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INFLUENCE OF MINERAL WATER ON THE COURSE OF EXPERIMENTAL GASTROPATHY

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The study was carried out in two groups of male Wistar rats: control (modeling of gastric ulcer disease) and experimental (modeling gastric ulcer + prophylactic seven-day use as a drink in free access to mineral water). The rats were euthanized, the stomachs extracted and the effectiveness of the gastroprotective action by macro and microscopic examination was evaluated.

The results of the study showed that in animals that received mineral water, as compared to the control of the disease, the amount of erosions of the gastric mucosa and the Pauls Index decreased by 3.3 times, the density of the inflammatory infiltrate decreased by 2.5 times and the thickness of the mucous membrane of the stomach increased by 1.3.

Thus, the seven-day use of mineral water as a drink in free access is accompanied by a significant simplification of the experimental «indomethacin» ulcer in rats.

***Key words**: mineral water, experimental gastropathy, prophylaxis.*

Gastritis and gastric ulcer continue to be one of the most common pathologies of the gastrointestinal tract [1]. Modern medicine has significantly advanced its capabilities in the treatment and prevention of gastropathy. There are a number of effective drugs with proven antiulcerogenic effect. Their use in accordance with generally accepted prescribing regimens makes it possible to achieve significant success in the treatment of gastritis and gastric ulcers [2].

At the same time, it is obvious that the issues of food and drink hygiene have a high relevance in the field of non-drug support of such patients. The scientifically based use of special drinking regimens may contribute to delaying the recurrence of gastropathy and to general improvement in the quality of life of patients. In this context, the attention of hygienists continues to be attracted by mineral and medicinal-table waters [3]. The study of their antiulcerogenic properties can make a significant contribution to the development of medical prevention and rehabilitation of patients with gastric ulcer and gastritis.

Altai Krai, due to its unique natural diversity of bioresources, possesses numerous sources of mineral waters that can potentially be used in gastroenterology. We were interested in studying the effect of mineral water extracted from the wells in Altai Krai on the course of experimental gastropathy in animals, which was the purpose of this study.

Materials and methods

The study was conducted on 20 male Wistar rats aged 2-3 months and weighing 250-280 g, divided equally into two groups: control (modeling of gastric ulcer) and experimental (modeling of gastric ulcer + preventive seven-day intake of "Zavyalovskaya" mineral water as a drink in free access). Animals were kept in standard conditions. The studies were carried out in compliance with the principles of humanity set forth in the directives of the European Community (86/609 / EEC) and the Helsinki Declaration, in accordance with the "Rules for work with experimental animals".

The non-steroidal anti-inflammatory drug indomethacin was used as an ulcer-forming factor. The model of indomethacin damage to the stomach of rats was reproduced by a single intragastric administration of indomethacin at a dose of 60 mg/kg in 1 ml of saline. Before the administration of indomethacin, 7 ml of distilled water was intragastrically administered to the control group for 7 days. Under similar conditions for 7 days, animals of the experimental group were given "Zavyalovskaya" mineral water as a drink in free access. 6 hours after the administration of the ulcer-forming factor, the rats were euthanized, the stomachs were removed, washed in distilled water, the material was fixed in a solution of 10% neutral formalin and the effectiveness of the gastroprotective action was assessed by macro- and microscopic examination.

Histological preparations were stained with hematoxylin and eosin. Histochemical detection of neutral mucopolysaccharides was performed using the Schiff reaction, acid mucopolysaccharides were detected using alcian blue staining (pH 2.5). The density of the inflammatory infiltrate was calculated in 1 mm^2 using Avtandilov G.G. ocular grid. Morphometric measurements of the gastric mucosa (GM) were performed using the morphometric program Image Tool 3.0. The Pauls index was calculated by multiplying the average

number of destruction in the stomach per animal by the percentage of lesions in the group and dividing by 100%.

Statistical analysis was performed using the program Statistica 6.0. Results are presented as mean values (M) and standard error of the mean (± m). Statistical processing of the results was performed using the non-parametric Mann-Whitney test. Differences were considered significant when the validity coefficient value was $p < 0.05$ [4].

Results and discussion

The results of the morphological study showed, that in animals of the control group, in the gastric mucosa (GM), there was a phenomenon of pronounced erosive gastritis. In a macroscopic study, the surface of GM looked bumpy with clearly visible destructive changes in the form of pulverized hemorrhages, surface erosion, point and strip-like ulcers and the phenomenon of hyperemia. Destructive changes were detected in all 10 experimental animals of the control group. The number of ulcers and erosions in the stomach varied from 8 to 17, averaging 13.0 ± 1.6. Pauls index was 13.

According to the results of a microscopic study, it was established that GM looked atrophic, the glandular and pathogenic epithelium was in a state of degeneration. The thickness of GM was 285.9 ± 10.3 microns. In histochemical studies using the Schick method, the columnar epithelium of the superficial divisions of GM fluid showed a weak reaction to neutral mucopolysaccharides. At the same time, acid mucopolysaccharides were poorly expressed in the cells of the deep sections of the gastric fossae. Necrotic changes in the ulcers reached the muscle layer. The bottom of the ulcers was represented by fibrin and purulent-necrotic masses (Figure 1). The density of the inflammatory infiltrate in the submucosal layer was 4,704.0 ± 313.3 cells in 1 mm^2. Inflammatory infiltrate is represented by lymphocytes, plasma cells and a large number of neutrophils.

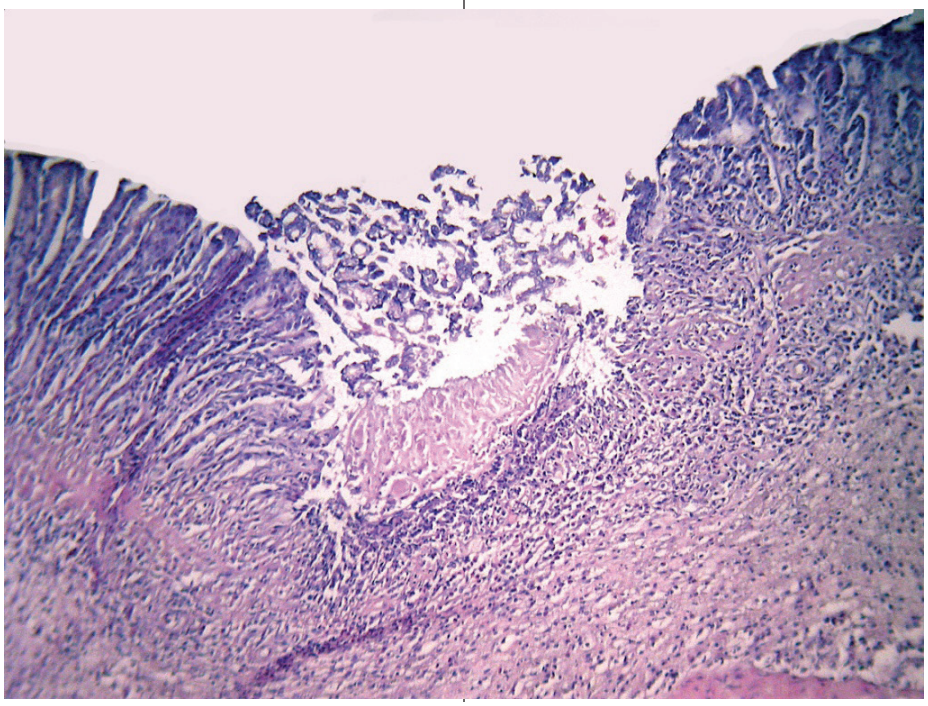

Figure 1 - Surface erosion in GM of rats by exposure to indomethacin. Stained with hematoxylin and eosin. Zoom x 100.

Against this background, in animals that received mineral water, the macroscopic GM on most sites looked even and smooth, in some areas weakly expressed destructive changes in the form of dust-like hemorrhages and small point erosions are determined. Such destructive changes were found in all 10 animals of the experimental group. The number of erosions was from 2 to 8, on average - 4.0 ± 0.9, which was 3.3 times less than in the control of the disease ($p < 0.001$). The Pauls index similarly decreased relative to the control group and amounted to 4 ($p < 0.001$).

Microscopic examination of GM in rats of the experimental group revealed signs of minor atrophy (Figure 2). The thickness of GM increased relative to the control of the disease by 1.3 times and amounted to 358.9 ± 10.8 μm ($p < 0.01$). In histochemical studies using the Schick method, the columnar epithelium of the superficial divisions of GM fluids gave a focal pronounced reaction to neutral mucopolysaccharides. Acid mucopolysaccharides were moderately detected in cells of the deep sections of the gastric fossae. Mild inflammation was observed in the submucosal layer. The density of the inflammatory submucosal infiltrate in animals of the experimental group was 2.5 times less than in the control of the disease, amounting to 1872.0 ± 225.7 mm^2 ($p < 0.001$).

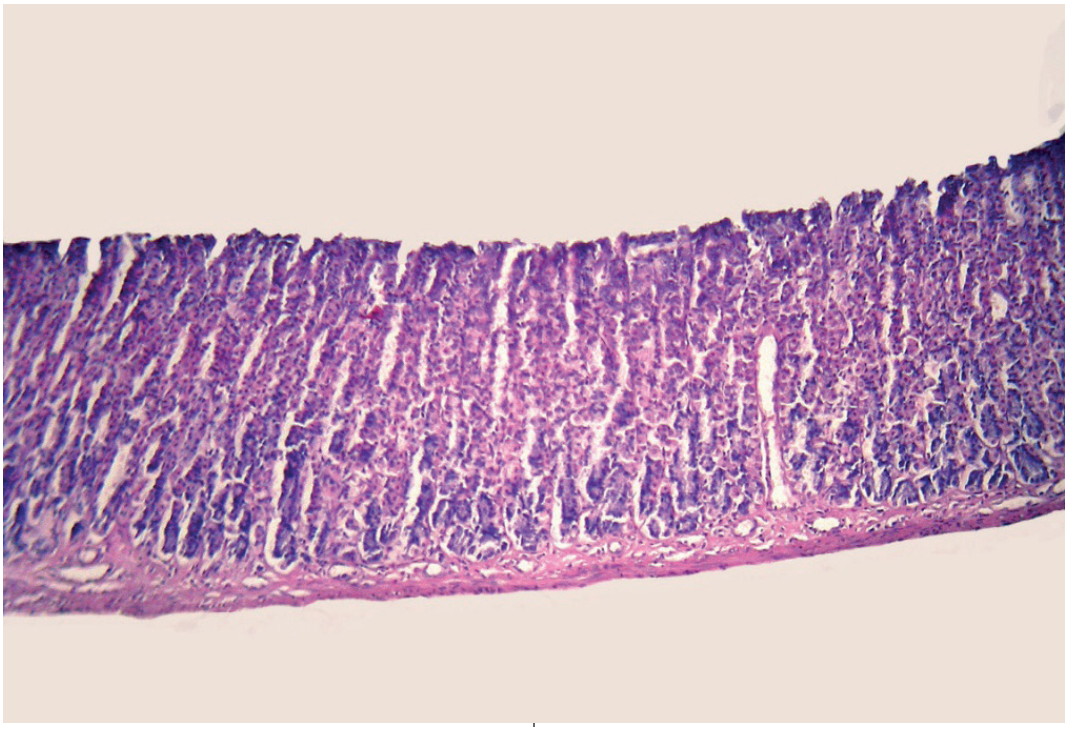

Figure 2 - Signs of atrophy and lack of erosion by the use of "Zavyalovskaya" mineral water. Stained with hematoxylin and eosin. Zoom x 100.

Thus, in the course of the study, a pronounced gastroprotective effect exerted by mineral water when consumed as a free-access drink by experimental "indomethacin" ulcer was established. This was evidenced by a significant decrease in the number and aggressiveness of erosion of GM, a decrease in the density of inflammatory infiltrate and an increase in the thickness of GM of rats compared to the control of the disease.

As is known, indomethacin is a non-steroidal anti-inflammatory agent, the basis of the mechanism of which pharmacological activity is the ability to inhibit cyclooxygenase and disrupt the synthesis of prostaglandins, including the so-called gastroprotective prostaglandins that regulate the synthesis of protective mucus in the stomach. Under its influence in the stomach, the synthesis of protective mucus is weakened, which leads to the damage of GM by hydrochloric acid produced in the stomach, and thus ulcerative damage to the stomach develops [5]. According to the manufacturer, the water is chloride-sulphate calcium-magnesium-sodium with a salinity of 4.5 - 6.5 g/l. High content of Mg^{2+} cations is especially noted. It is possible that, at least in part, this can explain the high gastroprotective activity of water identified in our experiments. It is well known, that magnesium ions in the stomach neutralize hydrochloric acid, thereby weakening its aggressive effect on GM. It should be added, that similar results regarding the antiulcerogenic action of magnesium salts have been obtained by other researchers [6].

Conclusion

The seven-day use of mineral water as a free-access drink is accompanied by a significant relief in experimental "indomethacin" ulcers in rats, as evidenced by a significant decrease in the number and aggressiveness of GM erosion, a decrease in the density of inflammatory infiltrate and an increase in the GM thickness.

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OPERATIONAL EXPERIENCE OF THE SCIENTIFIC LABORATORY OF THE ENVIRONMENTAL AND HYGIENIC PROFILE ON ACCREDITATION FOR THE COMPLIANCE WITH GOST ISO/IEC 17025-2009 "GENERAL REQUIREMENTS TO THE COMPETENCE OF TESTING AND CALIBRATING LABORATORIES"

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The article presents the analysis of the preparation of the laboratory for hygienic monitoring of the working conditions of the Institute of Occupational Health and Industrial Ecology of the Altai State Medical University for the accreditation procedure in the National Accreditation System for compliance with the interstate standard GOST ISO/IEC 17025-2009 "General requirements for the competence of testing and calibration laboratories". Particular attention is paid to the procedure for introducing methods for studying the physical, chemical and radiation factors of the environment and the production environment as a key component of the successful accreditation of the testing laboratory, as well as the need to integrate the quality management system of the scientific laboratory of the ecological and hygienic profile into the overall quality management system of the university.
Key words: *accreditation, testing laboratory, quality management system, methods for performing measurements, in-laboratory testing, interlaboratory comparative tests.*

Federal Law No. 412-FZ of December 28, 2013 "On Accreditation in the National Accreditation System" regulates the need for research by various laboratories, including scientific, at a high professional level, which guarantees reliable results and the provision of quality services to customers within established area of accreditation [1]. At the same time, the accreditation procedure in the Russian national accreditation system is a confirmation by the national accreditation body (currently the Federal Accreditation Service or RusAccreditation) that the legal entity meets the accreditation criteria, which is an official evidence of the competence of the testing laboratory to operate in a specific area of accreditation. In turn, the accreditation criteria, approved by Order No. 326 of the Ministry of Economic Development the Russian Federation dated May 30, 2014, imply the mandatory availability of a quality management system in the testing laboratory, necessary professional training of competent personnel, material and technical base, all necessary documents in the field of standardization, rules and methods of research, metrologically certified measuring instruments and test equipment, programs for conducting internal audit, laboratory testing and inter laboratory comparative tests (ICT), corrective and preventive action plans [2]. In this regard, along with the accreditation criteria, the main document regulating the activities of all laboratories regardless of the number of personnel and their test activities, and which should be used for accreditation in the national system is the interstate standard GOST ISO/IEC 17025-2009 "General requirements to the competence of testing and calibrating laboratories"[3]. The accreditation of the testing laboratory of the Institute of Occupational Hygiene and Industrial Ecology of the Federal State Budgetary Educational Institution of Higher Medical Education of the Altai State Medical University of the Ministry of Health of the Russian Federation was carried out on the basis of this standard. By the decision of the Federal Accreditation Service No.AA-471 of July 16, 2017, the laboratory of hygienic monitoring of the working conditions of the Institute of Occupational Health and Industrial Ecology of the ASMU was entered into the Register of Accredited Agents, including the National Part of the Unified Register of Certification Bodies and Testing Laboratories of the Customs Union. The laboratory has been assigned the number of the accreditation certificate RA.RU.21E94 dated July 16, 2018, which will be valid indefinitely with periodic confirmation of the competence of the laboratory to the accreditation criteria. For the first time in the history of ASMU, the testing laboratory of environmental, hygienic and labor protection directions received recognition of its independence and competence in the National Accreditation System, and the experience of accreditation will be taken into account by other laboratory units of the university.

Materials and methods

The field of accreditation of the laboratory of hygienic monitoring of working conditions contains 57 techniques that can be conventionally differentiated into five main groups of methods: 1) methods

for studying the physical factors of the production and environment (electromagnetic fields, microclimate parameters, ultraviolet, infrared and laser radiation, noise, vibration, ultrasound and infrasound, light environment, air ion and aerosol composition of air); 2) methods for studying ionizing radiation (gamma, alpha, beta, x-ray and neutron radiation, radon in water, indoor air and soil air); 3) research methods of chemical factors in the air of the working area (quantitative chemical analysis of manganese, iron, sulfuric acid, caustic alkalis, vitamin B6 and cephalosporin antibiotics, as well as measurement of concentrations of 22 chemicals in indoor air using an ANT-3M gas analyzer); 4) research methods of pharmaceutical indicators of the quality and safety of plant materials and medicinal plants (the amount of flavonoids in terms of rutin, humidity, total ash); 5) research methods of the severity and intensity of the labor process in accordance with the Order of the Ministry of Labor and Social Protection of the Russian Federation No. 33n dated January 24, 2014 "On Approval of the Methodology for conducting a special assessment of working conditions, Classifier of harmful and (or) hazardous production factors, report forms on conducting a special assessment of working conditions and instructions for filling it out." All 57 methods were officially introduced into the practice of the laboratory by a permanent commission of the Institute of Occupational Health and Industrial Ecology from December 2016 to July 2018 with the preparation of accompanying reporting documents - a program for introducing measurement techniques, an act of implementing a methodology, a working protocol for measuring and a final protocol for laboratory tests, which is made for the customer of laboratory tests. In terms of the implementation of scientific and laboratory research methods, 14,885 measurements of production environment factors (microclimate, light environment, air ion composition, electromagnetic radiation, noise, vibration, chemicals and aerosols) were carried out at 375 workplaces of employees of the Altai State Medical University. In addition, in accordance with the agreement on scientific and practical cooperation with the medical institution "Sanatorium of the Tsentrosoyuz of the Russian Federation in Belokurikha", 1550 measurements of the factors of the working environment at the workplaces of medical personnel, as well as a study of the equivalent dose rate of gamma radiation equivalent to the equilibrium volumetric activity of radon in indoor air (320 measurements) and the specific activity of radon in medicinal water (130 measurements).

Results and discussion

During the accreditation of the laboratory, the quality management system of laboratory tests, the material and technical base of the laboratory, measuring instruments, provision of necessary documentation, qualifications and experience of employees, as well as monitoring the implementation of the measurement procedure according to the main methods implemented in the laboratory were assessed: microclimate study, light environment, electromagnetic fields, noise, vibration, indicators of environmental radiation safety, harmful chemical substances in the air of the working area and medicinal plants. In particular, it was necessary to measure the parameters of microclimate, illumination, pulsation coefficient and brightness of the working surface, electromagnetic fields of industrial frequency (50 Hz), dose rates of gamma radiation, EEVA of radon -222 and toron-220 according to the guidelines of MU 2.6.1.2838 -11 "Radiation monitoring and sanitary-epidemiological assessment of residential, public and industrial buildings and structures after the completion of their construction, refurbishment, reconstruction according to radiation safety indicators", and also to perform a study of the local vibration of a hand-held mechanized instrument according to GOST 31192.2.-2005, an equivalent noise level according to the "work function" strategy according to GOST ISO 9612-2016, demonstrate procedures for conducting studies of harmful substances in a welding aerosol for manganese, examine the concentrations of cephalosporin antibiotics (cephalexin) in the air of the working area and determine the amount of flavonoids in terms of rutin in an elder black FS.2.5.0008.15. Of particular interest is the analysis of verification methods for conducting a quantitative chemical analysis of harmful substances in the air of the working area. The examination of the verification of technical competence of the laboratory in this area was carried out in the analytical hall. The expert examined the room, installed equipment, dishes, checked the inscriptions on the prepared solutions for photometric and potentiometric studies, the availability of methods for their implementation. Next, the expert checked the weighing room and the washing room, the storage place of the chemicals. Having walked around the premises, the expert assessed the declared analytical equipment in detail - the PE-5400UF spectrophotometer, making a note about the absence of a metrologically certified program for constructing calibration schedules for it in the laboratory. This remark was eliminated during the audit.

Then, the next day, the laboratory staff had to demonstrate the method of air sampling of the working area at the workplace of the welder in the laboratory premises declared in the field of accreditation and determining the concentration of manganese in the welding aerosol (MU 4945-88). Sampling was carried out using the electric aspirator PU-4E, in strict accordance with the method of selection without significant comments from the expert. Next, a sample of air was pre-

pared to determine the manganese concentration on the spectrophotometer. It was necessary to confirm the stability of the calibration dependence on the control solution. Stability has not been confirmed, and new graduation was built. The stability of the calibration characteristics directly depends on the quality of chemical reagents, which are used to obtain colored solutions. To increase the reliability of the obtained results, facilitate calculations and verify stability during accreditation, an application for the purchase of a metrologically certified calibration dependence creation program was submitted. The result of the study is less than the detection limit. To confirm the stability and accuracy of the analysis result, a control solution of manganese of unknown concentration prepared from a standard solution was measured. The result was accepted as satisfactory, taking into account the magnitude of the measurement error.

Further, the expert assessed the process of building a calibration dependence, according to the method for determining the content of cephalosporin antibiotics declared in the field of accreditation (MU 3994-85). Graduation is constructed without comment with a linear correlation coefficient close to 1.00.

The laboratory staff successfully completed all nine control tasks covering all areas of the accreditation field - the study of physical, chemical and radiation factors, as well as pharmaceutical indicators. The expert group was shown that the laboratory has a quality control system for laboratory tests that is integrated into the overall quality management system of the university and is a combination of the organizational structure, responsibility, procedures, types of work, capabilities and tools aimed at providing conditions that allow to obtain reliable measurements and objective results.

According to the results of the inspection, an on-site expert examination report was drawn up in which an unambiguous conclusion was drawn that the laboratory of occupational health and industrial ecology of the hygienic monitoring of working conditions of the Institute of Occupational Health and Industrial Ecology complies with the accreditation criteria and GOST ISO/IEC 17025-2009 "General requirements for the competence of testing and calibrating laboratories".

Conclusion

The main key component of the successful accreditation of an environmental-hygienic testing laboratory is the preparatory work in the following areas:

1) The phased development of a laboratory quality management system integrated into the university's overall quality management system.

2) Creating the necessary material and technical base and equipping the scientific laboratory with modern high-tech equipment to measure the physical, chemical, radiation factors of the surrounding and working environment, the severity and intensity of the labor process, the study of pharmaceutical indicators of the quality and safety of medicinal plant materials.

3) Timely metrological certification of measuring instruments and testing laboratory equipment.

4) Careful selection and placement of laboratory personnel, development and implementation of staff development programs.

5) Conduction of interlaboratory comparison tests at accredited laboratories on the basic research methods.

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1. Federal Law No. 412-FZ of December 28, 2013 "On Accreditation in the National Accreditation System".

2. Order of the Ministry of Economic Development of the Russian Federation of 30.05.2014 No. 326 "On approval of accreditation criteria, list of documents confirming compliance of an applicant, accredited person with accreditation criteria, and list of documents in the field of standardization, compliance of which with applicants, accredited persons ensures their compliance with the criteria of accreditation."

3. GOST ISO/IEC 17025-2009 "General requirements to the competence of testing and calibrating laboratories"

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PREVENTIVE ACTIVITIES CONCERNING TICK-BORNE VIRAL ENCEPHALITIS AMONG THE ADULT POPULATION OF THE SIBERIAN FEDERAL DISTRICT

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The article presents the results of the analysis of the effectiveness of specific and non-specific prophylaxis measures against tick-borne viral encephalitis among adults in the regions of the Siberian Federal District. As a result of the factor analysis, the leading factors determining the formation of a trend in the incidence of tick-borne encephalitis in adults in the regions of the Siberian Federal District have been identified, including: mite viral resistance (r = 0.44; p <0.001); acaricidal area (r = -0,33; p <0.001) and the adult immunodeficiency index (r = -0.41, p <0.001). Based on the results of the analysis, the authors developed recommendations for optimizing the package of preventive measures for this infection in the Siberian regions.

***Key words**: tick-borne encephalitis, prophylaxis, vaccination, immunization.*

Introduction

The epidemic process of tick-borne viral encephalitis in the regions of the Siberian Federal District (SFD) is characterized by the presence of pronounced natural and anthropogenic risk factors for the development of the disease. The high proportion of the rural population (30-50%), characteristic of the Siberian regions, provides an intensive frequency of contacts of the population with foci of infection and, consequently, cases of the disease in risk groups. In the overall morbidity structure, the majority of cases occur in the adult population of the SFO regions due to occupational risks and the nature of household activities. The possibility of the development of the chronic form of the disease, the severe course of the disease, the disability of the sick persons can cause the disability of the adult working population. The combination of the causes and consequences of the disease necessitates the development and practical implementation of regional immunization schedules that take into account the specific features of the tick-borne encephalitis epidemic process in each individual region and are based on the definition of risk groups for developing the disease.

The purpose of the study was to determine the characteristics of the epidemic process of TVE in the cohort of the adult population of the Siberian Federal District, as well as to analyze the effectiveness of specific and nonspecific prevention measures carried out in the regions.

Materials and methods

The study was based on a retrospective analysis of the data on the incidence of the adult cohort in the regions of the Siberian Federal District. The materials of the study included data from regional health services, the Federal State Statistics Service, the Federal Service for Supervision of Consumer Rights Protection and Human Welfare from 2000 to 2017. Forms of State statistical recording No. 5 "Data on preventive vaccinations" and No. 5 "Data on infectious and parasitic diseases" were used. Processing and analysis of the data was carried out in the program "Statistica 13.0".

Results and discussion

The study period of 2000-2017 was characterized by a pronounced trend aimed at reducing the incidence of TVE among the population of the Russian Federation. There was registered a decrease of 3.1 times from 4.1 ± 0.03 0/0000 to 1.3 ± 0.03 0/0000, respectively (p <0.001). Against the background of this trend, cases of lethal outcomes of the disease and the presence of its severe forms were recorded annually. During 2017, 1.9 thousand cases of KVE were detected in the Russian regions. The annual rate of decline in the study period was 3.7%, or 67.5% from 2000 to 2017. The prevailing features of the epidemic process were due to the increase in the volume of preventive measures, as well as the natural long-term cyclical nature of the disease.

The territory of Western Siberia, where the Siberian Federal District is located, is traditionally endemic in relation to endemic tick-borne infections, making the main contribution to the formation of the level of the annual incidence of TVE in Russia. In 2000-2017, in the regions of the Siberian Federal District, there was a downward trend with a decline of 62.3% or 3.9% per year. The average long-term incidence rate was 10.1 ± 0.23 $^{0}/_{0000}$. The largest share of cases of the disease was registered in Tomsk Oblast - 14.21 ± 0.19%, Novosibirsk Oblast - 9.88 ± 0.17%, Irkutsk Oblast - 8.45 ± 0.16%, Kemerovo Oblast - 10.38 ± 0.17% and Krasnoyarsk Krai - 33.64 ± 0.26%. The territories of these regions have favorable climatic conditions that are necessary for maintaining the active existence of natu-

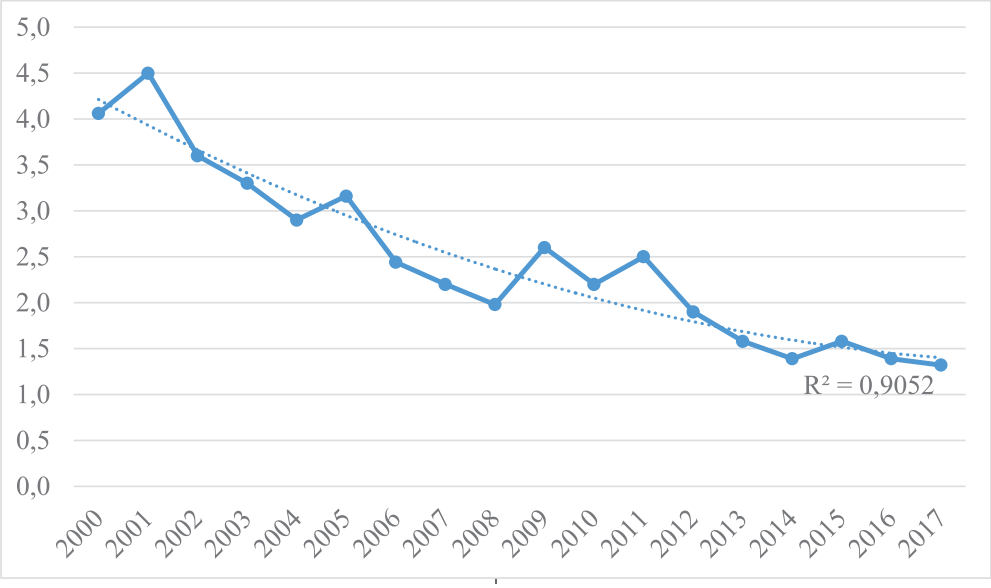

Figure 1 - Long-term dynamics of the incidence of tick-borne viral encephalitis in the Russian Federation (per 100 thousand population) in 2000-2017 with a polynomial trend line.

ral TVE foci. Within the boundaries of settlements in the regions of the Siberian Federal District, there are many anthropurgic foci, whose activity is manifested during the epidemic season and contributes to the occurrence of cases of illness among urban residents.

The main contribution to the formation of the overall TVE morbidity structure of the population of the SFD regions was made by a contingent of the population of 18 years and older, reaching 85.5%. The average long-term incidence rate in this age group during the study period from 2000 to 2017 constituted 8.7 ± 0.82 $^{0}/_{0000}$, which is 39.7% higher than the same indicator in the cohort of children and adolescents under the age of 17 years - 6.2 ± 1.39 $^{0}/_{0000}$, p <0.001. Adult morbidity rates exceeding the average long-term levels in the Siberian Federal District were observed in the Republic of Tyva, the Republic of Khakassia, the Republic of Altai, Tomsk Oblast and the Krasnoyarsk Krai. The data are presented in Figure 2.

An average of 1,321 cases of TVE among the adult population is observed annually in the regions of the Siberian Federal District. The number of average cases of the disease among children and adolescents is 5.9 times less. During 2000-2017, as part of the overall incidence of TVE in the SFD, the number of cases in 5 regions accounted for 77.8% of the total number of TVE cases among adults. Thus, in Kemerovo Oblast, an average of 165 cases were reported annually, in Irkutsk Oblast - 96 cases, in Novosibirsk Oblast - 140 cases, in Krasnoyarsk Krai - 442 cases, in Tomsk Oblast - 185 cases. In the territory of individual regions of the Siberian Federal District, in the overall structure of the average long-term incidence of TVE, from 52.9% to 94.1% accounted for the cohort of the adult population. The largest share of cases of TVE among adults in the regional structure of the incidence of TVE accounted for Omsk Oblast - 93.1%, Zabaykalsky Krai - 94.1%, Tomsk Oblast - 90.2%.

To analyze the territorial distribution of TVE incidence across the regions of the Siberian Federal District, cartograms were built using GIS-technologies of the ArcGIS software package. The results of data processing allowed us to estimate which areas are characterized with the highest risk of infection with TVE for the adult population. All subjects of the Siberian Federal District were ranked in three groups according to the level of the average long-term incidence rate of the population in 2000-2017: with a high potential risk of infection from 12.9 or more $^{0}/_{0000}$, average - from 6.7 to 12.8 $^{0}/_{0000}$, low - from 0.0 to 6.6 $^{0}/_{0000}$. In the regions of the Siberian Federal District with high and medium potential risk of infection of the adult population with TVE, there was a high viral infectivity of tick-carriers of the TVE virus, as well as forest, piedmont and mountain landscapes characterized by suitable conditions for the existence and development of activity of natural foci of infection. For example, the Republic of Buryatia, which belongs to the group of regions with the share of rural residents in the general structure of the population of 80%, was attributed to subjects with a low potential danger of infection with TVE. At the same time, despite the fact that the share of citizens in the structure of the population of Krasnoyarsk Krai is 70.3%, the region was included in the group of subjects of the Siberian Federal District which have a high potential danger of infection with TVE. The data are presented in Figure 3.

The set of factors that influence the formation of the dynamics direction of TVE epidemic process is individual for each region of the Siberian

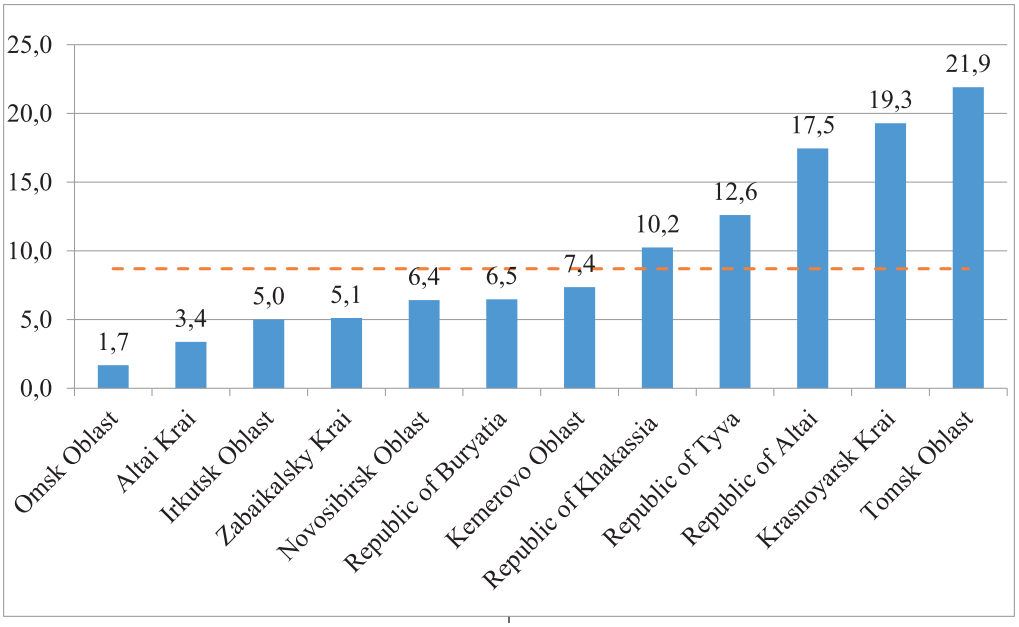

Figure 2 - Average long-term incidence of TVE of the adult population (18 years and older) in the regions of the Siberian Federal District and the average multi-year incidence of TVE in the Siberian Federal District (dotted line) in 2000-2017, per 100 thousand population.

of Western Siberia determines the varying severity of risk factors within the boundaries of one subject, which determines the regional characteristics of the epidemiology of TVE. In this regard, by epidemiological prediction of the incidence of certain groups of the population, it is necessary to take into account the multifactorial cumulative effect of the components of the natural and anthropogenic environment on the epidemic process of tick-borne infections. The analysis of multifactor influence will allow the sanitary-epidemiological service to make scientifically-based management tive measures in the areas of TVE endemic regions.

The multivariate analysis conducted in the course of this study allowed us to identify the leading components that have the most significant influence on the formation of trends in the TVE epidemic process. Such factors include the area of acaricidal treatments of the territory, the virus-pattern of tick-carriers of TVE virus, the indicator of the immune layer among the adult population of the regions of the Siberian Federal District. The tick-borne virophore index increases with advancing to the north and in the northern regions

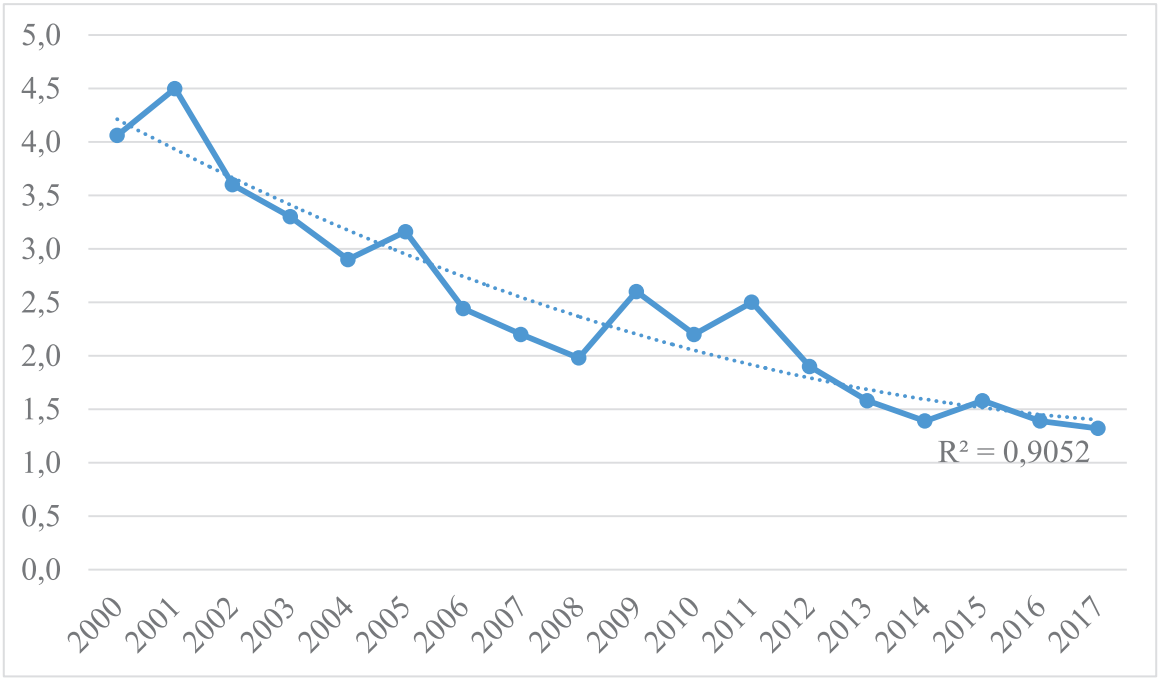

Figure 3 - A ranking cartogram of subjects of the Siberian Federal District according to the value of the average long-term incidence of TVE among people aged 18 and over in 2000-2017. (0/0000).

of the Siberian Federal District, reaches maximum values for Western Siberia. This factor is leading in the formation of morbidity rates due to the presence of the revealed direct correlation of the average power with the incidence rates in 2000-2017 ($r = 0.44$; $p < 0.001$). The effect on the virus-infectivity index of TVE tick-carriers is not possible, because of the constantly high frequency of contacts of the adult population with natural and anthropurgic foci of infection, each year causes high rates of people affected by ticks.

Acaricidal treatments are used as a measure for the non-specific prophylaxis of TVE in the endemic areas of Western Siberia, which make it possible to regulate the number of ticks in the areas under treatment. As a result of the factor analysis, an inverse correlation average force was found between this factor and the incidence of TVE in the adult population ($r = -0.33$; $p < 0.001$). Acaricidal treatments in the regions of the Siberian Federal District were conducted, as a rule, within the boundaries of settlements, including places of mass visits of people. This allowed reducing the number of ticks in the anthropurgic foci of infection.

Vaccination is a measure of specific prophylaxis that has a direct impact on the dynamics of the incidence of TVE in the endemic regions of Western Siberia. This factor is leading in the formation of the incidence rate of TVE, ensuring its reduction and the presence in the population of the immune layer. Despite the high frequency of public contact with natural and anthropurgic foci of TVE, vaccination prevents infection with the virus and, consequently, the development of the disease. The significant contribution of the factor to the formation of the average long-term levels of morbidity in the adult population of the Siberian Federal District is confirmed by the presence of an inverse correlation link of average strength ($r = -0.41$; $p < 0.001$).

Conclusion

The incidence of the adult population of the regions of the Siberian Federal District is characterized by an average long-term rate of 8.7 ± 0.82 $^0/_{0000}$ and is 39.7% higher than that of children under 17 years of age (6.2 ± 1.39 $^0/_{0000}$, $p < 0.001$). The contribution of the adult population to the formation of the overall TVE incidence in the SFD is 85.5%, which is determined by the presence of occupational risk groups, as well as the features of the household activity, suggesting a high probability of contact with natural and anthropurgic foci of infection. These circumstances form the annual incidence in all regions of Western Siberia.

The factor analysis allowed us to determine the leading factors causing the formation of trends in the incidence of TVE in the adult population cohort. The group of such factors includes the indicator of the immune layer of the adult population ($r = -0.41$; $p < 0.001$), the area of acaricidal treatment of the territory ($r = -0.33$; $p < 0.001$), the viral infectivity of TVE tick-carriers ($r = 0\ 44$; $p < 0.001$). Epidemiological prediction of the incidence of TVE in the endemic areas of the Siberian Federal District should include consideration and analysis of these leading factors, which will ensure timely adjustment of the likelihood of developing the disease in risk groups and, consequently, optimizing the volume of preventive measures.

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ASSESSMENT OF IMMUNIZATION AGAINST PNEUMOCOCCUS INFECTION OF CHILDREN UNDER 5 YEARS IN THE CITY OF BARNAUL OUTSIDE THE PREVENTIVE VACCINATION SCHEDULE

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Yu.A. Kozyanova, T.V. Safyanova

In December 2016, in terms of a retrospective cohort epidemiological study, 2 cohorts of children (vaccinated and unvaccinated) were chosen, each consisting of 156 children under the age of 60 months, to assess the effectiveness of vaccination with a 10-valent conjugate vaccine (PCV) against pneumococcal infection on the number of episodes of Cases respiratory and ENT diseases, as well as on the number of antibiotic courses prescribed for the treatment of these diseases. The results of the study showed that the use of polyvalent conjugate pneumococcal vaccine reduced the frequency of episodes of Cases of respiratory and ENT diseases by 34.1% (95% CI: 31.1-37.0), and the frequency of prescriptions of antibacterial drugs for their therapy by 52.7% (95% CI: 43.7-61.6).

Key words: *S. pneumoniae, respiratory diseases, diseases of the respiratory tract, conjugated pneumococcal vaccines, courses of antibacterial therapy.*

Introduction

Streptococcus pneumoniae is often a representative of the normal microflora of the upper respiratory tract [1]. According to the World Health Organization (WHO), pneumococcus occupies a leading position in the etiological structure of acute otitis media (AOM) and community-acquired pneumonia (CAP), especially in childhood [2, 3, 4, 5], which is a significant health problem in the whole world. Taking into account the existing antibiotic resistance of this microorganism [6, 7], vaccination, as one of the most effective preventive measures against this infection [5], is of particular relevance. In the Russian Federation, vaccination against pneumococcal infection was introduced in the National Vaccination Schedule in late 2014 [8]. In Altai Krai, the effectiveness of immunization has not been assessed, but there has been an experience with the use of vaccination against pneumococcal infection in an organized groups of children until 2014.

Research objective: to evaluate the epidemiological effectiveness of vaccination against pneumococcal infection outside the National Vaccination Schedule in relation to the number of cases of respiratory and ENT infections in children of Barnaul under the age of 5 years.

Materials and methods

In terms of a retrospective cohort epidemiological study, 2 groups of children born in 2012-2013 were chosen, each consisting of 156 children under the age of 60 months. One group included children vaccinated with 10-valent PCV according to the 2+0, 2+1 scheme before the start of mass immunization against pneumococcal infection, the other group - unvaccinated children of the same age (± 1-2 months), who were observed in the same clinic (who had not received a single dose of vaccine against pneumococcal infection). The observation period was since the last dose of vaccination + 1 month to 60 months.

The study consisted of a passive component: an independent study of the medical records of children in the cohort. According to the form number 112/u "History of child development" in the studied groups, there were tracked the doctor registered episodes of Cases of the upper respiratory tract and respiratory organs and the appointment of courses of antibiotics in the treatment of these nosologies.

To calculate the vaccine efficacy (VE), the formula VE = 1- RR was used, where RR = relative rate (relative frequency) of courses of antibiotics or episodes of Cases.

The quartiles method was used to estimate the distribution and description of the data. By asymmetric distribution of the feature, the assessment of the reliability of differences in the analyzed groups was made by the criterion $\chi 2$ (chi-square). In other cases, the arithmetic mean (X) and the standard error of the mean (± m) were calculated, followed by the Student's criterion assessment.

In all procedures of statistical analysis, the critical level of significance was assumed to be 0.05.

Results and discussion

According to the results of the study, it was found, that the average age of vaccination onset constituted 16.6±7.5 months, which differed from the recommended one - 2 months. Children vaccinated according to the 2+0 scheme were 141, according to the 2+1 – 15. The total follow-up duration (time of risk) for each cohort was 335.86 years (4030.3 months). On average, the duration of ob-

servation for each participant (time of risk) of both cohorts was 2.55±0.40 years (25.84 ± 4.76 months).

During the study period, the total number of episodes of the nosologies studied in a cohort of vaccinated children was 666, in a cohort of unvaccinated, 1011 episodes were recorded. The main proportion of vaccinated children was in the range from 0 to 4 times, unvaccinated - from 5 to 9 times (Table 1).

Table 1.

The proportion of vaccinated and unvaccinated children, depending on the number of episodes of Cases of the studied nosologies

Quartiles	Unvaccinated, %	Vaccinated, %
1 (0-4 episodes)	32,1	58,3
2 (5-9 episodes)	48,7	35,3
3 (10-14 episodes)	17,3	6,4
4 (15 and more episodes)	1,9	0,0

The maximum number of episodes during the observation period was recorded in an unvaccinated child and amounted to 23 cases. A similar number among the vaccinated was 14.

Therefore, the frequency of episodes of the studied diseases among vaccinated children is less than among unvaccinated ($p < 0.05$).

During the study period, 61 courses of antibacterial drugs were prescribed in a cohort of vaccinated children, 129 courses were given to a cohort of unvaccinated children. There was a large asymmetry in the frequency of prescription of antibiotics for the treatment of the studied diseases among vaccinated children: a greater proportion of children with a low frequency of prescription of courses of antibacterial drugs among those vaccinated compared with unvaccinated (Table 2).

Table 2.

The proportion of vaccinated and unvaccinated children, depending on the number of prescribed courses of antibacterial drugs for the treatment of the studied nosology

Quartiles	Unvaccinated, %	Vaccinated, %
1 (0-1 course)	78,2	94,2
2 (2-3 courses)	17,3	5,1
3 (4-5 courses)	3,2	0,6
4 (6 and more courses)	1,3	0,0

The effectiveness of vaccination of the given sample in relation to the reduction of prescribing courses of antibacterial drugs was 52.7% (95% CI: 43.7-61.6), in relation to the reduction of episodes of Cases of diseases studied - 34.1% (95% CI: 31.1 -37.0).

Conclusion

The results of the study showed that the use of conjugated pneumococcal vaccine reduced the incidence of respiratory organs and ENT organs in the cohort under study, as well as the frequency of prescribing antibacterial drugs during their treatment, which may be of significant importance in combating the development of antibiotic resistance.

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BIOCHEMICAL MARKERS OF DAMAGE TO THE KIDNEY TISSUES BY EXPERIMENTAL OXALATE NEPHROLITHIASIS

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The aim of the research was to study the dynamics of γ-glutamyltransferase activity and cyclooxygenase-1 concentration in urine and the dynamics of malonic dialdehyde concentration in rat kidney tissue by experimental oxalate nephrolithiasis.

Experiments were performed on 15 male Wistar rats aged 2-3 months and weighing 180-220g. Oxalate nephrolithiasis was modeled for 6 weeks in accordance with the generally accepted ethylene glycol model. Prior to the start of the experiment, and after 3 and 6 weeks, urine collection was performed, in which γ-glutamyl transferase (GGT) activity and cyclooxygenase-1 (COX-1) concentration were determined. At the end of 6 weeks, in the homogenate of renal tissue, the concentration of thiobarbituractivative products (TBRP) was determined. The results of the experiments showed that the activity of GGT in the urine after 6 weeks of nephrolithiasis simulation increased by 6.6 times, the concentration of TBRP in the kidney homogenate relative to the level of healthy rats increased by 1.3 times, and there was a tendency to the increase of COX-1 concentration in the urine by 1.7 times.

Experimental oxalate nephrolithiasis shows an increase in activity of biochemical markers of lithogenesis - GGT and TPBP. This indicates the development of a damaging factor in the kidneys, which is based on the activation of lipid peroxidation.

Key words: *oxalate nephrolithiasis, γ-glutamyltransferase, malonic dialdehyde, cyclooxygenase-1.*

Kidney stone disease is still one of the most common and serious diseases of the urinary system. The epidemiology of nephrolithiasis covers every 10th inhabitant of developed countries [1]. Modern ideas about the pathogenesis of nephrolithiasis indicate the initiating role in the stone formation process of the so-called "damaging factor" - destructive changes in the structure and function of the tubular epithelium of the kidney, resulting in formation of crystalline material from urine at a certain site, and the primary focus of lithogenesis is formed [2]. In this regard, it is considered that certain signaling molecules, indicating the development of a damaging factor, may be biochemical markers of lithogenesis. This may be important not only in the study of the pathogenesis of nephrolithiasis, but also in assessing the effectiveness of the developed methods of drug treatment of urolithiasis.

The results of a number of studies show that the cascade of reactions provoking the development of a damaging factor begins with the destruction of nephrocyte cell membranes under the influence of crystallization driving forces [3]. Therefore, we were interested in studying the dynamics of activity and/or content of γ-glutamyltransferase, malonic dialdehyde and type 1 cyclooxygenase in the kidneys by experimental nephrolithiasis. As is known, γ-glutamyltransferase is a membrane-bound enzyme that is present in large amounts in kidneys [4]. Malonic dialdehyde is the main product of peroxidation of membrane phospholipids [5]. Cyclo-oxygenase-1 is an enzyme involved in the catabolism of membrane phospholipids during the conversion of arachidonic acid to prostaglandins [6]. These signaling molecules are found in the kidneys in significant amounts, and therefore, they can potentially be biochemical markers of the development of oxalate nephrolithiasis.

Thus, the research objective is to study the dynamics of γ-glutamyltransferase activity and cyclooxygenase-1 concentrations in the urine and the dynamics of the concentration of malonic dialdehyde in the renal tissue of rats by experimental oxalate nephrolithiasis.

Materials and methods

The experiments were carried out on 15 male Wiatar rats, 2-3 months old and weighing 180-220g. The studies were carried out in accordance with the requirements of the "Rules of work with the use of experimental animals." Throughout the study, the animals were located in individual metabolic cages adapted for urine collection. Oxalate nephrolithiasis was modeled according to the generally accepted ethylene glycol model, according to which the rats were provided with free-access 1% ethylene glycol (EG) for 6 weeks [7].

Prior to the experiment, as well as after 3 and 6 weeks, urine was collected, in which the activity of γ-glutamyltransferase (GGT) and the concentration of cyclooxygenase-1 (COX-1) were determined. GGT activity (U/mg creatinine per day) was determined by an optimized kinetic method on a Vitalon 400 semi-automatic analyzer. Under the action of γ-glutamyltransferase in the transfer reaction of L-γ-glutamyl-3-carboxy-p-nitroanilide to glycylglycine, a colored 5-amino-2-nitrobenzo-

ate is formed. The rate of increase in the optical density of the sample at a wavelength of 405 nm is proportional to the activity of GGT in the analyzed sample. For the quantitative determination of cyclooxygenase-1 in the urine using enzyme immunoassay, a kit for the determination of prostaglandin-endoperoxide synthetase 1 (PTGS 1) by Cloud-Clone Corp was used.

By the end of 6 weeks of nephrolithiasis modeling, the animals were euthanized under ether anesthesia, both kidneys were removed, one of which served to determine the concentration of thiobarbiturate reactive products, the main representative of which is malondialdehyde, as well as to conduct morphological studies aimed at the confirmation of stone formation processes. The concentration of thiobarbiturate-reactive products (TBRP) in the homogenate of the renal tissue was determined by the colorimetric method, measuring the color intensity of the solution during the chemical reaction of the TBRP with thiobarbituric acid. For morphological studies, the kidneys were fixed in a 10% formalin solution, processed by a standard technique, and a 6 μm thick cross section was made through the renal papilla. The obtained sections were stained with hematoxylin and eosin. Calcium deposits were identified using the Koss histochemical method, and the number of calcium deposits in the field of view was calculated using a computer program, and their size was determined. Morphometric studies were performed using the ImageJ 1.43 and AxioVision 3.1 software packages.

The statistical processing of the results was carried out using the computer program "Statistica 12.0". The results are represented by the median (M) and interquartile range (25%, 75%) for GGT, COX-1, and TBRP, as well as the mean and standard error of the mean (M ± m) for morphometric parameters. Statistical comparisons of dependent samples were carried out using the Wilcoxon nonparametric test. The results were considered reliable by the value of the significance indicator $p < 0.05$.

Results and discussion

As a result of the experiments, it was established that the activity of GGT in the urine by the end of the 3rd week of the experiment increased from the initial level by 5.0 times. Subsequently, the growth of the value of the described indicator continued, as a result of which, at the end of 6 weeks, it already exceeded the initial figures by 6.6 times. Against this background, the concentration of COX-1 in the urine by the end of the 6th week of the observation period increased relative to the initial level by 1.7 times. This change was not statistically significant and only manifested itself in the form of a trend (Table 1).

Table 1

Indicators of GGT activity and COX-1 concentration in the urine by six-week experimental oxalate nephrolithiasis

Week	GGT activity (U/mg creatinine per day)	COX-1 Concentration (ng / ml)
Initial level	0,25 (0,05 ; 0,85) n=14	3,9 (2,18 ; 5,69) n=15
3rd week	1,24 (0,36 ; 2,10) n=14 p=0,0231	Not determined
6th week	1,64 (0,87 ; 4,63) n=11 p=0,0166	6,6 (6,0 ; 7,4) n=10 p=0,114

Note: n – number of urine samples for analysis; p – indicator of the significance of changes relative to the initial level.

As follows from Figure 1, the concentration of TBRP in the homogenate of the renal tissue of rats by a six-week experimental nephrolithiasis constituted 7.6 (7.23; 7.65) μmol, which is 1.3 times higher than in healthy rats - 6.1 (5.37; 6.91) μmol ($p = 0.00428$).

By histochemical staining on calcium by the Koss method, single crystals or groups of deposits of brownish-black stones of various shapes and sizes were observed in the tubules of the rat kidneys (Figure 2). The number of deposits in the gaps of the tubules ranged from 3 to 7 and averaged 4.6 ± 0.2 in the field of view with the zoom of × 400, with a modal value of 4. When performing computer morphometry, the area of stone deposits ranged from 31.5 μm^2 to 567, 9 μm^2 and averaged 171.9 ± 27.6 μm^2.

Discussing the results obtained, we note that under the conditions of a modeled oxalate nephrolithiasis, GGT activity underwent the most pronounced growth. As is known, GGT is a membrane enzyme that is localized on the outer side of the membrane of many cells of the body [4]. Therefore, on the one hand, an increase in its activity in the urine by nephrolithiasis may indicate the destruction of the cell membranes of nephrocytes and the release of the enzyme into the lumen of the tubules. On the other hand, it is known, that GGT activity is a marker of oxidative stress in body tissues [8]. It has been established, that the most

important function of GGT in the body is to maintain the physiological concentration of glutathione in the cytoplasm of cells, which is the main thiol antioxidant in the body [8]. Therefore, it is possible, that such a pronounced increase in GGT activity could be a compensatory response to the developing oxidative stress in the renal tissue by oxalate nephrolithiasis. This can be confirmed by the increase in the concentration of TBRP recorded in our experiments in the homogenate of the renal tissue. As is known, malonic dialdehyde, the main product of membrane phospholipid peroxidation, is the main representative of TBRP [5].

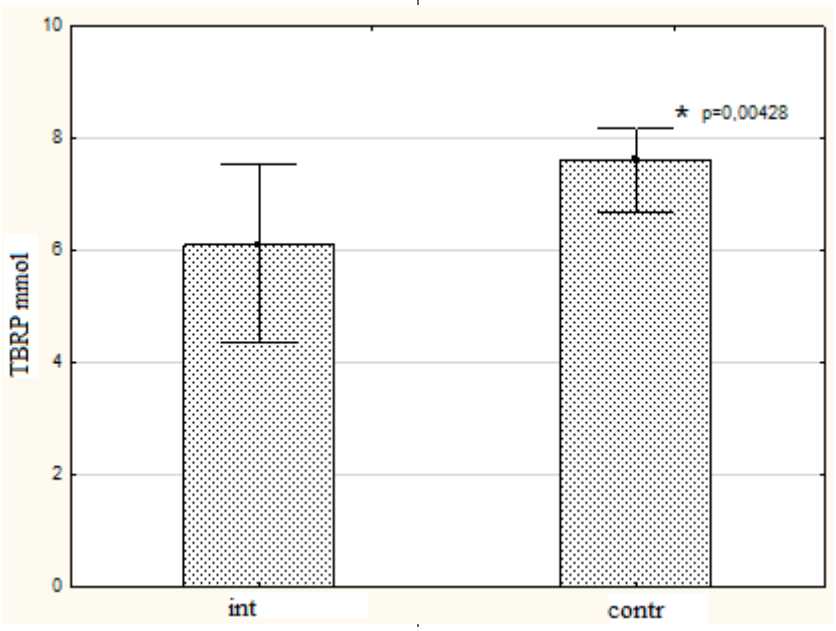

Figure 1 - The concentration of TBRP by experimental oxalate nephrolithiasis in relation to the group of intact rats.
Note: int - indicator of healthy rats; contr - indicator of rats with nephrolithiasis.

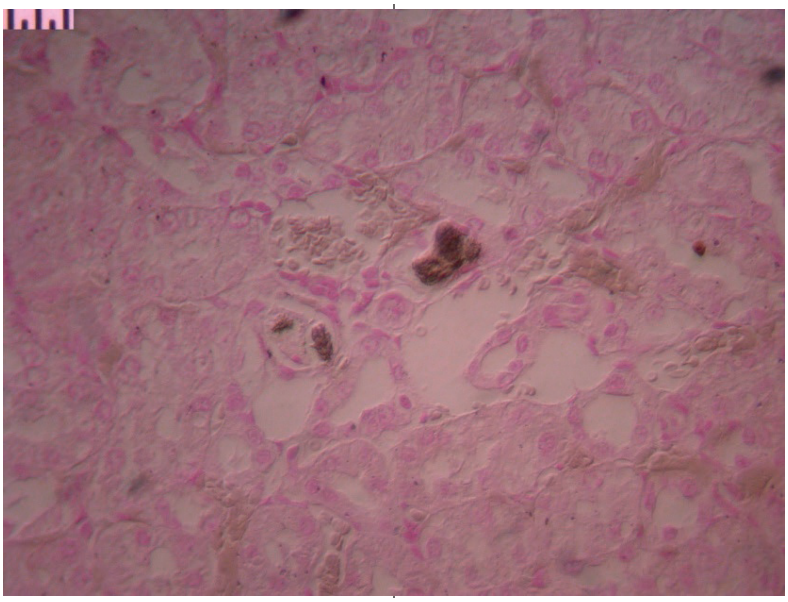

Figure 2 - Deposits in the tubules of the rat kidneys by experimental oxalate nephrolithiasis. Coloring according to Koss. Zoom × 400. Note: brown calcium deposits are in sight.

In addition, we should note the concentration of COX-1 in the urine recorded in our experiments. This enzyme is involved in the metabolism of arachidonic acid to prostaglandin H_2 - an intermediate product in the synthesis of a number of prostagladins [6]. The role of the cyclooxygenase metabolic pathway of membrane phospholipids in the pathogenesis of renal pathologies is quite complex and diverse. There is an opinion that COX-1 is involved in cytoprotection, the most striking example of which is the regulation of production of protective mucus in the stomach [6]. It is possible that COX-1 may have a similar function in the kidneys. However, of course, this issue is subject to further in-depth study.

Summarizing the above, we note that under conditions of a six-week simulation of experimental oxalate nephrolithiasis, characteristic changes in the dynamics of biochemical markers of the development of pathology were recorded. This was mainly evidenced by a nearly 7-fold increase in GGT activity during the experiment and a significant increase in the concentration of TBRP in the homogenate of the renal tissue by 30%. Confirmation of the development of nephrolithiasis were the results of morphological studies, which showed the formation of a significant amount of calcium deposits in the tubules of the kidneys of experimental rats.

Conclusion

By experimental oxalate nephrolithiasis, an increase in the activity of biochemical markers of lithogenesis — GGT and TBRP — is observed. This indicates the development of a damaging factor in the kidneys, which is based on the activation of lipid peroxidation.

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MORPHOFUNCTIONAL CHARACTERISTICS OF THE MAST CELL POPULATION OF THE LIVER OF WHITE RATS BY DEEP IMMERSION HYPOTHERMIA (EXPERIMENTAL RESEARCH)

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*The research objective was the estimation of the morphofunctional activity of mast cells of rat liver by hypothermia. The research was conducted on 20 Wistar rats. Hypothermia was modeled by means of immersion of the animals, caged individually, into the water of 5°C at the ambient temperature of 7°C. The criteria of the termination of the impact was rectal temperature 20-25 °**C**, that showed the deep stage of hypothermia. During the experiment, the animals were divided into 4 groups. The rats of the 1st group were killed immediately after the termination of cold impact, the animals of the 2nd group were killed 2 days after the experiment, the rats of the 3rd group were examined in 7 days and the 4th group animals were studied after 14 days of cold impact. The results of the research showed that the cold stress had an expressed influence on mast cells of rat liver. On the 2nd day of the experiment, the morphofunctional activity of mast cells increased, which coincided with the onset of regeneration of hepatic cells. On the 7th day (in the period of highest activity of adaptive processes) the quantative and morphometric parameters of mast cells were the highest. On the 14th day of the experiment, the activity of mast cells sufficiently decreased. Thus, hypothermia is a potent activator of the morphofunctional activity of tissue mast cells. Interstitial mast cells can be important factors in the activation of regenerative and adaptive processes in the liver in response to the damaging effect of the cold factor.*

Key words: *hypothermia, liver, mast cells, adaptation.*

Despite numerous studies devoted the death of people from cold, the diagnosis of cold death and fatal hypothermia continues to be relevant. At present, many aspects of the effect of cold on the human body have been thoroughly studied, and diagnostic and differential diagnostic criteria for death from hypothermia have been developed, but a whole view on compensatory-adaptive processes and adaptation of the organism to the cold factor in pathological anatomy and medical science is currently missing.

According to modern concepts, mast cells (MC) are the key link in the influence on metabolic processes, being of an important biological significance in tumor growth, regeneration, adaptation to stress and hypoxia [1, 2, 3, 4, 5, 6, 7 , 8, 9, 10].

The mast cell population in the tissues of the body is an independent regulatory system. This statement is confirmed by the fact that MC of various tissues of experimental animals are systematically included in the adaptive processes under the influence of extreme factors on it. At the same time, only a few works are devoted to the study of the value of MC by hypothermia. Thus, it has been shown that exposure to cold factor causes an increase in the number of MC and their degranulating forms in the skin, skeletal muscles and mesentery of the intestine of experimental animals [11, 12, 13].

The research objective was to study the effect of deep immersion hypothermia on the morphofunctional activity of the mast cell population of the rat liver in the experiment.

Materials and methods

The study was performed on 25 Wistar rats. The hypothermia was modeled by placing animals in individual cages in water at a temperature of 5 °C at an ambient temperature of 7 °C. The criterion for the termination of exposure was the achievement by the animals of a rectal temperature of 20–25 °C, which corresponded to a deep degree of hypothermia. The exposure time was individual and averaged 40±5 min. During the experiment, the rats were divided into 4 groups. Animal of the 1st group (n = 5) were derived from the experiment by decapitation immediately after the termination of cooling, animals of the 2nd group (n = 5) - after 2 days, animals of the 3rd group (n = 5) - after 7 days, and animals of the 4th group (n = 5) - after 14 days. A morphological study of the liver of intact animals (n = 5), which served as a control group, was also conducted.

For histological examination, liver samples were fixed in 10% neutral formalin, then the material was wired on a TISSUE-TEK VIPTM6 machine (Sakkura, Japan), embedded in Histomix paraffin (TISSUE-TEK TEC 5 paraffin filling station, Sakkura, Japan). Sections 5-7 μm thick were made on a Accu-Cut SRM rotary microtome (Sakkura, Japan), stained with hematoxylin and eosin

in a TISSUE-TEK Prisma automat (Sakkura, Japan) and placed under the film in a TISSUE-TEK Film automat (Sakkura, Japan). MC were revealed by toluidine blue (BioVitrum, Russia). Microscopic photographs of MC in the liver were obtained using a Nikon Eclipse E200 microscope (Japan) with a VIDI CAM digital video camera (Russia) at the zoom x 400. The distribution density of MC was calculated in five fields of view by the microscope zoom x 400 in the Image Tool. 3. The index of degranulation of TK (IDTK) was calculated (the percentage of cells in the state of degranulation of the total number of TK). The morphometry of MC was performed using the licensed morphometric program "VideoTest-Morphology 5.2". Statistical processing of the data was performed using the statistical package Statistica 6.0.

Results and discussion

The results of the study showed, that in the liver of rats of the control group, MC were located in the connective tissue of the portal tracts and along the sinusoids. They were small and round in shape (Figure 1). The number of cells varied from 1 to 2, their average number was 1.2 ± 0.2 in five fields of view by x 400 zoom. The MC area was 34.0 ± 0.7 μm^2. The granules were mainly located compactly in the cytoplasm of MC. The number of compact forms of MC was 83.2 ± 0.3%. The phenomenon of degranulation was noted in a small number of cells. DIMC (degranulation index) was equal to 16.8 ± 0.5% (Table 1).

Immediately after exposure to a single deep immersion hypothyroidism, MC in the liver tissue looked small, rounded or elongated, located alone in the portal tracts, localized near the vessels and bile ducts (Figure 2). The distribution density of MC was 4.0 ± 0.5 in five fields of view, the cell area was 40.2 ± 3.2 μm^2. Compact forms of MC equaled 84.5% ± 5.1. DIMC was 15.5% ± 5.1 (Table 1).

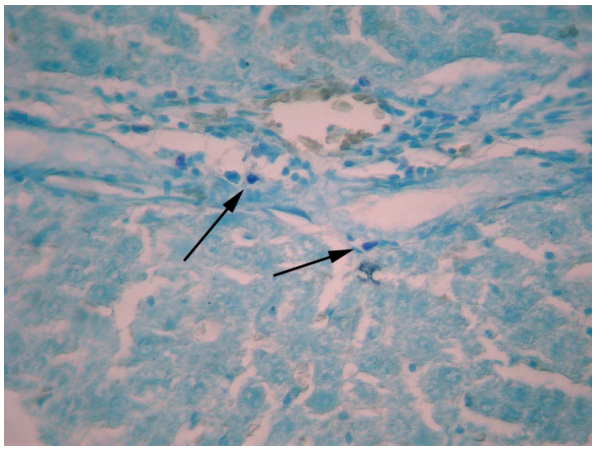

Figure 1 - Small number of small mast cells in the portal tracts of the liver of rats of the control group (shown by arrows). Staining by toluidine blue. Zoom x 400.

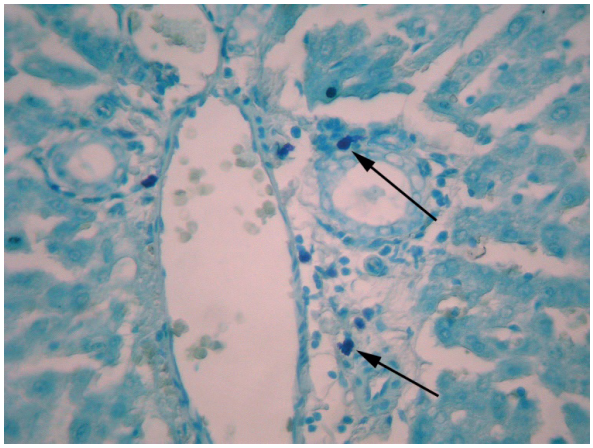

Figure 2 - Increase in the number of mast cells in the portal tracts of the rat liver immediately after conducting deep hyperthermia (shown by arrows). Staining by toluidine blue. Zoon x 400.

On the 2nd day after a single immersion hypothermia, MC in the liver were located in groups and singly in the portal tracts, in the stroma of the liver, near the vessels and bile ducts. MC looked polymorphic, with elongated cells predominating (Figure 3). The distribution density of MC at this time of the experiment was 10.2 ± 1.4, the area of MC – 51.5 ± 1.9 μm2. Compact forms of MC were 75.8% ± 0.9. DIMC was equal to 24.2% ± 0.9 (Table 1).

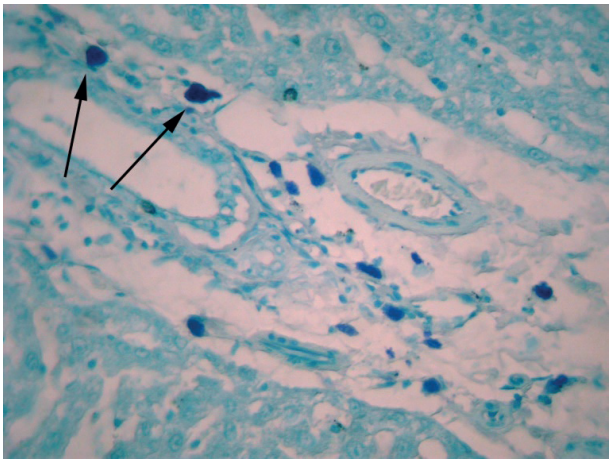

Figure 3 - 2 days after hypothermia, the number of mast cells in the portal tracts of the rat liver increases, the cells are enlarged in size, polymorphic (shown by arrows). Staining by toluidine blue. Zoon x 400.

On the 7th day after hypothermia, MC were located in groups and singly in the portal tracts, near the vessels, the bile ducts and in the connective tissue stroma. At this period of the experiment, by their morphological characteristics, MC differed significantly from the cells of the previous study: they had large sizes, elongated and irregularly shaped cells prevailed (Figure 4). The distribution density of MC at this time of the experiment was 10.8±0.9, their area increased to 73.3±3.2 μm^2.

Compact forms of MC were 67.2% ± 1.9. DIMC was equal to - 32.8% ± 0.9 (Table 1).

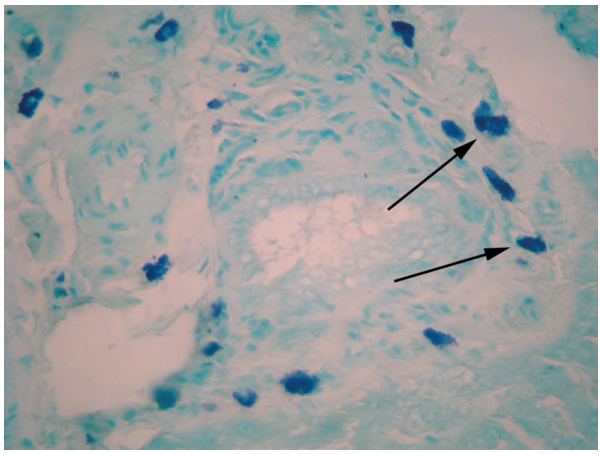

Figure 4 - 7 days after hypothermia, the number of mast cells in the portal tracts of the rat liver is high, the cells are of large size (shown by arrows). Staining by toluidine blue. Zoon x 400.

On the 14th day of the experiment, MC were located in the portal tracts, mainly singly, near the vessels and bile ducts. In comparison with the previous experiment, the size of MC decreased. Round-shaped cells prevailed (Figure 5). The distribution density of MC at this time of the experiment was 9.0±1.4, the cell area was 57.9±2.3 μm^2. Compact forms of MC were 84.9% ± 3.1. DIMC was 15.1% ± 3.1 (Table 1).

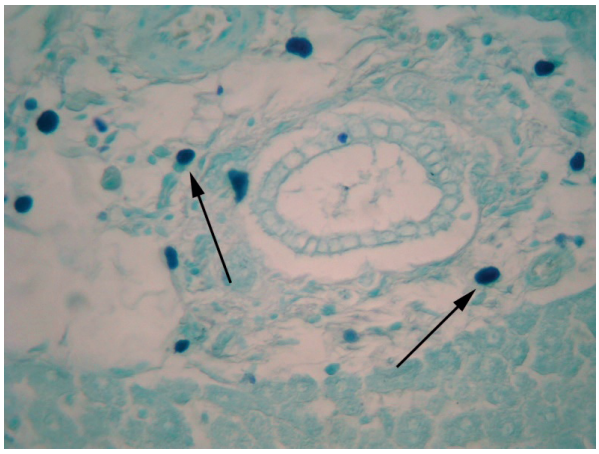

Figure 5 - 14 days after hypothermia, the number of mast cells in the portal tracts of the rat liver decreases (shown by arrows). Staining by toluidine blue. Zoon x 400.

Table 1

Quantitative and morphometric characteristics of mast cells, depending on the duration of the experimental single deep immersion hypothermia

Term of experiment	Mast cell parameters			
	MC number	Compact MC number (%)	DIMC	MC area (μm^2)
Control	1,2±0,2*	83,2±0,3	16,8±0,5	34,0±0,7*
Immediately after hypothermia	4,0±0,5*	84,5±5,1*	15,5±5,1*	40,2±3,2*
2 days after hypothermia	10,2±1,4*	75,8±0,9*	24,2±0,9*	51,5±1,9*
7 days after hypothermia	10,8±0,9	67,2±1,9*	32,8±0,9*	73,3±3,2*
14 days after hypothermia	9,0±1,4	84,9±3,1*	15,1±3,1*	57,9±2,3*

Note * - data are significant by <0.05.

Conclusion

The results of the study showed that a single deep immersion hypothermia had a significant impact on the morphofunctional activity of liver MC in experimental animals. The number and size of MC began to increase immediately after exposure to hypothermia, and after 7 days, during the active regeneration and adaptation of hepatocytes, the number of MC and their morphometric parameters were the highest, and the number of degranulating forms of these cells was maximum. By the 14th day of the experiment, the content of MC in the liver remained at the same level, but the area of MC and DIMC significantly decreased. The increase in the amount of MC in the liver tissue of animals on the 7th day of the experiment may be due to their migration from other organs. An increase in the morphofunctional activity of MC in the posthypothermic period leads to an acceleration of regenerative processes in the liver tissue after the damage caused by exposure to the cold factor. Thus, hypothermia is a potent activator of the activity of the interstitial liver MC. Interstitial MC serve as important stimulators of hepatocyte regeneration in the process of adaptation of the liver to hypothermia.

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THE ROLE OF ACUTE HYPOXIC HYPOXIA IN INCREASING THE CONCENTRATION OF AMNIOTIC LIQUID COMPONENTS IN RABBITS AT LATE TERMS OF PREGNANCY

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Research objective: to assess the influence of 60 minute exposure to acute hypoxic hypoxia (10,0 ± 2,0 % O_2) on the amniotic fluid (AF) of first-pregnant rabbits on the 27-28th day of gestation.
Materials and methods: Female rabbits were randomly divided into experimental (n = 9) and control groups (n = 6). Volume of amniotic fluid, osmolality and concentration of electrolytes – Na^+, K^+, Cl^-, non-organic phosphate (P_i), Ca^{2+} and organic components (creatinine, urea, protein) in AF were measured.
Results: It was found that acute hypoxic hypoxia causes an increase in volume and osmolality of AF, as well as an increase in AF's electrolytes and organic components. We suppose that changes in AF parameters were caused by an increase of AF formation and an acceleration of water resorption in fetus kidneys or fetus membranes.
Conclusion: It was found that an increase in AF osmolality during hypoxia is associated with an increasing concentration of normal components of AF, and it is possible that this phenomenon may increase the concentration of diagnostic biomolecules. We hypothesize that correcting the concentration of diagnostic biomolecules in AF according to it's osmolality (especially in hypoxia) could provide a more precise diagnostic technique than using raw concentrations.
Key words: *amniotic fluid, pregnancy, fetus, hypoxic hypoxia, rabbits.*

The volume and composition of the amniotic fluid (AF) reflects the homeostasis of the mother and the fetus, therefore, it is useful to monitor it during pregnancy with the definition of a number of important parameters [1]. However, the study of the regulation of the volume and composition of AF is hampered by the presence of a number of sources of its formation and outflow [2].

The regulation of the volume and composition of AF involves several mechanisms: fetal diuresis, intramembrane pathway (transfer of water and electrolytes from AF to fetal vessels), ingestion of AF to the fetus, secretion by the lungs and nasopharyngeal of the fetus, transmembrane pathway (absorption of liquid through the amnion into the mother's body), percutaneous pathway and absorption through the umbilical cord epithelium [3]. It should be borne in mind that by the 20th day of pregnancy, the skin of the fetus and the surface of the umbilical cord are keratinized, as a result of which the percutaneous and umbilical cord paths do not practically transport AF components [4]. The remaining six paths can be divided into pathways of formation (fetal urine, lung secretions and nasopharyngeal secretions) leading to an increase in AF volume, and outflow pathways (intramembrane pathway, ingestion and transmembrane pathway) leading to its decrease.

Fetal diuresis is the main way of forming AF [5]. There is shown a direct correlation between the speed of the urine flow of the fetus and the volume of the AF [6], which forms approximately three-quarters of the volume of the AF, being the main source of its ionic and organic composition [2]. It is also known, that the course of pregnancy largely depends on the provision of normal respiratory metabolism between the mother's body and the fetus. Recent studies show that chronic hypoxia does not cause changes in the volume of AF [7].

The purpose of this study was to study the effect of acute hypoxic hypoxia on the volume and electrolyte composition of the amniotic fluid on the 27-28th day of pregnancy.

Materials and methods

The study was conducted on primigravida rabbits (n = 15) weighing 4-5 kg at a period of gestation of 27-28 days (with a normal gestational age of 31 days). Fertilization was performed by various randomly selected males, after which the female rabbits were kept in single cages on a free diet. Rabbits were randomly divided into two groups: experimental (n = 9) and control (n = 6).

Rabbits from the experimental group were placed in an individual hermetic flow chamber for 60 minutes, where a gas mixture containing 10 ± 2% oxygen and 90 ± 2% nitrogen was injected with a compressor. The control of the gas composition in the chamber was carried out using a Microlux O_2+CO_2 gas analyzer (Mikrolux LLC, Yekaterinburg, Russia). Rabbits from the control group were placed in the same chamber for 60 minutes, containing atmospheric air.

After that, animals were killed by the method of cervical dislocation and in 15 minutes exposed

to midline laparotomy, and the uterus was removed. Amniotic bags with fetuses were isolated and removed from the uterine cavity and the maternal and fetal parts of the placenta were separated without disturbing the integrity of the amniotic sac. The amniotic fluid was removed from the amniotic sac by a single-use syringe.

Criteria for the inclusion of the rabbits in the study: 1) gestational age of 27-28 days; 2) the mass of pregnant rabbits 4-5 kg. The criterion for excluding the fetuses from the study is the weight of the fetus less than 20g. The general characteristics of the rabbits and their fetuses are presented in Table 1.

Table 1

Mass of the fetus and placenta on the 27-28th day of life

	Hypoxia (n = 43)	Control (n = 40)	P
Fetus weight, g	37,8 (30,6—50,3)	38,4 (31,0—41,8)	P = 0,280
Mass of the fetal part of placenta, g	3,37 (2,79—3,71)	3,47 (3,10—4,22)	P = 0,180

Note: data are presented in the form of - median (25-75%), P - the significance of intergroup differences according to the Mann-Whitney U-test.

Samples of amniotic fluid were obtained from the rabbits of the experimental group (n = 43) and the control group (n = 40). Samples were centrifuged for 15 minutes by 1200 g, frozen and stored at a temperature of minus 20 ° C no more than one month before the biochemical study. In samples of amniotic fluid, concentrations of Na^+, K^+, Cl^- ions, inorganic phosphate (Pi) and Ca^{2+}, creatinine, urea, protein and osmolality were determined.

Biochemical studies were performed on an automatic biochemical analyzer Dimension Xpand (Siemens, Germany). The concentration of Na^+, K^+ and Cl^- ions was determined by a potentiometric method using the QuikLyte Integrate Multisensor module (Siemens, cat. No. S600, USA); total concentration of Ca^{2+} - colorimetric method with CA Calcium Flex reagent cartridge reagent kit (Siemens, cat. No. EA4164, USA); the concentration of Pi - by the colorimetric method using the PHOS Phosphorus Flex reagent cartridge reagent kit (Siemens, cat. no. EA4172, USA). Osmolality of AF was determined using a Vapro osmometer (Wescor, United States). The concentration of creatinine in AF was determined by the modified Jaffe method using CREA Creatinine Flex reagent cartridge reagents (Siemens, cat. No. DA4254, USA); the concentration of urea - kinetic enzymatic (urease) method reagents BUN Urea Nitrogen Flex reagent cartridge ("Siemens", cat. No. EB4309, USA); protein concentration - by a colorimetric method with pyrogallol red using the Belok-PGK-Novo reagent kit (Vector-Best, Cat No. B-8084, Russia) using the Bellur-600 total protein analyzer (Tehnomedika Research and Production Enterprise, Russia). This work has been approved by the local ethical committee of the Federal State Budgetary Educational Institution of Higher Education of the Altai State Medical University.

Data processing

The statistical data processing was performed using the JMP 7.0 program (SAS Institute, USA). The median, 25 and 75 percentiles, the accuracy of intergroup differences were calculated by the Mann-Whitney test, multiple correlation analysis - by the Spearman test. The level of statistical significance was taken as 5% ($p < 0.05$).

Results and discussion

Acute hypoxic hypoxia caused an increase in the osmolality of the AF and the concentration of all the studied inorganic ions and organic components (Table 2).

There was found an increase in the concentration of all the components studied, as well as osmolality of the AF, including an increase in only the filtered components — creatinine and urea. This suggests that filtration in the kidneys of the fetus has increased, but water reabsorption has also increased, either at the stage of the formation of fetuses by the kidneys [7] or in the amniotic sac itself [8]. Considering the existence of two transport systems in the fetal membranes - water transport by aquaporins [9] and transcytosis [10], as well as the fact that the concentration of most of the studied components (except Na^+ and Cl^- ions) were exceeded by about a third, the most likely is the combination of the activation of reabsorption of AF components in fetal membranes by both of these mechanisms.

The volume of AF correlated with the concentration of creatinine and Ca^{2+} by acute hypoxic hypoxia (r = 0,460, p = 0,023; r = 0,445, p = 0,029, respectively), but did not correlate in the control group (r = 0.093, p = 0.597; r = 0.187, p = 0,286, respectively), which indicates an increase in the filtration process in the kidneys of the fetus. In addition, the volume of the amniotic fluid was normally inversely related to the urea concentration (r = -0.481, p = 0.0004), by acute hypoxic exposure, the connection became direct (r = 0.434, p = 0.034).

Conclusion

In accordance with the knowledge of the ways of AF formation (fetal urine, secretion by the lungs and nasopharynx, intramembrane pathway, swal-

lowing, transmembrane pathway), the revealed AF concentration on the background of acute hypoxic hypoxia is likely due to the removal of water from the resulting primary urine of the fetus or fetal membranes from the amniotic sac.

Table 2

Effect of acute hypoxic hypoxia on the volume and composition of amniotic liquid of female rabbits on 27-28 days of pregnancy

	Hypoxia (n = 43)	Control (n = 40)	p
Amniotic fluid volume, ml	0,36 (0,15—0,88)	0,46 (0,24—0,78)	0,357
Osmolality, mosmol/ kg	301,0 (295,8—307,3)	237,0 (223,0—245,3)	< 0,001
Na^+, mmol/l	144,0 (142,0—148,0)	132,0 (129,0—135,3)	< 0,001
K^+, mmol/l	10,50 (9,80—11,13)	7,40 (6,68—8,93)	< 0,001
Cl^-, mmol/l	109,0 (107,0—111,0)	102,0 (100,8—106,0)	<0,001
Ca^{2+}, mmol/l	2,99 (2,51—3,55)	2,13 (2,05—2,30)	< 0,001
P_i, mmol/l	1,32 (1,12—1,55)	1,00 (0,78—1,11)	< 0,001
Protein, g/l	5,15 (4,89—5,38)	3,65 (3,15—3,75)	< 0,001
Creatinine, mmol/l	0,200 (0,162—0,231)	0,155 (0,136—0,166)	< 0,001
Urea, mmol/l	8,20 (7,75—8,70)	6,50 (6,05—6,85)	< 0,001

Note: Data are presented in the form - median (25-75%); p - the reliability of intergroup differences according to the Mann-Whitney test.

These data show that an increase in the concentration of biomolecules in AF during hypoxia, for example, lactate [11] or interleukin-8 [12], may be associated with an increase in the osmolality of AF. To normalize the concentration of biomolecules in AF (especially during hypoxia), it may be important to calculate the relationship of the studied biomolecules to osmolality or the normal component of fetal urine, for example, creatinine [3].

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EFFICIENCY OF USE OF COMBINED VITAMIN COMPLEX: VITAMIN D AND VITAMIN K (LITERATURE REVIEW)

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In the present literature review, the data on the role of two vitamins D and K involved in the regulation of calcium metabolism, as well as other physiological and pathophysiological processes in the human body is provided. Taking into account the modern data, the relationship between the level and effects of vitamin K and vitamin D on calcium metabolism in bone tissue is discussed.

***Key words**: vitamin D, vitamin K, osteoporosis, extraosseous effects of vitamin D.*

Role of vitamin D in the regulation of bone structure

The amount of calcium contained in human bone tissue is in a state of direct dynamic equilibrium with the concentration of this ion in human plasma. Thus, a stable level of its circulation in the blood is maintained. At the same time, 99% of calcium is contained in bone tissue, and the remaining 1% is accounted for by blood plasma, muscles, brain cells and also skin. Despite such a small percentage of calcium in these organs, its biological activity is very high - it acts as a regulator of muscle contraction, including the heart muscle, participates in the mechanisms of synoptic transmission of impulses, changes nervous excitability, affects the permeability of cell membranes, participates in blood coagulation processes, etc.

By themselves, the bones of the skeleton are not an inert place of calcium deposition. Old calcium deposits in them are destroyed, the new ones are formed in their place. The speed of this process, called the turnover rate, varies significantly with age.

In infants, up to 100% of the calcium in the bones can be replaced during the first year of life. In older children, the calcium turnover is about 10% per year, while in adults, the figure does not exceed 2-3%. The increase in bone mass occurs mainly in childhood and adolescence, while the trabecular (spongy) bone reaches its peak (maximum) mass at 12-16 years, and cortical - at 20-24 years. The peak of bone mass is reached by 25-30 years, and in 40-50-year-old people, bone mass usually begins to decrease (up to about 1% per year), since bone resorption may begin to predominate over its formation. Bone mass loss accelerates during menopause - during the first 5 years of menopause, the bone mineral density (BMD) index may decrease to 5% per year. Peak bone mass is an important predictor of risk of fractures in the period of maturity and aging of the body. The magnitude of the peak of bone mass is influenced by genetic (60-85%) and hormonal, as well as environmental factors (in particular, physical activity and nutrition) [1]. A huge role in maintaining the desired bone density is played by hormones, including exogenous hormones: vitamins D and K.

Calcium is a hardly digestible element that enters the body with food through the small intestine, being absorbed mainly in the duodenum. Here, fatty acids form complex compounds with calcium salts, which are then absorbed by the villi of the intestines. Calcium absorption in the intestine occurs in two ways: transcellularly (transcellularly) and intercellularly (paracellularly). The first mechanism is mediated by the action of the active form of vitamin D (calcitriol) and its intestinal receptors. It plays an important role in low and moderate intake of calcium from food. With an increase in the calcium content in food, the intercellular transport of ion begins to play a leading role due to the significant gradient of its concentration.

For effective absorption of calcium, fat-soluble vitamin D is necessary. Without it, and also without fatty acids, calcium cannot overcome the barrier between the gastrointestinal tract and blood. Vitamin D enhances calcium absorption in the small intestine by inducing the synthesis of calcium-binding protein by enterocytes, and also increases calcium reabsorption in the renal tubules.

Vitamin D increases the permeability of the cytoplasmic membrane of cells of the intestinal epithelium for calcium, as a result of which it enters the enterocytes along an electrochemical gradient. This process of calcium transport can be mediated through the nuclei of target cells by stimulating of transcription of DNA and RNA by vitamin D [2], which is accompanied by an increase in the synthesis of specific transport proteins, for example, calcium binding protein [3], including calcium transport from enterocytes to the blood.

Influencing the kidneys, vitamin D enhances the reabsorption of calcium in them. In addition, vitamin D stimulates the absorption of phosphates and magnesium from the intestines, and also participates in the final differentiation and maturation of osteoblasts, without which normal bone tissue formation is impossible [4, 5].

Thus, maintaining a normal concentration of this vitamin in the body is of paramount impor-

tance for mineral metabolism. Thus, by hypocalcemia, vitamin D affects the bone architecture like parathyroid hormone (PTH), i.e. increases bone resorption and at the same time calcium absorption in the intestine. When vitamin D is deficient, only 10-15% of calcium and 60% of phosphorus from food are adsorbed in the intestine [6].

Extraosseous effects of Vitamin D

All of the above effects of vitamin D in the body is not limited. Recent studies have shown the effect of vitamin D on the immune system [7]: in particular, by stimulating transforming growth factor TGFbeta-1 and the production of interleukin 4 (IL-4), vitamin D suppresses the inflammatory activity of T-lymphocytes, allergic and autoimmune disorders, for example, such as juvenile diabetes, rheumatoid arthritis, etc. [8-14]. Vitamin D and calcium prevent the development of muscle weakness, is necessary for the functioning of the thyroid gland and normal blood clotting. A number of studies show that by improving the absorption of calcium and magnesium, vitamin D helps to restore the myelin sheaths of neurons [15–16], so it is included in complex therapy for multiple sclerosis and participates in the regulation of blood pressure and heart rate (in particular, by hypertension in pregnant women)). Its deficiency is associated with common diseases such as Alzheimer's disease and schizophrenia [17]. Vitamin D affects cell proliferation, differentiation and apoptosis, and also modulates the activity of the immune system. Apoptosis is important for the elimination of tumor cells. So, vitamin D through the immunomodulating activity of its own receptor causes the death of cancer cells. In this process, vitamin D affects the transcription of genes involved in the regulation of cell growth, division and apoptosis [18]. These effects make it effective in the prevention and treatment of cancers of the breast, ovaries, prostate, brain and leukemia [19-26].

Vitamin D realizes its biological effects through genomic and extragenomic mechanisms. Extragenomic mechanisms involve the effect of vitamin D on signaling pathways in cells of the immune and nervous systems. The mechanism mediated through the genetic material of the cell is the most important mechanism for the effects of vitamin D. The vitamin D receptor regulates the expression of several thousand genes in the human genome [27]. Thus, vitamin D is one of the key factors for maintaining genome stability.

Recent studies have shown that children with vitamin D deficiency are more likely to be obese [27]. It has been established, that in the development of obesity, the violation of the activity of insulin-like growth factor-1 (IGF-1) is significant. This regulatory peptide is one of the most important factors supporting the balance between adipose and muscle tissue. By the deficiency of IGF-1 activity, adipose tissue begins to predominate over the muscle one [28]. As a result, atherosclerosis and vascular calcification are accelerated. Vitamin D stimulates the synthesis of IGF-binding proteins, which prolongs the half-life of IGF-1, thereby enhancing anti-atherosclerotic effects. Consequently, vitamin D deficiency can be associated with obesity [29], high BMI [30], insulin resistance [31] and an adverse effect on insulin secretion [27].

There is a fairly large amount of data on the effects of vitamin D on the regeneration of skin and body tissues. It is known that diabetes mellitus occurs with characteristic changes in the skin: hyperpigmentation and dryness of the elbows, dull appearance of the skin of the face, itchy skin, a tendency to form pustular elements. Such changes are associated with impaired insulin receptor activity (insulin resistance). Active forms of vitamin D alter the expression of the insulin receptor gene, increasing their density and activity in the kidneys, liver and adipose tissue. At the same time, vitamin D regulates the transcription of fibroblast growth factor necessary for the implementation of the healing process of wounds [32], which is very important in the treatment and prevention of diabetic foot in patients with diabetes mellitus. The vitamin D receptor regulates the expression of interleukins, tumor necrosis factor, affects the production of antimicrobial peptides, which are endogenous "antibiotics", synthesized to maintain the immunity of the skin and other epithelial surfaces. Vitamin D contributes to wound healing, skin recovery by psoriasis and atopic dermatitis [27].

Vitamin D enters the human body in two ways: with food rich in this vitamin (fatty fish, fish oil, dairy products with normal fat) [4], and from the skin, where vitamin D is formed from a cholesterol-like substance under the influence of sunlight. If the body receives a sufficient amount of ultraviolet radiation on the open surface of the skin, the need for vitamin D is fully compensated [33, 34]. However, having considered this process in more detail, it is easy to see that it is quite difficult for a modern city dweller to avoid hypovitaminosis, because the amount of vitamin D synthesized in the malpighian and basal layers of the epidermis under the action of sunlight depends on factors such as:

- wavelength of visible light - the most effective is the average spectrum of UV-B waves, the wavelength is 290-315 nm;
- intensity of UV-B radiation. The sufficiency of UV-B radiation for the synthesis of vitamin D is observed only at certain times of the day: from about 11 to 14 hours [35]. Most of the territory of Russia is located in a zone of low insolation (north of 40 ° latitude), and most of the settlements are characterized by a small number of sunny days per year (from 40 to 70 days) [36]. At the same time, UV-B radiation, necessary for the synthesis of vi-

tamin D, does not reach the Earth's surface in all regions of the country [37];

- the level of the atmosphere pollution. Industrial emissions and dust do not transmit the spectrum of ultraviolet rays that potentiate the synthesis of vitamin D. This explains, in particular, the high prevalence of rickets in children living in industrial cities [38];

- initial pigmentation of the skin. The activity of the synthesis of vitamin D is inversely related to the degree of skin pigmentation [39, 40], the synthesis of vitamin D gradually decreases with increasing tanning [7];

- age. Aging skin loses its ability to synthesize vitamin D [41];

- physical activity. A rather active transition of the synthesized vitamin D from the epidermis into the bloodstream occurs during active exercise. Hypodynamia significantly reduces the content of cholecalciferol synthesized in the skin into the bloodstream [42].

In addition, it is known that calcitriol can be synthesized not only in the kidneys, but also in the cells of the pancreas, stomach, large intestine, epidermis, vascular endothelium, as well as in macrophages and placenta, which indicates para- and autocrine function.

Currently, there are many works proving the presence of vitamin D deficiency in the modern inhabitant of northern latitudes [43-52,37], as well as the deficit of Ca and osteoporosis. Low levels of vitamin D are associated with an increased risk of bone fractures. Vitamin D deficiency can contribute to weakness in the muscles of the proximal limbs, slowing down the walking speed, difficulty getting up from a sitting or squatting position, as well as lifting heavy objects [27].

Vitamin D with calcium supplements is used for the prevention and complex treatment of osteoporosis in a number of developed countries [53]. Vitamin D preparations are prescribed individually to patients, based on laboratory data on the concentration of this vitamin in the blood plasma [37]. In cases of improperly selected dosage or prescription of drugs without laboratory control, an overdose effect is possible. And at the same time, applied therapy does not always achieve the desired effect. Hypervitaminosis D is fraught with various consequences, the most important of which is abnormal calcification (deposits in vessels, kidneys and other tissues: deposits in the mammary glands, heel spurs) [54].

Depositing in the vessels and giving them rigidity, precious calcium seriously harms the body. In addition, calcium is often deposited in cholesterol plaques, making them very dense. As a result, the lumen of the vessel narrows, and its wall collapses. All this leads to thrombosis or rupture of blood vessels, and therefore to heart attacks, strokes and internal hemorrhages. Calcium in the arteries is now more dangerous than diabetes, elevated cholesterol and hypertension [55]. In such cases, calcium, having overcome with such difficulty the barrier of the gastrointestinal tract, can have an adverse effect on the body.

Exactly the disturbances in the distribution of calcium in the body (arising primarily due to calcium deficiency), and not some kind of imaginary "excess consumption" of calcium, can be the cause of both atherosclerosis and osteoporosis. Osteoporosis and vascular diseases are comorbid conditions (results of 25-year observations of the Framingham cohort) [56]. A large-scale study of intima wall thickness and density of lumbar bones showed that intimal wall thickness (an indicator of atherosclerosis progression) is inversely proportional to bone density (reflecting the state of the body's calcium depot) [57]. Calcium supplementation leads to an improvement in vasodilation of vessels and significantly reduces cardiovascular risk [58]. It should be noted that the intake of calcium preparations in doses that meet the recommended daily intake is completely safe [27].

The value of vitamin K

It is known that, having entered the blood, calcium should reach its destination - bone, muscle and other tissues, and not circulate infinitely in too large quantities in the bloodstream.

However, calcium alone cannot do this. It needs a carrier, which is a vitamin K-dependent protein. It delivers calcium to the bone tissue and organs - muscles, heart, brain. For a long time, vitamin K has not been given such great importance, since it was believed that there is no hypovitaminosis K, and the only purpose of vitamin K is to regulate the formation of vitamin K-dependent coagulation factors (II, VII, IX, X, proteins C and S), affecting blood clotting [59, 60].

In fact, the physiological function of vitamin K is much broader. Vitamin K is a fat-soluble vitamin that, in addition to hemostatic, plays a significant role in the metabolism in bone and connective tissues. To begin with, vitamin K consists of a mixture of several similar substances, the main of which are K_1 (phylloquinone) and K_2 (menaquinone) [61]. Phylloquinone is close to chlorophyll, is located in the green part of plants of vegetable greens (parsley, dill, spinach, sorrel, carrots, beets, turnips, as well as in all kinds of cabbage, zucchini, cucumbers, tomatoes, legumes, apples, nuts) and comes from the duodenum into the blood, where it presents for short, 2-3 hours, in contrast to K_2 (6-8 hours). Vitamin K_2 (menaquinone) is synthesized by the normal microflora (E. coli) of the large intestine, and also comes from fatty foods (fermented cheeses and soy products, beef liver, egg yolks, butter are rich in them). Both forms of vitamin K affect calcium metabolism, but K_2 has a more powerful effect than K_1 [62].

Vitamin K is involved in the synthesis of 16 proteins (ten in the liver and six other tissues). These proteins undergo carboxylation with the participation of the corresponding enzyme (vitamin K is a cofactor of this enzyme) [63-68], and only after that does the protein bind calcium. It is assumed that the form of vitamin K_2 has a greater affinity for these proteins compared to the form of vitamin K_1, which explains its greater efficiency [62].

Bone tissue is represented by cellular elements, an organic matrix and minerals. The organic matrix of 90% consists of collagen fibrils, and the remaining 10% are various non-collagen proteins. Osteocalcin (matrix Gla protein) is the main non-collagen protein synthesized predominantly by osteoblasts. At the same time, osteocalcin belongs to vitamin K-dependent proteins [69]. This protein, important in the formation of bone tissue, promotes the deposition of calcium salts in them. Vitamin K_2 provides carboxylation of osteocalcin. Slowing the carboxylation of this protein adversely affects its ability to bind to bone tissue and reduces bone mineralization. It is important that by the lack of vitamin K, less carboxylated forms of osteocalcin are formed. By a pronounced vitamin K deficiency, part of the osteocalcin remains completely non-carboxylated. These forms have a lower affinity for bone tissue. Thus, the more non-carboxylated osteocalcin in the blood, the lower the bone mineral density [70].

Another important protein is MGP, which, produced by chondrocytes and vascular smooth muscle cells, prevents the deposition of calcium in the vessels [71]. In the presence of a sufficient amount of vitamin K in the body, the process of bone calcification proceeds normally and calcium is distributed in the body correctly [72]. Therefore, vitamin K deficiency is a direct route to osteoporosis and atherosclerosis [73, 74].

It should be noted that not only the prescription of vitamin K_2, but simply the prescription of vitamin D reduces the level of non-carboxylated osteocalcin. In addition, this indicator is considered a risk factor for osteoporotic fractures.

Another mechanism of action of vitamin K on the bone system is the effect on osteoclasts: maintaining programmed death of osteoclasts (apoptosis) [75], vitamin K maintains a balance between the formation and death of these cells, thereby preventing excessive demineralization of bone tissue. Vitamin K also has other functions: it prevents the oxidative processes in the cells [76] (this effect is especially marked in the cells of the nervous tissue), participates in the synthesis of sphingolipids necessary for the myelin sheaths of the nerves [77], and also helps in regulating the body's inflammatory response (decreases release of some inflammatory mediators, for example, interleukin-6) [78].

Recent studies have shown the positive role of vitamin K in the prevention and treatment of a number of diseases: viral hepatitis, liver cancer, diabetes, Alzheimer's disease, rheumatoid arthritis [79].

Today we can note the presence of a deficiency of this essential vitamin [80, 81]. Vitamin K_1 is found in green leafy vegetables - cabbages, salads, as well as in wheat and other cereals. However, vitamin K_2 is not sufficient in them, it is synthesized from vitamin K_1 in the body of animals and birds that eat herbs and grains, namely in fatty tissues, and also gets into the milk and all the dairy products. The modern concept of healthy eating says that all foods must be low fatty, i.e. devoid of vitamin K. Animals and birds are almost not fed on green grass and cereals, transferring to forage, deprived of vitamin K_1. As a result, they also stopped synthesizing K_2 for us. Butter and other dairy products are mostly not useful.

In the low-fat cottage cheese and milk, vitamin K is completely absent. It turns out that healthy food, which we have been strongly promoting in recent years, only harms us. Low-fat cottage cheese has a high calcium content, but a substance that correctly distributes it throughout the body lacks in it. So, lovers of low-fat products who are afraid of the accumulation of cholesterol in the body, are going to suffer from the imbalance of substances in the body. Cholesterol participates in the creation of plaques on the walls of blood vessels, but together with calcium. However, since vitamin K prevents the deposition of calcium on the walls of blood vessels, by sufficient content of this vitamin, cholesterol does not accumulate there. It turns out that truly healthy food, although it contains cholesterol, also includes countering the harmful work of cholesterol.

Another source of vitamin K, which is the normal intestinal flora, also suffers. After all, any problems with digestion can contribute to vitamin K deficiency [82], the main of which today is dysbacteriosis.

Intake of a large number of different drugs, presence of preservatives, antibiotics in food, increased radiation background - all this leads to the fact that almost everyone is susceptible to disruption of the normal intestinal flora, and therefore, vitamin C deficiency. In addition, hypovitaminosis can cause: ulcerative colitis, celiac disease, shortened small bowel syndrome, gastrointestinal surgery, problems with the function of the pancreas, liver, gallbladder. Drugs, which not only cause dysbacteriosis, but also many others: anticoagulants, salicylates, promote hypovitaminosis [83]. Vitamin K deficiency often develops endogenously, caused by a violation of its formation in the intestine or a violation of absorption. There is some evidence that the aging process itself can contribute to vitamin K deficiency.

So, we can conclude that for most people today there is not only a deficiency of such an important and difficult to digest trace element as calcium, but

also a lack of substances that make its work effective, which creates conditions for the development of osteoporosis and atherosclerosis. Therefore, the prevention of these diseases is necessary, using calcium only in combination with them, i.e. with vitamins D and K [84-86].

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CONNECTION OF BIOLOGICAL AND SEASONAL FACTORS WITH INDICATORS OF THE AMOUNT AND ACTIVITY OF PLATELETS BY PHYSIOLOGICAL PREGNANCY IN THE CONDITIONS OF ALTAI KRAI

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A.E. Maltseva

The aim of this work was to study the dynamics of platelet number in pregnant women due to biological (weight, body length, age) and seasonal factors. The data of examination records of pregnant women served as a material for the study. Data on the number of platelets on the 12th and 28th weeks of pregnancy, growth, weight of patients before pregnancy are taken into account. The possible causes of platelet dynamics, taking into account the analysis of the results of other authors, are considered. The results indicate the influence of solar activity on the hemostatic system and the composition of peripheral blood. It is shown that, by normal platelet content, their dynamics during pregnancy depends on biological factors and is subject to intra-annual fluctuations, thus, seasonal shifts are observed.

***Key words**: platelets, pregnancy, mother's body composition, year season.*

During pregnancy, develop adaptive shifts in hemostasis, reflecting the state in the system that limits blood loss during labor. The hemostasiological status is characterized by an increase in the blood coagulation potential, an increase in the structural properties of the blood clot and inhibition of the enzymatic fibrinolytic activity [1]. These changes, together with an increase in circulating blood volume (CBV), prevent bleeding during the separation of the placenta, the formation of an intravascular thrombus and play an important role in preventing pregnancy complications such as thromboembolism and bleeding after the development of DIC syndrome. In normal pregnancy, there is an increase in the level of VII (proconvertin), VIII (anti-hemophilic globulin), X (Stuart factor) coagulation factors (from 50 to 100%), prothrombin level and IX factor (Christmas factor by 20-40% and especially plasma fibrinogen level.) The concentration of fibrinogen increases by 50%, which is the main cause of a significant increase in the erythrocyte sedimentation rate (ESR) during pregnancy. The prothrombin index also increases significantly by the 38th-40th week of pregnancy. The number of platelets decreases slightly due to their increased consumption. [2].

In the hemostasis system, platelets play a special role, containing thromboplastic and antiheparin factors, fibrinase, fibrinolytic agents, proteins that provide adhesion, aggregation and platelet release reactions. Information on the change in the number of platelets during the gestational process during physiological pregnancy is ambiguous.

In women, the normal level of platelets in the blood is 150-450 thousand/1 μl. With the onset of pregnancy, the level of platelets in the blood decreases slightly, which is called thrombocytopenia. In pregnant women, 100-415 thousand/1 μl of platelets is normal. This is due to the addition of the third circulation (placental) and some blood thinning. Thus, most authors point to a decrease in the number of platelets throughout pregnancy and associate this with both an increase in circulating blood volume and the consumption of platelets in the uteroplacental circulation. Other researchers do not detect changes in platelet count during pregnancy and even report cases of thrombocytosis [3] as a result of dehydration and blood clots or the presence of a hidden disease. The constitution of a pregnant woman is not taken into account.

It is known that the dynamics of hemostasis is subject to seasonal changes [4]. Knowledge of seasonal changes in platelets has a prognostic value, especially in cases where background indicators indicate thrombocytosis or thrombopenia.

The aim of the research was to study the dynamics of platelet count in pregnant women due to biological and seasonal factors.

Materials and methods

The material for the study was the data of examination records of 1154 pregnant women under observation at maternity hospital No. 2 in Barnaul during 1998. The age of those surveyed is 15-43 years old (average age is 24.6 ± 0.19 years). In order to verify the type and degree of identified dependencies, a repeated analysis was carried out on the available material of 2014 data (2150 women). The selection of materials for the analysis in these years was justified by the fact, that in 1998 and 2014, the indicators of environmental factors were the closest and typical for the climate of Altai Krai; The average annual indices of solar activity were comparable and corresponded to the middle of the ascending branch of the 11-year solar cycle. Seasons were classified as follows:

1. Astronomical seasons: counted from the points of the solstice (in winter - of December

22, in spring - of March 21, in summer - of June 22, in autumn - of September 22). Determined by the dynamics of the rotation of the Earth around the sun.

2. Calendar seasons: dividing the year into four seasons, three calendar months each: autumn - from mid-August to mid-November; winter - from mid-November to mid-February; spring - from mid-February to mid-May; Summer - from mid-May to mid-August.

3. Climatic seasons: dividing the year into four seasons according to the annual temperature of the environment of the region [1].

Among the patients were women whose pregnancy proceeded in rural and urban environments. The study took into account data on the number of platelets at the 12th (T12) and 28th (T28) weeks, pregnancy duration, height (average height 164.14 ± 0.24 cm), weight of patients before pregnancy (average weight 62, 14 ± 0.43 kg). The determination of body mass index (BMI) was carried out according to the formula: BMI = body weight (in kg)/ body length (in cm^2). The following grades of body mass index (BMI) taken for women were taken into account: body mass deficiency - BMI <19; normal body weight - 19 <BMI <24; overweight (pre-obesity) - BMI> 29. We used the methods of parametric statistics with the calculation of the mean and its errors and regression analysis using the "Statistica-6" package.

Results and discussion

The total sample included three people with a level of TP12 ≤ 150 thousand/1 mm^3 and one person with a level of TP28 ≤ 150 thousand/1 mm^3, and no persons with platelet count ≥400 thousand/1 mm^3 were found. Upon further analysis, cases of thrombocytopenia were excluded from the sample as atypical. Thus, in the pregnant women examined, the platelet count at the 12th and 28th weeks of gestation was within the physiological norm [5].

Often occurring extragenital pathology is a metabolic syndrome (excess of proper body weight). Among the disorders accompanying metabolic syndrome are the tendency to thrombosis and an increase in plasma levels of plasminogen activator inhibitor [4]. Metabolic syndrome is a combination of abdominal obesity, hyperglycemia, dyslipoproteinemia, arterial hypertension, impaired hemostasis and chronic subclinical inflammation, the pathogenetic essence of which is the phenomenon of insulin resistance (IR) [6]. Interest in this problem is also explained by the significant contribution of the metabolic syndrome to the development and progression of cardiovascular diseases [7].

In recent years, interest in studying the relationship of metabolic disorders, obesity and their attendant changes in the hemostasis system with an increase in cardiovascular complications in people with metabolic syndrome has grown significantly. To date, enough data has been accumulated indicating that insulin resistance (IR) and developing hyperinsulinemia (GI) may increase the risk of cardiovascular complications due to their coagulation disorders. Although, if the majority of researchers are of the same opinion about the nature of changes in the vascular and plasma components of the hemostasis system in the metabolic syndrome, then judgments about the state of the platelet level of the coagulation system are very contradictory. In this regard, we determined the platelet count and assessed the degree of physiological thrombocytopenia by the 28th week in pregnant women with different body mass index (BMI) values (Figure 1).

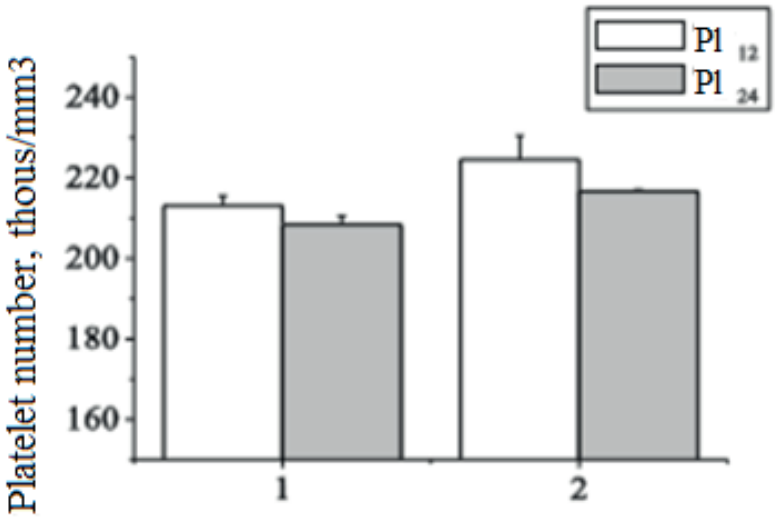

Figure 1 - The number of platelets during gestation in patients with normal weight (1) and obesity (2) All observed were divided into two groups: 1 - with a BMI ≤30 (84 people) and a BMI> 30 kg/m^2 (834 people).

According to WHO criteria, obese women significantly dominate in the sample. Both at the 12th and 28th weeks of pregnancy, platelet count is higher in the group of overweight women. The decrease in the number of platelets by the 28th week of gestation is more pronounced in women with obesity (4.9% vs. 2.2%). According to the authors, a correlation was found between the plasma lipid spectrum and the fatty acid composition of platelet membranes, due to changes in the content and ratio of omega-3 and omega-6 PUFAs against the background of atherogenic changes in the blood lipid profile in people with metabolic syndrome [5].

In their body, there is observed predominance of activated freely circulating morphological forms of platelets presented by disk echinocytes, spherocytes, sphere echinocyte, and a relationship between the morphological forms of platelets and fatty acid composition of the membranes, due to an increase in omega-6 PUFA, there has been revealed a relationship between the number of active morphological forms of platelets and the level of functional platelet activity determined by the intensity of the aggregation response to the action of different inducers.

In the literature, there is no data on the effect of age and body length on platelet count in both women outside of pregnancy and during it. Regression analysis showed that at the 28th week of pregnancy, the number of platelets is inversely proportional to the length of the body (P = 0.02) and the age of the woman (P = 0.001) (Figure 2).

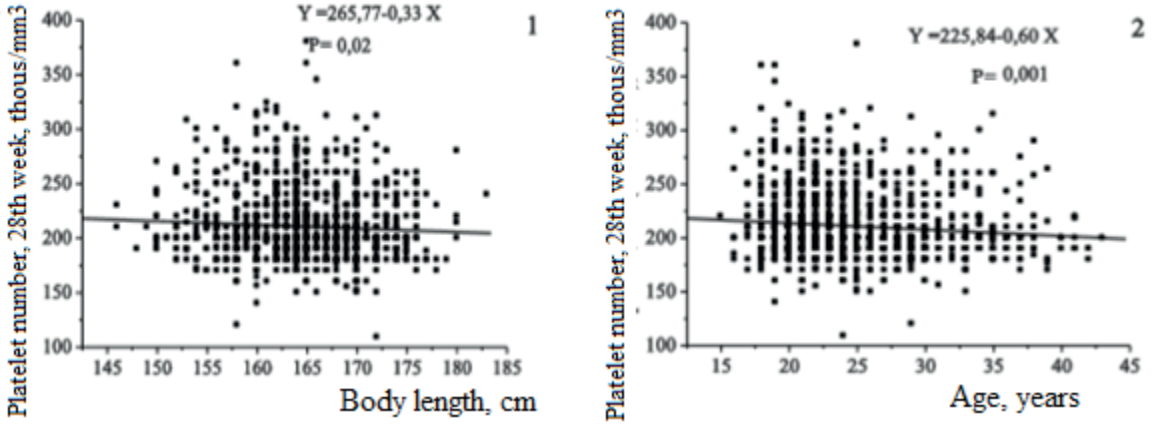

Figure 2 - Dependence of the number of platelets on the length of the body (1) and the age (2) of pregnant women at the 28th week of gestation.

The relationship between the degree of reduction in the number of platelets by the 28th week of pregnancy in relation to their number at the 12th week with age was not found. Moderate thrombocytopenia without a change in their functional activity may be associated with increased consumption of platelets in the peripheral blood circulation [7].

Wallaschofski H. et al. [8] explain the decrease in the number of platelets during physiological pregnancy by platelet aggregation, which occurs under the influence of progesterone and prolactin. These hormones can cause platelet aggregation without the addition of aggregation inducers. Similar platelet activity was noted by R. Lifenko. in I-II trimesters, when platelet aggregation practically corresponds to that of non-pregnant women in phase II of the menstrual cycle, but by the third trimester, this indicator may decrease [9].

The change in the seasons has a significant effect on the dynamics of blood parameters. Seasonal changes are subject to coagulation of blood. Activation of the blood coagulation system occurs more often in spring and much less in summer. It is shown, that platelet aggregation activity is subject to seasonal fluctuations, and all recorded aggregation indicators are higher in the autumn-winter period. The disaggregation component of the aggregation potential turned out to be the most variable, it prevails in the spring-summer time [5].

In our study, in meteorological conditions of Altai Krai, the maximum number of platelets at the 28th week of pregnancy was observed at conception in February, and the minimum - at conception in January and December. In May and August in pregnant women, the number of platelets increased, and in July - decreased (Figure 3).

The greatest manifestation of the physiological reduction in the number of platelets is noted by conception in December. The maximum severity of the physiological reduction in the number of platelets by the 28th week of pregnancy is observed in July (Figure 1). These results may have prognostic significance, especially in cases of the risk of thrombocytopenia or thrombocytosis. According to other authors, studies have shown that during the period of seasonal geomagnetic changes (winter - summer), the number of platelets decreases, mainly due to the redistribution of cell subpopulations. These results indicate the effect

of solar activity on hemostasis and peripheral blood composition. In a number of works, it has been shown that the state of microcirculatory hemostasis and the anti-aggregation activity of blood vessels in healthy people are associated with the seasonal content of antioxidants in the body. It is suggested that in the fall, when there are natural conditions for increasing the level of antioxidants (the diet is associated with the predominance of vegetables and fruits in the diet), the coagulation and aggregation ability of platelets is weakened. Apparently, solar disturbances, as well as other factors of the human environment, are not an exception, and a healthy body responds to their impact with an adequate adaptive response: the acceleration of the blood clotting process is compensated by the activation of the blood fibrinolytic system to maintain the balance of blood rheological properties and energy processes in cells [eleven]. It is possible that in this case the functional properties of platelets also change.

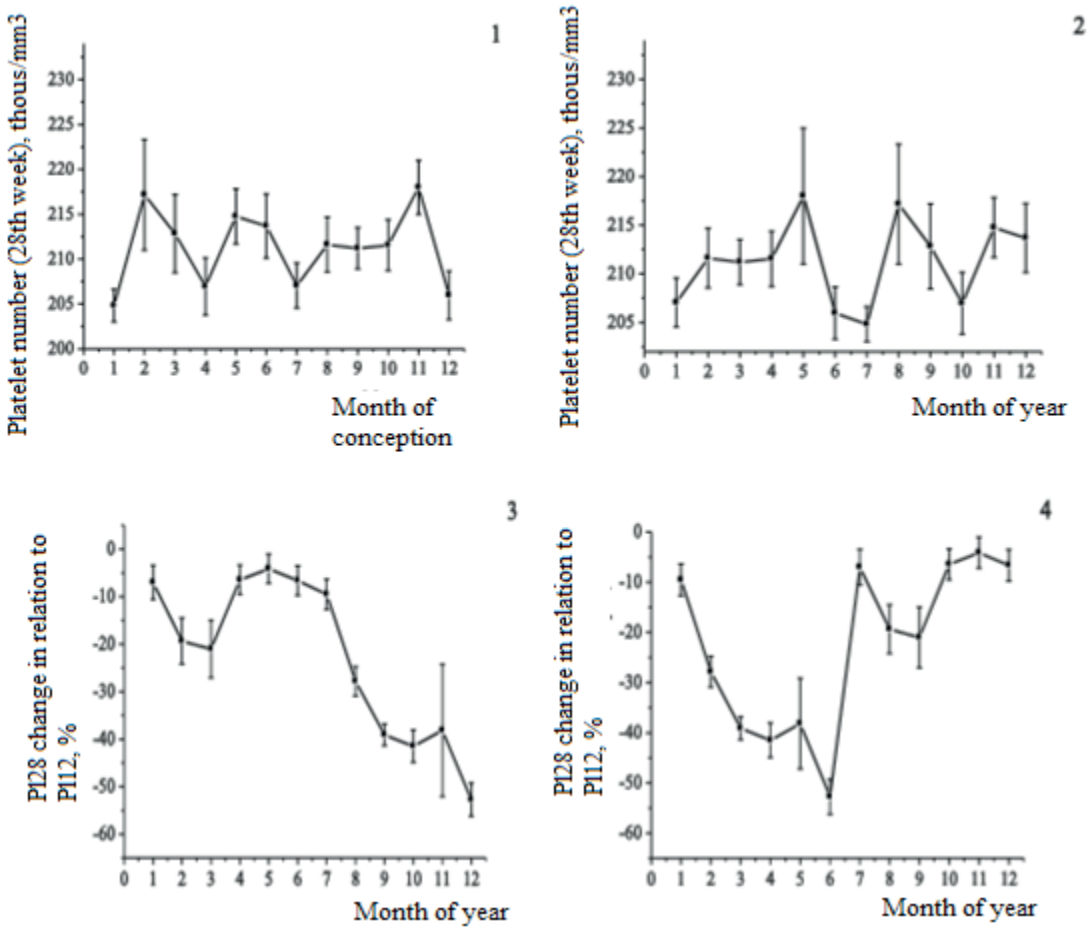

Figure 3 - The number of platelets at the 28th week of gestation depending on the month of conception (1) and in different months of the year (2), the physiological decrease in the number of platelets from the 12th to the 28th week of pregnancy depending on the month of conception (3) and different months of the year (4).

Conclusions

1. In overweight women, the number of platelets at the 12th and 28th weeks of gestation is increased compared with women who have normal body mass index.

2. At the 28th week of pregnancy, the number of platelets is inversely proportional to the length of the body and the age of the woman.

3. The maximum number of platelets at the 28th week of pregnancy is noted by conception in February, and the minimum - by conception in January and December. In August, the number of platelets in pregnant women increased, and in July - decreased. The greatest manifestation of the physiological reduction in the number of platelets by the 28th week of pregnancy is observed by conception in December, and during pregnancy in July.

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CLINICAL AND DYNAMIC MECHANISMS OF THE INITIAL PERIOD OF SCHIZOPHRENIA SUBTYPE WITH A LEADING NEGATIVE SYMPTOM COMPLEX

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The systematization of the manifestations of the initial period of simple schizophrenia as a subtype with a leading negative symptom complex with the determination of the clinical-dynamical mechanisms of the formation of each clinical pattern, taking into account its duration, was carried out. The aim was the systematization of clinical-dynamic mechanisms of the initial period of the schizophrenia subtype with a leading negative symptom complex. Material and methods: 168 patients with simple schizophrenia were examined, 128 (76.2%) men, 40 (23.8%) women. The average age of the sample was 28.1±1.4 years. A structured diagnostic interview was used, anamnestic information was collected on premorbid, initial period and the manifestation of the disease. Standardization was carried out using the ICD symptom checklist and the ICD-10 symptom glossary for mental disorders. Statistical processing was carried out using the Mann-Whitney criterion, the Spearman correlation coefficient and the Fisher criterion. Results: the initial period with prevalence of pseudo-neurotic disorders was noted in 73 patients (43.5%), in women it was significantly more frequent ($p < 0.05$). Behavioral disturbances prevailed in 70 patients (41.7%). Prodrome by the type of the "simplex"-syndrome proper was observed in 25 patients (14.8%). In most patients (75.6%), a gradual initial period prevailed. Subacute and acute prodrome was in 17.3% and 7.1%, respectively. There were no statistically significant differences in the frequency of occurrence of its types in terms of duration depending on gender. Discussion: clinical-dynamical mechanisms of the prodromal stage of the disorder were distinguished, differing in the prevalence of certain clinical manifestations, as well as in its duration. Conclusion: by simple schizophrenia, in most cases (75.6%), the initial period occurred with a gradual increase in pseudo-neurotic and behavioral disturbances, as well as manifestations of the "simplex"-syndrome. This trend is typical for both sexes, but in women, pseudo-neurotic disturbances (57.5%) were met statistically significantly more frequently ($p < 0.05$). The transition of the initial period to the debut of simple schizophrenia proceeded smoothly.
***Key words**: schizophrenia, symptom complex, diagnostics.*

In recent decades, the clinical picture of schizophrenia is considered mainly in a dichotomous model, in which the opposite poles occupy positive and negative symptom complexes, represented, respectively, by delusions and hallucinations - on the one hand, and emotional indifference and lack of will (abulia) on the other [1-2]. In the classical division of schizophrenia into subtypes, only two of them are fully manifested by the clinical picture, in which negative symptoms play a leading role - simple and residual. Residual schizophrenia is the outcome of paranoid, hebephrenic, catatonic and disorganized schizophrenia, and is usually fixed after several episodes of exacerbation of the subtypes listed. The focus of this work is simple schizophrenia as the only one of the negative pole subtypes that has an initial (prodromal) period.

The concept of ICD-10 [3] treats simple schizophrenia as an infrequent disorder difficult to diagnose. Its main features include the progressive development of oddities in behavior and the inability to meet the requirements of society. Characterized by the flattening of the affect, the loss of impulses without prior pronounced psychotic symptoms. The disorder is not as distinctly psychotic as other subtypes of schizophrenia. In general, individual subtypes in recent years act as objects of study less and less often due to the gradual change of the categorical paradigm in psychiatry to the dimensional one [4], and the simple form has practically dropped out of the research space due to the narrowing of the diagnostic boundaries of schizophrenia in the latest systematics of the DSM [5 -8], based in this section on first-rank symptoms described by K. Schneider [9]. At the same time, studies of recent years show the importance of scientific research in this direction [10-11], especially since in DSM-5 [8] there is still an indication of the need for further study of simple schizophrenia, which in this systematics is called "fuzzy psychosis syndrome. "

The initial period of schizophrenia, as a rule, remains out of sight of psychiatrists due to the low appealability of specialized care at this stage, however, even by appealing, arises a problem conditioned by difficulties of diagnosis, which leads to complications in the choice of therapy. Adequate treatment is more effective if it begins even before the manifestation of the schizophrenic pro-

cess. At the same time, it is necessary to clarify the diagnostic criteria and classify the initial period of schizophrenia, especially with its protracted variants [12].

The pre-manifest stage is represented by subclinical changes that affect adaptation opportunities and are manifested by a large number of absenteeism or cessation of studies, persistent changes in interests, prolonged social passivity, estrangement and isolation, prolonged changes in appearance and behavior [13]. Among the behavioral phenomena of the prodrome, suicidal occupies a special place [14-15]. Although it should be emphasized, that sometimes a suicide attempt during the initial period is a consequence of a real stressful situation and should not be considered a manifestation of a schizophrenic process [16], since linear determinism contributes to the erroneous qualification of mental status [17]. On the other hand, a suicide act, carried out in the absence of a traumatic situation or mood disorders can be classified as a part of behavioral disorders [18] or as dissociation of emotional manifestations in schizophrenia [19]. The most complete attempt to systematize the prodromal stage of simple schizophrenia was made by E.D. Kosenko [20], who divided it, depending on the dominant symptoms, into a psychopathic, apathoabulic, neurosis-like and painful sharpening of premorbid pathological character traits, with severe affective disorders, with phenomena of philosophical intoxication.

In accordance with the above, we have systematized the manifestations of the initial period of simple schizophrenia as a subtype with a leading negative symptom complex with the definition of the clinical and dynamic mechanisms of the formation of each clinical pattern, taking into account its duration.

The purpose of the study is to highlight the clinical and dynamic mechanisms of the initial period of a subtype of schizophrenia with a leading negative symptom complex.

Material and methods

The sample included 168 patients with simple schizophrenia, verified in accordance with the criteria for this ICD-10 subtype [3] and who were at the time of the study at the dispensary observation in the city of Tomsk. Among all surveyed, men constituted 128 (76.2%), women - 40 (23.8%), the average age of the sample was 28.1±1.4 years. The study was conducted in compliance with the protocol approved by the local ethical committee of the Mental Health research Institute, Tomsk National Research Medical Center.

Study design. This work is an observational cohort retrospective study, the subject of which are the clinical and dynamic mechanisms of the initial period of simple schizophrenia, manifesting chronic negative disorders. Taking into account the early age of the onset of schizophrenia, the pronounced manifestations of a decrease in the adaptive potential in the prodromal stage and its duration, the study was based on the clinical-typological [21], static-dynamic assessment [22] adopted for such samples. The survey was conducted using a structured diagnostic psychiatric interview, anamnestic information was collected on premorbid, initial period and disease manifestation. For standardization of clinical data, an estimated list [23] and a glossary of symptoms for mental disorders [24] for ICD-10 were used.

Data processing was carried out using the Statistica 8.0 package. To assess the reliability of differences in quantitative indicators, the criterion of Mann-Whitney was used. To assess the linear dependence of quantitative data, the Spearman correlation coefficient was calculated. Comparison of independent samples for the frequency of occurrence of variants of the initial period was performed using Fisher criterion. The values obtained during the analysis of the correlation were taken as reliable for the values of the correlation coefficient $r = 0.5\text{-}1.0$ with the significance level of differences $p < 0.05$.

Results and discussion

On the basis of the grouping of clinical manifestations, we have combined their polymorphism into three groups of symptom complexes of the initial period of simple schizophrenia: with the predominance of pseudo-neurotic disorders, the predominance of behavioral disorders and the manifestation of the "simplex" syndrome (Table 1).

The initial period with the predominance of pseudo-neurotic disorders was noted in 73 patients (43.5%). Moreover, in women, it occurred significantly more frequently ($p < 0.05$). It was clinically expressed in complaints of weakness, lethargy and feeling unrested. Distraction, disturbance of concentration appeared, there were "runaway" thoughts, "emptiness" in the head, "poverty of thoughts" and "absence" of memory. Along with this, irritability, chilliness of the limbs, hyperacusia. All this contributed to a decrease in activity, the emergence of subclinical hypochondria with fixation on various bodily sensations, which were of the most diverse nature. There were local or generalized amorphous, painful, sensations difficult to qualify, sometimes imitating somatic or neurological diseases. There was a feeling of change in the self, a sense of impending catastrophe, transient phenomena of auto- and allopsychic derealization, alienation of bodily sensations, a sense of the lifelessness of the surroundings. Chronic hypohedia developed. Pseudo-neurotic symptoms could also arise in the form of a somatic sensation, under the "veil" of physical distress. Such patients complained of pain in the area of the heart, disorders of the gastrointestinal tract. Objectively, there were vegetative-vascular disorders, nasal

bleeding, cardialgia, pseudo-neurotic attacks, reminiscent of panic attacks, but not meeting the criteria for the diagnosis of panic disorder in terms of severity, quantity and temporary factor. Behind the façade of various variants of pseudo-neurotic disorders, transient "influxes" of thoughts, their "sounding", fancy senestopathies, loss of a sense of continuity and integration of the mental process sometimes appeared. In general, the symptom complex consisted of a combination of hyperstetic disorders and mental exhaustion.

Table 1

Clinical characteristics of the initial period of simple schizophrenia

Initial period	Gender					
	Male		Female		Both	
	Abs.	%	Abs.	%	Abs.	%
1. Pseudoneurotic disorders	50	39,1	23*	57,5	73	43,5
2. Behavioral disorders	54	42,2	16	40,0	70	41,7
3. «Simplex» syndrome	24	18,7	1	2,5	25	14,8
Total	128	100,0	40	100,0	168	100,0

Note: * denotes data with a significance level of $p < 0.05$

Behavioral disorders prevailed in 70 patients (41.7%). They were manifested by disinhibition, abandonment of generally accepted forms of behavior, oppositional attitude towards the nearest environment, non-motivated outbursts of rage, gloominess, inactivity. Patients became alienated, inaccessible, treated their relatives with open hatred, became cold and callous. Since the debut of the disease in most cases was observed in the adolescent period, parents often viewed this behavior as part of a "transitional" age or as a defect in upbringing. The behavior was marked by pronounced asociality, penchant for the criminogenic groups. Sometimes, in the foreground there was disinhibition, hyperexcitability and fussiness. Due to the emotional coldness of the external affective discharges, actions, behavior were particularly ridiculous and brutal. Many patients were prone to dromemania, not having a specific range. They were incomprehensible and inexplicable to others. The weakness of volitional delays led to socially dangerous actions. The vague sense of change, "mental" discomfort and disorder of mental activity determined the emergence of craving for alcohol. In drunkenness, behavioral deviations and emotional-volitional disorders were even more pronounced.

The initial period, proceeding according to the type of the "simplex" syndrome itself, was observed in 25 patients (14.8%). For them, the characteristic was a decrease in mental productivity, the emergence of an opposition to relatives, heightened sensitivity or lethargy, laziness, gloominess, isolation, alienation. The patients felt their own "change", which was manifested by undiscovered ideas of the relationship and a sense of "catastrophe". Thinking became blurry, fuzzy. Appeared more or less pronounced phenomena of metaphysical intoxication. Against the background of depressed mood, there were occasionally "influxes", "delays", "breaks" of thoughts.

There was noted an unproductive tendency to analyze actions, abstract problems. The increasing thread of allopsychic emotional resonance with autopsychic orientation and the destruction of interpersonal relations passed through the red thread through all the initial simplex symptoms. Patients lost weight, closed, dropped out of school, work. The development of these disorders, as a rule, was gradual.

The mechanism of the formation of symptom complexes from the first signs of prodrome to manifestation is also determined by the length of the initial period. We estimated its duration to three months as the acute onset of the disease; from three months to one year as subacute and over one year as gradual. In the majority of patients examined - 75.6%, a gradual initial period prevailed (Table 2).

Subacute and acute prodrome occurred much less frequently - 17.3% and 7.1%, respectively. There were no statistically significant differences in the frequency of occurrence of its types in duration depending on gender.

In contrast to the subtypes of schizophrenia, characterized by episodic course, especially with the predominant positive symptom complex, with a simple form, with its "erased" onset and chronic nature, the allocation of the initial period is quite a difficult task. In the present study, the clinical and dynamic mechanisms of the prodromal stage of the disorder were distinguished, which differed in the prevalence of certain clinical manifestations, as well as its duration.

Table 2

The duration of the initial period of simple schizophrenia

Duration of the initial period	Gender					
	Female		Female		Female	
	Abs.	%	Abs.	%	Abs..	%
1. Acute	10	7,8	2	5	12	7,1
2. Subacute	20	15,6	9	22,5	29	17,3
3. Gradual	98	76,6	29	72,5	127	75,6
Total	128	100,0	40	100,0	168	100,0

"Simplex" syndrome, being the basis for the diagnosis of simple schizophrenia after the manifestation of the process, occurred only in 14.8% of cases in prodrome. The presence of pseudo-neurotic and behavioral disorders in the foreground in the majority of the examined people testifies in favor of the fact, that with this subtype more often in the initial period, non-specific psychopathological precursors of the disease appear, which extremely complicate the diagnosis. The data obtained open up prospects for further research primarily from the perspective of clinical dynamics from the prodromal stage to the completion of the first year of the disease in order to understand the mechanisms of syndrome formation.

The given types of prodromal periods were previously distinguished by other authors in terms of duration [25-26]. With regard to this study, it was necessary to establish the frequency of their occurrence in simple schizophrenia. The results obtained on the prevalence of a gradual initial period correspond to the traditional notions of inconspicuous, gradual onset of simple schizophrenia [27–31], and also give an understanding that the duration of prodrome does not depend on the sex of the patients. In the future, it seems necessary to analyze its relationship with other constitutional factors.

It should be emphasized that in routine clinical practice, a retrospective analysis of the initial period, the development of the disease is important for predicting the clinical manifestations of the disorder and the adaptive capacities of patients, which is evident from the present study and repeatedly emphasized in the psychiatric literature [20, 32-36].

Conclusion

The study showed, that by a simple subtype of schizophrenia, characterized by a predominance of a negative symptom complex in the clinical picture in most cases (75.6%), the initial period proceeded with a gradual increase in pseudo-neurotic and behavioral disorders, as well as manifestations of the "simplex" syndrome. This tendency is characteristic of both sexes, but in women, pseudo-neurotic disorders were statistically significantly more frequent ($p < 0.05$) (57.5%). The transition from the initial period to the debut of simple schizophrenia proceeded smoothly, which in some cases required additional differential diagnostics to verify the debut of the disease.

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THROMBOTIC EVENTS AND CONDITION OF THROMBOTIC READINESS IN PATIENTS WITH ISCHEMIC HEART DISEASE

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Retrospectively, n = 130 men with ischemic heart disease (main group) and n = 39 men without ischemic heart disease (control group) were included for the analysis of thrombotic events in the anamnesis and the study of the parameters of the hemostasis system in order to assess the thrombotic readiness status. It was found that for patients with IHD, in comparison with the control group, an increased content of fibrinogen levels, RFMC, HC, ET-1, increased activity of factor VIII and FV, indicating the presence of thrombotic readiness, are typical. Patients with IHD and multi-vessel CA lesions had a more pronounced activation of blood clotting at the levels of RFMC, PV and factor VIII activity, and also ArgpT was significantly higher than in patients with less severe CA atherosclerosis. In these patients, more frequent occurrence of Hmzg and Htzg forms of folate cycle genes was found. In young patients who underwent MI with "pure" CA, gene polymorphisms were detected not only in the folate cycle, but also in PAI-1 and F XIII.

Key words: *thrombotic events, thrombotic state, ischemic heart disease, coronary artery atherosclerosis.*

An important role in the development of atherosclerosis as the main cause of the ischemic heart disease (IHD) is played by disorders in the hemostasis system [1, 2]. A permanent hypercoagulable state is formed, predisposing to the development of thrombotic complications in the setting of atherosclerosis. These processes can be caused both by genetic defects by congenital thrombophilia and a consequence of exposure to adverse environmental factors and numerous diseases [3, 4]. In the past ten years, not only the genetic roots of most congenital types of pathologies have been identified, but also candidate genes for the overwhelming number of acquired diseases, including thrombogenic polymorphisms and mutations that create an innate tendency to increased thrombus formation, have been found [5]. Studies devoted to thrombogenic polymorphisms in patients with IHD show that some mutations can be independent risk factors (RF) in the development of coronary atherosclerosis and complications associated with them. However, some mutations can reduce the risk of coronary artery thrombosis, even in patients with severe coronary atherosclerosis [6, 7, 8, 9]. The phenomenon of the development of thrombosis of the coronary vessels as a cause of myocardial infarction (MI) in the absence of atherosclerotic lesions of the coronary arteries (CA) in young patients remains poorly studied.

Materials and methods

The study was conducted in a group of patients n = 130 people, selected from the register of chronic ischemic heart disease (HIBS) and examined at the Regional Clinical Hospital. These patients constituted the main group with an average age of 50.7 ± 10.4 years. The comparison group (control) was presented by 39 men who considered themselves healthy, without a personal and family thrombotic history, the average age was 47.5 ± 3.8 years. The groups were comparable in age and sex. In the regional hematological center of Z.S. Barkagan, in patients of both groups, there was carried out a laboratory study of blood using the methods of studying coagulation, anticoagulant, fibrinolytic units of the hemostasis system, vascular-platelet hemostasis, endotheliosis markers and molecular genetic testing using 12 parameters by the method of polymerase chain reaction. Statistical processing of the material was carried out using descriptive statistics, software packages STATISTICA 6.0. In the presence of a normal distribution, the statistical significance of the differences was determined using Student's t-test. For each of the values having a normal distribution, the mean (M), standard deviation (SD) are given. Upon detection of sample distributions other than normal, non-parametric data processing methods were used. Differences with the probability of an event $p \leq 0.05$ were considered statistically significant.

Results and discussion

The study of thrombotic history among patients with IHD showed that 77.7% of patients have a history of arterial thrombotic events (myocardial infarction, ischemic stroke), and their familial thrombotic events constituted 31.5%.

By the study of hemostasis parameters, it was found that for patients with IHD (main group) compared with the control group (Table 1), increased levels of fibrinogen and high-molecular components of the fibrinogen pool (SFC), increased activity of VIII and Willebrand (FV) factors, indicating a moderate activation of blood coagulation - a state of thrombotic readiness [10, 11], balanced by physiological anticoagulants, were typical. Thus, the level of SFC in patients with IHD was twice as high as its value in the control group, whereas the activity

of antithrombin (AT) III and protein C remained normal. In patients with coronary artery disease, the time of XII-a dependent fibrinolysis (XII-a ZF), activated through the triggers of the contact phase of blood coagulation and the kallikrein-kinin system, was almost twice as long. At the same time, the amount of the substrate of the fibrinolytic system – plasminogen - was sufficient.

Table 1

Comparative characteristics of the studied parameters of the hemostasis system and endotheliosis markers in patients of the main and control groups

Parameter	main group (IHD), n=130 (X±SD)	Control group (healthy), n=39 (X±SD)	p
Platelets,*10^9/l	229,80±55,87	249,66±49,03	0,3
PTT, c	33,83±4,96	35,02±2,03	0,1
Clotting time, c	13,42±2,16	13,36±1,26	0,8
Thrombin clotting time, c	20,02±3,51	18,84±1,20	0,04
Fibrinogen, g/l	4,09±1,41	2,98±0,74	<0,001
SFC, mg/100 ml	8,97±7,30	6,01±3,94	0,01
D-dimer, ng/ml	115,19±108,02	68,23±29,89	0,008
Antithrombin III activity, %	104,34±11,22	108,79±9,23	0,02
Screening for Disorders in the Protein C, HO System	1,01±0,18	1,09±0,14	0,007
XIIa-dependent fibrinolysis, min	25,82±13,99	14,35±9,26	<0,001
Plasminogen, %	118,86±21,63	114,48±15,82	0,2
Factor VIII activity, %	171,68±29,93	131,41±38,24	<0,001
AgrT - ADF, %	53,41±15,64	64,35±17,93	<0,001
AgrT - ristomycin, %	81,22±19,37	86,90±7,81	0,1
AgrT - adrenaline, %	34,94±23,56	36,64±28,20	0,7
AgrT - collagen, %	48,03±23,72	65,17±20,87	<0,001
WF, %	172,34±34,30	131,05±38,14	<0,001
HC, mkmol/l	13,64±5,87	9,86±2,22	0,04
ET-1, fmol/ml	0,79±0,90	0,26±0,02	<0,001

In addition, elevated levels of endothelin (ET) - 1 and homocysteine (HC) have been detected, indicating the presence of endothelial dysfunction [4, 12]. The difference in aggregation activity (platelet aggregation - AgrT) in the groups of patients with IHD and the control group, according to our data, was associated with the intake of antiplatelet agents.

According to the frequency of carriage of thrombogenic mutations and polymorphisms in the compared observation groups (Table 2), in patients with IHD, out of 12 studied genes, only the Htzg A66G genotype of the MTRR gene was found to predominate (p = 0.03).

Table 2

Polymorphic variants of coagulation factor genes and folate cycle system genes in the studied groups

Locus	Genotype	Main group n=130, n (%)	Control group n=39, n (%)	p
MTRR A66G	AA	31 (23,8)	12 (30,8)	0,4
	AG	68 (52,4)	13 (33,3)	0,03
	GG	31 (23,8)	14 (35,9)	0,1

Patients of the main group were divided into three subgroups depending on the number of atherosclerosis affected CA: n = 35 - single-vessel, n = 32 - two-vessel, and n = 58 - multi-vessel lesion of CA. Five patients of the main group were not included in any of these subgroups due to the absence of CA lesion according to the CAG data.

When comparing the parameters of the hemostasis system in patients with single- and multi-vessel lesions of CA, it turned out that a significant dif-

ference was found in the level of SFC (p = 0.03), FV (p = 0.03) and factor VIII activity (p <0.001), which were higher by more severe lesion to CA (Table 3). In comparison with the control group, a significant difference was found in patients with respect to the level of fibrinogen, the activity of AT III, XII-a ZF along with an increased level of SFC, D-dimer and activity of VIII factor. Thus, the greatest shifts in SFC were revealed in the case of the multi-vessel lesion. At the same time, there was no difference in the anticoagulant level and fibrinolysis when comparing the subgroups between themselves and the control group, indicating a deterrent effect of the anticoagulants and the fibrinolysis system in response to moderate thrombinemia detected in patients with IHD.

Table 3

Comparative characteristics of the studied parameters of the hemostasis system and endothelial dysfunction in patients with IHD, depending on the degree of CA lesion

Parameter	Main group n=130, (X±SD)			p1-2/1-3/2-3
	Single-vessel lesion n=35	Two-vessel lesion n=32	Multi-vessel lesion n=58	
	1	2	3	
SFC, mg/100 ml	8,27±5,19	8,57±4,83	11,05±6,33	0,8/0,03/0,06
Factor VIII activity,%	159,33±37,65	166,58±30,25	181,40±21,75	0,4/<0,001/0,009
AgrT - ADF, %	48,03±8,36	51,83±16,04	58,61±17,18	0,2/0,001/0,07
AgrT - adrenaline, %	29,32±19,11	32,38±24,34	43,02±24,73	0,6/0,01/0,1
AgrT - collagen, %	40,12±24,65	44,00±26,39	55,15±19,62	0,6/0,02/0,07
WF, %	159,90±38,83	176,00±27,80	176,12±33,14	0,06/0,03/0,9

The AgrT study on ADP, adrenaline and collagen showed a significant difference with greater aggregation in patients with multi-vessel lesions.

Comparison of subgroups showed that 52.9% of patients with the single-vessel lesion and 52.6% of patients with multi-vessel CA lesions received two-component antiplatelet therapy (p = 0.9). This fact indicates a lower efficacy of disaggregant therapy in patients with multi-vessel CA lesions.

When studying the parameters of the endothelium lesion, a significant difference was found in all subgroups compared with the control group for the level of WF, HC and ET-1. At the same time, the difference between the subgroups, as well as in the study of the hemostasis system, was found in patients with the multi-vessel lesion of the coronary artery, where there were more disorders than by the single-vessel process.

A comparative study of TP showed that in patients with the multi-vessel lesion, the Htzg genotype A1298C prevalence of the MTHFR gene is higher (53.5%) than in the control group (28.2%, p = 0.01) and in patients with the single(31, 4%, p = 0.04), two-vessel lesion of CA (31.2%, p = 0.04).

It should be noted that the Hmzg genotype G226A of the F XIII gene was less common in the subgroup with the multi-vessel CA lesion (3.5%), while this genotype was more common in the control group (15.4%, p = 0.03). This interesting fact may indicate the possible protective effect of the Hmzg carrier form of this gene in the development of MI. This hypothesis is supported by the messages of Andrienko E.Yu., Samokhodskfya L.M., Balatsky A.V., Makarevich P.I., Boytsov S.A. (2011) and Tsepokina A.V., Panasenko A.V. (2016) [6, 13].

According to our studies, out of 130 patients with IHD, only five (3.8%) did not have atherosclerotic CA leisons. These five patients had MI at the young age, confirmed laboratory and instrumentally (ECG, EchoCG). The average age of patients with "clean" CA was 36.6 ± 2.6 years.

In patients with "clean" CA, compared with patients with various types of CA, a tendency to a higher content of the components of the fibrinogen pool (SFC) was found - 13.30 ± 6.22 mg/100 ml (p <0.05); to an increase in time of XII-a dependent fibrinolysis - 29.80 ± 14.65 minutes and HC content - 14.52 ± 4.41 μmol/l. All five patients showed a carrier of the same type of thrombogenic polymorphisms - a combination of Hmzg and Htzg forms of PAI 1 genes and folate cycle genes (MTR, MTRR, MTHFR). And also, despite the history of MI, in all cases, the presence of the Hmzg or Htzg form of the F XIII gene is noted, which disproves the theory of the protective effect of this gene in patients with "clean" CA.

The phenomenon of "clean" coronary arteries and the presence of myocardial infarction in the history of young patients turned out to be interesting. This is partially confirmed by the discovery of a significantly lower Hmzg carrier frequency of the form of the F XIII gene in patients with the multi-vessel coronary lesion.

According to the accepted concepts, thrombophilia is characterized by: young age, familial TA, changes in the hemostasis system in combination with Hmzg or Htzg carrier forms of thrombogenic genes [13, 14, 15]. Consequently, the development of myocardial infarction in patients with "clean" CA, in our opinion, is due to thrombophilia.

Conclusions

1. Retrospectively, TS among patients with IHD was 77.7%, and familial TS - 31.5%.

2. In patients with IHD, a state of thrombotic readiness was detected, which is more pronounced in laboratory patients with multi-vessel lesions of CA. Against the background of the same disaggregant therapy, patients with the multi-vessel CA lesion, AgrT on ADP, adrenaline and collagen were higher than in patients with the single-vessel CA lesion.

3. The facts of the prevalence of the Htzg genotype A1298C of the MTHFR gene with a simultaneously low frequency of Hmzg G226A genotype of the F XIII gene by multivascular CA were found.

4. Among patients with "clean" CA and MI in history, in all cases, a carrier of Hmzg or Htzg forms of PAI 1, F XIII genes and folate cycle (MTR, MTRR, MTHFR) was found.

5. MI at a young age in patients with "clean" CA is associated with thrombophilia.

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DYNAMICS OF THE STATE OF HIGHER MENTAL FUNCTIONS BY DIFFERENT TYPES OF ANESTHESIA BY SURGICAL RECONSTRUCTION OF CAROTID ARTERIES FOR ATERO-SCLEROTIC OCCLUSION

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There were examined 278 patients who had undergone reconstructive operations on carotid arteries concerning atherosclerotic stenosis of a lesion with application of various kinds of anesthesia. In dynamics, there was conducted a neuropshychological inspection with the use of a short rating scale of the mental status, the battery of frontal dysfunction and the clock drawing test. It is shown that the least cognitive deficiency in patients with both symptomatic and asymptomatic stenoses is invoked by regional anesthesia in combination with the facilitated general anesthesia. In patients with asymptomatic stenoses, inhalation anesthesia with sevoflurane is accompanied with more expressed cognitive disorders in comparison with total intravenous anesthesia with propofol. Medicated correction of cognitive disorders with ceraxon during postoperative period is clinically effective, contributes to faster restoration of the highest mental functions and enriching of quality of life.

Key words: *propofol, sevoflurane, carotid endarterectomy, cognitive disorders, ceraxon.*

Recently, in anesthesiology practice, there has been a steady increase in the interest in the study of changes in higher mental functions (HMF) after performing operations under general anesthesia. This circumstance is responsible for the emergence of the concept of postoperative cognitive dysfunction (POCD) [1] in modern medical terminology, and the term "moderate cognitive disorders" (MCD) as a predimental state is included as an independent position in the 10th edition of the ICD. In this regard, of particular interest are patients with atherosclerotic lesions of the brachiocephalic vessels, who underwent carotid endarterectomy (CEE). The problem is that most patients with critical stenosis of the internal carotid artery (ICA) have initial disorders of higher mental functions due to chronic cerebral ischemia, which can be aggravated by intraoperative compression of the common carotid artery and the damaging effects of drugs for general anesthesia. Single clinical studies are devoted to these issues [2].

Of particular interest are the data on the medical correction of existing cognitive disorders, including those associated with general anesthesia. Promising in this regard is Ceraxon® (cytidine 5′-diphosphocholine, or CDP-choline) - a natural intermediate metabolite of membrane phospholipid biosynthesis. Being a precursor of acetylcholine, provides enhanced synthesis and release of acetylcholine with increased activity of the cholinergic system. In addition, it has a multimodal neuroprotective effect, protecting and restoring damaged cell membranes, blocking the toxic effect of glutamate, inhibits the activity of phospholipases, thereby preventing the formation of free fatty acids and free radicals [3, 4]. One of the few neuroprotectors with evidence in clinical trials included in the 2008 international guidelines for the treatment of ischemic stroke. Recently, data on the efficacy of the drug for the correction of postoperative cognitive disorders has appeared [5].

The purpose of the study is to study violations of higher mental functions in the application of various modern methods of CEE anesthesia and the search for possible ways of their drug prevention.

Materials and methods

A total of 278 patients aged 45 to 68 years (192 men and 80 women) were examined. All patients had ipsilateral stenosis of the carotid artery greater than 70%, or with an unstable atheromatous plaque, which is confirmed by the results of a duplex study. Depending on the method of anesthesia, the patients were divided into IV groups. According to the six main features (sex, age, nature of concomitant diseases, the volume of surgical intervention, the duration of the operation, the severity of neurological disorders), the compared groups were comparable. 60 patients of group I were exposed to total intravenous anesthesia based on propofol as a method of anesthesia (propofol infusion 5–6 mg/kg/hr with bolus administration of fentanyl 3-4 μg/kg/h). In group II (n = 60), after performing regional anesthesia of the cervical plexus according to Pashchuk (the latter was verified by neurostimulation) and the development of adequate analgesia, surgical intervention was performed under conditions of mechanical ventilation and intravenous infusion of thiopental Na. 70 patients of group III underwent low-flux inhalation anesthesia with sevoflurane (sevoran, Abbott Lab-

oratories Ltd, Great Britain) until reaching 1 MAC. For the potentiation of the analgesic effect, a bolus administration of fentanyl of 1-1.5 μg/kg/h was applied. Group IV consisted of 88 patients with asymptomatic stenoses, it was formed by an equal percentage of patients with each of the anesthesia methods used, who received a drug correction of cognitive impairment by ceraxone in the postoperative period.

To assess the overall severity of cognitive impairment, we used summary indicators of the main screening neuropsychological tests: Mini Mental State Examination - MMSE [6], Frontal Assessment Battery (FAB) [7], to estimate the spatial function, we used the clock drawing test [8]. Neuropsychological testing was carried out at the following stages: before the operation, on the 3-5th day of the postoperative period, 1 month after the operation.

Various methods of statistical processing were used in the work, depending on the type of random variables and the research task set [9].

To assess the normality of the distribution of characteristics, there were used indicators of kurtosis and asymmetry, characterizing the shape of the distribution curve. The distribution was considered normal when the values of these indicators ranged from -2 to 2. The equality of the sample variances was evaluated by the F-criterion.

The values of continuous quantities are given as M ± m, where M is the sample mean and m is the standard error of the mean.

In cases of normal distribution, as well as equality of selective variances, the Newman-Keuls q-test was used for multiple comparisons of means. To compare the main groups with the control group, Dunnett's q-test was used. For comparison of related samples, the paired Student's t-test with Bonferroni correction was used.

In case of distributions that do not correspond to the normal one, as well as for the inequality of variances, the non-parametric Dunn Q-test was used for multiple comparison. To compare the bound samples, the non-parametric Friedman test was used. To compare the complications between groups, the χ-square test is used.

The level of statistical significance by checking the null hypothesis was taken corresponding P <0.05. In all cases, bilateral criteria were used.

Processing and graphical presentation of data was performed using the computer software Statistica 6.0 and Excel 2003.

Results and discussion

Analysis of the results of neuropsychological testing revealed a different initial state of the HMF. As a result of a comparative assessment of cognitive functions in the studied groups, it was established that prior to the operation, there were no statistically significant differences in the assessment of neuro-psychological tests between groups. Moreover, in patients with symptomatic stenoses (most of whom had a concomitant neurological deficit), the results of neuropsychological testing allowed diagnosing the syndrome of MCD, and in patients with asymptomatic stenoses - a syndrome of light cognitive disorders (LCR).

On the 5-7th day of the postoperative period, subgroups of patients with symptomatic stenosis (Table 1) determined the development of POCD in the I and III groups of patients, the absence of statistically significant negative dynamics of the state of HMF - in the II group.

At this stage of the study, significant differences in the total score of the main neuropsychological tests between the I and III groups of patients were determined. This was confirmed by a decrease in the MMSE level by 1.4 points (p = 0.046), FAB by 0.9 points (p = 0.049) and scores on the clock drawing test by 1.1 points (p = 0.045) in patients of group III compared to I. There were no other statistically significant changes at this stage. After 1 month of the operative treatment, no significant differences between the analyzed groups were recorded, and the state of the HMF corresponded to the LCD syndrome.

Consequently, inhalation anesthesia with sevoflurane in patients with symptomatic stenosis is accompanied by the development of a more pronounced cognitive deficit compared with anesthesia with propofol and a regional technique, the latter being accompanied by the development of the least severe cognitive dysfunction.

More distinct changes in the studied parameters were observed in subgroups of patients with asymptomatic stenoses, in whom in the nearest postoperative period, there were significant differences in the analyzed parameters in all three groups (Table 2).

The highest average scores on the results of neuropsychological tests were observed in group II patients. Thus, the MMSE level was higher by 1.7 points (p = 0.037) compared with the I group and by 2.9 points (p <0.001) compared with the III group of patients. The FAB level in group II exceeded similar values in groups I and III by 1.4 points (p = 0.043) and by 2.5 points (p <0.001), respectively. Score for the clock drawing test of the II group was higher than in group III by 1.3 points (p = 0.039). At the same time, at this stage of research, there were statistically significant differences in the studied parameters between the I and III groups. The MMSE level in group I was 1.2 points (p = 0.041), and the FAB was 1.1 points (p = 0.049) higher than the values of similar indicators in group III. At the last stage of the research, no significant differences in indices between groups were registered.

Table 1

Comparative characteristics of the dynamics of cognitive functions between groups in patients with symptomatic stenosis (n = 96) (M ± m)

Neuropsychological test	Groups of patients	Stages of research		
		Before surgery	5-7th day	1 month
MMSE	I II III	25,8±0,7 25,7±0,6 25,5±0,6 p_1=0,999 p_2=0,984 p_3=0,999	24,9±0,4 25,6±0,5 24,2±0,4 p_1=0,624 p_2=0,525 **p_3=0,046**	27,2±0,3 27,8±0,4 27,8±0,6 p_1=0,999 p_2=0,755 p_3=0,550
FAB	I II III	14,1±0,5 13,9±0,5 13,6±0,3 p_1=0,989 p_2=0,778 p_3=0,940	13,2±0,7 13,8±0,7 12,9±0,5 p_1=0,907 p_2=0,980 **p_3=0,049**	17,1±0,6 17,0±0,8 16,5±0,4 p_1=0,999 p_2=0,793 p_3=0,925
Clock drawing test	I II III	8,2±0,4 8,4±0,6 8,9±0,5 p_1=0,990 p_2=0,624 p_3=0,893	8,1±0,6 8,6±0,5 7,5±0,3 p_1=0,893 p_2=0,755 **p_3=0,045**	8,9±0,5 9,0±0,3 9,1±0,4 p_1=0,998 p_2=0,986 p_3=0,996

Legends: p_1 - significance of differences in indicators between the I and II groups, p_2 - significance of differences in indicators between the I and III groups, p_3 - significance of differences in indicators between the II and III groups.

Table 2

Comparative characteristics of the dynamics of cognitive functions between groups in patients with asymptomatic stenosis (n = 94) (M ± m)

Neuropsychological test	Groups of patients	Stages of research		
		Before surgery	5-7th day	1 month
MMSE	I II III	27,5±0,4 27,5±0,4 27,6±0,5 p_1=0,999 p_2=0,998 p_3=0,998	26,1±0,3 27,8±0,6 24,9±0,4 **p_1=0,037** **p_2=0,041** **p_3<0,001**	28,7±0,4 28,9±0,3 28,9±0,6 p_1=0,687 p_2=0,979 p_3=0,999
FAB	I II III	15,8±0,6 15,9±0,2 16,1±0,2 p_1=0,998 p_2=0,952 p_3=0,861	14,2±0,4 15,6±0,4 13,1±0,6 **p_1=0,043** **p_2=0,049** **p_3<0,001**	17,4±0,5 17,5±0,2 17,1±0,4 p_1=0,997 p_2=0,954 p_3=0,755
Clock drawing test	I II III	9,1±0,3 9,0±0,6 9,1±0,4 p_1=0,998 p_2=0,999 p_3=0,999	8,2±0,5 8,9±0,8 7,6±0,2 p_1=0,544 p_2=0,699 **p_3=0,039**	9,3±0,6 9,3±0,4 9,4±0,2 p_1=0,999 p_2=0,998 p_3=0,995

Legends: p_1 - significance of differences in indicators between the I and II groups, p_2 - significance of differences in indicators between the I and III groups, p_3 - significance of differences in indicators between the II and III groups.

Summarizing the results of the conducted research, it can be concluded that against the background of the initial LCR syndrome in patients with asymptomatic stenoses in the immediate postoperative period in the first and third groups of patients, there developed POCD, which has the highest degree of severity in group III, whereas the results of the neuropsychological testing

in group II remained at the initial level. However, as early as 1 month after surgical treatment, all groups showed a positive dynamics of the state of HMF, indicating a practical elimination of cognitive deficit.

Thus, a comparative assessment of the results of neuropsychological testing between groups revealed the presence of marked differences in cognitive impairment in the immediate postoperative period. In patients of group II, who used combined anesthesia, combining a deep blockade of the cervical plexus with a lightweight general anesthesia with thiopental, HVF disorders were the least severe. This is explained by a significant reduction in dosages of drugs of central action and the known neuroprotective properties of thiopental. The greatest cognitive deficit was observed in the group of patients who underwent inhalation anesthesia with sevoflurane compared with total intravenous anesthesia based on propofol. In conclusion, it should be noted that at the last stage of the research (1 month after surgical treatment), there was a positive dynamics of the state of HMF in all groups, which did not show significant differences.

As shown by the results of our studies, in all analyzed patients due to atherosclerotic occlusion of the ICA, there was determined one or another level of initial cognitive disorders, and in patients of groups I and III, POCD developed in the postoperative period. For the prevention of identified cognitive impairments, the IV group of patients was identified (n = 88). All patients were exposed to CEE with an equal percentage of total intravenous anesthesia with propofol and inhalation with sevoran. The comparison group consisted of 94 patients from previously analyzed groups I and III, in whom no drug therapy for postoperative cognitive disorders was performed. Patients of group IV, in order to prevent the identified cognitive impairments, were treated with Ceraxon 2000 mg IV i.v. in drops during the first 7 days of the postoperative period, then the dose varied from 1000 to 2000 mg depending on the dynamics of the state of higher mental functions. This therapy lasted on average for 14 ± 2 days, the average course dose of ceraxone was 24.0 ± 2.0 g of the drug.

The analysis of the therapy showed that in 16 (17.0%) patients of the comparison group there was no positive dynamics, and a significant cognitive deficit remained after 1 month after surgical treatment, requiring long-term therapy and rehabilitation, postoperative cognitive dysfunction in almost all patients was compensated by the end of the 1st month after surgery. Only in 4 patients (4.5%) of the IV group, persistent cognitive dysfunction remained, in all other cases positive dynamics was noted, allowing to state the elimination of cognitive impairments by the end of the 2nd week of the postoperative period. Consequently, drug therapy, which was carried out in group IV to prevent the development of cognitive disorders, proved its clinical efficacy (with a significance level of 5%) compared with patients in the comparison group, contributed to a more rapid restoration of the HMF and improved quality of life.

Conclusions

1. Inhalation anesthesia with sevoran and total intravenous propofol-based anesthesia in patients with symptomatic stenosis is accompanied by the development of a more pronounced cognitive deficiency compared with combined anesthesia based on regional anesthesia of the cervical plexus.

2. In patients with asymptomatic stenosis, total intravenous anesthesia based on propofol and inhaled with sevoran is accompanied by the development of postoperative cognitive dysfunction, while combined anesthesia does not cause a worsening of cognitive deficit.

3. Prevention of the development of cognitive disorders of the postoperative period by ceraxone is clinically effective, contributes to a more rapid recovery of higher mental functions and improves the quality of life.

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APC-RESISTANCE ON THE BACKGROUND OF COMORBID STATES AS A POSSIBLE VTEC PREDICTOR IN THE CARRIERS OF FACTOR V LEIDEN MUTATION DURING PREGNANCY

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The aim of the study is to determine the role of factor Va resistance to activated protein C and comorbidity in carriers of the FVL (1691) GA mutation in the realization of venous thromboembolic complications (VTEC) during pregnancy. A prospective clinical cohort study of 1100 women of reproductive age was conducted, the course and outcomes of 2,707 pregnancies were analyzed. Two cohorts were identified: the main group – 500 patients with genotype FVL (1691) GA and control group – 600 women, with the genotype FVL (1691) GG. The carriage of the FVL (1691) GA mutation in pregnancy is statistically significantly associated with the development of VTEC compared with the genotype FVL (1691) GG (RR4.7, p <0.0001). In all cases, in the time period preceding the thrombosis episode, the APC resistance index for the normalized ratio (NR) was ≤0.49, while for APC resistance with NR ≥0.5, episodes of VTEC were not determined. When VTEC occurs during gestation, carriers of the FVL (1691) GA mutation are significantly more likely to suffer from comorbid conditions (RR4.5, p <0.0001). It was found that venous thromboses during gestation are realized under the condition of pronounced APC-resistance caused by the carriage of the FVL (1691) GA mutation and comorbidity. The indicator of APC-resistance can serve as an objective laboratory marker determining the feasibility of conducting antenatal thromboprophylaxis.
***Key words**: factor V Leiden mutation, genotype FVL (1691) GA, thromboembolic complications, pregnancy, comorbidity, APC-resistance.*

The discussion of issues related to the study of risk factors for thromboembolic events during pregnancy has invaluable practical significance. Accumulated knowledge suggests that today it is not possible to consider VTEC in isolation, in the context of either just a genetic predisposition or taking into account only certain proven risk factors. A thrombotic event represents the tip of the iceberg, the clinical implementation of the totality of the whole spectrum of congenital and acquired risk factors with their combined influence.

The gestation period is associated with a significant procoagulant shift in the balance of the hemostasis system, as well as with other metabolic changes [1-2]. The relative risk (standardized incidence rate) of VTEC [3-4] in pregnant women or women after childbirth ranges from 4 to 4.5. The first 6 weeks after birth are associated with a 22-fold increase in risk, with a peak observed in the first 3 weeks [5-6].

The carriage of mutations of factor V Leiden (*FVL(1961)GA*) is traditionally considered as a genetic, unmodifiable risk factor for the development of VTEC [7-9]. Moreover, the risk stratification is based on the zygosity of the mutation: *FVL(1961) AA* carriage is defined as high risk, and *FVL(1961) GA* carriage is moderate [10-12]. The implementation of VTEC in both carrier variants is most often associated with a provoking factor, such as surgery, trauma, postpartum period, immobilization, hormone therapy or chemotherapy, or the coexistence of other risk factors, such as pregnancy, age and comorbid conditions [13-16]. Examples of comorbidities that are associated with an increased risk of thrombosis in pregnant women include urinary tract infections [12], cardiovascular diseases [14], pregnancy-induced hypertension/pre-eclampsia [13,16], obesity [18-19], varicose veins [17].

Multiple risk factors increase the risk of thrombosis [20], however, to date, the available evidence does not allow an accurate assessment of the risk of developing VTEC based on a combination of various factors [9].

Thus, it is not always possible to predict the degree of probability of the implementation of VTEC with carriage of FVL(1691)GA mutation, based on the already proposed risk stratification. Considering the available data, it is not quite clear why, when predicting the development of clinically significant events, the laboratory phenotype of the *FVL(1691)AA/GA* mutation does not take into account the resistance of factor Va to activated protein C (APC-resistance), the magnitude of which, in fact, determines the tendency to intravascular thrombosis.

The ambiguous opinion of researchers about the significance of the heterozygous carriage of the *FVL(1961)GA* mutation, both independently and in combination with the known temporary risk factors for the development of VTEC, lack of data on the role of the laboratory phenotype in the form of APC-resistance and comorbidity in the implementation of thrombotic events determined the goal of our research.

Research objective: to determine the role of factor Va resistance to activated protein C and comorbidity in carriers of FVL(1691)GA mutation for the realization of venous thromboembolic complications.

Materials and methods

According to the goal, a prospective clinical cohort study of 1,100 women of reproductive age was conducted on the basis of clinical units of the Altai State Medical University of the Ministry of Health of the Russian Federation from 2009 to 2017, and the course and outcomes of 2707 pregnancies were analyzed. Two cohorts were identified: the main group — 500 patients with FVL(1691)GA genotype (mean age 30,2±4,7 years, total number of completed pregnancies — 1085) and the control group — 600 women, normozygous for the *FVL(1691)GG* mutation (mean age 30,3±3,9 years, total number of completed pregnancies - 1622). The groups were comparable in age (p> 0.05) and ethnicity: the main group was 91.2% Russian, the control group - 89.9% (p> 0.05).

Criteria for inclusion in the main group:

- female gender;
- carriage of FVL(1691)GA mutation;
- age from 18 to 45 years;
- confirmed uterine pregnancy with a period of 7-8 weeks, occurred in the natural cycle.

The criteria for inclusion in the control group were the same as in the main group, but the patients did not carry FVL(1691)GA /*AA* gene.

Exclusion criteria from study groups:

- multiple pregnancies;
- pregnancy occurring in assisted reproductive technology programs;
- somatic diseases in the stage of decompensation;
- autoimmune diseases, including antiphospholipid syndrome;
- presence of chromosomal aberrations.

The study was approved by the local ethical committee of the Altai State Medical University of the Ministry of Health of the Russian Federation (Records No. 5 of 06/25/2009).

Along with the standard methods of examination, regulated by the Order of the Ministry of Health of the Russian Federation dated November 1, 2012 No. 572n "On approval of the procedure for providing medical care in the profile "obstetrics and gynecology (except for using assisted reproductive technologies)", and determining of factor V Leiden mutation, all patients were studied for APC-resistance. Eight points were selected to assess APC-resistance, taking into account trophoblast invasion waves and reflecting the "critical" periods of pregnancy: 7-8 weeks, 12-13 weeks, 18-19 weeks, 22-23 weeks, 27-28 weeks, 32-33 weeks, 36-37 weeks and 2-3 days after delivery. It should be noted that this laboratory analysis was carried out in the absence of heparin prophylaxis. APC-resistance was determined using the "Factor V-PC-test" reagent kit (Technology-Standard LLC, Russia) by the value of the normalized ratio (NR).

Statistical data processing was performed using the MedCalc Version 17.9.7 statistical software package (license BU556-P12YT-BBS55-YAH5M-UBE51). Verification of variation series for normality was performed using the Shapiro-Wilcoxon test. Data of laboratory parameters are presented in the form of a median (Me), 95% confidence interval (95% CI) and interquartile range [25th and 75th percentiles]. Series comparison was performed using non-parametric methods. For indicators characterizing qualitative signs, the absolute value and the relative value in percent were indicated, the verification of statistical hypotheses about the coincidence of the observed and expected frequencies was performed using the $\chi 2$ criterion and Fisher's exact test. For binary signs, the relative risk (RR) and 95% confidence interval (95% CI) were calculated. The critical level of significance of differences (p) is defined as $p < 0.05$. To analyze the relationship between one qualitative sign (VTEC/absence of VTEC), acting as a dependent, resulting indicator, and a subset of quantitative and qualitative signs, we used a logistic regression model with a step-by-step predictor inclusion algorithm. The results of the evaluation of the logistic regression equations are represented by a set of regression coefficients, the achieved significance levels for each coefficient, as well as an assessment of the agreement index (Concordant) of the actual membership of the patient in one group or another.

Results and discussion

In the present study, out of 500 female carriers of FVL(1691)GA mutation, thrombotic events were recorded in 70 women (14.0% of 500) versus 9 (1.5% of 600) compared with the normozygous FVL(1691)GG genotype (control group), which possesses statistical significance [RR9,3; 95%Cl:4,7-18,5; p<0,0001].

In all nine cases of thrombosis, patients of the control group were diagnosed with DVT. In six of the patients, thrombosis was determined outside of pregnancy and was induced in five cases by taking combined hormonal contraceptives, in one case - by blocking intramedullary osteosynthesis for diaphyseal fracture of the tibia (second day of the postoperative period). In three cases, DVT was registered during pregnancy: one episode in the first trimester, two in the postpartum period (the third and sixth days).

In 70 patients-carriers of *FVL(1961)GA* mutation, 98 episodes of thrombotic events were recorded at various periods of life: in 45 women (64.3% of 70) - a single episode of VTEC; retrombosis (2 or more) in 25 (35.7% of 70) women. In total, thrombotic events occurred outside of pregnancy in 58 (11.6% of 500) women and accounted for 65

episodes, the incidence of retromboses was determined in 12.1% (7 out of 65) of cases. During pregnancy, heterozygous carriage of the *FVL(1961)GA* mutation was realized by thrombotic events in 33 patients, and in 2/3 (21 out of 33), these were episodes of retromboses.

In the next part of the work, in order to study the somatic status, more than 30 nosological forms were analyzed according to the International Classification of Diseases (ICD), X issue. Diagnosis of the selected conditions was carried out by related experts in accordance with the directive documents using laboratory, functional and clinical research methods. Analysis of the data showed that patients with a personal history of thrombosis with FVL(1691)GA mutation were statistically significantly more likely to have lower limb varicose veins (LLVV) (ICD code X - I83.9), hypertensive disorders (ICD code X - I11.9), overweight (ICD code X - E66) and chronic inflammatory diseases of the respiratory system (Table 1).

Table1

Somatic status of patients with a personal history of thrombosis with carriage of FVL(1691)GA mutation

Nosological form	Personal history of thromboses n=70		Personal history of thromboses lacks n=430		Statistics		
	абс	%	абс	%	p	RR	95%CL
Hypertensive heart disease	29	41,4	76	17,7	<0,0001	2,3	1,6-3,3
Obesity and other types of redundancy (BMI ≥25)	38	54,3	135	31,4	0,0001	1,7	1,3-2,2
Chronic inflammatory diseases of the respiratory system	38	54,3	142	33,0	0,0001	1,6	1,2-2,1
LLVV	39	55,7	155	36,0	0,0005	1,5	1,2-1,9

Further, the relation of comorbidity of the isolated (Table 1) states with the development of VTEC in carriers of FVL(1691)GA mutation was studied. Somatically healthy women with episodes of VTEC in a personal history were not identified in the study. Comorbid conditions by carriage of FVL(1691)GA mutations were detected in 95.7% (67 of 70) patients with thrombosis in their personal history and 24.4% (92 of 430) in the absence of those [RR 4,5; 95%Cl:3,7-5,4; p<0,0001] (Figure 1).

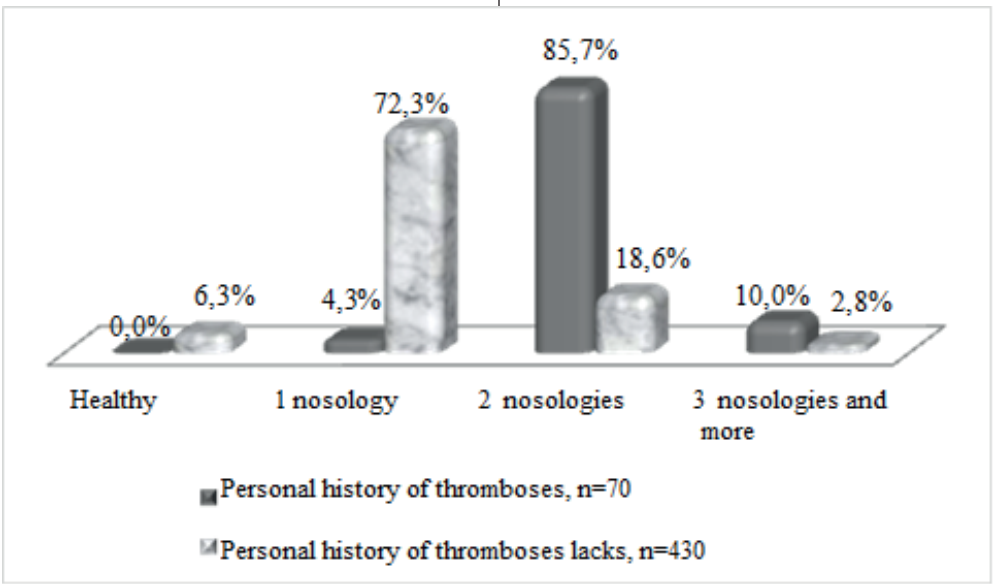
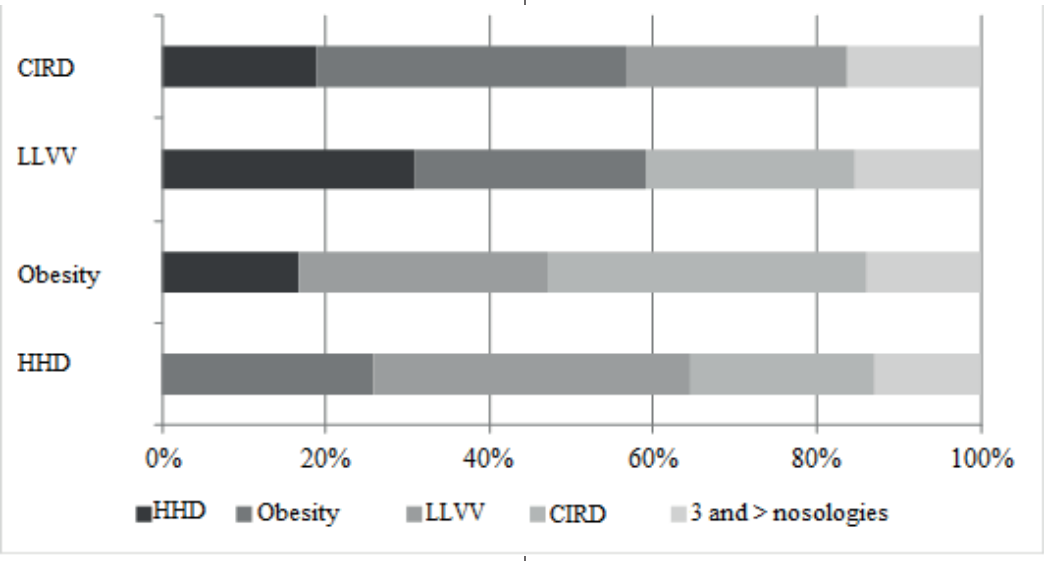


Figure 1 - Comorbidity in patients - carriers of FVL(1691)GA mutation, depending on the clinical implementation in the form of thrombotic events.

A more detailed analysis showed that by the implementation of a thrombotic event, hypertensive disorders in 27.6% of cases were combined with overweight, in 41.4% - with LLVV; varicose disease, in turn, was registered in 28.5% of cases against the background of overweight and in 25.6% against the background of chronic inflammatory diseases of the respiratory organs. The structure of comorbid conditions is shown in Figure 2. A study of comorbidity by the carriage of FVL(1691)GA mutation showed that a combination of the two nosologies considered increases the risk of thrombosis

during gestation by 4.6 times [RR4,6; 95%Cl:3,7-5,7; p<0,0001], and comorbidity, represented by three nosological forms, by 3.7 times [RR3,7; 95%Cl:1.5-9.2; p=0,039].



Note: HHD - hypertensive heart disease, CIRD - chronic inflammatory respiratory diseases.
Figure 2 - The structure of comorbidity in patients - carriers of FVL(1691)GA mutation by clinical implementation in the form of thrombotic events.

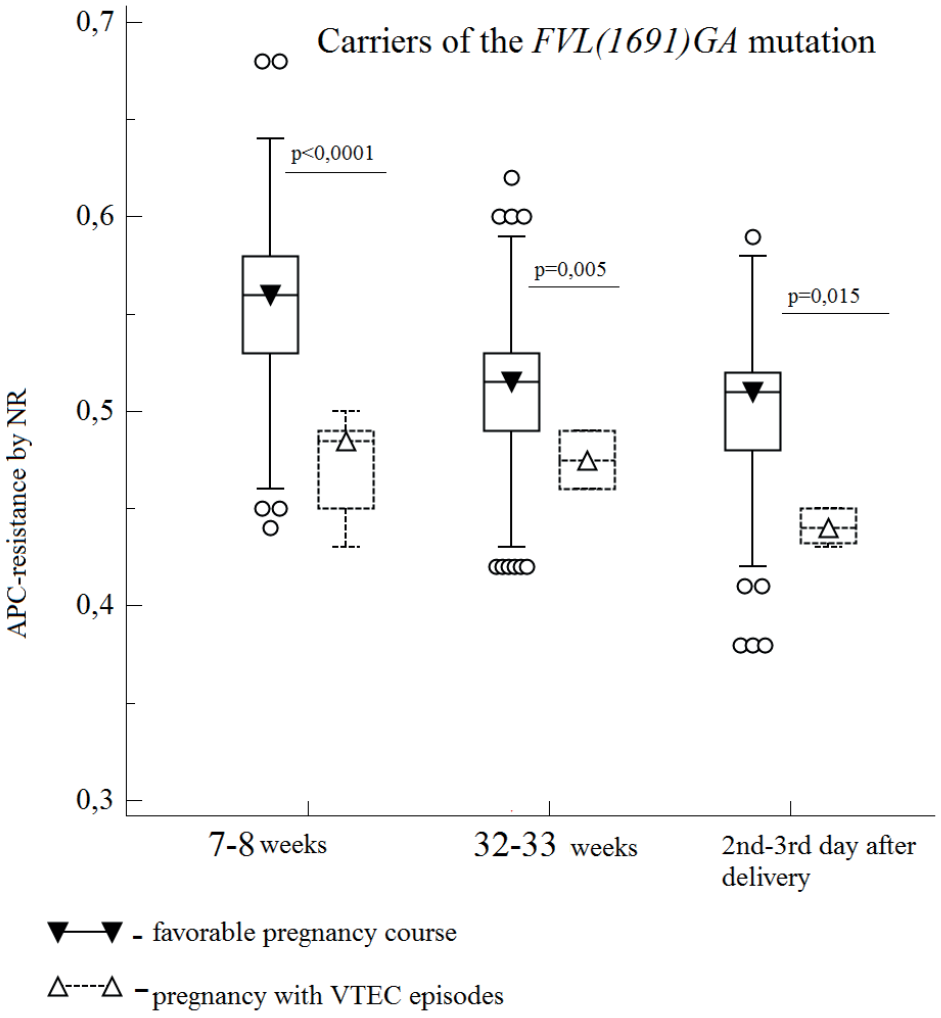
It should be noted that, despite the presence of an associative, statistically significant association between the carrier of FVL(1691)GA mutation in combination with the identified temporary risk factors, it is unlikely to predict the likelihood of thrombotic events. Given this circumstance, we attempted to consider the importance of the manifested laboratory phenotype of FVL(1691)GA mutation in the form of APC-resistance for the development of thrombotic complications. Since activated protein C is one of the main physiological anticoagulants that break down activated coagulation factors Va and VIIIa, by APC resistance, Va and VIIIa factors become insensitive to the inactivating effect of C protein, which leads to excessive thrombin formation and inhibition of fibrinolysis. The value of APC-resistance, as noted above, in fact, determines the propensity for intravascular thrombus formation [21-22].

In accordance with the data obtained, an explicit connection of APC-resistance in carriers of FVL(1691)GA mutation with the development of VTEC has been determined. Thus, the median NR indicator, reflecting the degree of APC-resistance, preceding the episode of phlebothrombosis in the first trimester (7-8 weeks), constituted 0,49 [95%Cl:0,43-0,49]; in the third trimester (32 weeks) - 0. 48 [95%Cl:0,46-0,49]; on 2nd-3rd day after delivery - 0.44 [95%Cl:0,43-0,48], and was statistically significantly lower than in the group of women with a favorable course of pregnancy (Figure 3).

A multiple logistic regression analysis was performed to rank the selected predictors of FVL(1691)GA mutation according to the degree of association with the implementation of VTEC during pregnancy. Several models were obtained in various clinical situations determined according to the recommended methodology for stratifying the risk of VTEC during pregnancy and in the postpartum period [9]: 1) Asymptomatic patients (who do not have thrombosis episodes); 2) One-time VTEC in history, associated with transient risk factors; 3) Multiple episodes of VTEC in history.

The models were formed through the gradual inclusion of predictor variables, in which five risk factors were selected, significantly more common in pregnant women with FVL(1691)GA mutations with a personal history of thrombosis: hypertensive states, lower limb varicose veins, BMI≥25, chronic inflammatory respiratory diseases and "APC-resistance with NR ≤0.49", preceding a thrombotic event. The categorical response variable is a VTEC fact (presented as a binary value: 1 - yes; 0 - no). Table 2 presents models that have concordant values of more than 80%.

The inclusion of somatic conditions as predictor variables in logit analysis of pregnant carriers of *FVL(1691)GA* mutation showed that a statistically significant additional risk factor for the development of VTEC is comorbidity, which is determined by the association of hypertensive disorders and LLVV. Thus, with the association of isolated nosologies, the implementation of thrombosis in asymptomatic carriers of *FVL(1691)GA* mutation mutation is predicted in 71% of cases, and when the predictive variable "phlebothrombosis in history" is added to the model, the implementation of VTEC during the period of gestation is predicted in 89% of observations.



Notes: median - marker; "box" - inter-quarter span between 25% and 75%; lines - values corresponding to 2.5 and 97.5 percentiles; free elements - emissions.

Figure 3 - Values of APC-resistance at control points preceding venous thrombosis by carriage of FVL(1691)GA mutation

It is noteworthy that, according to the predictive models obtained in pregnant women, when the variable "APC-resistance ≤0.49" preceding the thrombotic event was included, background somatic states lost their statistical significance. In these cases, the manifestation of the laboratory phenotype of *FVL(1961)GA* mutation was the decisive risk factor for the implementation of VTEC, and thrombosis is already predicted in 85% of asymptomatic women and in 93% of cases with an episode of VTEC in history.

This study showed that carriage of *FVL(1961)GA* mutation during pregnancy is associated with VTEC and is due to exposure to additional risk factors and/or background somatic pathology.

In accordance with the data obtained, along with the main factor inducing the implementation of thrombotic events (APC-resistance) in carriers of *FVL(1961)GA*, an important role belongs to background somatic pathology. At the same time, the dominant factors of thrombogenic risk during gestation are the comorbid conditions considered in the study in various combinations.

There are numerous papers in the scientific literature reflecting that the risk of VTEC developing is higher in patients with a non-infectious therapeutic profile, including: cancer, diseases of the cardiovascular system, chronic diseases of the upper respiratory tract and obesity [23–25], which is consistent with our study . However, we have not met any work that determines the risk of thrombosis when one patient combines two or more chronic diseases pathogenetically interrelated or coinciding in time, regardless of the activity of each of them, that is, by comorbid conditions [26].

Comorbid conditions of the cardiovascular system in the form of arterial hypertension and LLVV dominate in the present work, which, probably, along with pronounced APC-resistance, forms the Virchow's triad [27], which determines thrombus formation: endothelial damage (arterial hypertension), decrease in blood flow velocity varicose veins of the lower limbs and increased blood clotting (APC-resistance).

The obtained data on the structure of somatic pathology with comorbidity, in our opinion, are of practical interest from the point of view of the possibility of modifying these risk factors. For example, overweight, a proven risk factor for VTEC [28-29] is a controlled factor, weight correction can not only reduce the risk of developing VTEC [30], but also affect blood pressure indicators [31-34], thereby further reducing the risk of implementation of thrombotic events.

Interestingly, in previously published papers on the subject, APC-resistance and its severity by *FVL(1961)GA* carriage was not considered as a prognostic factor in the development of clinical events.

Given that in all cases in the period preceding an episode of thrombosis, the NR index was ≤0,49 [95%Cl:0,41→0,49], we can note the leading role of APC-resistance, which, together with clinical data, determines the appropriateness heparin prophylaxis.

Table 2

Logit models with risk factors for the implementation of VTEC by carriage of FVL(1691)GA mutations in various clinical situations

Variable	Coefficient (b)	Standard error	p -value	Adjusted odds ratio (OR)	95% confidence interval (95% Cl)
Asymptomatic carriers of FVL (1691) GA during pregnancy					
Constant term	-4,0538				
LLVV	1,12792	0,40290	0,0050	3,0892	1,4025- 6,8047
Hypertensive states	1,16268	0,38223	0,0024	3,1985	1,5121- 6,7656
Percentage of concordance 91,40 %					
Chi-squared – 29,307; P < 0,0001; AUC=0,71; 95%Cl 0,69-0,73					
Asymptomatic carriers of *FVL(1691)GA* during pregnancy with laboratory phenotype APC-resistance ≤0.49					
Constant term	-5,3221				
LLVV	0,72249	0,51437	0,1601	2,0596	0,7515 - 5,6444
Hypertensive states	0,60268	0,52296	0,2491	1,8270	0,6555 - 5,0921
APC-resistance ≤0,49	3,31762	1,04563	0,0015	27,5945	3,554 - 214,231
Percentage of concordance 93,60 %					
Chi-squared – 33,415; P < 0,0001; AUC=0,85; 95%Cl 0,80-0,90					
Pregnant carriers of *FVL(1691)GA* with episodes of thrombosis in history					
Constant term	-4,0054				
Hypertensive states	0,93798	0,42215	0,0263	2,5548	1,1169- 5,8439
LLVV	1,02765	0,43265	0,0175	2,7945	1,1968 - 6,5251
Episode of VTEC in history	2,50308	0,41229	<0,0001	12,2201	5,4467 - 27,417
Percentage of concordance 95,60 %					
Chi-squared - 29,307; P < 0,0001; AUC=0,89; 95%Cl 0,87-0,93					
Pregnant carriers of *FVL(1691)GA* with episodes of thrombosis in history with laboratory phenotype APC- by NR ≤0.49					
Constant term	-6,0866				
Hypertensive states	0,61156	0,57227	0,2852	1,8433	0,6004 - 5,6588
LLVV	0,64990	0,56564	0,2506	1,9153	0,6321 - 5,8040
Episode of VTEC in history	2,26496	0,57556	0,0001	9,6307	3,117 - 29,7568
APC-resistance ≤0,49	3,45147	1,06639	0,0012	31,5468	3,901 - 255,085
Percentage of concordance 96,80 %					
Chi-squared – 49,516; P < 0,0001; AUC=0,93; 95%Cl 0,89-0,98					

Conclusion

In modern clinical medicine, various diseases lose their mononosological nature, acquiring the status of comorbidity. The data obtained allow the formation of thrombosis-associated comorbidity, including arterial hypertension, LLVV, overweight (BMI> 25), chronic inflammatory respiratory diseases and the carriage of *FVL(1961)GA* mutation. Obviously, the objective laboratory criterion that determines the risk of implementation of VTEC in this group is the indicator of APC-resistance. Patients so stratified require lifelong clinical examination and allowable modification of controlled factors. The high degree of comorbidity

determines the need for a comprehensive individual approach to the management of each patient, the development of diagnostic criteria, prevention and treatment.

In terms of the preventive nature of personalized medicine, it is necessary to take into account that the heterozygous carriage of *FVL(1691)GA* mutation against the background of comorbid conditions is:

- risk factor for the development of VTEC in asymptomatic women during pregnancy [RR4,7; 95%Cl:1,5-14,7; p=0,0069];
- indications for determining the severity of APC-resistance.

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AUTOIMMUNE THYROIDITIS IN PATIENTS WITH CHRONIC HEPATITIS C

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Chronic viral hepatitis – is one of the most pressing health problems. In the meantime, autoimmune thyroiditis (AIT) is one of the most common diseases in endocrine practice. According to different researchers, in patients with HCV, antithyroid antibodies detection frequency ranges from 2,5 to 42,3%. The purpose of this study is a comparative evaluation of thyroid functional status in patients with chronic viral hepatitis C in combination with autoimmune thyroiditis. There was held a prospective, dynamic, clinical, laboratory and instrumental examination of 76 patients in three clinical groups: group I with autoimmune thyroiditis, group II with autoimmune thyroiditis in combination with chronic viral hepatitis C, group III with chronic viral hepatitis C. There was defined the structure of thyroid gland pathologies, evaluated the clinical course of autoimmune tyreopathies on the background of hepatitis C, investigated the performance of the functional condition of the thyroid system in this group of patients. It was found that hepatitis C virus affects thyroid status system that proves the role of HCV as a causative factor in the development of autoimmune thyroiditis.
***Key words**: chronic viral hepatitis, thyroid gland, autoimmune thyroiditis.*

Chronic viral hepatitis (CVH) is one of the most pressing health problems. The prevalence of chronic hepatitis C (CHC) ranks third among all infectious diseases [1, 2]. In the pathogenesis of organ damage during HCV infection, the direct cytopathic effect of the virus and the immunological reactions caused by it leading to liver damage are discussed, as well as viral replication outside the liver - in tissues of lymphoid and non-lymphoid origin [3, 4, 5, 6, 7, 8]. Viral hepatitis is not limited to liver damage and is a systemic disease that occurs with the regular development of extrahepatic manifestations [5, 6, 9, 10, 11]. Extrahepatic lesions can occur both clinically latently and in the form of expressed clinical syndromes or independent diseases characterized by a high frequency and originality of their spectrum. Despite the contradictory data in the literature concerning the relationship between HCV infection and autoimmune thyroiditis, the prevailing view is that HCV may play the role of one of the etiological factors of this disease, and that by chronic hepatitis C, its latent forms are often manifested under the influence of IFN-α. The incidence of symptoms of autoimmune thyroiditis (AIT) varies in different studies from 2.5% to 42% [5]. Chronic viral liver diseases often lead to the development of thyroid dysfunction (thyroid gland), in particular, an increase in the level of thyroxin-binding globulin (TBG) and thyroxin (T4) serum; a decrease in the level of triiodothyronine (T3) due to a decrease in its secretion, deiodination and assimilation by the T4 hepatocytes; increase in the level of reverse T3. The level of thyroxin can also decrease due to defective production of TBG or by reducing T4 binding at the periphery [12].

Thyroid dysfunction in patients with chronic hepatitis C is manifested in most cases of hypothyroidism and occurs in 3.5-7% of cases, according to a number of authors [13]. A much larger part of the surveyed (31-42.5%) revealed diagnostic significant levels of anti-thyroid antibodies (anti-microsomal, anti-peroxidase, antibodies to thyroglobulin) [14, 15]. The mechanisms for the development of the above complications in hepatitis C are not clear enough. There are various assumptions. According to some data, the hepatitis C virus directly affects such organs as the salivary glands, pancreas and thyroid gland [16], and according to other sources, the virus triggers autoimmune processes of tissue and organ damage [17]. The hepatitis C virus, localized in thyroid tissue, is likely to directly cause its damage [18, 19, 20]. On the other hand, it is possible that autoimmune reactions are caused by such a peculiarity of the virus as the ability to mimicry some components of thyroid tissue [19, 21]. In general, all authors indicate the presence of elevated titers of antithyroid antibodies, including antibodies to thyroglobulin, peroxidase, thyrotropin receptors and antimicrosomal antibodies [22, 23, 24]. A single prognostic parameter of the thyroid gland is not developed. This question requires further study, since this criterion is very important.

The research objective is to compare the functional state of the thyroid gland in patients with chronic viral hepatitis C in combination with and without autoimmune thyroiditis.

Materials and methods

The study included 76 patients divided into three clinical groups: group I with autoimmune thyroiditis (21), of which 9 men and 12 women, the average age was 49.0 ± 6.0 years; group II - 28 people (12 men and 16 women) with AIT in combi-

nation with CHC, average age - 36.70 ± 2.35 years; group III - 27 patients (12 men and 15 women) with chronic hepatitis C, average age - 37.10 ± 7.91 years.

All patients underwent a thorough medical examination (collection of complaints, anamnesis, physical examination, standard laboratory research methods). All patients were tested to determine the level of serum hormones - TSH, T3, T4, as well as antibodies to thyroperoxidase (AtTPO) and liver and thyroid gland ultrasound. All patients were examined by an endocrinologist. The level of hormones was determined on an automated bio-immunoassay analyzer "Bio-Rad", model 680 Reader (USA) using Alkibio Tiroid IFA reagent kits. Diagnosis of CHC was confirmed: by PCR with determination of HCV RNA (quantitative, genotype), ELISA (presence of antibodies to core, NS 3-5), blood biochemical parameters, fibrosis level according to Metavir scale (elastography and/or puncture biopsy of the liver.

Before the disease of viral hepatitis, thyroid pathology in patients had not been registered. Patients did not receive specific therapy for viral hepatitis. Statistical processing of research results was performed using parametric methods on a PC using Microsoft Excel, Statistica 6.0 for Windows. The data are presented as arithmetic means and errors of the mean (M ± m). To assess the significance of differences of compared means (relative values) there was used the Student's criterion ($p < 0.05$).

Results and discussion

Clinical symptoms in patients of group I (AIT) were manifested as asthenovegetative - 38.0% (weakness, decreased performance), dyspeptic - 14.2% (nausea, heartburn, bitter mouth, decreased appetite), cardiac - 24.0% (rapid heartbeat), dermatological - 19.0% (dry skin, excessive sweating) and hyperthermic - 4.7% of syndromes. In patients of group II (AIT with chronic hepatitis C), in addition to asthenovegetative (53.5%), dyspeptic (28.5%), dermatological (10.7%) syndromes, the arthralgic syndrome occurred (7.1%). Patients of group III (CHC) showed the presence of the asthenovegetative (48.1%) syndrome, in combination with dyspeptic (33.3%) and arthralgic (14.8%) syndromes, and in 3.7% of patients, subfebrile body temperature was registered.

According to biochemical blood tests, patients showed moderate signs of cytolytic and dysproteinemic syndromes. In particular, an increase in the activity of AlAT by two times (129.16 ± 58.48 U/l) and AsAT by two time (97.6 ± 40.16 U/l), a relative decrease in albumin in 42.8% of patients and an increase of γ-globulin in 46.4% (34.2 ± 4.2 g/l). An increase in the activity of AlAT and AsAT was observed in 98.3% of patients, GGT - in 53.3%, ALP - in 15.0%, thymol sample - in 9.1%. In patients of group I, no changes in the biochemical analysis of blood were detected (Table 1).

Table 1

Results of biochemical analysis of blood in the study groups

Parameter (MU)	group I n=21	group II n=28	group III n=27	Norm
Total bilirubin (μmol/l)	15,05±3,18	18,54±4,35	16,11±4,28	8,55-20,52
Direct bilirubin (μmol/l)	4,2±1,26	4,97±1,64	4,21±1,29	1-5,1
Indirect bilirubin (μmol/l)	11,24±2,46	11,47±3,66	12,94±2,43	15,4
ALAT (Un/l)	30,12±5,18	129,16±58,48	102,4±16,1	0-40
ASAT (Un/l)	30,5±4,12	97,6±40,16	81,8±41,04	0-40
GTP (Un/l)	40,10±5,12	63,29±15,06	59,5±25,9	11-63
ALP (Un/l)	130,11±26,17	191,37±64,22	152±48,12	0-350
PTI (%)	96,78±5,12	96,8±5,14	96,24±5,30	80-100
Albumin (g/l)	38,09±6,13	30,75±0,5	38,07±7,33	30-55
Globulins (g/l)	24,14±6,14	35,78±6,23	31,2±2,21	17-35

In patients of group I, levels of T_3 (2.29 ± 1.30 nmol/l), T_4 (112.7 ± 6.60 nmol/l), TSH (1.98 ± 1.04 mU/l) were within normal values, and the level of AtTPO (13.82 ± 2.76 IU/ml) slightly exceeded the reference values (Table 2). In patients of group II, T_3 levels (2.28 ± 1.57 nmol/l), T_4 (110.67 ± 3.09 nmol/l) and TSH (1.84 ± 1.43 mU/l) were also within normal values. Elevated values of AtTPO were detected in 18 (64.2%) patients of group II and averaged 16.03 ± 1.66 mU/ml, which was significantly ($p = 0.006$) higher than the values of patients of group I. Studies have shown an increase in the level of AtTPO in 17 (62.9%) patients of group III, on average it was 15.90 ± 2.43 IU/ml ($p = 0.03$), which is also significantly higher than in patients of group I. AtTPO increase in patients with mixed pathology with normal T_3 values (2.34 ± 1.51 nmol/l), T_4 (100.66 ± 4.91 nmol/l) and TSH (2.31 ± 1.84 mU/l), is possibly determined by an autoimmune process in the thyroid gland, the etiological factor of which may be HCV infection.

In 7 (33.3%) patients of group I, there was a slight increase in TSH and a decrease in T_3 total. In group II, 11 (39.2%) patients had an increase

in TSH, a decrease in T_3 total, in 5 patients - $T_{4\ free}$ and for 8 (29.6%) patients with chronic hepatitis C, increased TSH, a decrease of T_3 total and in 2 patients - $T_{4\ free}$.

According to the results of the study, it is seen that in patients of group II, a significant decrease in the level of triiodothyronine is observed (Table 3).

Table 2

Thyroid hormone and AtTPO levels

Parameter (MU)	group I n=21	group II n=28	group III n=27	Norm
TSH (μU/ml)	1,98±1,04	1,84±1,43	2,31±1,84	0,2-3,2
T_3 total (nmol/l)	2,29±1,30	2,28±1,57	2,34±1,51	1,1-3,0
T_4 total (nmol/l)	112,69±6,60	110,67±3,09	100,66±4,91	53-158
$T_{4\ free}$ (nmol/l)	14,07±5,39	15,68±5,93	14,27±5,66	10-25
AtTPO (U/ml)	13,82±2,76	**16,03±1,66** p=0,006**	**15,90±2,43** p=0,03**	До 15

Note: *p<0,05; **p<0,02

Table 3

Thyroid hormone levels by autoimmune thyroiditis (AIT) in combination with chronic viral hepatitis C

Parameter (MU)	AIT n=7	AIT and CHC n=11	CHC n=8	Norm
TSH (μMU / ml)	3,91±0,46	5,00±1,26***	4,45±0,44***	0,2-3,2
T_3 total (nmol/l)	1,09±1,01	0,93±0,04*	0,96±0,50	1,1-3,0
T_4 total (nmol/l)	105,22±7,75	117,38±9,72	110,07±4,71	53-158
$T_{4\ free}$ (nmol/l)	14,06±0,23	9,04±0,43*	10,25±0,76	10-25

Note: *p<0,05; **p<0,02; ***p<0,01

The functional activity of the thyroid gland mainly depends on the level of thyroid-stimulating (TSH) hormone of the pituitary gland in the blood. Secretion of TSH is regulated by the principle of "long chain" feedback and is determined primarily by the level of thyroid hormones. Triiodothyronine (T_3) is the main regulator of TSH secretion at the pituitary level. When examining the values of thyroid-stimulating hormone, the parameters in patients of groups II and III were most elevated and were significantly higher (p <0.01) than in group I patients, which indicates the activation of the thyroid-stimulating function of the pituitary against the background of CHC. Disruption of hepatotropic function by hepatitis led in parallel to a decrease in thyroid function (T_3 tot, T_4 free).

Thyroxin (T_4) is the main hormone, a kind of source or prohormone of triiodothyronine secreted by the thyroid gland, its significant decrease was noted only in the second group of patients (p <0.05). The level of T_3 is also reduced in patients of group II (p <0.05).

Thus, in patients with chronic hepatitis C, which occurs on the background of AIT, hypofunction of the thyroid gland and an increase in the level of thyroid stimulating hormone were revealed, which indicates the activation of the thyrotropic function of the pituitary gland. The concomitant reduction in thyroid hormone levels allows the diagnosis of subclinical hypothyroidism in the studied group of patients. By subclinical hypothyroidism, the presence of AtTPO may affect the decision to initiate replacement therapy, since the carrier status of AtTPO is a risk factor for the progression of subclinical hypothyroidism to obvious.

According to the results of thyroid ultrasound in all patients of group I, heterogeneous echogenicity was revealed, of which 16 (76.1%) patients had foci of fibrosis, 5 (23.8%) had foci of fibrosis and nodal inclusions. In patients of group II, on the background of inhomogeneous echogenicity, 12 (42.8%) had foci of fibrosis, 11 (39.2%) had foci of fibrosis and nodal inclusions. In group III, diffusely heterogeneous changes were observed in 9 (33.3%), nodal inclusions, cysts in 8 (29.6%) patients.

Conclusions

1. A significant increase in AtTPO level in patients with AIT in combination with CHC may be determined by the presence of an autoimmune inflammatory process in the thyroid gland, occurring against the background of moderate hepatitis activity, the etiological factor of which is HCV infection.

2. The combined course of CHC aggravates the course of AIT.

3. Autoimmune thyroiditis on the background of viral hepatitis significantly more often occurs with subclinical hypothyroidism.

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POSSIBILITIES OF SELECTED SEROLOGICAL TESTS IN THE NEUROSYPHILIS DIAGNOSTICS

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To date, despite the large number of serological methods for diagnosis of neurosyphilis, an unambiguous algorithm for the laboratory diagnosis of this infection does not exist. The article provides comparative characteristics of treponemal and non-treponemal tests and focuses on the methods that are most sensitive and specific. By comparison of different serological methods, there is traced a positive correlation of two treponemal tests used for the diagnosis of syphilitic pathology of the central nervous system, with the possibility of interchangeability.
Key words: neurosyphilis, serodiagnosis, treponemal tests.

The incidence of syphilis in Altai Krai in 2016 compared to 2013 decreased by 48%, but the observed intensive decrease in the incidence of syphilis does not indicate a full-fledged epidemiological well-being. Due to ongoing measures to combat STIs, high rates of reducing the incidence of syphilis were achieved. During the reporting period, despite a general decline in the incidence of syphilis, there is an increase in its late forms, including neurosyphilis, from 0.3 cases in 2013 to 1.9 cases in 2016 per 100,000 population (Figure 1).

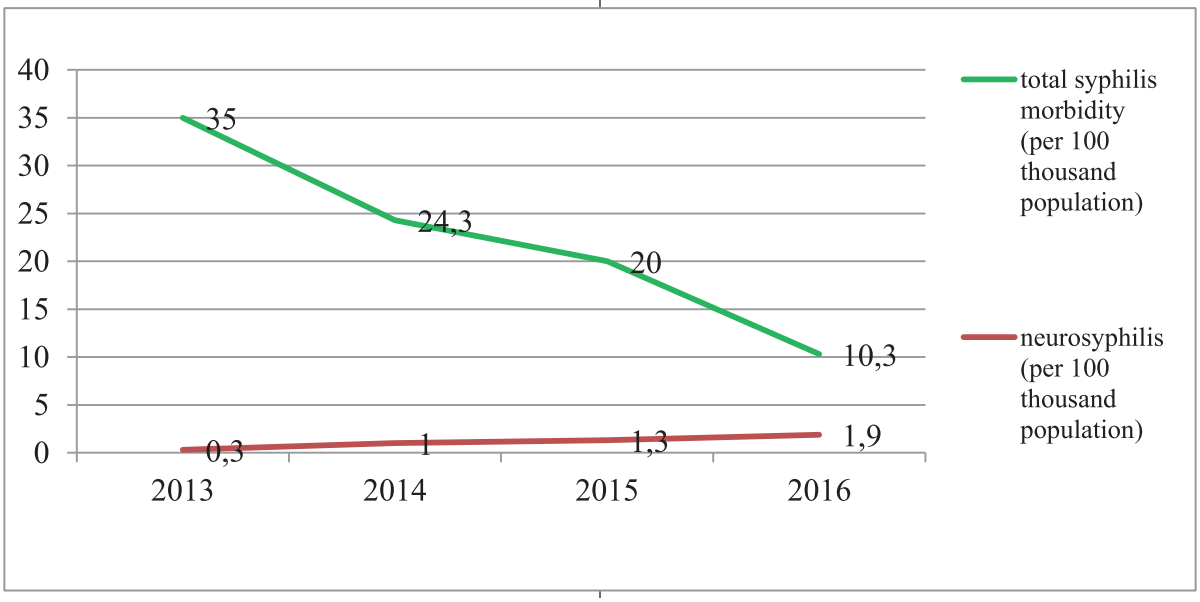

Figure 1 - Dynamics of syphilis and neurosyphilis morbidity in Altai Krai in 2013-2016

Diagnosis of neurosyphilis is often associated with significant difficulties due to the pathomorphism of the disease, causing the occurrence of erased, asymptomatic, latent, atypical forms. The change in the clinical picture of neurosyphilis in recent years has been influenced not only by the active use of chemotherapy for syphilis, but also by the use of antibiotics by the population by intercurrent diseases [1]. An important role in the development of neurosyphilis is given to inadequate therapy, caused both by the lack of sensitivity of the infecting strain of pale treponema to the antibiotics used (seroresistance) and by immunity deficiency, as well as by the virulence of the pathogen in the early stages of the syphilitic process [2]. The development of disease pathomorphism can also be associated with a change in the internal mechanisms of the response of the microorganism to infection with pale treponema and the evolution of the pathogenic properties of the infectious agent [1]. The identification of patients with neurosyphilis at the present time, as a rule, occurs in somatic and neuropsychiatric hospitals by receiving positive results of screening studies for syphilis. However, in the outpatient setting of the medical organizations of the psycho-neurological profile, serological screening for syphilis is often not performed, thus, it is not possible to exclude the syphilitic etiology of the psycho-neurological symptoms. This leads to an erroneous diagnosis and failure

to provide proper medical care. Neurosyphilis may be masked as a variety of neurological diseases with severe symptoms, as well as asymptomatic. Exploring the epidemiological characteristics of the dynamics of the incidence of syphilis, there can be seen a clear tendency to an increase in the incidence of neurosyphilis over the past years. This situation may accompany the post-epidemic period, when, against the background of a decrease in the total number of new cases of syphilis, there is an increase in the number of cases of late and latent syphilis, the number of seroresistant cases, when, most likely, favorable conditions are created for the occurrence of specific lesions of the nervous system [1]. This contingent of patients constitutes a reserve of undiagnosed neurosyphilis, plus catastrophic large-scale self-treatment of syphilis using "proven" methods from the Internet and other publicly available "social" information resources. All this gives prerequisites for the further growth of cases of neurosyphilis. Based on the polymorphism of the manifestation of neurosyphilis, there is an acute problem of mutual understanding between dermatovenereologists and doctors of various specialties in the full diagnosis and management of patients with positive specific and non-specific reactions to syphilis. Serodiagnosis of neurosyphilis in this situation requires an integrated approach, the use of various treponemal and non-treponemal tests.

Research objective: to evaluate the diagnostic efficacy, clinical sensitivity and specificity of various treponemal and non-treponemal tests in patients with suspected and defined neurosyphilis. To compare the tests between each other in terms of sensitivity and specificity with positive and negative values and determine the most optimal approach to serodiagnosis of neurosyphilis based on the results obtained. Analyze the experimental use of the indicator biochemical test to determine the level of protein in the liquor.

Materials and methods

90 samples of cerebrospinal fluid (CSF) of patients were examined. All samples were divided into two groups. The study group consisted of 26 samples (12 samples of the cerebrospinal fluid of patients with a previously confirmed diagnosis of neurosyphilis, 14 samples of the cerebrospinal fluid of patients with clinical and serological indications of seroresistance). The control group - 64 samples of the cerebrospinal fluid of patients from the neurological departments without indications for liquor diagnosis of syphilis with negative serological tests for syphilis in serum. Studies of samples of cerebrospinal fluid were performed by nine serological methods, including seven treponemal tests: 1) an enzyme immunoassay method with the detection of total antibodies for syphilis (EIA total antibodies) (Invitro-Sif-AT, Medical-Biological Union, Russia); 2) EIA with detection of class G antibodies to syphilis (IgG EIA) ("Melisa Sif-IgG-DS-strip", Medical-Biological Union, Russia); 3) EIA method with detection of class M antibodies to syphilis (IgM EIA) (Invitro-Cif-IgM, Medical-Biological Union, Russia); 4) the method of reaction of passive haemagglutination to syphilis (RPHA) ("Lewis RPHA-test", Nearmedic plus, Russia); 5) the method of reaction of passive agglutination of particles to syphilis (RPAP) ("SERODIA TP-PA", Fujirebio, Japan); 6) the method of immunofluorescence reaction to syphilis with whole liquor (RIF) ("LumiBest Antipallidum", Vector-Best, Russia); 7) the method of reaction of the linear immunoblot to syphilis (IB) ("INNO-LIA Syphilis Score", Fujirebio, Belgium) was used to selectively investigate 9 samples from both groups. As well as two non-treponemal tests: microprecipitation reaction to syphilis (RMP) (Syphilis-AgCl-RMP, Ecolab, Russia) and fast plasma reagin response to syphilis (RPR) (Lewis RPR test, Nearmedic plus, Russia). Additionally, qualitative and semiquantitative determination of protein by test strips (URIBEL, Biosensor AN, Russia) of all liquor samples from the study group and 5 liquor samples from the control group (samples that gave a positive result in the passive agglutination of particles (RPAP) were carried out.

Results and discussion

Positive results of studies of liquor by various serological methods, according to the studied groups, are presented in the table (Table 1).

In the study of cerebrospinal fluid with test strips for protein in the study group (n = 26), five results were obtained, exceeding the protein level of 0.3 g/l. In the control group of five selected samples, four samples showed a similar result (the protein level exceeds 0.3 g/l). High rates of detection of positive results in liquor diagnosis by the RPAP method (clinical sensitivity of the method is 96.2%, specificity - 92.1%) allow using this method as a reference in the diagnosis of neurosyphilis along with RIF (clinical sensitivity of the method - 88.4%, specificity - 96.8 %), which is regulated by regulatory documents on the management of patients with syphilis [3, 4]. The positive correlation of diagnostic treponemal tests of passive agglutination of particles (RPAP) and immunofluorescence reaction with whole liquor (RIF) allows using these methods to improve diagnostic efficiency in the diagnosis of neurosyphilis in combination with other treponemal and non-treponemal tests in different variations: RPHA + EIA +RMP(RPR), RIF + RPHA + RMP (RPR), RIF + EIA + RMP (RPR), RPAP + RIF + RMP (RPR). The fact that in the study group, 19.2% of the samples of the liquor in the study of test strips for protein showed an excess of the protein level over the norm, allows us to recommend this method as an auxiliary in the study of the liquor.

The probability that in the control group, a similar result was obtained only in samples in which antibodies to pale treponema were detected indicates a relatively high specificity of this method by its low sensitivity.

Table 1

Comparative evaluation of clinical sensitivity, specificity and diagnostic efficacy of serological tests in the study of cerebrospinal fluid for syphilis

	Study group (n=26)	Control group (n=64)	Sensitivity %	Specificity %	diagnostic strength %
RPAP	25	5	96,2	92,1	93,3
RIF	23	2	88,4	96,8	94,4
RPHA	21	2	80,7	96,8	92,2
EIAtot	14	2	53,8	96,8	84,4
EIA IgM	-	-	0	100	-
EIA IgG	12	2	46,1	96,8	82,2
RMP	3	1	11,5	98,4	73,3
RPR	2	1	7,6	98,4	72,2
IB	2 neg, 4 ND	2 neg, 1 нND			

Conclusions

None of the presented and reviewed serological tests has 100% sensitivity and specificity. The high detectability of the RPAP method makes it possible to use it as a reference. It is possible to increase the diagnostic effectiveness of liquor diagnosis by combining various serological tests. The ability to use the methods of EIA IgM and IB in liquor diagnosis of syphilis, as well as the study of the qualitative and semi-quantitative determination of protein in the cerebrospinal fluid with test strips requires additional clinical trials.

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LIQOUR LEVEL OF TUMOR NECROSIS FACTOR ALPHA AS A PROGNOSTIC BIOMARKER OF FREQUENCY OF ACUTE MULTIPLE SCRLEROSIS

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In a study involving 60 patients with remitting multiple sclerosis (disability 3.5±1.6 points according to Expanded Disability Status Scale, EDSS) it has been found that the relative risk of exacerbation over the next three years is associated with the liquor level of tumor necrosis factor alpha in the period of the disease exacerbation. The relative risk of rapid progression of neurological deficits (over 0.75 points EDSS per year) is not associated with pleocytosis, protein concentration or alpha tumor necrosis factor in the liquor.

***Key words**: multiple sclerosis, liquor, alpha tumor necrosis factor.*

The problem of disclosing the mechanisms of reversible damage to the central nervous system (CNS) to irreversible and their progression in multiple sclerosis (MS) - a multifactorial chronic demyelinating disease of the central nervous system, which is characterized by the progression of neurological deficit and disability mainly at working age, is becoming of increasingly important socio-economic significance. [1, 2, 3]. This is due to the increasing incidence of MS throughout the world and the urgency of developing approaches to the rational use of immunomodulatory drugs, which in the early stages of MS can prevent the exacerbation and slow down the development of disability [2, 3, 4, 5, 6].

According to modern concepts, the basis of MS pathogenesis is immune inflammation in the CNS with axonal demyelination during the period of exacerbation and partial remyelination of them into remission, as well as oligodendrocyte apoptosis leading to axon death and irreversible impairment of nerve impulses [1]. In the most common remittent type of MS in the early years of the disease, immune inflammation predominates with alternating clinical exacerbations and remissions, often incomplete, and later neurodegeneration with the rapid and irreversible development of diffuse neurological symptoms [5]. The frequency of exacerbations and the rate of progression of MS vary widely. The causes and patterns of these differences in the course of MS are not disclosed, although they are the subject of numerous studies of recent years.

There remains an unresolved issue of the extent to which the severity of immune inflammation in the CNS determines the dynamics of the accumulation of irreversible neurological disorders. Data on the association of different types of MS with immune inflammation indices are contradictory and obtained mainly in the study of the blood, and not the cerebrospinal fluid of patients. So, in patients with remitting MS in the period of exacerbation, different researchers revealed multidirectional changes in the level of stimulating the inflammatory response of cytokines - tumor necrosis factor alpha (TNF-α), pro-inflammatory interleukins and others [7, 8]. There is no consensus about the possibility of objectification with the help of laboratory biomarkers of inflammation activity in the central nervous system in MS [9].

All of the above defined the purpose of the study: to evaluate the prognostic value of the TNF-α liquor level in relation to the frequency of exacerbations and progression of neurological disorders in MS.

Materials and methods

The study involved 60 patients with MS. Criteria for inclusion in the group were: remitting type of MS; disease duration of at least 5 years; disability not more than 6.5 points according to the Expanded Disability Status Scale, EDSS) [10]. Exclusion criteria: prior to the inclusion in the study, the use of drugs that change the course of MS, other autoimmune diseases. The clinical characteristics of patients are presented in Table 1.

Patients were included in the study in the acute stage of MS. Liquor was taken before treatment. In the period of exacerbation, pulse-therapy with methylpredinzolone was performed (course dose 3.0-5.0 g.). A repeated study of liquor was performed in remission, not earlier than three months after completion of the course of exacerbation therapy.

The relationship of the cerebrospinal fluid indices with the frequency of exacerbations was assessed in a three-year prospective observation of patients, with the rate of development of neurological disorders - according to the results of a retrospective analysis.

MS was diagnosed according to the 2005 McDonald criteria [11]. Magnetic resonance imaging was performed on an Impact tomograph (Siemens Magneticom, Japan) with a magnetic field strength of 1T using T1 and T2 images, as well as TIRM mode. For contrasting, Gadovist (Baer Schering Pharma, Germany) was administered in a standard dose of 0.1-0.3 mmol/kg body weight.

Table 1

Characteristics of the group of patients with multiple sclerosis included in the study (n = 60)

Parameter	
Age, years (M ± SD)	37,3 ± 10,4
Men: women, abs. number	18 : 42
Debut age, years (M ± SD)	28,8 ± 8,9
Disability by EDSS, points (M ± SD)	3,5 ± 1,6
The rate of progression, points/year (M ± SD)	0,57 ± 0,19
Disease duration, years (M ± SD)	7,2 ± 1,4
The duration of the first remission, months (M ± SD)	37,8 ± 23,4

The rate of MS progression was calculated by the ratio of EDSS at the time of the survey to the duration of the disease and regarded as slow with an increase in EDSS ≤ 0.25 points/year, an average - with an increase in EDSS in the range of 0.25-0.75 points/year, high - with an increase in EDSS > 0.75 points/year [4].

Liquor was taken by lumbar puncture. General clinical analysis of cerebrospinal fluid was performed by standard methods [12]. The concentration of factor TNF-α was measured by enzyme immunoassay using Bender Medsystems reagents (Austria). Research is allowed by the ethical committee of the Altai State Medical University, Ministry of Health of the Russian Federation.

Statistical analysis of the data was carried out in the Statistica program (v. 6.0) using the methods for estimating correlations (Spearman coefficient), comparing paired observations was performed by means of the Newman-Keuls test. The odds ratio (OR) was calculated by logistic regression analysis. The clinical informativity of the tests (operational characteristics) was calculated by the ROC analysis method (receiver operating characteristic analysis) in the JMP program (v.5.1). For quantitative variables, the results are presented as a sample mean (M) with an indication of the standard deviation (± SD), in some cases - 95% confidence interval (CI). For all statistical criteria used, a critical significance level was adopted, $p < 0.05$.

Results and discussion

It was established that during the period of MS exacerbation in the cerebrospinal fluid of patients, pleocytosis was slightly expressed and practically absent in the period of MS remission. The proportion of lymphocytes in the leukogram of cerebrospinal fluid varied from 58 to 100%. The protein concentration was low and did not significantly change during the exacerbation of the disease (Table 2).

Table 2

Liquor indicators of inflammation in patients with multiple sclerosis in the period of exacerbation and remission

Indicator	Exacerbation (n=60)	Remission (n=58)	Level of significance, p
Pleocytosis, mln cells/l Norm: 0-5 mln cells/l	11,3 ± 1,8	1,8 ± 0,2	0,012
Protein mg/l Norm: less than 450 mg/l	356 ± 187	352 ± 131	0,739
TNF-α, pg/ml	21,8 ± 5,2	4,2 ± 3,1	0,031

During exacerbation, compared with the period of remission, the concentration of TNF-α was increased by 5.2 times (Table 2). This is consistent with the idea of the activation of inflammation in the CNS during exacerbation of MS [13].

A positive correlation was found between the level of TNF-α and the number of exacerbations for three years (r_s = 0.583, p = 0.041). According to the results of logistic regression analysis, of all the studied inflammation indicators, only TNF-α is significantly associated with an increased risk of MS exacerbations in the next three years, whereas none of the studied parameters can be considered as a prognostic marker of rapid MS progression (Table 3).

A common method for evaluating the clinical informativity of diagnostic and prognostic tests - ROC-analysis (receiver operating characteristic analysis) showed that the liquor TNF-α level over 23 pg/ml as an indicator of the likelihood of MS exacerbation in the next three years had an acceptable practical use sensitivity (72,2; CI 46,5-90,3) and specificity (77.8; CI 52.4–93.6) with an area under the ROC curve of 0.80 (CI 0.65–0.96).

The results of the study suggest that the concentration of TNF-α in liquor in the period of exacerbation is promising for further study as a prognostic biomarker for the exacerbation of remitting MS.

Table 3

*Relative risk of high rate of progression and exacerbation of multiple sclerosis depending on the level of inflammation in liquor in the period of exacerbation**

Indicator	Median	Odds ratio mean value (CI)	Level of significance, p
Risk of exacerbations of multiple sclerosis			
Pleocytosis, mln cells/l	10,9	0,89 (0,37-2,11)	0,535
Protein mg/l	353	0,73 (0,31-1,39)	0,345
TNFa, pg/ml	22,3	3,05 (1,91-6,88)	0,023
Risk of high progression rate of multiple sclerosis			
Pleocytosis, mln cells/l	10,9	1,17 (0,47-2,91)	0,633
Protein mg/l	353	0,84 (0,32-2,20)	0,724
TNFa, pg/ml	22,3	1,95 (0,9-2,58)	0,082

*Note: * - median is used as the threshold level.*

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