at individual protection of their respiratory system.

Measures of administrative control include:

- regular evaluations of the risk of spread of infection in different departments, different procedures and manipulations and for different categories of employees and visitors;
- development, approval, execution and regular adjustment plan for infection control the institution as a whole and/or its individual units;
- use of the protocols of detection, isolation, inspection and effective treatment of potentially infectious TB patients; division of streams of patients (sort) at admission and split their placement in the hospital (for the first time identified active forms of smear positive, MDR TB, the combination of TB with HIV and parenteral hepatitis, chronic forms of TB without-negative and etc);
- development of algorithms of safe working process; the implementation and monitoring of its implementation by staff, patients and visitors of the institution (for example, restrictions for patients of MBT + cases, observance of requirements of safety in laboratories to work with infectious hazardous material in biological safety cabinets etc);
- regular training of all the employees of health facilities and sanitary-educational work with the patients and the population;
- employee survey to detect infection and active TB disease.

Activities in the source of infection include the development Plan for the prevention of nosocomial TB infection in TB institutions, containing, including planning work in the premises, excluding the intersection of dangerous infectious patients (materials) with healthy or abacillary people (clean material), a survey of outpatient TB suspects, isolation, sorting and treatment TB patients, training of health workers, health education for patients and the population [2,5].

The plan of measures for infection control should describe in detail the whole complex of measures aimed at minimizing the risk of infection with tuberculosis medical and other personnel, as well as persons receiving medical care in TB hospitals. The plan should identify deadlines and benchmarks of efficiency and timelines of its key components (number of new cases among personnel, the number of patients who left the territory of the hospital, the number

of purchased respirators and surgical masks, the share of employees not using respirators in high-risk areas, etc).

The plan approved by the head of the institution where also appointed persons responsible for execution of the plan IR in General and, if necessary, its separate components.

Directions of the engineering control [2,5] include: ventilation and air conditioning; ultraviolet irradiation; stand-alone air cleaners of various types (UV filter - HEPA filters, electrostatic, electromagnetic, etc.).

Priority attention when carrying out control over the state of environmental give premises and areas with a high risk of tuberculosis transmission:

boxes and rooms for TB bacillary patients, especially with MDR; intensive care; rooms for induced sputum; bronhoscopic, dental, X-ray; operating rooms; section rooms; laboratory for tuberculosis control. The specific choice of measures for environmental control in each setting depends on the layout of the latter, local climatic conditions.

Despite the implementation of measures of administrative and engineering controls, in hospitals often occur areas of increased risk of M. tuberculosis infection with high concentrations in the air of infectious aerosols. In these cases, to prevent infection, measures of individual respiratory protection are applied - the third level of infection control. It is realized through the application by medical workers of certified respirators and patients - surgical masks. Personal respiratory protection should be used by health workers in the premises or conditions of high concentration of infectious aerosols or aerosol MDR or XDR infections, and bacillary patients – at the contact with healthy people or other, abacillary patients.

The application of the personal respiratory organs protection measures significantly reduces the risk of inhaling infected with Mycobacterium tuberculosis air. Effective use of this component of infection control is impossible without the necessary administrative support. Development and introduction of infection control in TB institutions proves the necessity of using similar approaches in General health care institutions, especially outpatient clinics.

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Evaluation of Eating Behavior Study of a Representative Sample of Students

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ABSTRACT

The paper presents the results of studying the organization of diet, eating habits, food quality in a representative sample of the 1st year students of all North Eastern Federal University named after M.K. Ammosov faculties and institutes. Studying of existing nutrition disorders allowed investigating the initial state of health of students and identifying individual risk factors of diseases of the gastrointestinal tract.

Keywords: students, diet, eating habits, eating behavior, nutrition, eating disorders, risk factors, morbidity structure, digestive diseases, healthy lifestyle.

INTRODUCTION

The usefulness of the diet determines the health of the population affecting the growth and physical development, efficiency, adaptive capabilities, morbidity and life expectancy.

Malnutrition problems of young people are alarming not only health professionals but the Russian government. Thus, the Russian Consumer's Inspection together with the Institute of Nutrition has prepared a bill "Policy Framework of the Russian Federation in the field of healthy nutrition of the Russian Population for the period up to 2025."

In this document the special attention is focused not only on technical regulations preparation to ensure the quality and safety of food products and the system of educational programs in the field of healthy lifestyle and nutrition [6].

Diseases of the digestive system are on the one of the leading places in the morbidity structure of NEFU students. Modern nutriciology attaches a great importance to nourishing factors in digestive diseases development.

Both quantitative and qualitative nutritional disorders strongly change the digestive system job, being one of the risk factors of chronic diseases of the digestive system.

Therefore, our studies on the profiling of dietary regime disorders among 1st year students of the NEFU will become the basis for curative measures, a healthy diet and lifestyle propagation.

The aim of the investigation was to study the eating pattern, dietary habits of students, and prevalence of the digestive system diseases.

MATERIALS AND METHODS

The work was performed in the Institute of Health NEFU named after M. K. Ammosov. Clinical and functional studies were carried out in the Institute of Health of NEFU at the Department of Therapy with endocrinology in cooperation with Department of therapeutic physical training of NEFU. The study was approved by a local ethics Committee of Yakut Scientific Centre of Complex Medical Problems of Siberian Department of Russian Academy of Medical Sciences.

The Work was performed 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 «Adaptive capacity and health of the indigenous population of Yakutia in conditions of modernization of the socio-economic system». The informed consent was obtained prior to study at the students, the questionnaire was conducted anonymous.

A representative sample of 800 people from a representative group of 1st year students of all faculties and institutes of the NEFU (3400 pers.) was created by random numbers method in Excel program. 649 people of the representative sample took part in investigation that represents 81,1%. There were 292 Male (45%) aged 15 to 26 years (18,8±1,5), Female – 357 (55%) aged 16 to 30 years (18,8±1,5) of 649 paticipants. The ratio of male and female was equal which corresponds with the general population of 1st year students of all faculties and institutes of the NEFU.

Digestive system questionnare contained 26 questions to implement the diet, presence of complaints and risk factors for diseases of the digestive system.

RESULTS AND DISCUSSION

The results of the studies are evidenced of sufficiently high prevalence of such chronic diseases of the gastrointestinal tract as chronic gastritis, cholecystitis, colitis between students. The morbidity of the irritable bowel syndrome, biliary dyskinesia, intestinal dysbiosis, chronic viral hepatitis is reasonably high.

Diseases of the digestive system ranked the 2nd place in the structure of the total incidence (143,3 per 1,000 students).

Chronic gastritis, irritable bowel syndrome, biliary dyskinesia and intestinal dysbiosis provided substantive input into the structure of digestive diseases (tabl. 1).

Results of the survey revealed gross violations of the diet. Breakfasts and meals was absent to 36,4% and 2,5% of the students, hot meals – to 64,4% and 44,2% of the students noted the presence of the late dinner in the mode of the day.

Table 1

Class XI Diseases of the digestive system to 1000 students

Name of nosological form	Index
Chronic gastritis	61,6
Functional disorders of the stomach	1,5
Gastric ulcer	1,5
Esophagitis	1,5
Biliary dyskinesia	16,9
Chronic cholecystitus	7,7
Intestinal disbiosis	16,9
Irritable bowel syndrome	20
Chronic colitis	7,7
Chronic enteritis	1,5
Chronic pancreatitis	1,5
Chronic viral hepatitis	3,1
Total	143,3

Carbohydrates were dominated in the students' diet that represents 69,4%, proteins – 50,5%, fats – 29,9%.

Results of investigation of preferred students dishes are shown in figure 1.

The young people mostly preferred the fried foods: cakes, chips, grilled meat and fish. Steamed water food (soups, cereals), was only 32,4%.

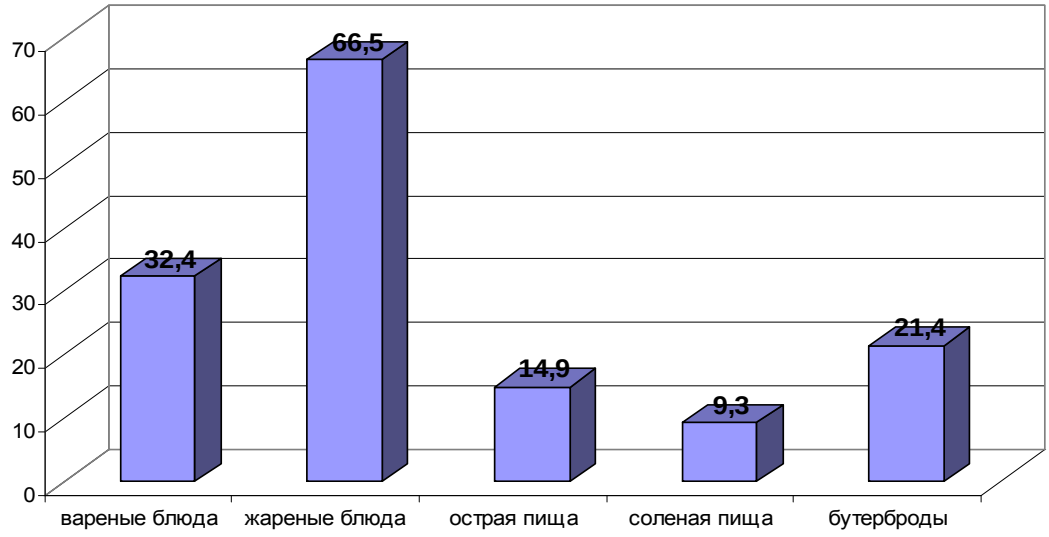

Figure 1 Food Quality.

The maintenance of the water regime is reflected in figure 4. The water and carbonated beverages was consumed by 70,5%, 70,2%.

Study of the complaints and objective examination of the students revealed the presence of dyspeptic disorders and pain in the epigastric area, in the right upper quadrant and along the large intestine. 27,9% of respondents complained of the presence of epigastric pain, the right and left upper quadrant and umbilical area –19,1%, the right iliac area – 11,8%, the left iliac area – 0,3%. Pains were mainly nagging (42,7 %), sometimes stabbing (30%) and obtuse (27,3%).

There were the following dyspeptic disorders: problems of gulping (3,8%), vomiting (13%), regurgitation (13,6%), salivation (16,6 %), nausea (26,9%), epigastric burning (38,1%) burping (42,6%), hoove (57%), obstruction (18,4%), diarrhea (20,2%).

12,4% of respondents noted a weight loss, 37,2% – generalized weakness among the students presented problems of dyspepsia in the epigastric area.

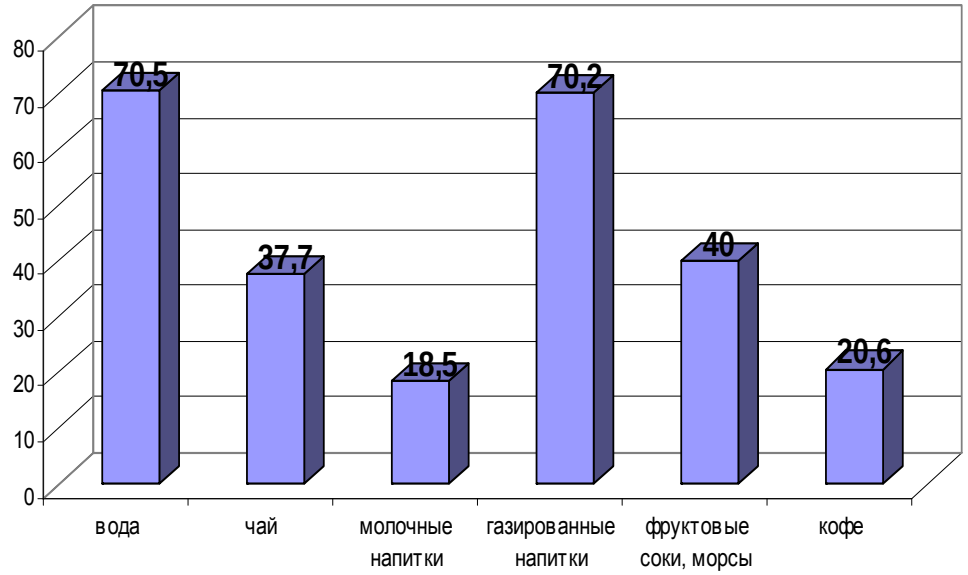

Figure 2 The maintenance of the water regime

Analysis of the pain factors showed the pain syndrome was associated with consuming of fat (33,1%), indigestible (20,7%), dairy (7,6%), sweets (6,2%), fancy bread (4,8%), spicy (14,5%) and fried food (13,1%).

The results analysis indicated the diseases of the digestive system in 1st year students of the NEFU took the 2nd place in general structure of morbidity and amounted to 143,3 per 1,000 students.

High incidence of diseases of the digestive system identified in 1st year students of the NEFU agrees with those of other authors who conducted a similar study among university students [1, 2, 4, 6].

It should be noted that adolescents and young people tend to irregular food intake for various reasons. They are characterized by large breaks between food intake, dry diet, monotonous nutrition, etc. Thus, our analysis of the data showed that the main disorders were associated with irregular food intake, violations of repetition factors and diversity of food. The bulk of the students prefered to "bite" sandwiches, desserts (cakes, candy, chocolate, etc.) that is causing functional disorders and the development of digestive diseases. There were serious abnormalities in adherence to diet (no breakfast, hot lunch and, conversely, the presence of a late dinner) [8].

O.S. Sushko analyzed catering among university students in Tomsk, which confirms that the nutrition of young people is characterized as irrational, i.e. unbalanced and with disturbed dietary regime.

It is known that a change of the quality of food has the negative impact on the health of the students. So modern young man consumes a lot of refined and easily digestible protein and carbohydrates, canned foods, which leads to disruption of meals balance for basic food ingredients. It is noted that the students eat randomly with long breaks during the day, and a percentage of people eating in the evening mostly at bedtime with plenty of food high. Most students said that they have no desire to cook, preferring fast foods, chips, juice packs, fizzy drinks. A lack of fresh fruits and vegetables riched in vitamins, macro-structural (calcium, phosphorus, magnesium), essential trace elements (zinc, copper, selenium) and fiber is observed in eating habits of the students. This fact is consistent with a number of other modern scientific data [2, 3, 5, 10, 11] examined in other regions of the Russian Federation regarding malnutrition, lack of vitamins and essential elements in the diet.

Anamnesis study and clinical examination suggests that an abnormality and violation of food quality in 1st year students involves a violation of the functional state of the digestive system, and then to the occurrence of chronic diseases, which is confirmed by similar studies devoted to the relationship of diet and gastroenteric symptomatology [7, 9, 12, 13, 14].

CONCLUSION

Preliminary study of the diet and dietary habits of the 1st year students of the NEFU showed the presence of gross violations in their dietary habits and food intake. Irregular eating, big breaks between meals, dry diet, monotonous diet, eating primarily of carbohydrate products, carbonated drinks are typical for young people, improper cooking of food is widespread.

The highest rate of nutrition-related morbidity of the digestive, endocrine, sexual and other system diseases is observed as a result of an unbalanced diet that requires in-depth study of actual nutrition and introduction of wellness activities.

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Development of Mobile Ophthalmologic Aid in Yakutia

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ABSTRACT

The article presents the data about first mobile eye groups founded in Russia at the end of XIX for fight against trachoma that was a prototype of modern mobile crews for solving other problems. In order to approach the highly skilled ophthalmologic help to a residence of patients as well as to ensure its availability to any patient mobile operational modules on the basis of different types of transport were established. The similar module in the Republic of Sakha (Yakutia) was created in 2002 that allowed approaching the qualified ophthalmologic help to the population of remote areas of the republic, to reduce economic expenses and patient's psycho-emotional stress, and also to liquidate the list for planned treatment for cataract patients in RS (Y).

Keywords: trachoma, eye groups, mobile ophthalmosurgical team.

Eye illnesses were widespread among the population of Russia even before the World War I. In 1910 there were 300 thousands patients all over the country rated at 21,4% with trachoma, 19,2% with blindness due to glaucoma, 13,5% with cornea diseases, 12,1% with smallpox, 4,9% with eye blennorrhea of newborns, 4,8% with optic nerve atrophy, 3,9% with diseases of central nervous system and vascular periorbita, 3,7% with syphilis and eye injuries and 1,8% with congenital blindness [1].

The Mariinsky blinds' protectorate founded in 1881 by the state secretary K.K. Grotom, named after the empress Maria Aleksandrovna was engaged in organizing ophthalmologic aid in the country. It worked as a charitable organization. The protectorate established clinics, organized permanent "oculist" aid posts, supplied them with tool kits and glasses, drugs [8]. By the initiative of the Prof. L. G. Bellyarminov, the chairman of the blinds' protectorate

department, in 1893 a project of organization of eye groups (EG) was worked out fighting against blindness prevalence among the population and implementation of surgical help to patients with eye diseases [12]. At the same year first seven flying EG started working, in 1894 they numbered 21, in 1895 − 24, in 1896 − 31, in 1897 − 33. In four years (1893-1896) on behalf of the protectorate 150016 patients received medical aid and 38867 eye operations [8] were performed. Further the number of groups began to increase gradually and reached the maximum in 1900 (38 EG). Then this number decreased and fluctuated between 30 and 32 (except 1904-1905 when it went down to 17-19). In reports of the protectorate about blinds it was informed that for 20-year term 535 groups were working in the European Russia, Siberia and the Caucasus. 1008564 primary patients, on the average 1885 per one group were accepted. EGs performed 177384 larger and 142316 small operations and operational grants. EGs were to prepare personnel for further permanent job on local places [5] as well.

In the Soviet period People's Health Committee of RSFUR, the Russian Society of the Red Cross and People's Health Committee of some republics took part in supplying equipment and supporting flying EG. EGs went to autonomous republics and districts which had been mainly affected with trachoma or in such places, where no satisfactory eye help was conducted.

The first eye group was sent to Yakutia by the blinds' protectorate on the petition of the Yakut governor Kraft and the Yakut bishop Makariy in 1908. The group worked in Olyokma, Yakutsk and Namsky village, 2446 people [2] were examined.

In Yakutsk a special eye aid post was opened in 1910, it started functioning at the Yakut civil hospital, as a constant office. Since then the head Grinyova – Pleskaya conducted periodic journeys to other districts. During the Civil war all these measures fell apart and only in 1924 in Yakutia the project of organizing the eye groups was recreated. The main activity of this group organized by the Siberian Office of the Red Cross concentrated in the Yakutsk district , in uluses (regions) where the group led by Dr. Titov S. performed mass inspections of the population along with out-patient and stationary activity. In 1925 high mortality of the Yakut population and enormous distribution of trachoma induced the Commission of Academy of Sciences of the USSR to organize medical and sanitary groups for studying health of the population of the Yakut Autonomous Socialist republic (YaASSR). The group staff was headed by the ophthalmologist Dorofeyev V. N., they tending to work for 1,5 years. Due to remote areas of the territory in the republic, the study was concentrated first of all in the central regions of the republic: Vilyuysky and Olyokma. As a result of the conducted research, among 2667 patients with pathology of eyes, high incidence of trachoma at indigenous people, especially among

women was revealed. The prevalence of trachoma among Yakuts in the Vilyuysky district amounted to 49,22%, in Olyokma 27,75%. Trachoma was the main reason of one eye blindness, in the Vilyuysky district making 72%, in Olyokma - 33,3%. As a result of the research, several measures were taken to prevent trachoma [2]. In 1925 in Yakutsk the trachoma clinic with in-patient department on 15 beds was established, it involving the organization of network of ophthalmologic establishments in Yakutia, having changed the quality of the work profile. Besides rendering the medical aid, diagnostic and prophylactic measures were carried out thanks to EG in areas of the republic. EGs went out to settlements annually since 1925 to 1942, rendering ophthalmologic help to the population of remote areas of the republic. Within the clinic there was a permanent EG having own staff and budget as well. As a result of such fruitful work such severe disease as trachoma, being one of the common diseases of the past, had been liquidated by 1962 [13].

After eliminating trachoma the time revealed new, not less complex problems: glaucoma was identified in most cases as pathology of the vision organ and has become the main reason of blindness for the last years; cataract as a disease of people at senior age; protection of children's eyes; medical aid to patients with eye injury; providing all types of correction; introduction of modern technologies in diagnostics and treatment of eye diseases; preparation of highly qualified specialists; extended preventive measures among the population at the level of new and modern requirements.

Till 1957 in the republic patients with glaucoma were not officially reported. According to data of the outpatient department within the trachoma clinic in 1955 314 patients with glaucoma were registered, in 1961 their number increased up to 910 persons. Glaucoma was seriously taken into account after adopting the order of Ministry of Health of RUFSR No. 275 "About measures on early reveal of patients with glaucoma". In order to prevent glaucoma at early stages mass inspections of the population were conducted at people of 40 years old and over. [13].

With the purpose to approach the highly skilled ophthalmologic help to remote residence of patients, ensuring its availability to everyone with eye diseases, in 1986 S. N. Fedorov worked out a project of establishing intersectoral scientific and technical complex "Eye Microsurgery" ISTC "EM"). The ophthalmologic clinic equipped with modern equipment – the scientific research institute ISTC "EM" and its branches in 11 cities of Russia became material resources of a new form of the ophthalmologic help: Cheboksary, Khabarovsk, Novosibirsk, Irkutsk, Krasnodar, St. Petersburg, Ekaterinburg, Orenburg, Volgograd, Tambov, Kaluga [3].

The idea of forehanded introduction and distribution of the latest technologies to remote settlements as well as available ophthalmologic help led to creation of mobile operational modules. Firstly such ophthalmologic operations with modules were carried out in the 70-s of the XX century by American surgeons in clinic "Orbis" on a basis of the plane. In our country the similar operation was performed in 1978 in ISTC "EM". According to the project of the research laboratory of experimental and clinical eye surgery the Finnish firm "Kiitokori" constructed a mobile operational bus [14]. Further there were three generations of such modules. The latest is executed by Carrus firm on the basis of Volvo buses where a modern operational and a diagnostic compartment are located. Such modules allow carrying out about 70% of all operations worked out in ISTC.

Now each ISTC branch in its regions has medical and diagnostic offices, advisory offices, rehabilitation and diagnostic posts, mobile diagnostic laboratories and operational on the basis of different transports. The main purposes of regional offices are to approach the qualitative ophthalmologic help to the residence of patients, to provide early identification of eye diseases, to carry out out-patient treatment and rehabilitation [7].

Despite the huge potential of ISTC "EM» with mobile structures, there is badly need for high-tech medical care (HMC) for regions of Russia. For the purpose of HMC availability, clinics of the republican level began to apply the experience of mobile structures in their work. As an example, the mobile ophthalmologic crew of the Kemerovo regional clinical ophthalmologic hospital carries out 100 departures of medical and sisterly crews to the remote rural areas [10-4]; the mobile medical microsurgical group of the Regional ophthalmologic clinic of the Tyumen region carried out 145 departures [6].

The territory of the Russian Federation is extensive and, despite efforts of experts of the health care organization, it is actually difficult to carry out qualitative medical care with the use of modern medical and diagnostic equipment to inhabitants of the remote settlements, and as well as with use of the latest techniques and approaches to prevention and treatment of various eye diseases.

The Republic of Sakha (Yakutia) is the subject of Russia, not having analogs on natural and territorial conditions on the planet. Considering features of the region, the huge territory (3,1 million sq.km), being in the permafrost zone, with remote means of communication and small population density (0,3 on 1 sq.km) for rendering the organizational and methodical, advisory and treatment-and-prophylactic help to the population of the remote areas of the republic in 2002 mobile teams including 2 surgeons and the 1 nurse were organized on the basis of the Yakut

Republican Ophthalmologic Hospital (YROH). During the period from 2002 to 2008 the mobile groups were carried out to the areas where on the basis of central regional hospitals there were equipped ophthalmologic offices and an ophthalmologist who was revealing and preparing patients to operation as well as keeping a close watch on patients after operational treatment. The surgery of cataract was carried out by a method of extra capsular extraction with implantation of a rigid intraocular lens (RIL), with subsequent seam overcasting. A patient with glaucoma had surgical infiltration – deep sclerectomy with back sclera trepanation. For that period 1111 operations were performed concerning cataract of 74%, glaucoma – 8,5%, others – 17,5%. Due to high cost of work in mobile teams, such activities were carried out within the voluntary medical insurance (VMI). The date 2008 was considered to be of great significance in the work of mobile crews in RS (Ya), when in cataract surgery the technique of ultrasonic facoemulsification (FE) with implantation of IOL flexible models was introduced. In glaucoma surgery due to long postoperative rehabilitation of patients, not infiltrating deep sclerectomy was selected for operations. As this type of operation is carried out only at an open-angle form and ineffective at a closed-angle form, the number of glaucoma operations carried out was reduced. With introduction of hi-tech methods in the work of mobile crews, the total amount of mobile operations carried out in 2010 as compared with data of 2008 increased up to 131%, including cataract - 195%, while glaucoma surgery decreased by 17% [10].

Organizational forms of providing mobile ophthalmologic services are difficult, but are quite feasible. Besides advantages of high-quality and qualified ophthalmologic help, social and economic aspects that allow patients to save considerable funds on travel expenses are of great importance and promote the availability of high-quality medical care to social and unprotected parts of the population [11].

Despite the growth of surgical activity, requirements for the expeditious treatment of cataract remained insufficient as the mobile crews worked within VHI, that causing to a long-lasting list for planned surgical treatment. In order to increase the availability of hi-tech ophthalmologic help to the republic population on February 10, 2011 the order of the Ministry of Health (MH) of RS (Y) No. 01-8/4-117 "About organization of a mobile ophthalmologic team rendering medical help within the Territorial Program of Obligatory Medical Insurance (TP OMI) of RS (Ya)" was approved. The crew consisted of 2 ophthalmologist-surgeons, 1 ophthalmologist-diagnostician , 1 anesthesiologist, 2 operational nurses, 1 anesthesia nurse, 1 medical technician, 2 drivers. Financing of the medical services rendered by mobile ophthalmologic surgical teams (MOST) was made by medical insurance companies on tariffs

and methods of payment, approved by the General agreement on payment of the medical services within TP OMI in RS(Y). Payment options of medical services for “completed case” of treatment were made according to the standards of medical care approved by MH RS (Y). In the work of MOST standards on the following nosology were approved: cataract, glaucoma, progression and position of eyelid anomalies, pterigium. Since 2011 to 2012 the mobile crew performed 1078 operations. Introduction of the standards and "the case" treatments performed by MOST on free charge allowed to increase the amount of operations up to 31%; cataract surgery for 44,2%, glaucoma for 24,2%. During 11 years 3011 operations were performed by MOST YaROH, 84,3% of them concerning cataract. The number of operations carried out in a year has increased on 3 times. Since 2011 for rendering the consultative assistance to the population in remote areas of the republic doctors of the policlinic of YaROH have carried out mobile aid, having examined 1781 patients from 2011 to 2012 [9].

Approaching the qualified ophthalmologic aid to the population of the remote republic settlements promoted the reduction of economic expenses and patents’ psycho-emotional stress, as well as liquidating the list for planned treatment of cataract patients in RS (Ya). Considering high working costs of the mobile structures, in the remote areas of the Arctic region it is necessary to organize ongoing mobile specialized service on the basis of the Yakutsk Republican Ophthalmologic Hospital, giving medical aid within the Territorial Program of Obligatory Medical Insurance (TP OMI) of RS (Ya).

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Some Aspects of quality of Rendering of Specialized Help to Patients with Combined Craniocerebral Trauma in Multifield Hospital

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ABSTRACT

The treatment of patients with combined craniocerebral trauma (CCT) is associated with clinical difficulties, because its symptoms are very different from other combined trauma. Nowadays the standards in treatment of such patients are not developed fully. Earlier, in 1970-80 years both domestic and foreign researchers have proposed the different scales and schemes for the estimation of such injuries. The State Hospital No 1 named by Pirogov N.I. in Moscow is considered as a multiple trauma centre with powerful diagnostic and clinical base. The article deals with the analysis of disease histories of 400 patients with combined craniocerebral trauma which were treated in this hospital for last five years. The most of the patients was brought to the hospital for the first days after trauma. The quality of pre-hospital and clinical aid was assessed; the mistakes of Reception Department were analyzed. Based on the results of analysis the system of complex inspection of patients with high-energy trauma was embedded in the State Hospital No 1.

The tactics of treatment of long bone fractures was performed depending on the severity of head injury. The computer tomography (CT) with the so-called program «combined injury» was embedded. It means the complex approach: patients, who received high-energy and heavy criminal injury, were examined by CT of the brain, chest and pelvis at the same time. It revealed that 13.7% of patients had fractures of the ribs, 8.3% of cases - fractures of the pelvis, 22.8% of cases - signs of brain injury. For the last 10 years the clinic actively applies the principle of one-stage surgical treatment by the participation of multiple surgical teams. Preliminary results of this study let us to conclude that using of this modern approach and sufficient technical equipment allow considering the head injury as not a contraindication to the active choice of tactics of treatment of fractures of long tubular bones.

The key words: emergency medical aid combined craniocerebral trauma, quality of medical help.

One of the key points of modern traumatology is the problem of treatment of combined injuries.

It has been noted that in the age period between 20 to 60 years, the mortality rate from injuries is twice than the numbers of mortality cases after cardiovascular diseases and cancer [6]. The most common type of combined injuries is the combined craniocerebral trauma (CCT), which is caused not only by the mechanical destruction of the skull and brain, but also others parts of human body. It is about 80-85% of all combined injuries and it is about 18.3% to 68.5% of the mortality [3].

One of the priorities in the concept of development of health system of the Russian Federation to 2020 is to maintain and strengthen public health and improving the quality of medical care [1]. The treatment of patients with CCT is associated not only with certain clinical difficulties, but also with significant material costs [5]. That's why the main aim faced by doctors in the treatment of this group of patients is the assessment of the damage that allows predicting the nature of pathological changes, to plan and develop an optimal strategy of rendering qualified medical aid to such patients.

Currently, the standardization of treatment of patients with CCT is not sufficiently developed. As it was noted by many researchers, in order to use any standards in treatment, first of all, it is necessary to elaborate common terminology and classification correctness. The state hospital No 1 of Moscow, according to the systematization of staff composition and its work with patients, proposed by V.V.Zhedrenok and E.K.Gumanenko in 2008, is a trauma centre of the first level, i.e. represents a multi-profile hospital with powerful diagnostic and clinical base. The teams on duty consist of specialists of different profile. The hospital is provided by all conditions for rendering of specialized medical care in case of any damage [7].

The analysis of disease histories of 400 patients with combined craniocerebral trauma which were treated in this hospital for last five years, was done. The most of the patients was brought to the hospital for the first days after trauma. The main reason of trauma is traffic accidents (56,75%). It is necessary to underline that the type and localization of injury depend on kind of transport and the role of victim during this accident. For example, the multiple injuries outside the scull are often among pedestrians (30,3%). Katatrauma is on the second place among the reasons (35.3%), primarily due to increasing of occupational injuries in the construction of tall buildings and a failure. Not less frequent reason of CCT (21.5%) becomes criminal injuries (beating), and the so-called «alcohol trauma», i.e. injuries sustained by a person in alcoholic intoxication.

The classification of CCT is based on two principles:

1. Localization of damages outside the skull

2. The ratio of craniocerebral and outside the skull damage on their severity.

The patients were classified considering localization of outside the skull damages that puts its mark on the clinical picture and surgical tactics (Fig.1).

Except the local factors, the diagnostic characteristics, therapy and result depend on the ratio of damages on their severity. It is obliged to divide every type of CCT on four groups:

1. 7,0% - hard CCT (brain injury of nor very hard and hard degree, the compression of the brain depressed skull fractures, intracranial haematomas, hydromas) and heavy outside if skull damages (broken hip, pelvis and internal injuries).
2. 18,0% - hard CCT and non-hard outside the skull damages (closed fractures of forearm bones, feet, 1-3 ribs without damage to the lung).
3. 7,0% - non-hard CCT and hard outside of skull damages.
4. 48,0% - non-hard CCT and non-hard outside of skull damages.

The symptoms of CCT are different significantly from the features of combined injuries of other localizations. The traumatic shock of the patients with intracranial lesions is rather peculiar due to the fact that the mechanisms of brain injury and traumatic shock are different. That is the reason why this pathological process was called as the «syndrome of mutual complication» in the literature [2].

Thus, edema and swelling of the brain tissue are showing its traumatic defeat. So, when CCT central nervous system (CNS) gets like «double blow» [4].

That is why the unit system of independent assessment is needed in order to get the adequate assessment of the severity of the victim with combined injuries and methods of treatment that allows comparing the result of the treatment of such patients.

In 70-80 years of the 20th century, the different scales and schemes of the estimation of the injuries have been proposed by our and foreign researchers. The scale of gravity TS (Trauma Score), is used in this paper. This scale was proposed by the American researcher H.Champion in 1982. It allows estimating only the total condition of the patient, without taking into account the nature and location of damage. Total scores can be from 1 to 16, the estimation of patient's condition is expressed in percent. This scale is convenient in everyday practical use. It is necessary to underline that this scale reflects the state of the Central nervous system, as it includes scale Glasgow. For characteristics of patients with CCT and fractures of the long bones, it is important to give the general description of the injury, taking into account the presence or absence of the victim of traumatic shock and severity of damage to the Central nervous system.

The estimation of the quality of prehospital and hospital care was done. The assessment of the quality of primary care is carried out on the analysis of 200 accompanying sheets of the emergency ambulance of the patients with CCT. The following errors were revealed: no immobilization of fractures in patients with CCT (15.0% of cases); no indication on trauma of the skeleton system with its presence in 23% of cases; underestimation of the severity of head injury in 7% of cases. These errors are related to the lack of complaints from patients because of loss of consciousness and objective examination of the patients.

Next, the analysis of the main errors in the work of the admission department was done. It showed that no inspection of traumatologist in the documents of the disease was reported in 5.5% histories, underestimation of the severity of head injury - in 7.0% histories. “Small” fractures of skeleton system were missed by a trauma specialist at survey in 9,0% of cases.

Because of these problems at the level of the admission department of the state hospital №1 the system of complex examination of patients with high-energy trauma was introduced: traffic accident, Calatrava and criminal injury. Also these patients were examined by the neurosurgeon and surgeon, This system has been used for the last five years and has significantly reduced the number of medical errors associated with under-reading damage in patients with CCT.

The introduction of computed tomography (CT) with the so-called program «combined injury» (when the patients who received high-energy and heavy criminal injury occurs CT of the brain, chest and pelvis) revealed fractures of the ribs with 13.7% of patients, with fractures of the pelvis in 8.3% of cases, having CT signs of brain injury in 22.8% of cases.

The tactics of treatment of long bone fractures was performed depending on the severity of head injury. Treatment of fractures in patients with mild head injury is not difficult. In this category of patients the stabilization of damaged fractures was made with the correction of the treatment of brain injury on the recommendations of a neurosurgeon, using standard methods of osteosynthesis, regardless of the presence of head injury.

The special approach is needed for the category of severely head-injured patients, accompanied by compression of the brain hematoma and requiring operative neurosurgical intervention. For the last 10 years the principle of one-stage surgical treatment of multiple surgical teams, with preliminary agreement tactics of operational manuals and consultation of specialists such a resuscitator, an anaesthetist, a trauma surgeon, neurosurgeon is used actively in our clinic.

The operational risks, the volume of estimate blood loss, duration of operational benefits are analyzed. If the condition of the patient on a scale TS is ot less than 10 points, the volume of estimate for intracranial hemorrhage intervention does not exceed 500 ml, and there is damage of one segment, the full osteosynthesis using standard of maloinvazivnogo techniques is applied. The amount of head injury in this category of patients was as follows: a bruise of a brain moderate and severe with compression of sub - and epidural hematomas 24 patient depressed fractures of the bones of the cranial vault - 26 patients with fractures of the skull base with profuse basal liquor - 4 patients. Single-stage operations performed serially or in parallel. In severe head injury with injury of the brain in the form of significant outbreaks of injury and intracerebral hematomas methods of osteosynthesis were used that do not require large expenditures of time and are not accompanied by loss of blood is of plate osteosynthesis, the imposition of a rod devices of external fixation, regardless of the state on the scale of TS. Moreover, we consider this type of intervention as a necessary life-saving manipulation, 32 operations for patients with severe head injury.

The case of the surgical treatment of the patient X., 25 years old, who was injured in a traffic accident was analyzed in the article. Diagnosis: hard combined trauma, open head injury, hard brain contusion, depressed comminuted fracture of the parietal and temporal bones left with transition on the basis of the anterior and middle cranial fossa, epidural hematoma (Fig.2), an open fracture of both the right shin with severe soft tissue injury (IIB extent by Kaplan). The condition of the patient was estimated in 10 degree according to the scale TS, level of consciousness – 9 degree to the scale Glasgow. For the first days after trauma simultaneously surgery was performed: resection craniotomy to remove fragments and epidural hematoma by volume of 80 ml, PHO wounds leg and fixation of fragments rod apparatus. In subsequent produced bandaging wounds Shin water-soluble ointment, skin plasty with local tissues and split-flap (Fig. 3). As a result, the system was dismantled by the end of 3 weeks, the patient is on trial at the end of three weeks stabilized (Fig. 4). Made stabilizing the osteosynthesis of the tibia intramedullary pin rod with distal and proximal block (Fig. 5).

Thus, the preliminary results of the conducted researches permit to conclude that the head injury is not contraindication to the active choice of tactics of treatment of fractures of long tubular bones of the patient because of using the modern approach and sufficient technical equipment. This choice is determined by a set of parameters, which are the severity of the injury and the extent of the disorder of the vital functions of the victim, that allows not only to improve the quality of life of the patient, but also to reduce the duration of his stay in hospital.

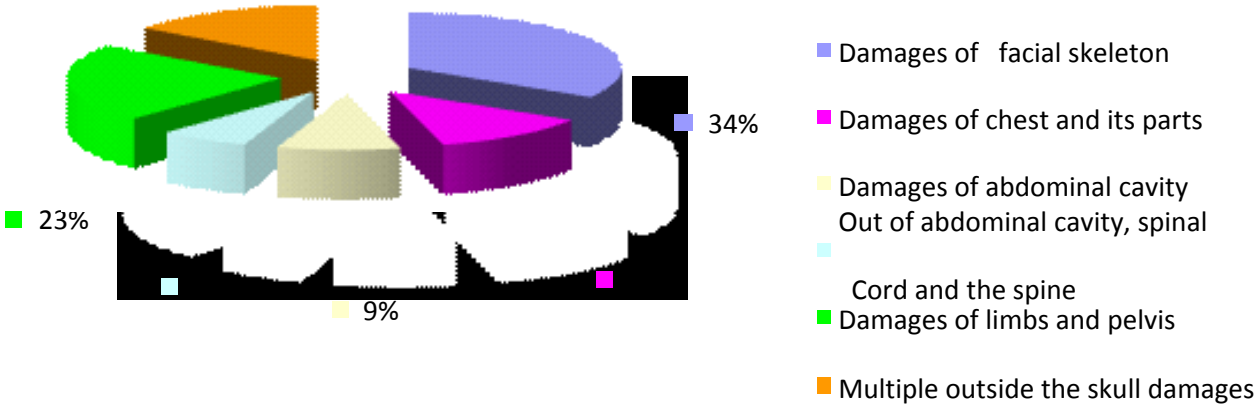


Fig.1. The distribution of patients with different places of their outside the skull' injuries.

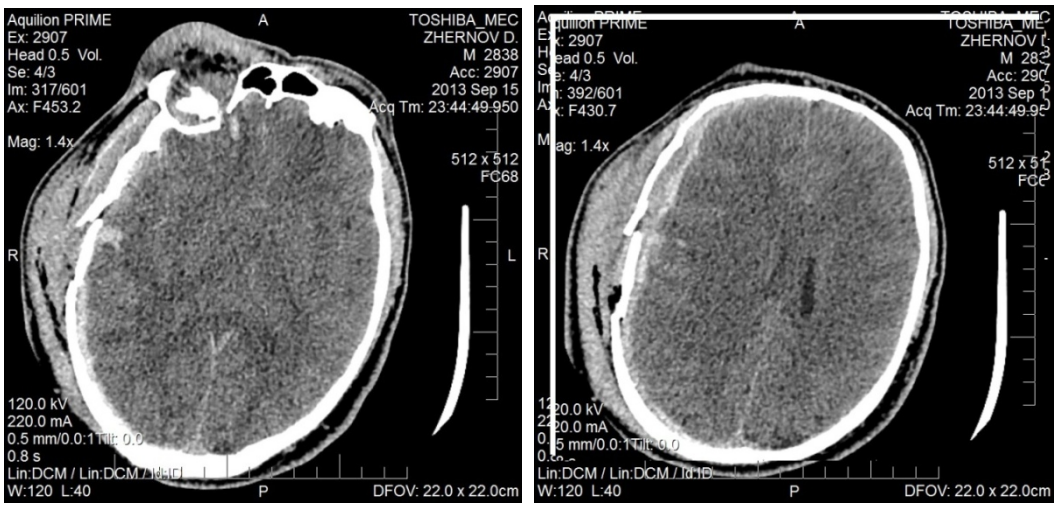
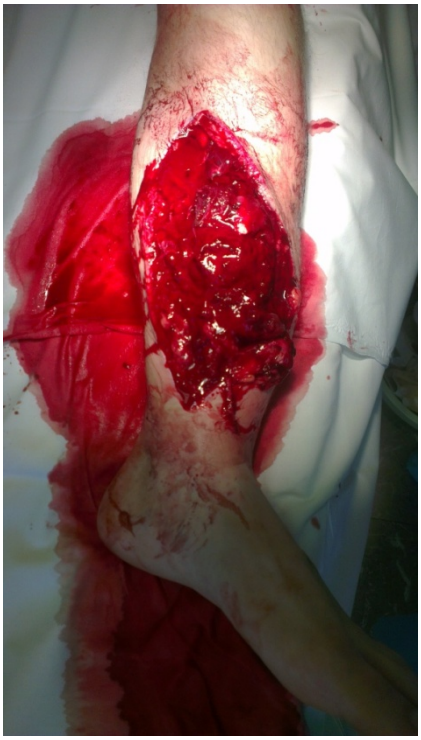
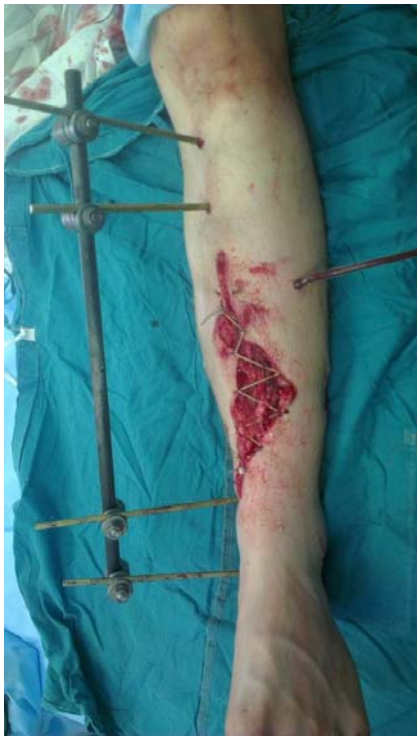


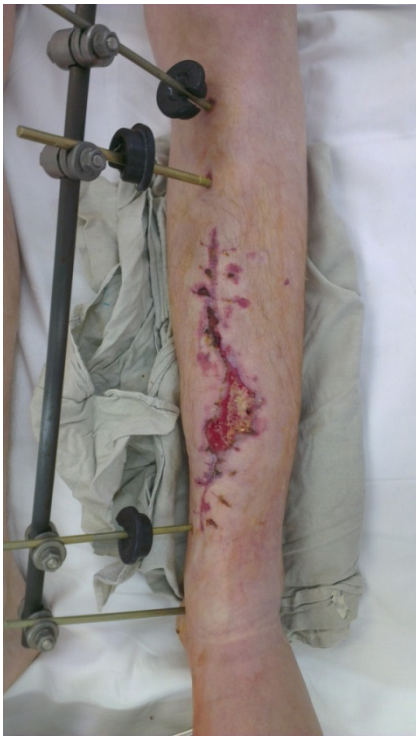
Fig. 2. CT of the patient X, of 25 years old



a

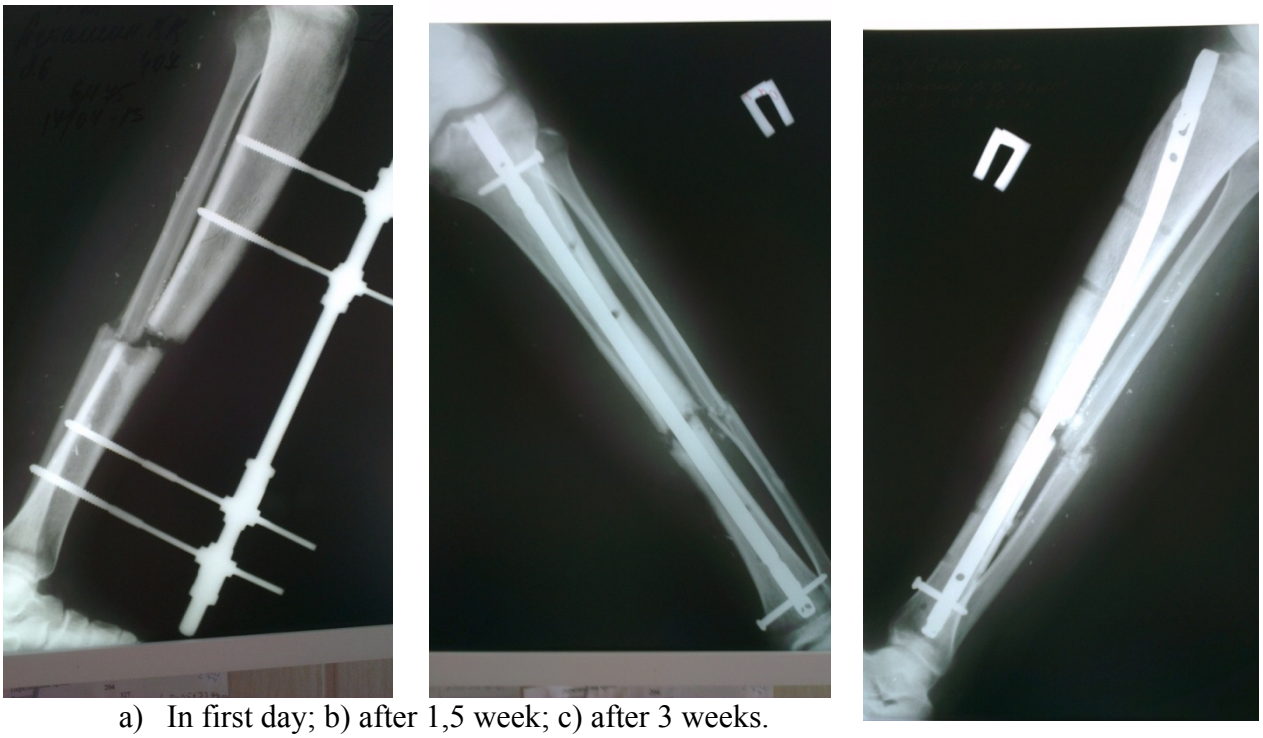


b



c

Fig. 3. The appearance of lower leg of the patient X, of the 25 years old:



a) In first day; b) after 1,5 week; c) after 3 weeks.

Fig.4. CT of the brain of the patient X, of the 25 years old, after the craniotomy for the first three days

a b c

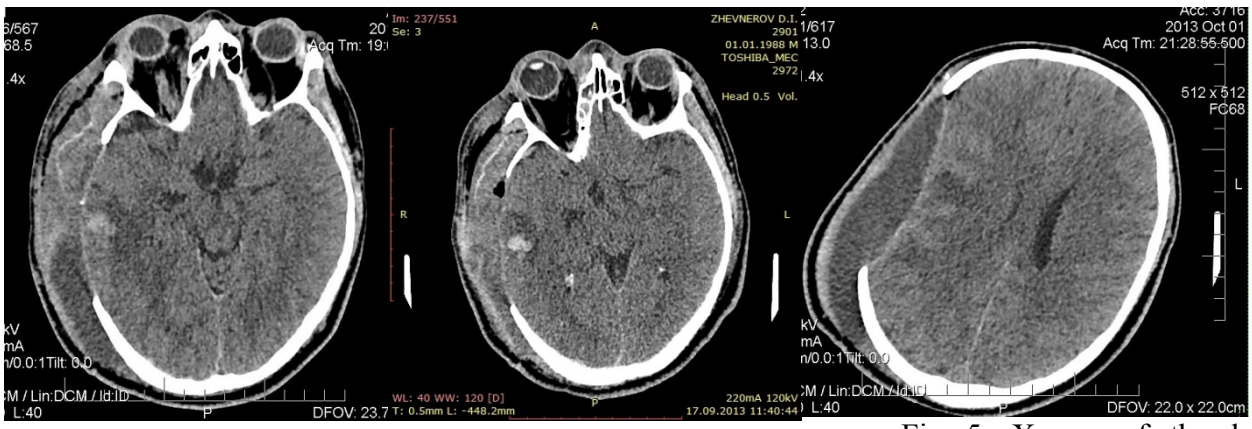


Fig 5. X-rays of the lower bones of the patient X, of the 25 years old, after the imposition of external fixation device (a), after running stable osteosynthesis pin lock (б,в)

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Actuality of Studying the Epidemiology of Osteoporosis and Bone Metabolism at the Regional Level

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ABSTRACT

We present the review of current literature on epidemiology and diagnostic challenges of osteoporosis in Russia and other countries. Abnormalities of peak bone mass formation are one of the risk factors of osteoporotic fractures. This review points out the importance of osteoporosis patient database creation, as well as reference database of mineral bone density on the regional level.

Keywords: osteoporosis, epidemiology, bone mineral density.

Osteoporosis it's a chronic systemic and progressive metabolic bone disease or clinical symptom at the other diseases, which is characterized by a decrease in bone density, violation of bone microacrchitectonics and increase of bone fragility due to imbalance in bone metabolism with predominance of destruction process over the formation process, decreasing bone strength and increasing risk of fractures [18].

THE ACTUALITY OF THE RESEARCH

Osteoporosis in Russia and in the world is a very prevalent disease. Every three woman and four men over 50 years old suffer from osteoporosis. Social significance of osteoporosis is determined by its consequences - bone fractures, which increase disability, mortality and large financial costs. Among urban population of Russia in 24% of woman and 13% of men aged 50 years and older at least one osteoporosis fracture is indicated [8].

Hip fractures have the most severe social and medical consequences. As shown by epidemiological research, the hip fractures among the Russian population in different age groups is on average 174.78 per 100 000 population (174.78 males and 275.92 for women), the frequency of the distal forearm fractures - 426 2 (201.1 in men and 563.8 for women) [1]. Frequency of osteoporotic fracture increases with age, and the frequency of hip fractures is growing exponentially [8].

In the USA hip fracture is more than 432 000 hospitalizations, 2. 5 million visits to doctor per year. Cost of treatment of hip fracture in 2005 year was estimated in 17 milliards USD. Hip fracture is 14% of all fractures, arising in result of falling and 72% of all costs allocated to fractures treatment. According to opinion of American doctors due to the aging population the

number of hip fractures and associated with osteoporosis fractures could be doubled or tripled by 2040 year [19].

Cost of one year hip fracture treatment with rehabilitation in Belgium is 15000 EUR, in Britain 12000 GBP, in Canada 26.5 CAD. Cost of only hospital patient treatment with hip fractures in 2000 is 1 166 765 RUB per year [8].

According to the Department of Health of the Government of Moscow patient life duration with 40% risk of osteoporotic fracture is equal to patient life duration with cardiovascular disease [9].

In the regions of Russian Federation, particularly in Saratov region, Saratov city, according to the research [10], osteoporosis disease was increased per year. In 2006 with osteoporosis 255 people were revealed, in 2007 the number of patients with osteoporosis increased by 138.4% (353 patients). Further in 2008 the number of patients increases by 165.8% (423 patients). Hip fracture dynamic in Saratov region have undulant form, in 2007 compared with 2006 (4315 patient) number of patient with forearm fracture was decrease by 44% (2849 patient). In 2008 forearm fracture was increase by 4.3% in compared with 2006 (2907 patient).

In four Russian cities (Bryansk, Vladimir, Pervouralsk and Yaroslavl), who was participated in multicenter epidemiological hip fracture research [1] (including pertrochanteric and subtrochanteric fractures) compared with previous research is noted an hip fracture increase by 2.5 times.

According to A.N. Kommisarov and G.A. Pal'shin [11] research in Republic of Sakha (Yakutia) during 1995-2010 for 15 year research, hip fracture increases in 4.8 times. An increase of frequency of fractures with age was revealed. We marked increase rates of woman in age group over 65 and men over 75 years. Morbidity is increasing amid growing proportion of the number of older persons in the population with simultaneous reducing of population total number.

Russia's territory is located mostly to north of 55 parallel of north latitude, which is a significant risk factor in the population of vitamin D deficiency due to lack of sun exposure in the winter months.

Russia's population consumes not enough calcium from food, as demonstrated by various research groups. 10-15 years old children on the average consume less than a glass of milk or milk product a day [2]. In Moscow population only 6% boys have normal calcium level; there were no girls with normal level of calcium. Calcium level affected to bone mineral density [6]. Significant decrease of calcium consumption was noted among student [17], woman-doctors in

reproductive age [4] and postmenopausal woman [14]. Lowest level of calcium was in the days of the Orthodox Church fasting.

The Institute of Nutrition of Russian Academy of Medical Sciences analyzed the nutrition of Russia population (more than 9000 people) in different age groups during the 1994-2003. The lowest level of calcium consumption has been revealed in men and women over 55 years, and also in groups of 18-30 years [7].

Yakutsk is located on the 62 parallel of north latitude, where the winter duration reaches up to 7 months in the year, with short light day. Such climatic features do not contribute enough insolation and production of vitamin D that have a negative effect to the assimilation of consumed calcium [11]. Middle level of vitamin D in children and teenagers in Yakutia is 14 ng/ml. In winter 60% healthy children have vitamin D deficit, in summer – 10%. In 32.5% healthy children the secondary hyperparathyroidism was detected [3], which corresponds to the rachitis disease prevalence among young Russia children from 54 to 66%.

In Russia in 2010 the social program called “Osteoscreening Russia” started; the main premise for its start was a discrepancy of official statistics on the prevalence of osteoporosis and the results of epidemiological research. Risk factors for osteoporosis were studied, using a questionnaire; screening of bone mineral density was done. The analysis showed that 20% of the researched people had osteoporosis and 28% - osteopenia in forearm. The decreased bone density for 1 standard deviation increases the risk of vertebral fracture for 1.7 times, hip fracture for 1.8 times [20]. It was also found that among the population in different regions of Russia in 50 years and older have 3 and more risk factors of osteoporotic fracture. Furthermore, it was found that the majority of the surveyed consumed less than half of the required daily by age of calcium with food that needs diet correction or calcium prescription in the form of pharmacological supplements [12].

According to recommendation of Russia Association of Osteoporosis (2009) the standard method for bone density research is a dual energy x-ray absorptiometry (DXA) [16].

In Russian Federation there are 167 densitometers. The device was unevenly distributed in country: Half of devices (52%) were installed in Moscow and others – in central hospitals of region centers. Even in Moscow region only 63% of the doctors are able to guide the patients for densitometry [4]. In the Cities of Siberia and Far East are working only 16 devices. Equipment is in Moscow densitometers is 8.6 in other Russia regions – 0.6 per 1 million of population [12].

In modern clinical practice the bone mineral density is compared with reference database which was installed by densitometer manufacturer (USA). Most acceptable method of the bone mineral density measurement is use of the T- and Z- parameters [5].

Use of U.S. database standards in other countries can be reason for distorting data of the epidemiology of osteoporosis (According to S.S. Rodionova and A.V. Krivova) [13].

According to Y.V. Khrapova results of research in 2007 [15] variability of mineral bone density middle value in different age and gender population groups of Novosibirsk, Moscow, St. Petersburg and white population of USA was revealed.

Thus, today in Russia is not enough objective data on the true prevalence of osteoporosis required for proper and thorough planning and organization of medical care.

There is a need to create a reference database of patients with osteoporosis at the regional level. Studying bone mineral density among the population of Republic of Sakha (Yakutia) by comparing it with standard values of the population of white USA citizens (Lunar database) can identify groups of risk and to develop a regional program for the prevention of osteoporosis and osteoporotic fractures.

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