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ORIGINAL STUDIES

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THE CONCENTRATION OF SERUM NEOPTERIN IN PATIENTS WITH SURGICAL TREATMENT OF SPONDYLOARTHROSIS COMPLICATED BY FACET SYNDROME

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КОНЦЕНТРАЦИЯ НЕОПТЕРИНА СЫВОРОТКИ КРОВИ У ПАЦИЕНТОВ ПРИ ХИРУРГИЧЕСКОМ ЛЕЧЕНИИ СПОНДИЛОАРТРОЗА, ОСЛОЖНИВШЕГОСЯ РАЗВИТИЕМ ФАСЕТ-СИНДРОМА

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Objective. To study the concentration of serum neopterin in patients before and after surgical treatment of pain syndrome caused by the formation of facet syndrome associated with spondyloarthrosis.
Materials and methods. The study involved 52 patients (32 men and 20 women) hospitalized to the neurosurgical department of the City Clinical Hospital No. 4. The main group consisted of 26 patients with

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spondyloarthrosis complicated by chronic pain associated with facet syndrome. The comparison group (n=26) consisted of patients with pain syndrome in radiculopathy. The control group (comparable in gender and age) consisted of 10 people who underwent periodic examination. The concentration of neopterin in blood serum was determined by solid-phase enzyme immunoassay (ELISA) using the Neopterin ELISA kit (cat. No. 59321; lot ENO230) ("IBL", Germany).

Results. Statistically significant differences in the level of serum neopterin concentration (p = 0.0016) were revealed between the studied groups. The highest concentration of neopterin was noted in the comparison group, where it was 10.31 ± 2.02 nmol/l, exceeding the results of the control group (p=0.02256) and the main group (p=0.04996). Three months after surgical treatment of patients in the main group, the median content of serum neopterin decreased by 1.161 times (p = 0.049029).

Conclusion. Further studies to clarify the influence of pain on metabolic processes and neopterin in particular are required.

Keywords. Neopterin, spondyloarthrosis, radiculopathy, pain.

Цель. Изучить концентрацию неоптерина сыворотки крови у пациентов до и после хирургического лечения болевого синдрома обусловленного формированием фасеточного синдрома на фоне спондилоартроза.

Материалы и методы. В исследовании участвовали 52 человека (32 мужчины и 20 женщин), госпитализированных в нейрохирургическое отделение ГКБ № 4. Из них 26 пациентов со спондилоартрозом, осложнённым хронической болью, составили основную группу. Сравнительный анализ проводили с пациентами с болевым синдромом при радикулопатии (n = 26). Контрольную группу, сопоставимую по возрастному и половому составу, составили 10 человек, проходившие периодический осмотр. Для определения концентрации неоптерина в сыворотке крови использовали метод твердофазного иммуноферментного анализа (ИФА) (набор Neopterin ELISA, кат. No. 59321; лот ENO230, «IBL», Германия).

Результаты. Между исследованными группами выявлены статистически значимые различия в уровне сывороточной концентрации неоптерина (p = 0,0016). Наибольшая концентрация неоптерина отмечена в группе сравнения, где она составила 10,31 ± 2,02 нмоль/л, превысив результаты контрольной группы (p = 0,02256) и основной (p = 0,04996). Через три месяца после хирургического лечения пациентов основной группы медиана содержания неоптерина сыворотки крови снизилась в 1,161 раза (p = 0,049029).

Выводы. Необходимы дальнейшие исследования для уточнения влияния боли на процессы обмена веществ и, в частности, неоптерина.

Ключевые слова. Неоптерин, спондилоартроз, радикулопатия, боль.

INTRODUCTION

Spondyloarthrosis is manifested by degenerative damage to the intervertebral joints involving cartilage, bones, ligaments, and muscles. A specific symptom of spondyloarthrosis is chronic pain, which intensifies with movement, which is accompanied by limited mobility and unstable morning stiffness [1; 2]. In some cases, lumbar pain is caused by radiculopathy [3]. Pain syndrome associated with damage to the facet joints of the lumbar spine is less known. Its occurrence may be associated with trauma [4; 5] and effusion [6].

Spondyloarthrosis is associated with static–dynamic loads, age-related degenerative–dystrophic processes in osteochondral tissue, and metabolic disorders in the human body. A study described a change in the pool of amino acids in the blood plasma during severe chronic pain syndrome [7]. The study of the serum concentrations of not only nitrogenous compounds related to

amino acids, but also metabolites of nucleic acid metabolism, particularly derivatives of purines and pyrimidines, is of particular interest. Neopterin, a derivative of pterins, is one of the metabolites of purine metabolism, intensively studied in recent decades, and synthesized by cells of the monocyte–macrophage series under the influence of interferons [8; 9]. Changes in neopterin concentration are mainly caused by diseases that occur with the activation of cytotoxic immune response, particularly infectious diseases caused by viruses, autoimmune diseases, and tumor processes [10–13].

Previously, we performed a preliminary assessment of the concentrations of neopterin in the blood serum of patients with pain syndrome related to spondyloarthrosis of the lumbar spine before and after treatment [14]. However, the group of patients examined was quite heterogeneous and had various concomitant diseases. Thus, examining a more homogeneous sample with the inclusion of patients with pain syndrome that developed in patients with spondyloarthrosis or spinal cord root entrapment appears appropriate.

This study aimed to analyze the dynamics of changes in serum neopterin concentrations in patients before and after surgical treatment of chronic pain syndrome related with spondyloarthrosis-associated facet syndrome.

MATERIALS AND METHODS

This observational, cross-sectional case-control study was performed in compliance with the ethical principles of medical research involving human subjects as set out in the Declaration of Helsinki of the World Health Organization.

The study enrolled 52 patients (male, $n = 32$; female, $n = 20$) hospitalized in the neurosurgical department of City Clinical Hospital No. 4. The main group consisted of 26 patients hospitalized for spondyloarthrosis complicated by chronic pain due to facet syndrome. Considering that this group of patients received surgical treatment, the serum concentration of neopterin was determined twice, that is, before surgery during hospitalization and 6 months after surgical treatment. Treatment consisted of high-frequency denervation of the facet joints at the L4–S1 level on both sides.

The inclusion criteria were as follows: (1) pain syndrome, (2) facet syndrome (spondyloarthrosis) confirmed by MRI data, (3) radiculopathy ruled out, (4) ruled out concomitant diseases such as hypertension and diabetes mellitus, in the stage of decompensation, and (5) normal results of a general blood test and a general urinalysis, as well as biochemical blood tests.

The exclusion criteria were as follows: (1) oncological diseases, (2) viral and infectious diseases in the acute phase or <4 weeks after recovery; (3) autoimmune diseases; (4) stage of decompensation of diabetes mellitus, hypertension, and liver and kidney diseases; (5) age <18 years; (6) incapacity; (7) pregnancy; and (8) patient refusal to participate in the study.

The comparison group (n = 26) consisted of patients with pain syndrome caused by to radiculopathy. The control group consisted of 10 patients who underwent periodic examinations, did not present any complaints at the time of the examination, and had normal results of a general analysis of urine, blood, and biochemical blood parameters (glucose and total cholesterol). The groups were comparable in terms of sex ratio and age ($p > 0.05$). Their comparative characteristics are presented in Table 1.

Blood samples were collected by vein puncture into vacuum tubes with a coagulation activator. After clot formation, the samples were centrifuged for 15 min at 3000 rpm using an Elekon CLMN-R-10-02 centrifuge. After performing all necessary tests, the remaining serum samples were separated and aliquoted into microtubes (Eppendorf – 0.7 mL) and stored at Ÿ20 °C until further studies.

The concentration of neopterin was determined by enzyme-linked immunosorbent assay (ELISA) using the Neopterin ELISA test system (Cat. No. 59321; lot ENO230, IBL, Germany), with manufactured-reported sensitivity of at least 0.7 nmol/L. The optical density of the samples was measured on a StatFax 3200 vertical photometer (Awareness, USA). Data accuracy was monitored based on the measurement results of two control samples that were part of the test systems. The neopterin content in control sample 1 was 5.48 nmol/L, with an acceptable range of results of 3.5–8.1 nmol/l, and in sample 2, it was 23.34 nmol/L with an acceptable range of 13.6–28.2 nmol/L.

For statistical processing of the study results, Statistica version 7 (StatSoft Inc., USA) was used. For the results obtained in each group, arithmetic mean (*M*) and standard deviation (*SD)* were calculated for a normal distribution of the trait and *Me* (median) and interquartile range (25–75th percentile) for a nonnormal distribution. To assess the distribution of results within the sample, the Shapiro–Wilk test was used. Therefore, for further calculations,

Table 1

Characteristics of the study groups

Characteristics of patients	Main group	Comparison group	Control group
Number of patients examined	26	26	10
Sex ratio (men/women)	15/11	17/9	6/4
Age of subjects, years	39.35 ± 6.56 38 (35; 44) 27–53	39.69 ± 10.9 36.5 (31; 47) 20–68	35.1 ± 5.59 35 (33; 37) 25–46

N o t e: *– the numerator shows the mean value (*M*) ± standard deviation (*SD*), the denominator shows the median (*Me*) and interquartile range (25 % quartile; 75 % quartile); under the slant line, there are highest and lowest results.

Table 2

Concentration of neopetrin (nmol/L) in the study groups

Characteristics of patients	Main group		Comparison group	Control group
	before surgery	after surgery		
Number of patients examined, n	26	26	26	10
Neopterin concentration, nmol/L	9.03 ± 1.39 8.87 (7.93; 9.7) 8.87–13.7	7.93 ± 2.36 7.64 (6.47; 9.68) 3.19–11.91	10.31 ± 2.02 9.82 (8.71; 11.87) 8.15–14.89	8.21 ± 0.85 8.21 (7.36; 8.71) 7.07–9.72
Shapiro–Wilk *W* test	0.88573 ($p = 0.00762$)	0.96754 ($p = 0.56065$)	0.88409 ($p = 0.00703$)	0.94675 ($p = 0.63022$)
Kruskal–Wallis *H* test	*H* test = 12.86557; $p = 0.0016$ Median test: $(p_{1,3}) = 0.338734$; $(p_{1,2}) = 0.049955$; $(p_{2,3}) = 0.02256$			
Wilcoxon test	$p = 0.049029$		-	-

N o t e: * – the numerator shows the mean value (*M*) ± standard deviation (*SD*), and the denominator shows the median (*Me*) and interquartile range (25 % quartile; 75 % quartile); under the slant line, there are highest and lowest results.

nonparametric statistics methods were used (Table 2). To compare neopterin content, the Wilcoxon and Kruskal–Wallis tests were used in related and unrelated groups, respectively ($n > 3$). The median test was used to compare further results between individual unrelated groups. A statistical significance level value ≤0.05 was taken as the maximum permissible probability of a type 1 error (p).

RESULTS AND DISCUSSION

Descriptive statistics characterizing the concentrations of neopterin in the studied groups are presented in Table 2. Statistically significant differences in the serum levels of neopterin were revealed between the studied groups (Fig. 1).

Before treatment, when comparing neopterin levels between patients, a statistically significant increase ($p < 0.05$) was revealed in the comparison group in patients with radicular pain syndrome. The median serum level of neopterin in the comparison group was 1.088 times higher than that in the main group ($p = 0.049955$) and 1.197 times higher than the data in the control group ($p = 0.02256$) (Table 2). Thus, statistically significant between-group differences were detected.

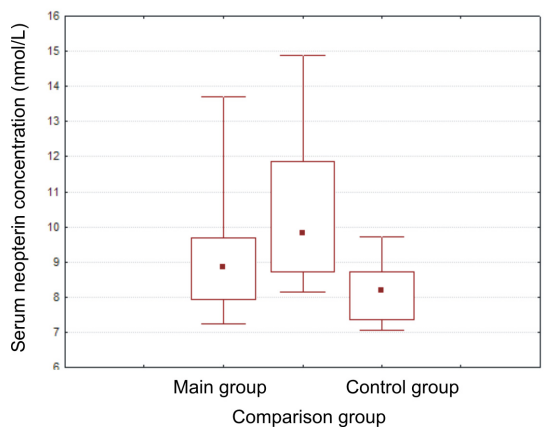

Fig. 1. Neopterin concentration (nmol/L) in the blood serum of the studied groups

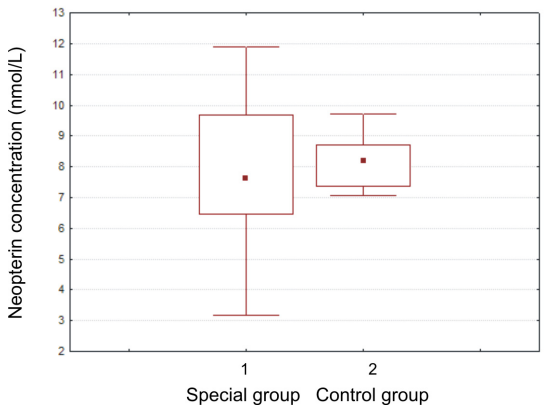

Fig. 2. Neopterin concentration (nmol/L) in the blood serum of patients of the main group after surgical treatment and control group

After surgical treatment, the serum concentration of neopterin in the main group with facet syndrome decreased statistically significantly (Wilcoxon test, $p = 0.049029$) and approached the indicators of the control group (Mann – Whitney *U* test, 114.5; $p = 0.589812$) (Fig. 2).

The higher concentration of neopterin in the blood serum of patients with pain syndrome caused by radiculopathy can be explained by the presence of neurogenic inflammation, which is accompanied by a greater release of proinflammatory factors that activate the antinociceptive purinergic system, in comparison with pain syndrome that develops in patients with spondyloarthrosis of the lumbar spine with facet syndrome development [15; 16]. In our opinion, this mechanism can lead to an increase in the serum concentration of neopterin [17; 18]. The prognostic value of studying the postoperative neopterin was identified in the authors' works when predicting postcholecystectomy syndrome[1] and other diseases [19].

Data are in poor correspondence with the preliminary results of the examination of patients who received high-frequency denervation of the facet joints [14]. However, with a more stringent selection of groups and an increase in the follow-up period to 3 months, this study revealed a statistically significant decrease in the concentration of neopterin in the blood serum.

An increase in the serum concentration of neopterin in the presence of diseases may be significant in the somatization of symptoms of diseases of internal organs [20]. In general, more studies are needed to clarify the role and pathogenesis of the effects of pain on the metabolism of tissues and organs.

CONCLUSIONS

1. The initial concentration of neopterin in spondyloarthrosis with the emergence of acute pain syndrome (facet syndrome) is not statistically significantly different from the examination results of the control group ($p = 0.338734$).

2. An effective treatment of pain syndrome caused by the compression of nerves in the area of the facet joints in spondyloarthrosis is accompanied by a statistically significant decrease not only in pain but also in the serum concentration of neopterin ($p = 0.049029$).

[1] O.V. Boyko, Yu.V. Kondrashova, A.V. Zhurikhin, and V.I. Boyko, RU patent No. 2498303.

3. Further research is necessary to clarify the effect of pain on metabolic processes.

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DYNAMICS OF FORMATION OF ANTIBODIES TO SARS-CoV-2 AFTER CORONAVIRUS INFECTION IN CHILDREN

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ДИНАМИКА ОБРАЗОВАНИЯ АНТИТЕЛ К SARS-CoV-2 ПОСЛЕ ПЕРЕНЕСЕННОЙ КОРОНАВИРУСНОЙ ИНФЕКЦИИ У ДЕТЕЙ

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Objective. To estimate the level of antibodies to SARS-CoV-2-IgM and SARS-CoV-2-IgG in children with COVID-19 in acute period and during 1 year period of follow-up after coronavirus infection.
Materials and methods. Blood samples for the presence of IgM and IgG antibodies to SARS-CoV-2 were analyzed in 119 children aged 11.0 [10.1; 11.2] with COVID-19 in the acute period (29.4 % asymptomatic, 51.3 % mild and 19.3 % moderate), and SARS–CoV-2-IgG in the dynamics of the follow-up after 1 (*n*=55), 6 (*n*=33) and 12 (*n*=32) months from the moment of discharge from the hospital in a prospective cohort study. The levels of SARS-CoV-2 surface glycoprotein S, including the receptor-binding domain – RBD were measured at different time by using enzyme-linked immunosorbent assay.
Results. The level of IgM positive rate for SARS-CoV-2 was initially negative in 86.6 % of children with COVID-19. The original seroconversion (on admission to the hospital) was 38.7 % and it increased to 96.7 % in 1 month and to 100 % in 12 months of observation. There were no statistically significant differences in IgG persistence depending on the age and course of COVID-19.
Conclusions. The new coronavirus infection causes a long-term response of IgG antibodies to SARS-CoV-2 which persists for one year of observation and increases by 12 months after the infection regardless of the severity of COVID-19.
Key words. COVID-19, new coronavirus infection, SARS-CoV-2, IgG and IgM antibodies, children.

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Цель. Оценить уровень антител SARS-CoV-2-IgM и SARS-CoV-2-IgG у детей с COVID-19 в острый период и на протяжении одного года наблюдения после перенесенной коронавирусной инфекции.

Материалы и методы. В проспективном когортном исследовании проанализированы образцы крови на наличие антител класса IgM и IgG к SARS-CoV-2 от 119 детей в возрасте 11 [10,1; 11,2] лет с COVID-19 в остром периоде (29,4 % бессимптомная, 51,3 % – легкая и 19,3 % среднетяжелая форма), а также определены SARS-CoV-2-IgG в динамике наблюдения за пациентами через один ($n = 55$), 6 ($n = 33$) и 12 ($n = 32$) месяцев от момента выписки из стационара. Уровни поверхностного гликопротеина S SARS-CoV-2, включая рецептор-связывающий домен – RBD, измерялись в различные моменты времени с помощью иммуноферментного анализа.

Результаты. Уровень коэффициента позитивности IgM на SARS-CoV-2 изначально оказался отрицательным у 86,6 % детей с COVID-19. Исходная (на момент поступления в стационар) сероконверсия составила 38,7 %, которая увеличилась до 96,7 % через месяц и до 100 % через 12 месяцев соответствующего последующего наблюдения. Не обнаружено статистически значимых различий в отношении персистенции IgG в зависимости от возраста и степени тяжести COVID-19.

Выводы. Коронавирусная инфекция вызывает длительный ответ антител класса IgG к SARS-CoV-2, который сохраняется на протяжении года наблюдения и усиливается к 12 месяцам после инфекции независимо от степени тяжести COVID-19.

Ключевые слова. COVID-19, коронавирусная инфекция, SARS-CoV-2, антитела IgG и IgM, дети.

INTRODUCTION

The novel human coronavirus SARS-CoV-2 is highly contagious, and the disease it causes, COVID-19, can lead to significant morbidity and mortality in some patients [1]. In children, SARS-CoV-2 infection is asymptomatic or mild; however, children are susceptible to severe COVID-19 manifestations and the associated post-infectious multisystem inflammatory syndrome [2]. Moreover, the severity of COVID-19 is associated with the host's immune response against SARS-CoV-2, and children and adolescents with mild or asymptomatic coronavirus infection can produce a strong and persistent antibody response [3].

Laboratory methods for diagnosing SARS-CoV-2 are based on the detection of viral RNA in patient secretions using the polymerase chain reaction (PCR) method and of antibodies (immunoglobulins M and G) in blood serum [4].

The immune response against SARS-CoV-2 plays a critical role in determining clinical outcome in adults and children [5]. Immunity to SARS-CoV-2 induced by natural infection may be mediated by a combination of humoral and cellular immunity [6]. A study comparing children to adults revealed different immune profiles during coronavirus infection, indicating less severe outcomes in pediatric patients [7].

Immune protective factors against SARS-CoV-2 have not been identified, although neutralizing antibodies are increasingly recognized as major mediators of protection [8].

Studies of severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome showed that virus-specific antibodies were detected in 80 %–100 % of patients 2 weeks after symptom onset [9; 10].

Most adults with coronavirus infection produce an IgG response that can persist for at least 12 months [11]. Recovered seropositive adults have up to 89 % protection against reinfection with the same strain [12]. In contrast, the proportion of children infected with

SARS-CoV-2 with seroconvertion is unknown, particularly among patients with asymptomatic or mild COVID-19. Furthermore, children may have distinct immune responses that modulate clinical severity [7]. Additionally, preexisting antibodies to seasonal human coronaviruses may contribute to some level of protection against SARS-CoV-2 in children [13]. Several innate and adaptive immune response aspects in children are not yet fully characterized owing to the limited number of studies in this population. The humoral response to SARS-CoV-2 remains poorly understood, whereas the immunological response to vaccines is well described in clinical trials, the characteristics and persistence of seroconversion are unclear, and follow-up data on IgG levels after COVID-19 are limited.

This study aimed to assess the level of SARS-CoV-2-IgM and SARS-CoV-2-IgG in pediatric patients with COVID-19 in the acute period and during 1-year follow-up after coronavirus infection.

MATERIALS AND METHODS

The prospective cohort study involved 119 children with COVID-19 from the City Clinical Hospital No. 1 of Chita, which at the time of the work was the single hospital accepting patients with coronavirus infection in the Trans-Baikal Territory.

Government authorities have taken preventive measures and given recommendations for the hospitalization of all pediatric patients with COVID-19, including asymptomatic forms to maintain social distance between people and prevent the spread of the SARS-CoV-2 virus.

Inclusion criteria were age 0–17 years, positive result for SARS-CoV-2, and parent/child consent to participate in the study. Non-inclusion criteria were children aged >17 years, negative test result for SARS-CoV-2, and refusal of parents (legal representatives) or children to participate in the study.

The diagnosis of COVID-19 is based on the criteria according to the methodological recommendations “Aspects of clinical manifestations and treatment of the disease caused by a new coronavirus infection (COVID-19) in pediatric patients” (version 1 dated April 24, 2020).

Demographic data, information about positive PCR tests for SARS-COV-2, medical history, comorbidities, and heredity were obtained from the electronic database of medical records.

All cases were confirmed by PCR using nasal and oropharyngeal swabs. During the study period, children and adolescents were not vaccinated against COVID-19. No reinfections were recorded during the follow-up period.

The severity of coronavirus infection was classified as asymptomatic but with a positive test for SARS-CoV-2 in 35 (29.4 %) pediatric patients (group I), as mild in 61 (51.3 %) pediatric patients (group II), and moderate in 23 (19.3 %) patients (group III). The median age of the pediatric patients was 11 (10.1; 11.2) years old; 53.8 % of those examined were boys. Additionally, children were distributed by age (subgroup 1, 0–6 years (n = 21); subgroup 2, 7–17 years (n = 98)).

To analyze the intensity of humoral immunity after coronavirus infection, SARS-

CoV-2-IgG was determined by monitoring patients 1 month (n = 55), 6 months (n = 33), and 12 (n = 32) months after discharge from the hospital.

Blood samples were collected within 48 hours of hospitalization from all 119 pediatric patients and tested for the presence of IgM and IgG antibodies to the surface glycoprotein S of SARS-CoV-2, including the receptor-binding domain (RBD) in the blood serum. The study was conducted using an enzyme immunoassay with a set of reagents SARS-CoV-2-IgM-ELISA-BEST and SARS-CoV-2-IgG-ELISA-BEST (Vector-Best, Novosibirsk region) according to manufacturer instructions. Antibody levels were expressed in S/CO (Signal/Cut-off) as signal/critical value: positive, results higher than 1.1 S/CO; questionable, those higher than 0.8 S/CO and lower than 1.1 S/CO; and negative, results lower than 0.8 S/CO.

Written voluntary informed consent to participate in the study was obtained from participants aged >15 years or from parents (legal representatives) of pediatric patients aged <15 years.

The study was approved by the local ethics committee of the Chita State Medical Academy (protocol no. 101; April 15, 2020) and was conducted in accordance with the Declaration of Helsinki.

Statistical processing of the research results was performed using IBM SPSS Statistics Version 25.0 software (International Business Machines Corporation, USA). The normality of distribution was tested using the Kolmogorov–Smirnov test. Quantitative variables were presented as median (Me) and interquartile range (Q_1; Q_3) and categorical variables as absolute values and percentages (%). The assessment of variations in the levels of the studied markers in the blood serum between two independent groups was performed using the Mann–Whitney test (U). Intergroup differences in one quantitative sign for comparisons of three or more independent groups were analyzed using the Kruskal–Wallis rank analysis of variance (H). The significant differences in pairwise comparisons between dependent samples were assessed using the Wilcoxon test. $P < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Serological testing for SARS-CoV-2 upon hospital admission demonstrated a lack of IgM antibody response in pediatric patients with COVID-19 in majority (86.6 %) of cases (Table 1).

Positive test results for IgM antibodies to the surface glycoprotein S (Spike) of SARS-CoV-2 in the blood serum were detected in 6.7 % of patients with confirmed SARS-CoV-2 infection, whereas 4.3 % of them were asymptomatic and 4.9 % and 11.4 % of patients had a mild and moderate form of the disease, respectively.

The seroprevalence (positive IgG antibodies to SARS-CoV-2) at the time of hospitalization was 38.7 %. Additionally, 56.3 % of patients with positive PCR for SARS-CoV-2 did not have IgG antibodies, and questionable results were recorded in 5 % of pediatric patients. It was revealed that 34.8 % of patients in group 1, 40.9 % of patients in group 2, and 37.1 % of pediatric patients in group 3 tested positive for SARS-CoV-2-IgG.

Table 1

Level of positivity rate of SARS-CoV-2-IgM and SARS-CoV-2-IgG depending on the severity of COVID-19 in pediatric patients of different ages upon admission

COVID-19 severity	IgM PC* level, *n*/ %			IgG PC level, *n*/ %		
	Negative < 0.8 S/CO	Questionable ≥ 0.8 and < 1.1 S/CO	Positive > 1.1 S/CO	Negative < 0.8 S/CO	Questionable ≥ 0.8 and < 1.1 S/CO	Positive > 1.1 S/CO
Asymptomatic, *n* = 23	19/82.6	3/13	1/4.3	15/65.2	0/0	8/34.8
Mild, *n* = 61	54/88.5	4/6.6	3/4.9	35/57.4	1/1.6	25/40.9
Moderate, *n* = 35	30/85.7	1/2.9	4/11.4	17/48.6	5/14.3	13/37.1
Total, *n* = 119	103/86.6	8/6.7	8/6.7	67/56.3	6/5.0	46/38.7

Note: *, level of positivity coefficient (PC).

The median level of SARS-CoV-2-IgM in asymptomatic coronavirus infection was 0.37 [0.35; 0.55] S/CO and corresponded to the values for mild (0.27 [0.27; 0.56] S/CO; $p = 0.533$) and moderate (0.28 [0.27; 0.73] S/CO; $p = 0.661$) severity of COVID-19. The asymptomatic course of SARS-COV-2 infection was characterized by a serum IgG concentration of 0.41 [0.39; 2.86] and did not differ from the indicators for mild (0.49 [0.37, 2.99] S/CO; $p = 0.439$) and moderate (0.75 [1.76, 3.14] S/ CO; $p = 0.551$) forms. No statistical difference was found in the IgM and IgG levels between patients of groups II and III ($p = 0.734$ and $p = 0.894$ S/CO, respectively).

Table 2 presents the antibody titers used depending on the coronavirus infection severity in pediatric patients of different ages. The study results showed an increase in the titer of IgG antibodies to SARS-CoV-2 in patients aged 0–6 years with moderate COVID-19 compared to that in asymptomatic patients and those with mild forms of the disease, whereas no significant differences were noted in the levels of antibodies of the IgM and IgG classes depending on the age of the pediatric patients and disease severity ($p > 0.05$). Because no significant differences were observed in IgM and IgG levels between pediatric patients of different ages, further dynamic follow-up of the study groups was performed among pediatric patients aged 0–17 years.

Median level of SARS-CoV-2-IgG during dynamic follow-up 1 month after coronavirus infection was 9.55 [8.15; 10.90], which is significantly higher by 12 times than similar data in the acute period (0.81 [0.79, 3.74]; $p = 0.000$). By month 6 from the date of discharge from the hospital, the IgG level to SARS-CoV-2 was 10.38 [8.97; 10.25], which is significantly higher than that in the initial study (0.92 [0.87, 2.48]; $p = 0.000$).

The proportion of seropositive patients (IgG to SARS-CoV-2) reached its maximum (100 %) values of 10.12 [8.63; 10.34] when determined after 12 months, compared to the results obtained 1 month and 6 months after coronavirus infection (96.4 % and 96.9 %, respectively; Table 3).

Table 2

Antibody titers upon admission depending on the severity of coronavirus infection in pediatric patients of different ages

Ig (S/CO)	Study group						Test statistics		
	I, $n = 23$		II, $n = 61$		III, $n = 35$				
	0–6 years, $n = 7$	7–17 years, $n = 16$	0–6 years, $n = 7$	7–17 years, $n = 54$	0–6 years, $n = 7$	7–17 years, $n = 28$	Kruskal–Wallis, $df = 3$	Mann–Whitney	
								Intragroup comparison	Comparison of subgroups studied
	1	2	3	4	5	6			
IgM	0.49 [0.40; 0.65]	0.30 [0.30; 0.55]	0.27 [0.,27; 0.54]	0.27 [0.27; 0.58]	0.50 [0.50; 1.28]	0.28 [0.28; 0.67]	$U = 3.45$; $p = 0.632$	$U_{1-2} = 43.5$, $p_{1-2} = 0.402$; $U_{3-4} = 185.5$, $p_{3-4} = 0.937$; $U_{5-6} = 65.0$, $p_{5-6} = 0.172$	$U_{1-3} = 20.0$, $p_{1-3} = 0.562$; $U_{1-5} = 21.5$, $p_{1-5} = 0.701$; $U_{3-5} = 18.0$, $p_{3-5} = 0.404$; $U_{2-4} = 427.5$, $p_{2-4} = 0.950$; $U_{2-6} = 216.0$, $p_{2-6} = 0.845$; $U_{4-6} = 739.0$, $p_{4-6} = 0.868$
IgG	0.44 [0.44; 4.08]	0.37 [0.36; 2.85]	0.31 [0.30; 1.68]	0.49 [0.49; 3.23]	3.30 [3.20; 8.35]	0.69 [0.67; 2.13]	$U = 3.90$; $p = 0.564$	$U_{1-2} = 46.5$, $p_{1-2} = 0.524$; $U_{3-4} = 151.5$, $p_{3-4} = 0.396$; $U_{5-6} = 62.5$, $p_{5-6} = 0.143$	$U_{1-3} = 20.0$, $p_{1-3} = 0.564$; $U_{1-5} = 16.0$, $p_{1-5} = 0.277$; $U_{3-5} = 15.0$, $p_{3-5} = 0.225$; $U_{2-4} = 363.0$, $p_{2-4} = 0.334$; $U_{2-6} = 211.5$, $p_{2-6} = 0.760$; $U_{4-6} = 696.0$, $p_{4-6} = 0.557$

Table 3

Dynamics of SARS-CoV-2-IgG antibody titer in pediatric patients after COVID-19

Post-COVID term	IgG PC level, n/%		
	Negative < 0.8 S/CO	Questionable ≥0.8 and < 1.1 S/CO	Positive > 1.1 S/CO
1 month, $n = 55$	2/3.6	0/0	53/96.4
6 months, $n = 33$	1/3,0	0/0	32/96.9
12 months, $n = 32$	0/0	0/0	32/100.0

Serological testing for COVID-19 demonstrated IgG seroconversion in all pediatric patients after 12-month follow-up, including asymptomatic patients, and in the acute period of the disease, SARS-CoV-2-IgG was recorded in 38.7 %.

IgM provides the first line of defense in viral infections, whereas IgG production lags behind IgM and is responsible for long-term immunity and memory [14]. According to a previous report on SARS in 2003, IgM was detected in the blood of patients 3–6 days after disease onset, and IgG could be detected 8 days after the onset of infection [15]. Other studies [16; 17] on seropositivity for antibodies to SARS-CoV-2 in pediatric patients reported lower rates than those detected in this study. Thus, in a meta-analysis, Rostami et al. revealed that the prevalence of antibodies to SARS-CoV-2 in the population aged <19 years was 2.3 % [16]. According to the Spanish national registry, seropositivity for SARS-CoV-2 was registered in 12.5 % of adults and 7.7 % of children [17]. The present study showed that the level of anti-SARS-CoV-2-IgG at the time of hospitalization is already relatively high (38.7 %), which is consistent with a previous study that found an early and high level of IgG response against SARS-CoV-2 [18]. The high incidence of positive IgG in the early stage of SARS-CoV-2 infection may be due to the fact that some patients with COVID-19 are asymptomatic in the first days after infection [19]. A study showed that in 97.5 % of people, symptoms appear within 11.5 days [20]. The recorded date of disease onset may be later than the date of infection because of the asymptomatic course, which explains the high level of IgG during week 1 of illness [21].

The long-term immune response of SARS-CoV-2, demonstrated during the dynamic follow-up of pediatric patients who had coronavirus infection in our study, coincides with that of the coronavirus associated with SARS-CoV [22]. The researchers reported that IgG antibodies were continuously detected for 2 years in patients who recovered from SARS-CoV [23].

Additionally, previous studies confirm our findings of high IgG positivity rate for SARS-CoV-2 [21; 24; 25]. Thus, IgG antibody titers remained elevated against protein S and RBD in 96 % and 99 % of cases, respectively [24]. In 95.3 % of patients included in the study by Li, IgG against SARS-CoV-2 was detected 5 weeks after symptom onset [21]. Iyer et al. examined 343 patients and showed that IgG persisted for 90 days after symptom onset [25]. Moreover, Zhu et al. reported that over 60 % of adults remained IgG positive 7 months after symptom onset, regardless of COVID-19 severity [26]. In the study by Whitcombe, 96 % of patients had anti-S protein IgG levels above baseline 4–8 months after infection [24]. In children in the Irkutsk Region, after recovery from a confirmed new coronavirus infection, antibodies were detected in 66.1 % of cases and persisted for up to 10–15 months to the nucleocapsid and up to 15–18 months to the RBD of SARS-CoV-2 [27]. An assessment of IgG levels to SARS-CoV-2 after COVID-19 in pediatric patients demonstrated that the highest antibody levels persisted for 2–4 months after the illness [28].

Thus, longer follow-up of pediatric patients infected with SARS-CoV-2 is crucial to establish the duration of humoral protection in this population. Further research is required to elucidate the role of long-term humoral responses in pediatric patients following SARS-CoV-2 infection and their relationship with protection against recurrent infections.

Tracking dynamic changes in SARS-CoV-2-IgG can provide additional information for diagnosing, monitoring, and predicting COVID-19 and developing new vaccines.

Conclusions

In 6.7 % of pediatric patients with coronavirus infection at the time of hospitalization, positive levels of the positivity coefficient of IgM antibodies to SARS-CoV-2 were detected, whereas the overall seroconversion rate was 38.7 %. Children who have had COVID-19, starting from month 1 after discharge from the hospital, demonstrated increased SARS-CoV-2-IgG antibody titer. Most pediatric patients with SARS-CoV-2 infection remain serologically positive 6 months after infection. IgG antibodies to SARS-CoV-2 persist up to 12 months after infection, regardless of the severity of COVID-19.

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CERVICAL INTRAEPITHELIAL NEOPLASIA: THE CURRENT STATE OF THE PROBLEM IN YEKATERINBURG

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ЦЕРВИКАЛЬНАЯ ИНТРАЭПИТЕЛИАЛЬНАЯ НЕОПЛАЗИЯ: СОВРЕМЕННОЕ СОСТОЯНИЕ ПРОБЛЕМЫ В Г. ЕКАТЕРИНБУРГЕ

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Objective. To determine the frequency of cervical intraepithelial neoplasia among female citizens in Yekaterinburg.

Materials and methods. The retrospective analysis of the report forms of the cervical pathology office in Yekaterinburg Perinatal Center from January to September 2022 was carried out. Statistical data processing was conducted using descriptive statistical methods in the Excel programme.

Results. 255 cases of precancerous cervical diseases and 7 cases of cervical cancer were revealed within 9 months of monitoring, among them 24 patients with precancerous pathology and 4 with cervical cancer had previously been followed up by a specialist. Most cases of cervical pathology were revealed in patients over 40.

Conclusions. The women's consultations of Verkh-Isetsky and Leninsky districts refer patients to the specialized cervical pathology office most actively. Patients of the cervical pathology office are provided with the full range of therapeutic and diagnostic procedures, with colposcopy being most often performed (27 % of cases). The dispensary group of patients of the cervical pathology office is mainly represented by women with cervical dysplasia of the 2nd degree (HSIL, CIN II) – 43.5 %.

Keywords. Cervical intraepithelial neoplasia, cervical cancer, biopsy, colposcopy, excision.

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Цель. Выявить частоту цервикальной интраэпителиальной неоплазии среди жительниц г. Екатеринбурга.
Материалы и методы. Проведен ретроспективный анализ отчетных форм кабинета патологии шейки матки Екатеринбургского перинатального центра в период с января по сентябрь 2022 г. Статистическая обработка осуществлялась с применением описательных методов статистики в программе Excel.
Результаты. За девять месяцев наблюдения было выявлено 255 случая предраковых заболеваний шейки матки и 7 случаев рака шейки матки, при этом 24 пациентки с впервые выявленной предраковой патологией и 4 с раком шейки матки ранее уже наблюдались у специалиста. Больше всего случаев патологии шейки матки было обнаружено у пациенток старше 40 лет.
Выводы. Наиболее активно направляют пациенток в специализированный кабинет патологии шейки матки женские консультации Верх-Исетского и Ленинского районов. Пациенткам кабинета патологии шейки матки выполняется весь объем лечебно-диагностических мероприятий, но чаще всего осуществляется кольпоскопия – в 27 % случаев. Диспансерная группа пациенток кабинета патологии шейки матки преимущественно представлена женщинами с дисплазией шейки матки 2-й степени (HSIL, CIN II) – 43,5 %.
Ключевые слова. Цервикальная интраэпителиальная неоплазия, рак шейки матки, биопсия, кольпоскопия, эксцизия.

INTRODUCTION

Cervical intraepithelial neoplasia (CIN) is an atypical transformation of squamous epithelium without stromal invasion and is a precursor of cervical cancer (CC). According to the literature, in women of reproductive age, CIN accounts for from 10.7 to 38.8 % in the structure of gynecological diseases. In addition, a number of studies show that pronounced cervical dysplasia progresses with the development of carcinomas in 0.2–0.4 % of cases within a year [1]. CC is the fourth most common cancer in women worldwide with an estimated incidence of 569,847 cases and 311,365 deaths according to the latest Globocan report [2]. In the Russian Federation, as of 2018, CC ranks 5th in the structure of oncopathology [1].

The leading role in the pathogenesis of CIN and CC development belongs to the human papillomavirus (HPV). More than 80 % of sexually active men and women become infected with HPV during their lifetime. Despite this, most cervical HPV infections resolve spontaneously; however, in a minority of women, the virus persists and progresses to cervical dysplasia and cancer. Known risk factors for the development of CIN are: early onset of sexual activity, a large number of sexual partners, long-term use of combined oral contraceptives, smoking, presence of mixed infections associated with herpes simplex virus type 2 or human immunodeficiency virus, as well as other sexually transmitted infections, bacterial vaginosis, history of vulvar and anal dysplasia [3].

Based on the extent of tissue involvement, CIN can be classified as CIN I (poorly differentiated neoplasia), CIN II, and CIN III (the most severe form). Although CIN I can regress, it can also progress to high-grade dysplasia and, even worse, to CC [1]. According to the World Health Organization (WHO), 99 % of high-grade intraepithelial lesions and invasive forms of CC can be detected at an early stage using well-organized screening programs [4]. WHO has approved three main methods: virological, cytological and visual [5]. Cervical cytology is the pre-

ferred method of screening for CC and its precursors in many countries, as it is a cheap and accessible test. Despite its high specificity, it has low sensitivity. False-negative results can be caused by inadequate collection and fixation of material [4]. Double staining for p16 and Ki67 helps identify truly malignant cells. Compared with HPV testing or single p16 staining, the sensitivity of double staining for detecting CIN II and above is significantly increased while maintaining the same specificity. Women with HPV+/p16+ were at high risk of developing CIN III+ after three years of persistent infection. Data from a large Italian screening study suggested immediate referral for colposcopy in women with HPV 16/18+ in combination with double staining of positive p16 and Ki67 tests. This may reduce the rate of false-positive HPV testing and allow effective identification of patients with HPV who require surgical treatment to prevent CC.

Persistent infection can lead to integration of the HPV genome into the host chromosome, causing cessation of the normal viral life cycle and overexpression of E6 and E7 oncoproteins through methylation of the 5'-C-phosphate-G-3' CpG sites. HPV integration often occurs early in CIN. PCR-based testing of E6/E7 mRNA not only provides a quantification of viral load, but also indicates its transcriptional activity, meaning that E6/E7 mRNA testing has prognostic value. It is a biomarker of significant dysplasia and CC [5]. In 2012, the American Society of Colposcopy and Cervical Pathology (ASCCP) published guidelines for screening tests for CC and precursors of cancer, which introduced a new concept: using a patient's risk of cancer progression and chance of recovery from HPV based on age and HPV subtype (HPV-16, HPV-18 and other high-risk HPV strains) for clinical decision-making when referring for colposcopy and planning follow-up. These recommendations were summarized in algorithms that began with cytology results. In April 2020, the ASCCP published 2019 guidelines for screening tests for CC and cancer precursors of cancer, which require a full shift to risk-based decision making and address the growing evidence that persistent HPV infection is a leading cause for CC developing risk. Colposcopy is now recommended for “any combination of history and current test results that gives a 4 % or greater chance of detecting CIN III or worse.” The biopsy should target any lesion present on the cervix, for example, 2–4 targeted biopsies (i.e., biopsies of tissue with abnormal appearance or acetowhite epithelium) within the squamous-glandular junction improve detection of CIN II or others. Targeted biopsies are 8 to 12 times more likely to detect CIN III and more severe lesions than random biopsies. If there are no lesions or visible squamous metaplasia at colposcopy, random biopsies or biopsy samples at the squamous-glandular junction should be considered for patients at greatest risk of CIN II or worser. When the colposcopy biopsy is abnormal, random biopsies at the squamous-glandular junction from unse-

lected quadrants should be considered, in addition to the recommended 2 to 4 targeted biopsies. The ASCCP does not recommend random biopsy in low-risk patients with a normal colposcopic biopsy and no squamous metaplasia (squamous metaplasia is normal but may be confused with changes in the acetowhite epithelium) [6].

Identified diseases are subject to conservative and surgical treatment methods in the form of ablation, excision and cervical conization, however, the latter can have a negative impact on fertility, the incidence of premature birth, and bacterial infection of the endo- and exocervix. In addition, it was found that women with CIN who underwent surgical treatment were still at risk of CC. Currently, therapeutic measures are carried out differentially depending on the stage of damage to the cervical epithelium and the woman's reproductive plans [7].

The purpose of the study is to identify the frequency of cervical intraepithelial neoplasia among female residents of Yekaterinburg.

MATERIALS AND METHODS

To achieve this purpose, we conducted a retrospective analysis of the report forms of the cervical pathology office in Yekaterinburg Clinical Perinatal Center (ECPC) from January to September 2022.

From March 16, 2022, by order of the Ministry of Health of the Sverdlovsk Region, patients with suspected and diagnosed CIN I–II degrees are subject to observation in the cervical pathology office of the ECPC. Doctors at antenatal clinics in Yekaterinburg have the opportunity to refer the patient to the office to perform colposcopy, cervical biopsy, excision of the transformation zone, cervical conization, polypectomy, hysteroscopy and some other manipulations. Patients with suspected CIN III, CC are subject to routing to the Regional Oncology Dispensary.

After surgical treatment of cervical pathology at the ECPC, patients are subject to dispensary observation at the antenatal clinic at their place of residence.

Statistical processing was conducted using descriptive statistical methods in Excel. Fisher's test was used; differences were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

In the ECPC cervical pathology office for the period from January to September 2022, a total of 255 cases of precancerous cervical diseases and 7 cases of cervical cancer were revealed. Among them, 24 patients with newly diagnosed precancerous pathology and 4 with CC had previously been followed up by a specialist. Primary cervical pathology most often occurred in patients over 40 years old – 93 cases (38 %), least often – in patients under 20 years old – 5 cases (2 %). There were no statistically significant differences in the incidence of CIN in women under 40 years old ($p > 0.05$).

But after 40 years, the prevalence of precancerous diseases is significantly higher than in patients under 20 years of age ($p = 0.017$), from 21 to 25 years ($p = 0.0017$) and from 26 to 30 years ($p = 0.017$). The age distribution of patients with precancerous cervical diseases is presented in Fig. 1.

The largest number of cervical diseases cases was identified in the Verkh-Isetsky district of Yekaterinburg – 68, the fewest cases of cervical pathologies were in the Oktyabrsky district and in the Sverdlovsk region – 3 (Fig. 2). Districts of Yekaterinburg, where the detection of precancerous diseases is low (such as Oktyabrsky and Kirovsky), have a full range of capabilities (cervical pathology office, appropriate equipment and trained personnel) for carrying out therapeutic and diagnostic procedures in women with CIN and patients are sent only in extremely difficult cases. The remaining areas of the city have the opportunity to provide medical care with equal interest and need. According to the routing order, only patients living in the territory of Yekaterinburg are subject to medical care. This explains the small percentage of patients from the Sverdlovsk region in the ECPC cervical pathology office – 1.5 % (3 people), in comparison with the Verkh-Isetsky and Leninsky districts of Yekaterinburg, this figure is significantly lower ($p < 0.0001$).

The Kirovsky and Oktyabrsky districts of the city referred patients to the cervical pathology office significantly less often ($p = 0.01$ and $p < 0.001$, respectively) (compared to the Verkh-Isetsky district) due to the presence of a well-equipped operating room on the basis of these institutions and highly qualified obstetrician-gynecologist.

Among the manipulations performed in the ECPC during this period, the predominant ones were (Fig. 3): colposcopy – 27 % (832), biopsy, excision and cervical conization – 21 % (652), ablative treatment procedures – 20.2 % (619), cervical argon plasma coagulation – 15.5 % (475), ultrasonic cavitation of the vagina – 14.6 % (450); the most rear manipulations were cervical diathermocoagulation – 0.2 % (4), removal of intrauterine devices and condylomas – 0.1 % (2 cases each).

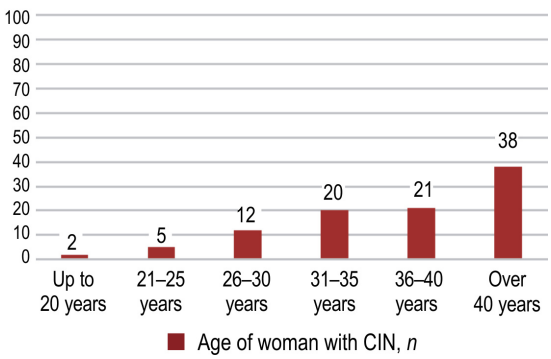

Fig. 1. Distribution of patients in the cervical pathology office by age groups, %

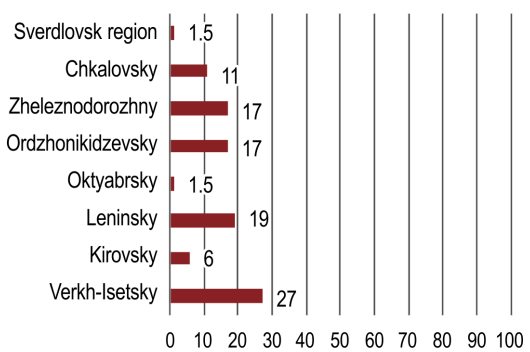

Fig. 2. Distribution of cervical pathologies cases detected for the first time by district of Yekaterinburg for the period from January to September 2022, %

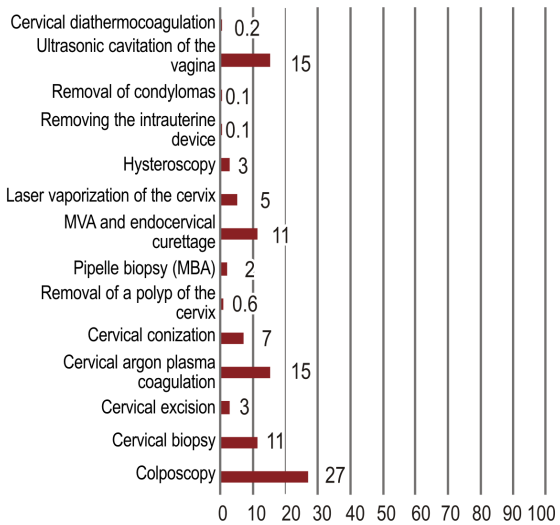

Fig. 3. Manipulations carried out in the ECPC cervical pathology office for the period from January to September 2022, %

By the end of September 2022, 258 women were registered at the cervical pathology office (Fig. 4). The largest dispensary group was patients with CIN II – 43.5 % (112), a slightly smaller group of women with CIN I – 28 % (71), patients with CIN III accounted for 17 % (45). 2.7 % (7) of patients were registered with cervicitis, VIN, CC, 2.3 % of women – with cervical leukoplakia (6). The smallest dispensary groups are patients with cervical ectopia 0.7 % (2) and polyps of the cervix 0.4 % (1).

The study confirmed the literature data on the high percentage of CIN (38 %) in patients over 40 years old [1]. However, it is also noteworthy that there are cases of precancerous cervical diseases in young patients, and even if this is only 2 % of cases (which in absolute terms corresponds to 5 patients), we understand that in the future these women remain at risk for the development of cervical cancer within two years after surgical treatment, they may have an increased rate of miscarriage (in particular, due to the development of isthmic-cervical insufficiency), labor activity anomalies (secondary uterine inertia) [8].

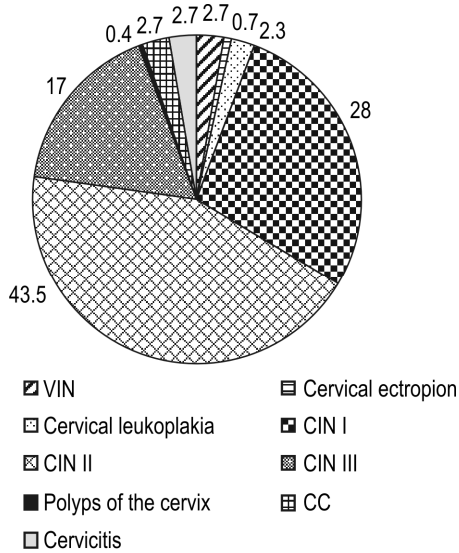

Fig. 4. Distribution of patients with cervical pathology within the dispensary group

The order on the work of antenatal clinics in Yekaterinburg made it possible to centralize patients with CIN in one institution for a full diagnosis and adequate surgical treatment if necessary. All antenatal clinics in the metropolis refer patients to the cervical pathology office according to the order. At the same time, we see that in the city institution (ECPC) patients with CIN began to appear, living in the Sverdlovsk region (1.5 %). Currently, this is only a small part of those living in these settlements, but we understand that in the future it may be necessary to form similar structures with the possibility of centralizing specialized

medical care in offices in the cities of the Sverdlovsk region.

Most often, precancerous diseases of the cervix in residents of Yekaterinburg are detected at stage CIN II (43.5 %), slightly less often at stage CIN I (28 %). CC was detected in 2.7 % (n = 7). Thus, we believe that the main goal of creating a cervical pathology office on the basis of the ECPC in the metropolis has been achieved: the centralization of patients with CIN has been carried out, the identification of precancerous cervical diseases in the early stages for the purpose of timely treatment and prevention of the CC development [9–11].

Conclusions

1. Most often (in 38 % of cases) precancerous cervical diseases are detected in the group of women over 40 years old.

2. All districts of Yekaterinburg refer patients to a specialized cervical pathology office; antenatal clinics in the Verkh-Isetsky and Leninsky districts are especially active.

3. Patients in the cervical pathology office are provided with the full range of therapeutic and diagnostic procedures: biopsy, cervical excision and conization – in 21 % of cases, colposcopy – in 27 %, ablative treatment procedures – in 20.2 %, cervical argon plasma coagulation and ultrasound vaginal cavitation – 15.5 and 14.6 %; the most rear manipulations was cervical diathermocoagulation – 0.2 % (4).

4. The dispensary group of patients of the cervical pathology office is mainly represented by women with CIN II – 43.5 %, with CIN I – 28 % and with CIN III – 17 %.

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HYPERTENSIVE DISORDERS IN PREGNANCY: PROBLEMS OF CLASSIFICATION, DIFFERENTIAL DIAGNOSIS AND MANAGEMENT

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ГИПЕРТЕНЗИВНЫЕ РАССТРОЙСТВА ПРИ БЕРЕМЕННОСТИ: ПРОБЛЕМЫ КЛАССИФИКАЦИИ, ДИФФЕРЕНЦИАЛЬНОЙ ДИАГНОСТИКИ И ТАКТИКИ ВЕДЕНИЯ

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Objective. To study the features of hypertensive disorders formation in pregnancy depending on the period and clinical form on the example of the population of Sverdlovsk region.
Materials and methods. The outcomes of 217 pregnant women with moderate and severe preeclampsia and gestational hypertension were analyzed (41, 74, and 102 cases, respectively).
Results. The severe preeclampsia group had the highest rate of fetal growth restriction (14.6 %), low birth weight -2045 g (1640–2650), preterm delivery (63.4 %), and cesarean delivery (87.8 %). A detailed analysis of 28 cases of gestational hypertension lasting up to 34 weeks was performed. The analysis revealed significant challenges in the differential diagnosis of this pregnancy complication, not only in terms of clinical manifestations, but also in terms of timely diagnosis in general.
Conclusions. The findings of this study spark a debate over the use of severity-based classification in clinical practice, which lessens the doctor's vigilance in milder forms that, however, result in equally se-

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rious complications. This raises the question of whether unified tactical approaches should be used in this pathology.
Keywords. Preeclampsia, hypertensive disorders in pregnancy, gestational hypertension, HELLP syndrome.

Цель. Изучение особенностей формирования гипертензивных расстройств при беременности в зависимости от срока и клинической формы на примере популяции Свердловской области.
Материалы и методы. Проведен анализ исходов у 217 беременных среди следующих клинических форм: умеренной и тяжелой преэклампсии, гестационной артериальной гипертензии, что составило 41, 74 и 102 случая соответственно.
Результаты. В группе с тяжелой преэклампсией наблюдался самый высокий процент реализации задержки роста плода (14,6 %) и дети с более низкой массой тела при рождении – 2045 (1640–2650) г. преждевременных родов (63,4 %) и частоты родоразрешения способом операции кесарева сечения (87,8 %). Проведен детальный разбор 28 случаев из категории «гестационная артериальная гипертензия» с манифестацией до 34 недель, который продемонстрировал большие сложности в подходах дифференциальной диагностики данного осложнения беременности не только согласно клиническим формам, но и своевременной постановки диагноза в целом.
Выводы. По результатам сравнительной характеристики не выявлено различий по большинству перечисленных выше показателей между группами с умеренной преэклампсией и гестационной артериальной гипертензией, что лишний раз доказывает необходимость пересмотра существующей клинической классификации и усовершенствования алгоритмов ведения беременности.
Ключевые слова. Преэклампсия, гипертензивные расстройства при беременности, гестационная артериальная гипертензия, HELLP-синдром.

INTRODUCTION

Preeclampsia (PE) is a pathological condition characterized by multisystem damage to the body of a pregnant woman and fetoplacental complex. The classic presentation of PE occurs in late pregnancy, less often in the postpartum period, and is manifested by elevated blood pressure in previously normotensive patients in combination with or without significant proteinuria. Preeclampsia is the leading cause of maternal mortality, increasing the risks of adverse perinatal complications, and induce long-term consequences in both the woman and child in the future [1–5].

It is customary to distinguish two degrees of PE severity: moderate and severe. Blood pressure (BP) level, the severity of proteinuria, and the presence or absence of clinical and/or laboratory manifestations of multiple-organ failure are used as diagnostic criteria to distinguish between these forms. The latter is a clinical variant of severe preeclampsia in the form of hemolysis, elevated liver enzymes, and low platelets (HELLP) syndrome. According to the literature, the overall prevalence of this pathological condition during pregnancy ranges from 0.2 % to 0.9 %, and as a symptom complex that complements the clinical presentation of preeclampsia, it ranges from 4 % to 24 % [6; 7]. This variability in statistical data is probably due to different classification approaches of hypertensive disorders during pregnancy in different countries and considering (or underestimating) incomplete variants of HELLP syndrome. Moreover, this pathology can progress to eclampsia in about 1 % of pregnant women

with hypertensive disorders. A decrease in the incidence of seizures associated with preeclampsia was noted, especially in developed countries, which can be associated with the use of clinical protocols that indicate basic therapy with the use of magnesium sulfate, which is known as a first-line anticonvulsant drug [8].

Since the introduction of clinical protocols to routine medical practice, the diagnostics of the above conditions has reached a higher level. Clinical recommendations include all generally accepted aspects of pregnancy management, treatment, and patient routing, including diagrams and algorithms, which enables doctors to navigate and make decisions quickly. Currently, during the first trimester of pregnancy, the risk of preeclampsia and other pregnancy complications from the group of "major obstetric syndromes" is assessed using the analysis of risk factors, biochemical indicators of prenatal screening in the first trimester, and Doppler ultrasound of the uterine arteries [9; 10]. If a high risk of preeclampsia is determined, according to modern clinical guidelines, drug prophylaxis with acetylsalicylic acid (ASA) and calcium supplements (in case of low calcium intake by the pregnant woman) are prescribed to pregnant women [9]. These measures have been proven to reduce the risk of developing PE for up to 34 weeks of pregnancy. Moreover, in several cases, the prescription of ASA is ineffective or the high risk of PE is not determined, and its prevention is not prescribed [11]. Therefore, scientific research aimed at developing alternative preventive measures [12] and new ways to predict PE and other hypertensive disorders during pregnancy continues [10; 13; 14].

Most classifications of hypertensive disorders during pregnancy include not only preeclampsia as such and the above-mentioned aspects of its clinical course (eclampsia, HELLP syndrome) but also such nosologies as chronic, preexisting arterial hypertension (AH) and gestational (pregnancy-related) AH which occurred after week 20 of pregnancy [9]. Moreover, these pathological conditions can be challenging to combine, aggravating the clinical manifestations of the main nosology, namely, preeclampsia. In clinical practice, white coat hypertension and transient hypertension caused by the emotional state of the pregnant woman are not rare; their differential diagnostics is of fundamental importance and seems to be a difficult task.

This study aimed to analyze the features of the formation of hypertensive disorders during pregnancy depending on the term and clinical form using the example of the Sverdlovsk region population.

MATERIALS AND METHODS

As part of a study on risk stratification for predicting hypertensive disorders in pregnancy (HDP), 217 patients were identified among 1089 pregnant women who had various forms of HDP, namely, severe and moderate PE, and gestational AH. Outcomes

were assessed retrospectively based on an analysis of the pregnant woman's electronic record of the Regional Obstetric Monitoring of the Sverdlovsk Region. The study received approval from the local ethics committee at the Ural State Medical University of the Russian Ministry of Health.

The working hypothesis regarding HDP implies the following. The earlier HDP occurs, the more severe is the form and the higher the risk of complications and adverse perinatal outcomes [1; 9]. In this regard, the patients were distributed into three groups, according to the available forms: severe preeclampsia (SPE), 41 cases; moderate preeclampsia (MPE), 74 cases, and gestational arterial hypertension (GAH), 102 cases, which accounted for 3.76 %, 6.79 %, and 9.36 %, respectively, of the total number of pregnant women who participated in the study.

Statistical processing of the results obtained was performed using Excel 2016 (Microsoft, USA) and StatTech 3.1.6 (Stattech, Russia).

Quantitative indicators were assessed for compliance with normal distribution using the Kolmogorov–Smirnov test. In case quantitative data were not normally distributed, they were described using the median (*Me*) and lower and upper quartiles (Q_1–Q_3). Categorical data were determined using absolute values and percentages. Comparison of three groups for quantitative indicators, which distribution differed from normal, was performed using the Kruskal–Wallis test, and post hoc comparisons were conducted using Dunn's test with Holm's correction. Comparison of percentages in the analysis of four-field contingency tables was performed using the Pearson chi-square test (χ^2).

Results and discussion

The parameters characterizing the course of pregnancy and perinatal outcomes were analyzed, including the frequency of fetal growth restriction (FGR), preterm delivery (PD), cesarean section (CS), and the average weight of the newborn. The results are presented in Fig. 1.

The presented graph shows that the lowest average newborn weight (*Me* (Q_1–Q_3)) was recorded in the group with severe preeclampsia (2045 g (1640–2650)) and the highest weight in the GAH group (3300 g (2992–3668)), which is an expected result confirmed by a statistically significant difference when comparing three groups ($p < 0.001$).

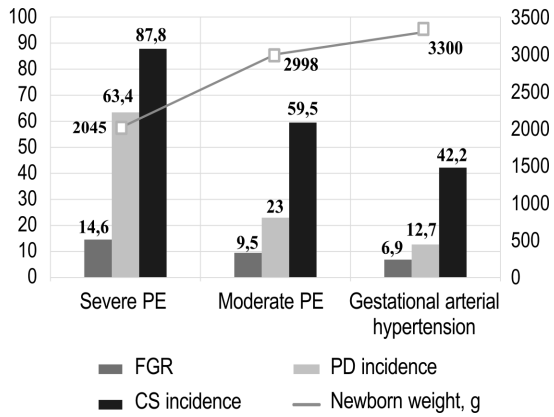

Fig. 1. Characteristics of the assessed indicators in the HDP groups

Notably, the incidence of FGR was highest in pregnant women with SPE, and the lowest FGR was in the group with GAH; however, the differences were not statistically significant. When comparing severe and moderate PE $p = 0.540$ (OR = 0.609; 95 %: 0.190–1.953), MPE and GAH $p = 0.580$ (OR = 0.705; 95 %: 0.236–2.105), SPE and GAH $p = 0.196$ (OR = 0.430; 95 %: 0.135–1.367).

The odds of PD in the MPE group were 5.8 times lower compared to those in the SPE group, which was a statistically significant indicator ($p < 0.001$, OR = 0.172; 95 %: 0.075–0.397), and expectedly lower in the GAH group by 11.8 times ($p < 0.001$, OR = 0.084; 95 %: 0.036–0.199). Despite the fact that the odds of PD in the GAH group were two times lower compared to those in the MPE group, no significant differences were revealed between the groups ($p = 0.075$, OR = 0.490; 95 %: 0.221–1.085).

When assessing the method of delivery, CS ranked first, which frequency in the SPE group was 36 (87.8 %), and a slightly smaller number was noted in the MPE group (44 cases; 59.5 %), whereas in GAH patients, it was 43 (42.2 %). No significant differences were found in the mode of delivery between the last two groups ($p = 0.101$).

For most of the parameters assessed, no significant difference was noted between GAH and MPE. Whether this indicates the difficulties of differential diagnostics and overdiagnosis in obstetrics or, conversely, underestimation of the existing status is unclear. Because proteinuria is not an obligatory component of the preeclampsia symptom complex, differential diagnostics of these two conditions seems impractical, as GAH is naturally included in the concept of "preeclampsia".

Citing as an example the criteria for gestational AH, the authors of the clinical protocol for the management of patients with hypertensive disorders during pregnancy, published in 2020, under the auspices of the American College of Obstetricians and Gynecologists, emphasize its similarity with preeclampsia without complications and do not note significant differences in monitoring such patients. Additionally, it highlights the serious impact of GAH on perinatal outcomes and cites statistics that demonstrate that approximately half of GAH patients will eventually develop proteinuria and other manifestations of organ dysfunction. It should be further noted that patients with GAH not accompanied by proteinuria are more probable to experience changes in clinical and laboratory parameters that characterize HELLP syndrome, and, generally, according to cohort studies, pregnancy more often ends in premature delivery [15; 16].

The International Society for the Study of Hypertension in Pregnancy classification divides preeclampsia into premature, birth before week 37; full-term, delivery after week 37; and postpartum preeclampsia. Among modern classifications, preeclampsia was characterized as early onset (delivery at a term less than 34 weeks) and late

onset (delivery at a term more than 37 weeks). However, how such a classification is applicable in clinical practice and, at the same time, a classification reflecting the timing of HDP manifestation is extremely useful for development of laboratory diagnostic tests, taking into account the different pathogenesis of early and late forms of preeclampsia, remains debatable [17; 18].

The next stage of the study was dividing the patients with HDP according to the period of manifestation, based on which the following data were obtained: 53 cases before week 34, 50 cases before week 37, and 114 cases after week 37, which amounted to 23.3 %, 22 %, and 50.2 %, respectively, among all pregnant women with HDP included in the study. Indicators considering the ranking by forms of HDP are presented in Fig. 2.

Based on the data obtained, the focus of attention of the attending physicians shifted to the group of patients in whom the manifestation of GAH occurred before week 34, which amounted to 28 cases (27.5 %), higher than with moderate PE where there were only 10.8 % of such patients. A detailed study of 28 records of patients with AH manifestations before week 34 and a final diagnosis of GAH yielded the following data. Twelve patients (42.8 %) had an outpatient record of elevated BP at a term earlier than 20 weeks or an indication of a periodic increase in BP before pregnancy. This group of pregnant women should most probably be specified as having pre-pregnancy chronic AH perhaps with superimposed gestational hypertension or preeclampsia; however, such a diagnosis has not been made in these and other cases of HDP. Meanwhile, 47 (21.7 %) of 217 patients had indications of chronic diseases of the cardiovascular system in the form of chronic AH or hypertensive disease.

In 17 (60.7 %) patients, according to the case diaries, the diagnosis changed from 2 to 5 times or was completely absent at the next visit according to the principle of no diagnosis if there was no high BP. The well-known

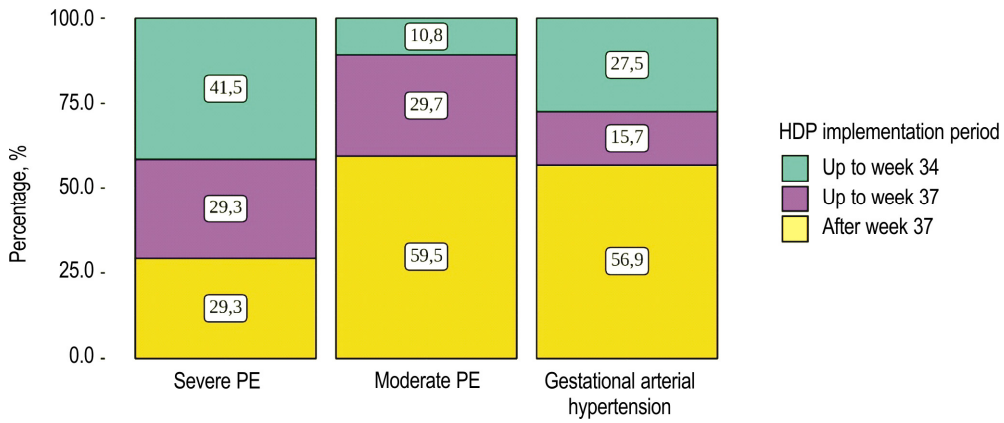

Fig. 2. Ranking of patients according to the form and timing of HDP manifestation

variants included vegetative-vascular dystonia of the hypertensive or mixed type, neurocirculatory dystonia, situational AH, and AH outside of a crisis. These conclusions should be considered incorrect owing to noncompliance with the generally accepted criteria for diagnosing AH during pregnancy, which leads to defects in treatment approach, for example, untimely prescription of hypertensive drugs. In several cases, various types of neurological diagnoses were practiced, which in most countries worldwide are not used at all. Limited appropriate treatment is another problem that arises with delayed diagnostics of preexisting chronic forms of AH, where the question of using combination antihypertensive therapy should often be raised.

In 7 of 28 patients (25 %), in parallel with AH, pregnancy complications, such as FGR and thrombocytopenia, developed; one case of antenatal fetal death was recorded, and episodes of rising systolic BP to 150–160 mmHg and diastolic BP to 90–100 mmHg were noted, which calls into question the final diagnosis of gestational AH.

Almost all the prescriptions outlined in the current clinical guidelines "Preeclampsia. Eclampsia. Edema, Proteinuria and Hypertensive Disorders during Pregnancy, Childbirth and the Postpartum Period" cover two conditions, namely, moderate and severe PE. However, as can be seen in practice and in most studies, the diagnosis of GAH raises the greatest difficulties and various questions, especially regarding differential diagnostics. It seems that in most cases, the conclusion of gestational AH shifts the focus of the doctor's vigilance towards the normal course of pregnancy, rather than towards the development of possible complications.

The existing classifications will be repeatedly reviewed. The American College of Cardiology has revised the criteria for stage 1 AH, in which figures now are 130–139/80–89 mmHg and not 140/90 mmHg [19]. Both international and Russian authors discussed the feasibility of dividing HDP by severity and time of manifestation; such an approach should improve diagnostics and ensure maximum timeliness in treatment [17; 20].

In anticipation of the revision of existing Russian clinical recommendations, the existing algorithms for the management of pregnancy in HDP should be improves. In particular, it is crucial to specify clearly the indications for consultation with a cardiologist, 24-hour BP monitoring, and subsequent collegial discussion of the results obtained, which does not seem difficult in the era of telemedicine. The American College of Cardiology guidelines regarding suspected transient AH or white coat hypertension recommend the use of "out-of-office" BP measurement to confirm the diagnosis and select therapy, with an emphasis on the possibility of telemedicine consultations [19]. However, this approach requires training patients to correct measurement of blood pressure at home; thus, it is proposed to include in the clinical recommendations a universal instruction leaflet for the patient, which

will indicate the criteria for correct BP measurement.

Ranking of patients according to the time of manifestation of HDP is significant, since most of the existing tests that can predict preeclampsia are most sensitive to earlier forms; hence, the risk groups identified as a result of calculating the combined risk in the first trimester should not be neglected.

Conclusions

1. Isolating GAH as an independent form of hypertensive disorders during pregnancy seems inappropriate owing to the lack of pathogenetic differences with preeclampsia.

2. Prognostically, GAH should be considered as preeclampsia with all the ensuing consequences regarding pregnancy management approach and preventive and therapeutic measures.

3. Maternal and perinatal outcomes with moderate preeclampsia and GAH do not differ significantly, indicating another argument in favor of the absence of the need to distinguish between these symptom complexes.

4. Preeclampsia and GAH show comparable tendencies towards worsening clinical manifestations during pregnancy up to the occurrence of multiple-organ lesions, which requires the use of uniform approaches in the formation of this pathology.

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DYNAMICS OF MAIN MARKERS OF PROTEIN METABOLISM AND MICROELEMENTS IN PATIENTS WITH NEW CORONAVIRUS INFECTION

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ДИНАМИКА ОСНОВНЫХ МАРКЕРОВ БЕЛКОВОГО ОБМЕНА И МИКРОЭЛЕМЕНТОВ У ПАЦИЕНТОВ С НОВОЙ КОРОНАВИРУСНОЙ ИНФЕКЦИЕЙ

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Objective. To study the dynamics of the main markers of protein metabolism and microelements in patients with a new coronavirus infection.
Materials and methods. A prospective clinical and laboratory study of the dynamics of the main markers of protein metabolism and microelements was carried out in 62 patients with a new coronavirus infection complicated by the development of pneumonia with a moderate course on the basis of Perm regional clinical hospital of infectious diseases in 2021–2022. On the 1st and 14th days of hospitalization all patients underwent general clinical and biochemical studies. The concentrations of total protein, albumin, transferrin, Fe, Zn and Cu was also determined. Statistic processing of the results was carried out using Microsoft Excel 2000 and "Statistica 10".
Results. A decrease in visceral pool proteins was revealed. It indicated their active consumption and formation of hypermetabolism – hypercatabolism syndrome in a moderate course of the infection. A progressive deficiency of Fe and Zn, correlating with life-threatening complications and deaths was revealed.
Conclusions. The course of a new coronavirus infection contributes to the development of metabolic disorders, and the identified deficiency of macro- and microelements is a predictor of a severe course of the infection.
Keywords. New coronavirus infection, markers of protein metabolism, microelements.

Цель. Исследование динамики основных маркеров белкового обмена и микроэлементов у пациентов при новой коронавирусной инфекции.
Материалы и методы. На базе Краевой клинической инфекционной больницы г. Перми в 2021–2022 гг. проведено проспективное клинико-лабораторное исследование динамики основных маркеров белкового обмена и микроэлементов у 62 пациентов при новой коронавирусной инфекции, осложненной развитием пневмоний со среднетяжелым течением. На 1-й и 14-й дни госпитализации у всех пациентов выполнялись общие клинико-биохимические исследования, а также определение концентрации общего белка, альбумина, трансферрина, Fe, Zn и Cu. Статистическую обработку результатов проводили с использованием Microsoft Excel 2000 и Statistica 10.
Результаты. Зафиксировано снижение белков висцерального пула, указывающее на их активное потребление и формирование синдрома гиперметаболизма – гиперкатаболизма при среднетяжелом течении инфекции. Выявлен прогрессирующий дефицит Fe и Zn, коррелирующий с наличием жизнеугрожающих осложнений и летальными исходами.
Выводы. Таким образом, течение новой коронавирусной инфекции способствует развитию нарушений обмена веществ, а выявленный дефицит макро- и микроэлементов является предиктором тяжелого течения инфекции.
Ключевые слова. Новая коронавирусная инфекция, маркеры белкового обмена, микроэлементы.

INTRODUCTION

Since the beginning of the Autumn season of this year, the incidence of new coronavirus infection (NCVI, COVID-19) has increased in Russia, which has been associated with the continuation of the pandemic that has affected more than 110 countries worldwide[1]. According to official statistics, in Autumn of 2023, more than 600 million individuals suffered this pathology, and lethal outcomes have been reported in 6 million patients. In the Perm region, a seasonal increase in the incidence of NCVI[2] has been recorded. According to the literature, various infectious lesions, changes in the metabolism of macro and microelements, and malnutrition contrib-

[1] Federal Service for Supervision of Consumer Rights Protection and Human Welfare, available at: https://www.rospotrebnadzor.ru/activities/recommendations.

[2] Federal Service for Supervision of Consumer Rights Protection and Human Welfare in the Perm Territory, available at: https://59.ru/text/health/2023/07/28/72544472.

ute to the formation of multiple-organ failure, leading to the most severe form of systemic inflammatory reaction[3] [1–3]. Additionally, several scientific studies noted the significant role of specific pathogens (viruses, bacteria, protozoa) in changes in the concentrations of microelements and vitamins in the dynamics of the disease courses, which contributes to disruption of the immune system and leads to irreversible outcomes [4–6]. Currently, the issues of the COVID-19 pathogenesis and the dynamics of changes in the main markers of protein metabolism and microelements remain insufficiently transparent. Moreover, there are assumptions that studying the concentration of basic proteins and microelements is beneficial in performing qualitative adjustments to changes in metabolic processes, and timely replenishment of energy deficiency will improve the results of therapy of the respiratory system in case of COVID-19[4] [7].

The study aimed to analyze the dynamics of the main markers of protein metabolism and trace elements in patients with a NCVI.

MATERIALS AND METHODS

A prospective clinical and laboratory examination of 492 patients with NCVI, complicated by the development of moderately severe pneumonia, was conducted at a regional clinical infectious diseases hospital in Perm in 2021–2022. The examined group included patients aged 20–75 years with viral pneumonia confirmed by X-ray or CT examination, with laboratory verification of the RNA of the SARS-CoV-2 virus by the molecular biological method (PCR) using the nucleic acid amplification test, and meeting the criteria for moderate NCVI according to the current versions of methodological recommendations of the Russian Ministry of Health. The study excluded patients aged < 20 years, persons hospitalized with decompensation of concomitant chronic diseases, those who received the vaccine, and pregnant women. Subsequently, using a mechanical selection method, a group of 62 people was formed, for whom, in addition to the recommended examination methods[5], the concentrations of total protein, albumin, transferrin, and Fe, Zn, and Cu were determined on hospitalization days 1 and 14.

Statistical processing of the results was performed using Microsoft Excel 2000 and Statistica 10. The values of indicators for qualitative characteristics were presented as % ± m and those for quantitative characteristics as Me (Q_1; Q_3). Significant differences

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[4] Rocco Barazzoni, Stephan CBischoff, Zeljko Krznaric, Matthias Pirlich, and Pierre Singer, Practical Guidance on Nutrition for Persons with SARS-CoV-2 Infection and Other Statements from Experts of the European Association of Clinical Nutrition and Metabolism; approved by the ESPEN Council, available at: https://cardioweb.ru/files/covid19/en/guide_to_nutrition_for_patients_COVID_19.pdf

[5] Ministry of Health of the Russian Federation. Temporary guidelines. Prevention, diagnosis and treatment of new coronavirus infection. Version 17. (12/14/2022). 259.

were assessed using the Mann–Whitney and Chi-square tests; the differences were considered significant at $p < 0.05$. The relationship between the quantities was studied using the pair correlation coefficient r.

Results and discussion

Patients aged 20–73 years (average: 58.6 years [54.7; 61.4]) were examined; there were 63.0 % ± 6.1 % men (39 individuals) and 37.0 % ± 6.1 % women (23 individuals). Hospitalization was performed on days 1–3 of the disease in 19 patients (30.6 % ± 5.9 %), days 4–5 in 35 patients (56.4 % ± 6.3 %), and later than day 6 in 8 patients (13.0 % ± 4.3 %). There were on average 22 (19.5; 24.5) bed days. At the time of hospitalization, 45 patients (72.5 % ± 5.7 %) had concomitant pathology, and 16 patients (25.8 % ± 5.6 %) had combined lesions of various systems. Most chronic lesions were registered in the cardiovascular system (hypertension, coronary heart disease, angina pectoris, varicose veins, $p < 0.05$), as well as chronic pathology of the endocrine, respiratory, urinary, and digestive systems (Fig. 1). The C-reactive protein level expectedly increased from 55.1 (15.3; 85.6) mg/l at the time of hospitalization to 68.9 (52.3; 126.1) mg/l by hospitalization day 14. As a result, the relationship between high concentrations of this protein and disease severity was confirmed ($r = 0.641$; $p < 0.001$), which was characterized by the volume of pulmonary damage and prevalence of inflammatory infiltration. The number of leukocytes, which initially had low levels in week 2 of the disease, increased from $3.5 \cdot 10^9$/l (3.1; 4.3) to $5.0 \cdot 10^9$/l (3.9; 6.4). When assessing body mass index, normal indicators were recorded in 27 patients (43.5 % ± 6.3 %), increased nutrition in 22 cases (35.4 ± 6.1 %), obesity at degrees 1 and 2 in 9 cases (14.6 % ± 4.5 %), and malnutrition in 4 patients (6.5 % ± 3.1 %). Thus, the identified laboratory data met the criteria for moderate severity of COVID-19 at the time of hospitalization. Considering the opinion of Russian authors that risk factors for an unfavorable outcome of NCVI include age over 35 years, a history of chronic diseases, lack of specific prevention, and correction of macronutrient metabolic disorders that does not meet the body's needs [8–10], the next stage of our study was investigating the main indicators of protein metabolism.

In the examined patients, the concentration of total protein at the time of hospitalization was determined below the reference values, whereas albumin and transferrin were recorded within normal limits (Fig. 2). However, by hospitalization day 14, a decrease was noted in the indicators of the high-molecular organic substances we studied, indicating their high consumption by the macroorganism during NCVI and the lack of rapid replenishment, despite the fact that in the hospital, all patients received traditional hospital nutrition. Negative dynamics revealed by hospitalization day 14 indicated the development of hypermetabolism–hypercatabolism syndrome with

the breakdown of tissue proteins and worsening of the course of COVID-19. In week 2 of hospitalization, chronic disease exacerbations were noted, and a relationship was observed between a decrease in the main markers of the protein pool and the complications that occurred ($r = 0.512$; $p = 0.013$). At the end of week 2 and beginning of week 3, due to the progression of multiple-organ dysfunction syndrome against NCVI with a significant decrease in macroelements, lethal outcomes were recorded in four patients (3.9 % ± 2.5 %).

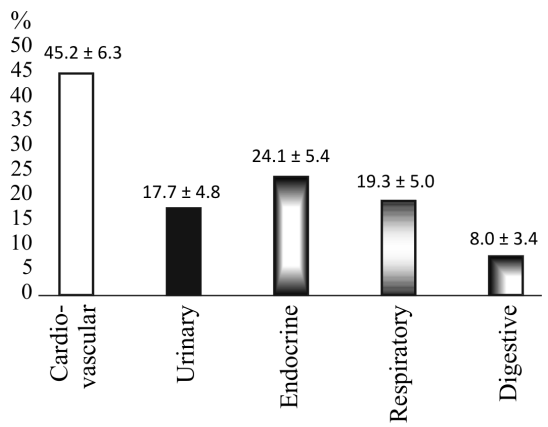

Fig. 1. Concomitant system lesions in patients with COVID-19, %

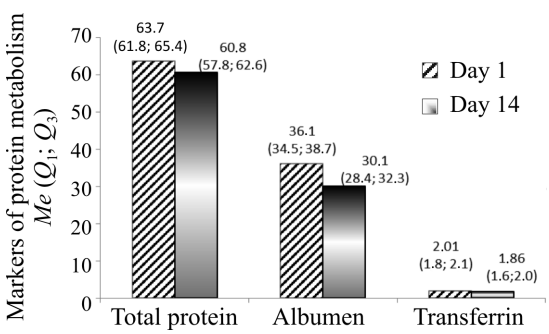

Fig. 2. Dynamics of levels of protein metabolism markers on the days 1 and 14 of hospitalization Me (Q_1; Q_3)

In the Russian literature, studies on the developing deficiency of visceral pool proteins in HIV-positive patients and those in intensive care units were found [2, 10]. Notably, a recognized risk group for a decrease in muscle mass and an increase in a systemic inflammatory response with a predominance of macronutrient breakdown are patients in intensive care units with respiratory failure of various etiologies [11, 12] and in patients hospitalized with moderate NCVI; such studies have not been previously conducted.

The next stage of our study was evaluating the dynamics of the content of microelements during NCVI. On hospitalization day 1, Fe indicators, according to WHO[6], were determined within acceptable limits (Fig. 3); erythrocytes at this time were $3.6 \cdot 10^{12}$/l (3.4; 3.6) and hemoglobin was 113 (110; 116) g/l, which corresponded to a deficiency of these indicators in males and lower limits of acceptable values in females. By day 14 of the disease, iron deficiency increased ($p < 0.05$), and its relationship with the negative dynamics of transferrin was revealed ($r = 0.801$; $p = 0.000$).

A crucial microelement in our body is zinc, which is used in various biological cycles of macroorganisms. Its deficiency can

[6] World Health Organization. Maternal Health and Safe Motherhood Program World Health Organization. Nutrition Program (1992). The prevalence of anemia in women: a tabulation of available information, 2nd ed. World Health Organization, available at: https://iris.who.int/bitstream/handle/10665/59705/WHO_MSM_92.5.pdf?sequence=1

lead to increased production of interleukin-6 proteins, decreased local immunity in the lung tissue, and a "cytokine storm" [13, 14]. In the examined patients, already on hospitalization day 1, a reduced concentration of this microelement was determined with <10.4 μmol/l (Fig. 3); by day 14, a progressive decrease in its values was revealed ($p < 0.05$). In patients with zinc levels < 9.9 μmol/l, the course of COVID-19 was aggravated by the development of multiple-organ failure and decompensation of comorbid diseases, and therefore, a relationship was registered between Zn deficiency and infection severity ($r = 0.603$; $p = 0.008$). In the group of patients examined, lethal outcomes were registered in seven patients (11.2 % ± 4.0 %), with Zn values of 8.0 (7.5; 8.1) μmol/l and Fe values of 7.4 (7.0; 7.6) μmol/l, which enabled to identify a relationship between low levels of microelements and lethal outcomes in NCVI patients ($r = 0.596$; $p = 0.004$).

When studying the concentration of copper during NCVI, we did not obtain significant differences on hospitalization days 1 and 14 ($p > 0.05$); its values remained within the reference range (Fig. 3). Several authors indicated that with an unfavorable course of COVID-19, the occurrence of complications and *exitus letalis* and Cu levels above normal were recorded, and this should be regarded as an independent parameter of the severity of NCVI [15]. However, in this study, the above statement could not be confirmed.

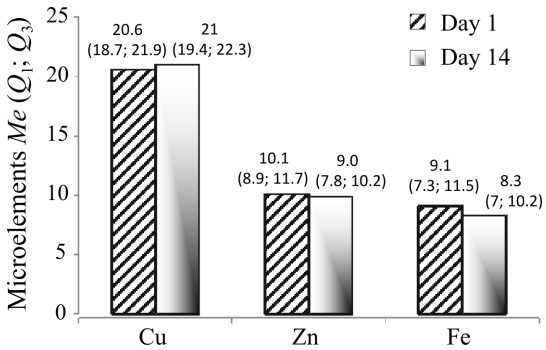

Fig. 3. Dynamics of microelements concentration on hospitalization days 1 and 14, Me (Q_1; Q_3)

Thus, in the dynamics of the disease, decreased Fe and Zn concentrations and macroelements were noted, which indicated the influence of the infectious agent on the development of metabolic disorders in patients with moderate NCVI.

Conclusions

1. In patients with moderate COVID-19, the progression of the deficiency of the main markers of the protein pool and vital microelements is determined over time.

2. Macro and microelement deficiency is a predictor of severe COVID-19.

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PREDICTORS OF CHANGES IN THE COURSE OF BREAST CANCER: THE RESULTS OF LONGITUDINAL STUDY

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ПРЕДИКТОРЫ ИЗМЕНЕНИЯ ТЕЧЕНИЯ РАКА МОЛОЧНОЙ ЖЕЛЕЗЫ: РЕЗУЛЬТАТЫ ЛОНГИТЮДНОГО ИССЛЕДОВАНИЯ

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Objective. To investigate the predictors of changes in the course of breast cancer in a longitudinal study.
Materials and methods. Women with the diagnosis of breast cancer underwent psychological diagnostics at the stage of diagnosis (*n*=201 at the first section of the longitudinal study, *n*=149 at the second section, *n*=94 at the third section, *n*=81 at the fourth section five years after the diagnosis). Methods of research: scale

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of basic beliefs, life orientations test, questionnaire “Ways of coping behavior”, test of vitality, questionnaire of subjective control, personal helplessness questionnaire, quality of life questionnaire.
Results. Predictors of changes in the course of cancer are the basic belief “I-Image”, the level of vitality, the locus of control in relation to health, the course and stage of the disease.
Conclusions. While conducting the study, it was found out that the course of the disease is associated with medical and psychological indicators. The data obtained as a result of the study are necessary for solving practical problems of increasing life expectancy and providing psychological care to women with breast cancer who are a socially vulnerable category of the population of the Russian Federation.
Keywords. Medical psychology; breast cancer; oncopsychology; course of the disease; predictors of the course of the disease; longitudinal study.

Цель. Изучить предикторы изменения течения рака молочной железы в ходе лонгитюдного исследования.
Материалы и методы. Психологическую диагностику прошли женщины с диагнозом рака молочной железы на этапе постановки диагноза ($n = 201$ при первом срезе лонгитюдного исследования, $n = 149$ при втором срезе, $n = 94$ на третьем срезе, $n = 81$ на четвертом срезе по прошествии пяти лет с момента постановки диагноза). Методики исследования: шкала базисных убеждений, тест жизненной ориентаций, опросник «Способы совладающего поведения», тест жизнестойкости, тест-опросник субъективного контроля, опросник личностной беспомощности, опросник качества жизни.
Результаты. Предикторами изменения течения онкологического заболевания выступают базисное убеждение «Образ “Я”», уровень жизнестойкости, показатель локуса контроля в отношении здоровья, течение и стадия заболевания.
Выводы. В результате проведенного исследования обнаружено, что динамика течения болезни связана с медицинскими и психологическими показателями. Полученные данные являются необходимыми для решения практических задач увеличения продолжительности жизни и оказания психологической помощи социально уязвимой категории населения РФ – женщинам с раком молочной железы.
Ключевые слова. Медицинская психология, рак молочной железы, онкопсихология, течение болезни, предикторы течения болезни, лонгитюдное исследование.

Introduction

Breast cancer is the most common cancer among women worldwide, with 2.3 million women diagnosed with breast cancer in 2020. Several factors increase the risk of breast cancer, including older age, obesity, family history of breast cancer, postmenopausal hormone therapy, history of radiation exposure, smoking, and alcohol consumption. Family history increases the risk of breast cancer; however, most women with this diagnosis have no known family history of the disease. The absence of a known family history does not automatically indicate that a woman is at less risk. Risk factors for poor outcome in breast cancer include the size of the primary tumor, histological grade, hormone receptor status, and the presence of regional lymph nodes and distant metastases [1]. Other crucial risk factors for an unfavorable course of cancer include obesity, smoking, alcohol consumption, social factors (e.g., marital status, standard of living, access to medical care), and certain psychological variables [1].

An integrated approach to breast cancer treatment involves linking the aspects of other sciences, including psychology, to medical research. The relationship between the psychological char-

acteristics of patients with breast cancer and disease course and survival has been confirmed in several studies. Cerezo et al. emphasized that "personality traits may influence the psychological adjustment and subjective well-being of breast cancer survivors" [2].

Increased survival is associated with depression [3], role functioning [4], social support [4–7], extraversion [8], participation in religious/non-religious groups [9], having hobbies [10], and having children and a spouse [10], whereas decreased survival with stressful events [11], anxiety/stress [12], hopelessness [12], and depression [7; 13–16]. A meta-analysis by Kim et al. showed a significant effect of depression and/or anxiety on cancer mortality [1].

Presently, the study of the relationship between medical, psychosocial factors, and survival is an urgent problem. However, the results of studying psychological, social, and sociodemographic variables affecting survival and disease outcome do not coincide in different studies.

Therefore, *this study aimed to* examine predictors of change in the course of breast cancer in a longitudinal study.

Verification of predictors of changes in the course of cancer contributes to better understanding of the needs of individual patients and is beneficial for providing appropriate psychological assistance. Investigating predictors of change in breast cancer course over a 5-year period may help in identifying patients at risk for poor disease outcomes.

MATERIALS AND METHODS

A study of the relationship between the psychological characteristics of patients with lung cancer with early diagnosis and subsequent disease course was performed at the Chelyabinsk Regional Clinical Center of Oncology and Nuclear Medicine (Chelyabinsk, Russia). This study assumes the use of a longitudinal method, which can be used to solve the significant problem of the dynamics of the course of the disease in connection with the individual characteristics of the patient with malignant neoplasms. The longitudinal nature of this study enables to note changes in the course of the disease associated with the sociodemographic, medical, and psychological characteristics of women with breast cancer. The longer the period of longitudinal research, the greater the probability and reliability of assessing the effects of the studied predictors of changes in the disease course of women with breast cancer.

During the entire period of the study, considerable unique empirical data were collected and processed. The study involved women aged 45–78 years diagnosed with breast cancer (n = 201 at section 1 of the longitudinal study, n = 149 at section 2, n = 94 at section 3, and n = 81 at section 4, 5 years after diagnosis establishment). When studying psychological predictors of changes in the course of breast cancer, data from section 4 of a longitudinal study were used. Initially, a large sample size of the longitudinal study was planned (201 respon-

dents) owing to the predicted decrease in the number of study participants in subsequent sections due to possible changes in life circumstances (e.g., change of place of residence, etc.), disease outcome (death), and changes in attitude towards treatment (e.g., refusal of any stage of antitumor treatment), and/or research (i.e., refusal of psychodiagnostics). In addition to psychological diagnostics, the sample collected data on the course of the disease over a 5-year period. Then, to study predictors of changes in the course of breast cancer, the sample of respondents was combined into three groups.

1) Patients whose disease course remained unchanged throughout the study (n = 35), including women with stable remission

2) Patients whose disease course changed from unfavorable (generalization, tumor progression) to favorable (remission, stabilization followed by remission) (n = 25)

3) Patients whose disease course changed from favorable (remission) to unfavorable (generalization, relapse, progression, diagnosis of a second concomitant cancer, death due to cancer) (n = 21)

We considered predictors of changes in the course of cancer, namely, disease stage, extent of surgical intervention (i.e., sectoral resection or mastectomy), sociodemographic indicators (i.e., marital status, work status, level of education), behavioral characteristics, and cognitive parameters (i.e., vitality, basic beliefs, locus of control, life orientation), as indicators of personal helplessness/independence.

This study included women with luminal breast cancer who underwent radical surgery. Patients with advanced unresectable breast cancer were excluded.

The study was conducted using standardized authoritative questionnaires that had undergone preliminary psychometric preparation, namely, Janoff-Bulman's Basic Beliefs Scale (adapted by Padun and Kotelnikova), Scheier and Carver's Life Orientation Test (adapted by Tsiring and Evnina), Lazarus' "Methods of coping behavior" questionnaire (adapted by Kryukova, Zamyshlyaeva, and Kuftyak), Maddi's Resilience test (adapted by Leontyev and Rasskazova), Rotter's subjective control test questionnaire (adapted by Bazhin, Golynkina, and Etkind), Tsiring and Stepanenko's questionnaire of personal helplessness, and quality of life questionnaire (SF-36).

The methods of mathematical statistics were methods of descriptive statistics, discriminant analysis (method of step-by-step selection of predictors based on the value of ʎ (Wilks' lambda)). Data on the course of the disease and its changes and the stage of diagnosing breast cancer were obtained from patient medical records.

The study was approved by the Bioethics Committee of the Tomsk National Research State University (Tomsk, Russia) (no. 5, dated February 11, 2021). Patients were informed of the aims and objectives of the study and signed written informed consent for participation.

RESULTS AND DISCUSSION

Discriminant analysis was used to determine the set of predictors associated with changes in the course of breast cancer, which resulted in a list of variables classifying the sample of respondents with different courses of cancer over 5 years. The predictors were methods of coping behavior, cognitive characteristics, systemic personal characteristics (personal helplessness/independence), and sociodemographic and medical parameters, and the grouping factor was the subject's assignment to a group depending on whether the course of the disease had changed or not.

Discriminant analysis results revealed that 92.1 % of the original grouped cases were classified correctly. Table 1 presents the coefficient ϧ (Wilks' lambda), *F*-criterion, and significance level, characterizing significant differences for each of the studied variables for groups of patients with different disease courses.

Moreover, the results showed with a high degree of significance that belonging to groups of subjects, depending on changes in the course of the disease over 5 years, is determined by indicators of resilience, the pronouncement of the basic belief "self-image", internality in relation to health, as well as the course and stage of the disease.

According to the data obtained, the sociodemographic variables considered in this study were not predictors influencing changes in the course of cancer. The characteristics used as discriminant variables (Table 1) were used as variables, and the differences in which between groups of women with different disease courses over 5 years were significant.

Table 2 presents the values of the discriminant functions.

Table 1

Discriminant analysis results: Wilks' lambda coefficients, *F*-test values, and significance level

Parameter	Wilks' lambda	*F*	*P*
Disease course	0.234	44.151	< 0.001
Basic belief "self-image"	0.105	17.368	< 0.001
Resilience	0.07	16.714	< 0.001
Disease stage	0.026	18.968	< 0.001
Internality in the field of health	0.017	20.077	< 0.001

Table 2

Discriminant analysis results: basic statistics of the canonical discriminant function

Function	Personal value	Proportion of explained variance, %	Wilks' lambda	Chi-square	*p*
1	16.959	88.1	0.017	97.93	< 0.001
2	12.295	81.9	0.303	88.61	< 0.001

Research results showed that the presented discriminant functions are informative and explain 88.1 % and 81.9 % of the variance. Considering the value of ϰ = 0.017 and ϰ = 0.303 with statistical significance $p < 0.001$, we conclude that the set of discriminant variables has good discriminative ability. Thus, changes in the course of the disease in women with breast cancer depend on the level of resilience, self-image, internal locus of control regarding health, and stage and course of the disease.

Furthermore, the study results revealed that women with stable remission (5 years) have a more pronounced belief in their own significance, are convinced that they are worthy of love and respect compared to patients whose disease course changed during the study ($M_1 = 31$; $M_2 = 20$; $M_3 = 29.5$ [hereinafter, M_1 is the average value of the indicator for patients whose disease course has not changed, M_2 is the average value of the indicator for patients whose disease course has changed from favorable to unfavorable, and M_3 is the average value of the indicator for patients whose disease course has changed from unfavorable to favorable]). Women with breast cancer, whose disease course changed from favorable (remission) to unfavorable (generalization, relapse, progression, diagnosis of a second concomitant cancer, death due to cancer) during a longitudinal study, are less likely to consider themselves attractive and interesting, have low opinion about themselves, and pay attention primarily to their own shortcomings, and not to their merits. Nevertheless, this group of patients has high resilience indicators ($M_1 = 80.48$; $M_2 = 81$; $M_3 = 65.25$). Regarding resilience, attention is drawn to the fact that patients whose disease course has changed from unfavorable to favorable have a low degree of resilience compared to those whose disease course has not changed or has changed from favorable to unfavorable. Women who have coped with unfavorable disease course have a low level of resilience. According to Leontiev, "resilience is a trait characterized by the degree to which a person overcomes given circumstances, and ultimately by the degree to which a person overcomes himself" [17]. However, the present study found conflicting findings regarding resilience, which requires further consideration. Most likely, in combination with other psychological characteristics, particularly with a positive self-image and externality in relation to health, a low level of resilience enables women to trust specialists and follow recommendations while maintaining a positive image of themselves. These features appear to protect against psychological maladaptation, helping patients cope more effectively with the cancer experience.

Locus of control is another predictor of changes in the course of breast cancer ($M_1 = 1.56$; $M_2 = 12$; $M_3 = 3.5$). According to the data obtained, internality in relation to health was expressed in patients with a change in the course of the disease from favorable to unfavorable compared to those whose course of the disease has not changed or has changed from unfavorable

Table 3

Results of discriminant analysis: coefficients of the canonical discriminant function

Function	Self-image	Internality in relation to health	Resilience	Disease stage	Disease course
1	−2.968	0.668	2.042	0.987	1.802
2	−1.803	0.441	1.605	0.594	1.292

to favorable. Women with breast cancer, characterized by an unfavorable course of the disease by the year 5 of the study, tend to rely on themselves in matters of health, consider themselves responsible for the state of their health, usually find the causes of the disease in themselves, and believe that recovery largely depends from their own actions. The listed features, combined with negative self-image and high level of resilience, are predictors of changes in the course of breast cancer from favorable to unfavorable.

Table 3 presents the coefficients of the standardized canonical discriminant function, which characterize the contribution of each variable to the value of the discriminant function, considering the influence of other variables.

The basic belief of self-image, resilience, and the course and stage of the disease greatly contribute to the value of the discriminant function that separates women depending on the change in disease course. Thus, in addition to psychological predictors of changes in the course of breast cancer, the set of discriminant variables that determine belonging to groups of subjects depending on changes in the disease course includes medical parameters of the stage and course of the disease. Patients whose disease course has changed from favorable to unfavorable are at stages III and IV of the disease and are characterized by an unfavorable course of the disease (mainly generalization and progression of the disease) when compared with data from patients whose disease course has not changed or changed to favorable during the longitudinal study.

CONCLUSIONS

The study of predictors of changes in the course of breast cancer is longitudinal, which shows the dynamics of the disease course associated with subjective psychological factors and the individual characteristics of the sick person over a 5-year period. The longer the period of longitudinal research, the greater the probability and reliability of assessing the effects of the studied predictors during the disease course of women with breast cancer.

The study results established that the dynamics of the disease are associated with medical and psychological indicators. The stage and course of the disease, basic belief about the significance and value of own "self", and level of resilience and locus of

control in relation to health are significant predictors of changes in the course of breast cancer.

The data obtained are crucial for solving problems of increasing life expectancy and providing psychological assistance to a socially vulnerable category of the population of Russia, namely, women with breast cancer. Understanding the mechanisms underlying psychological factors in survival is key to helping patients. The results can serve as basis for developing a system of psychological support for patients with malignant neoplasms at various stages of diagnostics and treatment.

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ASSOCIATION OF POLYMORPHIC VARIANTS OF HHIP, ADRB2 AND IL-33 GENES WITH CLINICAL MANIFESTATIONS OF BRONCHIAL ASTHMA IN CHILDREN

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АССОЦИАЦИЯ ПОЛИМОРФНЫХ ВАРИАНТОВ ГЕНОВ HHIP, ADRB2 И IL-33 С КЛИНИЧЕСКИМИ ПРОЯВЛЕНИЯМИ БРОНХИАЛЬНОЙ АСТМЫ У ДЕТЕЙ

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Objective. To study the association of polymorphic variants HHIP, ADRB2 and IL-33 genes with phenotypes of clinical course of bronchial asthma in children and effective treatment.
Materials and methods. 90 patients aged from 5 to 17 with the diagnosis of bronchial asthma were included in the investigation. Diagnostic procedures were carried out in all the patients. They included the study of genetic polymorphism of HHIP, ADRB2 and IL-33 genes to establish the association with the clinical phenotypes, findings of laboratory and instrumental study determining the course of bronchial asthma and the degree of its control.
Results. The study of polymorphism of HHIP, ADRB2 and IL-33 genes in children with bronchial asthma with different phenotypes of the disease revealed the association of genetic polymorphism with the severity of course of the disease as well as concomitant diseases. It was determined that allele T of genetic variant rs12504628 (T>C) of HHIP gene reduces the risk of a severe course of BA. Its protective role in the development of drug allergy was also proved. Genotype AA of ADRB2 gene is associated with reduced risks of the development of congenital defects of the tracheobronchial tree in BA. Polymorphic variants in the 4th and 6th exon of IL-33 gene are more frequently associated with moderate and severe course of asthma and base substitution in the 4th and 6th exon are associated with the severe course.
Conclusions. Associations of polymorphic variants of HHIP, ADRB2 and IL-33 genes with clinical manifestations of BA in children are determined in this study. They can be considered in a personalized monitoring of the patients and can help to control the disease totally.
Keywords. Genetic polymorphism, bronchial asthma, children, severity of asthma, level of control.

Цель. Изучение ассоциации полиморфных вариантов генов HHIP, ADRB2 и IL-33 с фенотипами клинического течения бронхиальной астмы (БА) у детей и эффективностью терапии заболевания.
Материалы и методы. В исследование включены 90 пациентов в возрасте от 5 до 17 лет с установленным диагнозом бронхиальной астмы. Всем пациентам были проведены диагностические процедуры, включающие исследование генетического полиморфизма генов HHIP, ADRB2 и IL-33 для установления связи с клиническими фенотипами, показателями лабораторных, инструментальных исследований, определяющими течение бронхиальной астмы и степень контроля заболевания.
Результаты. Проведенное исследование полиморфизмов генов HHIP, ADRB2 и IL-33 у детей, страдающих БА, с разными фенотипами заболевания выявило ассоциацию между полиморфизмами генов и тяжестью заболевания, а также с сопутствующими заболеваниями. Установлено, что аллель Т генетического варианта rs12504628 (Т > С) гена HHIP снижает риск реализации тяжелой БА, а также доказана его протективная роль в отношении реализации лекарственной аллергии. Генотипа АА гена ADRB2 ассоциирован со снижением риска реализации врожденных пороков развития трахеобронхиального дерева на фоне БА. Полиморфные варианты в 4-м и 6-м экзонах гена IL-33 чаще сочетаются со среднетяжелой и тяжелой астмой, а замены нуклеотидов в экзонах 4 и 6 ассоциированы с тяжелым течением БА.

Выводы. В данном исследовании установлены ассоциации полиморфных вариантов генов HHIP, ADRB2 и IL-33 с клиническими проявлениями бронхиальной астмы у детей, которые могут учитываться при персонализированном наблюдении за этими пациентами, и помочь в достижении полного контроля над заболеванием.
Ключевые слова. Полиморфизм генов, бронхиальная астма, дети степень тяжести астмы, уровень контроля.

INTRODUCTION

Bronchial asthma (BA) is a crucial problem of modern theoretical and practical medicine. The Global Asthma Network reported that approximately 348 million people currently suffer from BA, and at least 14 % of them are children[1]. In 2019, there were estimated 262 million people with BA and 461,000 deaths from the disease [1].

BA is a heterogeneous disease involving chronic inflammation of the airways and characterized by reversible broncho-obstructive syndrome, repeated episodes of wheezing, dyspnea, chest congestion, and cough. Its symptoms vary in time and intensity [2; 3]. BA treatment is mainly aimed at achieving and maintaining optimal control of the disease and preventing exacerbations [4].

Despite the wide availability of inhaled glucocorticosteroids (IGC) and standardized recommendations for asthma treatment, disease control remains suboptimal in most children. More than 50 % of pediatric patients with BA experience at least one exacerbation each year, including those with mild asthma. In Russia, the problem of asthma control is critical, because only 23 % of patients achieve complete control of the disease. The causes of insufficient control of BA include low adherence to therapy (43 %), lack of elimination of triggers (29 %), presence of concomitant diseases (15 %), smoking (15 %), and others [5].

Single-nucleotide substitutions in the genome can determine the influence of genetic polymorphism on the disease phenotype and predict differences in clinical manifestations of the disease, including symptom control.

A characteristic aspect of molecular medicine as a science based on data on the molecular structure of the human genome is its focus on correcting the pathological process in every individual, considering his unique genetic characteristics. Another feature is a preventive focus, when information about the genome obtained long before the obvious manifestations of the disease can prevent its development. Genetic predisposition can manifest through interaction with environmental factors, which forms a pathological phenotype. A common method for studying the contribution of genetic mechanisms to the development of BA is the search for associations of the disease and its phenotypes with polymorphic markers of candidate genes.

Over the past decade, genetic studies have identified several candidate genes de-

[1] Global Initiative for Asthma. Global Strategy for Asthma Management and Prevention, 2020, available at: https://ginasthma.org

termining susceptibility to BA. However, the results are often contradictory, confirming the need for further study of BA associations with polymorphic markers of candidate genes.

Some polymorphic candidate gene markers may lead to airway obstruction due to loss of lung elasticity, whereas others contribute to the formation of chronic inflammation resulting in airway obstruction or poor response to drugs such as β_2-agonists or IGC [6].

Genetic aspects of BA control in pediatric patients continue to be studied. In particular, polymorphism of the β_2-adrenergic receptor gene (*ADRB2* gene) is associated with the therapeutic response of patients to bronchodilators, β_2-agonists. Stimulation of β_2-adrenergic receptors leads to bronchodilation and improvement of bronchial conductance, affects the functioning of T cells and eosinophilic inflammation, and causes a decrease in the secretion of proinflammatory mediators from mast cells [7].

The product of the Hedgehog-interacting protein (*HHIP*) gene is an evolutionarily conserved signaling protein that is significant in various processes. There is evidence of an association of the rs1828591 single-nucleotide polymorphism of the *HHIP* gene with a predisposition to the development of bronchial obstruction [8]. The association of single-nucleotide polymorphism rs1512288 of the *HHIP* gene with bronchial obstruction reversibility is indicated, whereas such association has not been revealed with bronchial hyperreactivity [9].

Among the BA candidate genes, the cytokine alarmins are highlighted, which play a key role at all stages of allergic reactions. As regards molecular genetic methods, the gene for the cytokine alarmin interleukin (IL)-33 is promising for research. IL-33 is one of the central signaling molecules of immune responses in BA [10]. Its role has been confirmed in the pathogenesis of BA in children [11; 12]. IL-33 levels have been found to be elevated in sputum and bronchial biopsies of patients with asthma [13]. IL-33 is a tissue-derived cytokine that induces and enhances eosinophilic inflammation and has become a new target for the treatment of asthma and allergic diseases [14].

The binding of IL-33 to its receptor (ST2) enhances the expression of several proinflammatory mediators (IL-5, IL-4, and IL-13) and affects Th2 cell-mediated eosinophilic airway inflammation. Increased IL-33 was associated with the presence of the rs1342326 allele of the *IL-33* gene. The *IL-33* gene polymorphism rs1342326 was associated with a lower risk of asthma in children in Tunisia and higher cytokine IL-33 expression [15]. Single-nucleotide polymorphism rs992969 of the *IL-33* gene was found to be associated with blood eosinophil levels, asthma, and eosinophilic asthma. The rs4008366 polymorphism of the *IL-33* gene showed a weak association with eosinophilic asthma [16].

Using whole-genome sequencing, a rare variant of the *IL-33* gene (NM_001199640: exon7: c.487-1G > C (rs146597587-C), allele frequency = 0.65 %) was discovered in an Ice-

landic population, which disrupts the canonical acceptor splice site before the last coding exon of the gene [17]. This variant occurs at low frequency in European populations and is associated with lower eosinophil counts and reduced risk of asthma in Europeans (*OR* = 0.47, 95 %). In heterozygotes, the overall IL-33 mRNA expression is approximately 40 % lower than in noncarriers. This polymorphism results in a shortened form of the IL-33 protein. The shortened version does not form the IL-33R/ST2 complex and does not activate ST2-expressing cells. These data demonstrate that rs146597587-C is a loss-of-function cytokine mutation [17].

Despite several presented studies of polymorphisms of the *HHIP, ADRB2*, and *IL-33* genes in BA, the significance of the association of polymorphisms of the above-mentioned genes with the clinical course of BA in pediatric patients remains unclear. Hence, there is a need to improve a comprehensive assessment of the degree of BA control and determine the influence of clinical, laboratory, functional, and genetic characteristics on it.

This study aimed to analyze the association between the polymorphic variants of the *HHIP, ADRB2*, and *IL-33* genes and clinical phenotypes of BA in children and efficiency of the disease therapy.

MATERIALS AND METHODS

A single-center cohort study was conducted on 90 patients aged 5–17 years diagnosed with bronchial asthma of varying degrees of severity and control between November 2019 and March 2021. The diagnosis of BA was made based on current clinical guidelines[2]. The study was conducted at the Regional Children's Clinical Hospital of Perm and polyclinics of the Perm region within a scientific grant from the Russian Center for Scientific Information, formerly the Russian Foundation for Basic Research.

Inclusion criteria were children aged 5–17 years with an established BA diagnosis and signed informed consent.

Exclusion criteria were any acute respiratory infections during the examination period and age <5 years (owing to the impossibility of spirography in this age group).

The clinical condition and external respiratory function of all participants were examined. Subsequently, a set of diagnostic procedures was implemented, including the study of genetic polymorphism of the *HHIP, ADRB2*, and *IL-33* genes to establish an association between clinical phenotypes, indicators of laboratory and instrumental studies that determine the course of bronchial asthma, and the degree of disease control. The medical history of all patients was obtained, including allergic history, general blood test, rhinocytogram, the level of general and specific IgE, and immunogram as indicated. Spirography, pulse oximetry, chest X-ray, and peak expiratory flow rate (PEFR) test were performed. Based on clinical data and indicators of external respiratory function, exacerbation severity was determined according to clinical recommen-

[2] Bronchial asthma: clinical guidelines. 2021, available at: https://cr.minzdrav.gov.ru/recommend/359_2.

dations. The severity of BA exacerbations was determined according to the clinical criteria of clinical symptoms, PEFR, respiratory rate, pulse rate, frequency of use of emergency medications, and night awakenings. The BA control level was determined according to the Asthma Control Test, C-ACT, and Composite Asthma Severity Index.

The material for the molecular genetic study was DNA isolated from dried capillary blood spots in 90 children. A study was conducted on the frequencies of alleles and genotypes of polymorphic gene loci rs12551256-A and rs146597587-G of the *IL-33* gene in 70 children and rs12504628 of the *HHIP* gene and ARG16GLY rs1042713 of the *ADRB2* gene in 90 patients with BA, considering the severity and control of the disease. To identify mutant gene alleles, the polymerase chain reaction method was used. In pediatric patients with severe BA, and in children with poorly controlled/uncontrolled asthma ($n = 26$), the entire coding sequence of the *IL-33* gene, located on the ninth chromosome in the 9p24.1 region, was additionally sequenced (search for mutations in nine exons).

Cases were selected using the continuous sampling method, and the sample size was determined using a specialized equation for an unknown general population size:

$$n = t^2 \cdot p \cdot q / \Delta^2,$$

where n is the sample size; t, a coefficient depending on the confidence level chosen by the researcher; p, the proportion of respondents with the presence of the studied characteristic; $q = 1–p$, the proportion of respondents who do not have the studied characteristic; and Δ, the maximum sampling error.

Statistical processing of the results was performed using statistical software packages Microsoft Excel 2010.

The hypothesis about the normal distribution of the studied indicators was tested using the Shapiro–Wilk test. To extend the conclusions to general populations (95 % confidence), some indicators were presented as $M \pm 2m$ ($\% \pm 2m$).

When comparing dependent and independent groups of signs characterizing the level of control and/or severity of the disease (depending on the type of distribution of the analyzed indicators), a two-sample Student's t-test or the Mann – Whitney U test was used. In analysis of contingency tables of characteristics, Pearson's χ^2 criterion was used. The relationship between variables was studied using the correlation analysis. The significance level for the hypotheses being tested was set to 0.05.

The expected distribution of genotypes was estimated using the Hardy – Weinberg equation:

$$(q + p)^2 = q^2 + 2pq + p^2,$$

where q is the incidence of the recessive gene; p, the incidence of the dominant gene; q^2, the incidence of the aa genotype; p^2, the incidence of the AA genotype; and $2pq$, the incidence of Aa genotype.

To assess the correspondence of the observed distribution of genotypes to the ex-

pected one based on the Hardy – Weinberg equilibrium, the χ^2 criterion was used.

For statistically significant parameters, the relative risk (RR) was calculated using the equation:

$$\text{OP} = \frac{A \cdot (C + D)}{C \cdot (A + B)}.$$

The confidence (95 %) interval for the RR was calculated using the equations:

upper limit $e^{\ln(\text{OP})+1{,}96\cdot\sqrt{\frac{B}{A\cdot(A+B)}+\frac{D}{C\cdot(C+D)}}}$,

lower limit $e^{\ln(\text{OP})-1{,}96\cdot\sqrt{\frac{B}{A\cdot(A+B)}+\frac{D}{C\cdot(C+D)}}}$,

where A, B, C, and D in the equations were the number of cases in the cells of the four-field contingency table.

This study was conducted in accordance with the principles of the Declaration of Helsinki of the World Medical Association. Before including a pediatric patient in the study, written informed consent was obtained from his legal representative in accordance with local laws and regulations. Conclusion of the local ethics committee at Perm State Medical University no. 5/20 dated August 4, 2020, was obtained.

RESULTS AND DISCUSSION

All children in the study group were under regular medical check-up by a pulmonologist owing to a diagnosis of bronchial asthma. The median age of the patients was 13 years [Q_1–Q_3: 9; 15] years. Overall, 100 children participated in the study, including 72 boys (72 %) and 28 girls (28 %). Sixty-two patients had mild asthma, 27 had moderate BA, and 11 had severe asthma. The largest number of patients in the study cohort (67 patients) had incomplete control of bronchial asthma; lack of control was detected in 7 children, and 26 had complete control.

When collecting anamnestic data from 87 % of children, factors for violating the hypoallergenic regime were identified (i.e., the presence of animals in the house, carpets, flowering indoor plants, mold, passive smoking, pollution of the place of residence with exhaust fumes from cars or nearby industrial enterprises).

Outpatient medical records of children undergoing dispensary follow-up by a local pediatrician were analyzed to assess the comorbid background. Evaluation of the concomitant diseases of the examined children revealed that the largest percentage was accounted for by allergic rhinitis (80.0 %), atopic dermatitis (32.0 %), and pollinosis (40.4 %) (Table 1). Most children had two or more concomitant diseases.

The structure of complaints of the examined children was dominated by dyspnea during physical activity (70.0 ± 9.0), dyspnea on the street in the spring–summer period (34.0 ± 9.3) and with a respiratory infection (42.0 ± 9.7), difficulty breathing with wheezing (45.0 ± 9.8), and dry paroxysmal cough (48.0 ± 9.8).

Examination of the external respiratory function using the spirography method identified obstructive-type disorders in 29 children, and peak flowmetry control data showed significant deterioration in indicators

Table 1

Comorbidities in BA pediatric patients

Concomitant disease	Number of pediatric patients with an established diagnosis, *n*
Allergic rhinitis	80
Atopic dermatitis	32
Pollinosis	40
Food allergy	20
Diseases of the gastrointestinal tract (chronic gastroduodenitis/biliary tract dysfunction/chronic constipation)	17
Overweight/obesity	15
Congenital malformations of the tracheobronchial tree: accessory bronchus/ bronchial transposition	16
Urticaria fever	14

in 18 % of the patients and indicated their shift to the "red" zone, which confirms the hypothesis that the disease is not controlled in BA patients in the course of baseline therapy.

Characteristics of the studied genotypes. The distribution of genotypes in BA patients corresponded to the Hardy – Weinberg equilibrium, with the exception of the genetic variant rs146597587 of the *IL-33* gene (G > C), with only carriers of one genotype GG (Table 2).

Search for associations of genetic markers of the *HHIP, ADRB2*, and *IL-33* genes with the clinical course of bronchial asthma. Comparison of genetic markers in patients with severe BA (sBA) and mild/moderate BA revealed a tendency to reduce the risk of severe disease among individuals carrying the TT genotype (*OR* = 0.221 (95 % *CI*: 0.059–0.828; χ^2 = 5.759; p = 0.056)) and T allele (*OR* = 0.491 (95 % *CI*: 0.190–1.269; χ^2 = 4.270; p = 0.039)) of the studied genetic variant rs12504628 (T > C) of the *HHIP* gene; the frequency of the CC genotype in severe BA was 64 % versus 28 % for non-severe BA and that of C allele was 77 % versus 52 % (Table 3).

Table 2

Frequency distribution of genotypes of the studied polymorphisms (rs12504628 (T > C), rs1042713 (G > A), rs12551256, rs146597587 (G > C)) in a group of patients with bronchial asthma

Gene/ polymorphism	Genotype	N.O.	N.E.	χ^2 df = 1	Allele frequency	$h_{obs+\pm}$SE $h_{exp\pm}$SE	*D*
HHIP gene rs12504628 (T > C)	TT	21	18.68	0.254 p = 0.614	T = 0.455 C = 0.544	h_{obs} = 0.444 ± 0.052 h_{exp} = 0.101 ± 0.012	–0.586
	TC	40	44.64				
	CC	29	26.68				
	T	82	45.56				
	C	98	54.44				
ADRB2 gene rs1042713 (G > A)	GG	40	39.34	0.004 p = 0.952	G = 0.661 A = 0.339	h_{obs} = 0.433 ± 0.052 h_{exp} = 0.685 ± 0.049	–0.368
	GA	39	40.33				
	AA	11	10.34				
	G	119	66.11				
	A	61	33.89				

End of the Table 2

Gene/ polymorphism	Genotype	N.O.	N.E.	χ^2 $df = 1$	Allele frequency	$h_{obs+\pm}$SE $h_{exp\pm}$SE	*D*
IL-33 gene rs12551256 (A > G)	GG	24	22.56	0.095 $p = 0.758$	G = 0.593 A = 0.407	$h_{obs} = 0.438 \pm 0.062$ $h_{exp} = 0.825 \pm 0.047$	−0.470
	GA	28	30.88				
	AA	12	10.56				
	G	76	59.38				
	A	52	40.63				
IL-33 gene rs146597587 (G > C)	GG	40	100		G = 1.0 C = 0	$h_{obs} = 0$ $h_{exp} = 0$	0
	CG	0	0				
	CC	0	0				
	G	80	100				
	C	0	0				

Note: N.O., observed number of genotypes; N.E., expected number of genotypes. The χ^2 criterion was used to assess the correspondence of the observed distribution of genotypes to the expected one based on the Hardy – Weinberg equilibrium. *df,* the number of degrees of freedom; $h_{obs} \pm$ s.e. and $h_{exp} \pm$s.e., observed and expected heterozygosity, respectively, with error; *D,* the relative deviation of the observed heterozygosity from the expected one.

Table 3

Case-control analysis of the studied genetic variants for severe and non-severe BA

Gene/ polymorphism	Genotypes/ alleles	severe BA		non-severe BA		χ^2	*p*	*OR*
		N	%	*N*	%			
HHIP gene rs12504628 (T > C) 1-T/C, 2-T/T, 3-C/C	**TT**	1	9	20	**25**	5.759	0.056	**0.221 (0.059 < *OR* < 0.828)**
	TC	3	27	37	47			
	CC	7	64	22	28			
	T	5	***23***	77	***48***	**4.270**	**0.039**	**0.309 (0.109 < *OR* < 0.880)**
	C	17	*77*	81	*52*			
ADRB2 gene rs1042713 (G > A) 1-G/A, 2-G/G, 3-A/A	GG	5	45	35	44	1.898	0.387	1.048 (0.295 < *OR* < 3.720)
	GA	6	55	33	42			
	AA	0	0	11	14			
	G	16	73	103	65	0.211	0.646	1.424 (0.527 < *OR* < 3.847)
	A	6	27	55	35			
IL-33 gene rs12551256 (A > G) 1-A/G, 2-A/A, 3-G/G	GG	3	11	21	14	4.345	0.114	0.470 (0.190 < *OR* < 1.166)
	GA	c	45	28	40			
	AA	c	44	12	46			
	G	6	*33*	70	*34*	0.027	0.870	0.950 (0.646 < *OR* < 1.396)
	A	0	*67*	52	*66*			
IL-33 gene rs146597587 (G > C) 1-G/C, 2-G/G, 3-C/C	GG	8	11	22	14	4.345	0.114	0.470 (0.190 < *OR* < 1.166)
	GC	0	45	0	40			
	CC	0	44	0	46			
	G	16	*33*	44	*34*	0.027	0.870	0.950 (0.646 < *OR* < 1.396)
	C	0	*67*	0	*66*			

Note: *N*, the absolute number of observed genotypes; *p*, given for the χ^2 test; *c,* sequencing.

Comparison of genetic markers in patients with a combination of atopic dermatitis (AD) and bronchial asthma (BA + AD) and BA without AD (BA without AD) revealed an increased risk of a combination of asthma and dermatitis among individuals carrying the TT genotype [*OR* = 2.875 (95 % *CI*: 1.130–7.316; $\chi^2 = 5.751$; $p = 0.056$)] of genetic variant rs12504628 (T > C) of the *HHIP* gene, but without a significant difference.

Analysis of genetic markers in patients with a combination of congenital malformations of the tracheobronchial tree with asthma (BA + CM) and bronchial asthma without malformations (BA without CM) revealed associations with the combination of asthma and CM with the AA genotype [*OR* = 0.182 (95 % *CI*: 0.051–0.646; $\chi^2 = 8.567$; $p = 0.014$)] of the genetic variant rs1042713 (G > A) of the *ADRB2* gene, which has a protective effect against CM; it was found that carriers of the AA genotype have CM of the bronchial tree in 40 % of cases, versus 11 % of carriers of genotype AA without CM.

Analysis of genetic markers in BA patients and an aggravated allergic history among first-degree relatives (BA + hereditary history) and bronchial asthma without an aggravated hereditary allergic history (BA without a hereditary history) showed that carriers of the AA genotype [*OR* = 0.112 (95 % *CI*: 0.013 –0.932; $\chi^2 = 5.554$; $p = 0.062$)] and A allele (OR = 0.453 (95 % *CI*: 0.213–0.964; $\chi^2 = 3.537$; $p = 0.059$) of the genetic variant rs1042713 (G > A) of the *ADRB2* gene tend to have a lower incidence of an aggravated allergic anamnesis among first-degree relatives (4 % vs 26 % for the AA genotype and 26 % vs 44 % for the A allele). However, further study of genetic markers in a large cohort of patients is required to confirm the above statements.

Analysis of genetic markers in patients with BA and drug allergies (BA + DA) and bronchial asthma without drug allergies (BA without DA) revealed that carriers of the CC+TC genotype tend to have combined asthma and drug allergies (*OR* = 2.917 (95 % *CI*: 1.009–8.427; $\chi^2 = 4.984$; $p = 0.083$) of the genetic variant rs12504628 (T > C) of the *HHIP* gene; the T allele was shown to have a protective role against drug allergies (31 % vs. 52 %) (*OR* = 0.416 (95 %) *CI*: 0.190–0.909; $\chi^2 = 4.204$; $p = 0.040$).

Analysis of genetic markers in patients with uncontrolled BA and bronchial asthma with partial and complete control of the disease symptoms did not reveal associations with the studied genetic variants.

Carriage of the AA genotype of the *ADRB2* gene is associated with a reduced risk of aggravated allergic anamnesis among first-degree relatives and of congenital malformations of the tracheobronchial tree against BA (Table 4).

Sequencing and exome analysis of the *IL-33* gene showed a significant positive relationship between the frequency of damage in exons 4 ($r = 0.417$; $p = 0.034$) and 6 ($r = 0.593$; $p = 0.001$) and severity of BA. Nucleotide substitutions in these exons are more often associated with severe bronchial asthma.

Table 4

Associations of bronchial asthma with studied genetic variants

Gene/ polymorphism	Genotype/ allele comparison	*OR* (95 % *CI*)	Clinical associations
HHIP gene rs12504628 (T > C)	C vs T	0.309 (0.109–0.880)	Reduced risk of severe BA for carriers of the T allele
	TT vs CC+TC	2.875 (1.130–7.316)	Increased risk of a combination of BA and atopic dermatitis for carriers of the TT genotype
	C vs T	0.416 (0.190–0.909)	Reduced risk of drug allergies associated with BA for carriers of the T allele
ADRB2 gene rs1042713 (G > A)	AA vs GA+GG	0.182 (0.051–0.646)	Reduced risk of bronchial tree CM associated with BA for carriers of the AA genotype
	AA vs GA+GG	0.112 (0.013–0.932)	Reduced incidence of aggravated allergic anamnesis associated with BA for carriers of the AA genotype
	A vs G	0.453 (0.213–0.964)	Reduced incidence of aggravated allergic anamnesis associated with BA for carriers of the A allele

A study showed that the Gly16Gly genotype of the *ADRB2* gene is associated with an increased risk of a more severe form of bronchial asthma, as well as its nocturnal form, compared to the ADRB2 Arg16Arg genotype [13]. Analysis of 28 studies on the association of beta-2 adrenergic receptor gene polymorphisms with BA phenotypes confirmed the link between Gly16 polymorphism and nocturnal asthma; however, it did not reveal an association between the Arg16Gly variant and bronchial hyperresponsiveness [18].

Notably, in our sample, children with the indicated genotype AA of the *ADRB2* gene did not receive long-acting b_2-agonists as monotherapy as baseline therapy and did not use monotherapy with short-acting b_2-agonists as emergency medications over the past 6 months. Therefore, it was not possible to assess the risk of exacerbation of the disease in carriers of the Arg16 genotype who resort to the use of b_2-agonists.

Currently, it has been established that the *HHIP* gene affects the condition of both small and large airways [19]. The presence of A allele of the rs13118928 polymorphism of the *HHIP* gene may be associated with the emphysema–hyperinflation phenotype in patients with chronic obstructive pulmonary disease [20]. The state of external respiration function is the most crucial criterion for the severity of bronchial asthma. The presence of CC + CT genotypes increases the risk of drug allergies associated with BA by 2.9 times.

We did not identify an association between genetic variants of the rs12551256 polymorphism of the *IL-33* gene and features of the clinical course of BA. When analyzing the genotypes of the rs146597587 (G > C) polymorphism of the *IL-33* gene, all children were carriers of the same genotype. However, nucleotide substitutions in exons 4 and 6 of the *IL-33* gene were found to be associated with severe bronchial asthma. This indicates the need for further

studies with larger sample sizes on the exon polymorphism of the *IL-33* gene and its associations with the clinical course of bronchial asthma in pediatric patients.

Study limitations. The association of polymorphisms of the *HHIP*, *ADRB2*, and *IL-33* genes in pediatric patients cannot be concluded to the entire population of Russian children owing to the small study sample size. The distributions of genotypes and alleles of these genes will possibly differ from those given in this article with an increase in sample size. We have not conducted a multivariate analysis adjusted for the detected gene associations, considering the carriage of polymorphic variants of other genes and environmental factors, which may affect the results of assessing the effect of the genes under study.

Conclusions

A study of polymorphisms of the *HHIP*, *ADRB2*, and *IL-33* genes in BA pediatric patients with different phenotypes of the disease revealed an association between gene polymorphisms and the disease severity, as well as with concomitant diseases.

It was observed that the TT genotype of the genetic variant rs12504628 (T > C) of the *HHIP* gene reduces the risk of severe BA; however, it increases the risk of atopic dermatitis combined with BA by 2.8 times. The CC + CT genotype of the *HHIP* gene increases the risk of drug allergies associated with BA by 2.9 times.

The AA genotype of the *ADRB2* gene is associated with the absence of an aggravated allergy history among first-degree relatives and a reduced risk of congenital malformations of the tracheobronchial tree in the presence of BA. Nucleotide substitutions in exons 4 and 6 of the *IL-33* gene are associated with severe BA. Thus, attention should be paid to exons 4 and 6 to predict the course of the disease and for timely correction of baseline therapy.

Thus, this study established associations of polymorphic variants of the *HHIP, ADRB2*, and *IL-33* genes with the clinical manifestations of bronchial asthma in children, which can be considered in personalized monitoring of these patients and help in achieving complete control of the disease.

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INTEGRATED APPROACH TO THE STUDY OF BIODEGRADATION OF COMPOSITE MATERIALS FOR THE RESTORATION OF HARD DENTAL TISSUES

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КОМПЛЕКСНЫЙ ПОДХОД К ИЗУЧЕНИЮ БИОДЕГРАДАЦИИ КОМПОЗИТНЫХ МАТЕРИАЛОВ ДЛЯ ВОССТАНОВЛЕНИЯ ТВЕРДЫХ ТКАНЕЙ ЗУБОВ

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Objective. To study the stress-strain state of demineralized tooth enamel after its impregnation with a low-viscosity composite in a long-term period of functioning in the oral cavity in a series of equivalent cyclic tests; to study the correlation of the experimental results with clinical observations of the treatment of enamel caries using a modified infiltration technique.
Materials and methods. The parameters of laser holographic interferometry and atomic force microscopy, reflecting the timing, type and severity of deformation defects in samples of demineralized tooth enamel were analyzed in a series of equivalent cyclic tests. During the experiment the samples underwent classical infiltration with composite using ICON technology (main group) and a 2-stage enamel caries treatment technique, including carrying out a time-modified conditioning of classical infiltration and the final stage (stages) of enamel coating with a bioactive hybrid glass ionomer (comparison group).
Results. The first signs of the deformation defects generation in demineralized enamel filtered on the classical method (the appearance of perifocal microcracks) were recorded in 11.2 % of the tested samples after $0.9 \cdot 10^6$ cycles (equivalent to 9 months of functioning of a tooth with treated enamel caries in the oral cavity), during further cyclic tests the percentage of samples with local defects increased progressively. Signs of a violation of the stress-strain state in the enamel after combined treatment (samples of the comparison group) were revealed in cycles corresponding to 2–3 years of being in an artificial environment of the oral cavity. Experimental data correlated with the clinical observations both in time and frequency of complications (secondary/recurrent caries) in the long term after treatment of enamel caries by various methods.
Conclusions. In the process of equivalent cyclic tests, heterogeneous elastic-plastic deformations develop in the thickness of a section of demineralized enamel impregnated with a flowing composite and along its perifocal zones, most pronounced at the interface between intact and treated enamel, which leads to the development of local defects, delaminations, cracks, and destruction of the structurally heterogeneous environment of tooth enamel. According to the results of the comparative analysis, the most favorable results in terms of the timing of development, frequency and severity of deformation defects were obtained in samples of demineralized enamel after its treatment using a modified caries infiltration technique followed by coating with a bioactive hybrid glass ionomer.
Keywords. Composite materials, aesthetic and functional restoration, enamel caries, stress-strain state, equivalent cyclic tests.

Цель. В серии эквивалентных циклических испытаний изучить напряженно-деформированное состояние деминерализованной эмали зуба после ее импрегнации композитом низкой вязкости в сроки, эквивалентные длительному периоду функционирования в полости рта; соотнести результаты экспериментов с клиническими наблюдениями лечения кариеса эмали по модифицированной инфильтрационной методике.
Материалы и методы. Проанализированы параметры лазерной голографической интерферометрии и атомно-силовой микроскопии, отражающие сроки появления, тип и выраженность деформационных дефектов в образцах деминерализованной эмали зубов, на которых в процессе эксперимента воспроизведена классическая инфильтрация композитом по технологии ICON (основная группа) и двухэтапная методика лечения кариеса эмали, включающая проведение модифицированной по времени кондиционирования классической инфильтрации и заключительный этап (этапы) покрытия эмали биоактивным гибридным стеклоиономером (группа сравнения).
Результаты. Первые признаки генерации деформационных дефектов в проинфильтрированной по классической методике деминерализованной эмали (появление перифокальных микротрещин) зафиксированы у 11,2 % тестируемых образцов через $0{,}9 \cdot 10^6$ циклов (эквивалент 9 месяцев функционирования зуба с пролеченным кариесом эмали в полости рта), в ходе дальнейших цикловых испытаний процент образцов с локальными дефектами прогрессивно нарастал. Признаки нарушения напряженно-деформированного состояния в эмали после комбинированного лечения (образцы группы сравнения) выявлены в циклах, соответствующих 2–3 годам нахождения в условиях искусственной среды полости рта. Экспериментальные данные коррелировали с клиническими наблюдениями по времени и частоте проявления осложнений (вторичного / рецидивного кариеса) в отдаленные сроки после лечения кариеса эмали разными методами.

Выводы. В процессе эквивалентных циклических испытаний в толще участка деминерализованной эмали, проимпрегнированной текучим композитом, и по его перифокальным зонам развиваются неоднородные упругопластические деформации, наиболее выраженные на границах раздела интактной и пролеченной эмали, что приводит к развитию локальных дефектов, расслоений, трещин, разрушению структурно-неоднородной среды эмали зуба. По итогам сравнительного анализа наиболее благоприятные результаты в части сроков развития, частоты и степени выраженности деформационных дефектов получены у образцов деминерализованной эмали после её лечения по модифицированной методике кариес-инфильтрации с последующим покрытием биоактивным гибридным стеклоиономером.
Ключевые слова. Композитные материалы, эстетико-функциональная реставрация, кариес эмали, напряженно-деформированное состояние, эквивалентные циклические испытания.

INTRODUCTION

Aesthetic-functional restoration (AFR) of lost dental tissue (dentine, enamel, cement) is the most common treatment procedure in an outpatient dentistry clinic. Modern composite materials (CM) play a key role in the AFR of teeth, the requirements for which, despite their diversity in the dental market, are constantly increasing.

Modern dental materials science and conservative preventive dentistry highlight the following promising directions for the development of new composite materials for AFR: bioactive composites with antimicrobial/remineralizing ingredients; strengthened composites, including fiber-reinforced ones; fast-hardening composites; and CM with higher adhesive properties and increased crack resistance to ensure operational survivability of the material in the long term after AFR [1; 2]. An optimal balance of strength and viscosity properties enables improving the adhesion/marginal adaptation of CM to hard dental tissues (HDT) and its handling characteristics and ensuring the stability of the obtained AFR results over time. A modern trend in dental materials science regarding AFR are technologies for improving the physical and mechanical properties of CM by thermal, vibration, or mechanical activation of the material or their combination [3; 4]. Furthermore, modernization of AFR is progressing along the path of creating new more advanced adhesive and polymerization systems and technologies for conditioning HDT before filling [5; 6]. Notably, CMs are traditionally in demand for replacing actual dentin or cement defects. Experimental and clinical studies [7] indicate that a separate group of unfilled, high-flow composites with low viscosity (low-viscosity resin) is used for ultraconservative treatment of enamel focal demineralization, used in combination with traditional restorative treatment or teeth whitening [8; 9]. As an example of such CM with low-viscosity properties, an infiltrant is considered a classic and an improved variant of caries infiltration (C–I) using ICON technology (DMG, Germany), which in experimental and clinical conditions has demonstrated advantages compared to traditional remineralizing therapy [10].

The long-term functioning conditions of CM in the oral cavity, which determine the durability of restoration/infiltration of HDT, are largely affected by the development of the stress–strain state at the border of CM and tooth tissues (enamel, dentin, cement). The study of the patterns of multifactorial (mechanical, chemical, biomechanical, thermodynamic) biodegradation of infiltrating CM, simulating its behavior in dental tissues during the long-term functioning of the composite–enamel binary system in the oral cavity, is theoretically significant and practically justified; however, such studies are few. Testing equipment manufactured in other countries (Willytec, MTC-simulator, etc.) designed for these purposes are installed in several large world-class biomaterials science centers. Russian developments of such simulation systems and equipment are warranted, their bringing to ISO and GOST standards for qualified testing of new dental materials (including CM) and treatment and prophylactic technologies.

This study aimed to analyze, in a series of equivalent cyclic tests, the stress–strain state of demineralized tooth enamel after its impregnation with a low-viscosity composite in a long period of functioning in the oral cavity and correlate the experiment results with clinical cases of the treatment of enamel caries using a modified infiltration technique.

MATERIALS AND METHODS

Experimental and clinical studies were approved by the local ethical committee and conducted at the Department of Therapeutic Dentistry and Propaedeutics of Dental Diseases, E.A. Wagner Perm State Medical University of the Ministry of Health of Russia, and Department of General Physics, Perm State National Research University, in specialized laboratories of "ODK-STAR" (Perm). Forty premolars removed with an intact crown and root according to indications were selected, on which enamel caries was modeled using the original method[1] (ICDAS code 1, 2), confirming its compliance with the data of microcomputed tomography, atomic force microscopy (AFM), and scanning electron microscopy. The teeth were randomly distributed into two equal groups: the main group (the classical C–I technique was modeled on teeth with artificial caries) and comparison group, where artificial enamel caries on teeth was "treated" using a two-stage method, including the stages of impregnation of the demineralization focus with a composite with exposure to enamel conditioning increased to 4 minutes and of coating the impregnated enamel with a layer of bioactive hybrid glass ionomer[2].

To study the patterns of biodegradation of demineralized tooth enamel im-

[1] O.S. Gileva, M.A. Muravyova, E.S. Gileva, V.A. Valtsifer, and A.I. Nechaev, A Method for Modeling a Focus of Tooth Enamel Demineralization, Patent for invention RU 2503067 C1, 12/27/2013, application no. 2012147965/14; dated November 12, 2012.

[2] O.S. Gileva, M.A. Shakulya, A.D. Levitskaya, E.S. Syutkina, and E.V. Serebrennikova, Method for Treating Focal Demineralization of Tooth Enamel, Patent for invention RU 2571334 C1, 12/20/2015, application no. 2014146961/14 dated November 21, 2014.

pregnated with low-viscosity CM using the classical and combined C–I methods we developed, the study used a new Russian-made simulation complex of qualification equipment for conducting tribological tests of dental materials and technologies, developed jointly with engineering specialists[3].

In measuring nanodisplacements in infiltrated enamel and its microdeformations during equivalent cyclic tests of materials, the laser holographic interferometry (LHI) method with a counter-directional action scheme with double exposure was used, synchronized with the analysis of the enamel surface using AFM and calculation of its quantitative parameters to identify microcracks, ruptures, etc. in the material interface zone [11]. The hologram after each cycle of cyclic tests was compared to the original.

RESULTS AND DISCUSSION

Based on the results of cyclic tests of teeth of the main group, where enamel caries was treated according to the classical C-I method following manufacturer recommendations, after 0.9 x 10^6 cycles, equivalent to 9 months of functioning of the treated tooth as part of the dentofacial system, microcracks with branches (thinning) were determined in 11.2 % of cases using the LHI method synchronously with AFM analysis of the enamel surface at the border of healthy and impregnated enamel. After $1.2 \cdot 10^6$ cycles (equivalent to 1 year post-treatment), the proportion of detected cracks in the enamel increased to 55.5 %. At similar stages of equivalent cyclic tests, corresponding to 9- and 12-month follow-ups, on the surface of the tested teeth samples treated under experimental conditions according to the original two-stage method (impregnation with a low-viscosity composite followed by coating with a hybrid glass ionomer), microcracks or other defects were not detected in the cases, and the marginal adhesion of the impregnation zone to the apparently intact enamel was not disturbed. Moreover, after 60 minutes of testing (equivalent to a 3-year "operation" in the oral cavity of enamel impregnated with a composite), the first signs of microdeformation of its surface layers (an increase in the curvature of interference fringes) were observed; however, even at this stage, delaminations and microcracks in border areas of enamel were not noted. Disturbances in the deformation of enamel infiltrated with a composite using the classical method, identified under experimental conditions, corresponded to the cases of development of secondary caries, visible

[3] A.D. Levitskaya, O.S. Gileva, A Device for Producing a Damping Replica of the Occlusal Surface of a Tooth *in vitro* for Conducting Cyclic Tests of the Tooth for Axial Compression. Utility model patent RU 191943 U1, 08/28/2019. Application no. 2018145620 dated December 20, 2018. A.D. Levitskaya, O.S. Gileva, A Device for Bonding a Tooth *in vitro* to Conduct Cyclic Testing of a Tooth for Axial Compression. Utility model patent RU 190383 U1, 06/28/2019, application no. 2018145617 dated December 20, 2018. A.D. Levitskaya, O.S. Gileva, *In vitro* Tooth Centering Device for Cyclic Axial Compression Testing of Teeth. Utility model patent RU 191894 U1, 08/26/2019 application no. 2018145619 dated December 20, 2018. A.D. Levitskaya, O.S. Gileva, A Device for Performing Cyclic Tests of Teeth *in vitro* for Axial Compression, Patent for invention RU 2704208 C1, 10/24/2019, application no. 2018145123 dated December 18, 2018.

linear defects, and violations of the marginal adaptation of the composite in the thickness of the enamel in the long-term follow-up in 23.9 % of 67 patients with focal demineralization of enamel (12.18 months post-treatment). The experimental assessment results of the "functional survivability" of a special structurally heterogeneous environment (demineralized enamel impregnated with a composite, laminated with a layer of bioactive glass ionomer) in the tested samples of the comparison group corresponded to the data of clinical cases (no signs of secondary/recurrent caries for 1.5 years after treatment) and isolated cases of the appearance of defects (violations of border adaptation) on the enamel surface after 18 months of monitoring.

Notably, based on the test results, the Russian-made simulation system of equivalent cyclic tests of tested samples used in this study has demonstrated high qualification characteristics, which is characterized by the originality of design solutions, methods for assessing the performance characteristics of tested samples, approaches to regulating the rate of load increase, choice of temperature conditions, and methods of maintaining liquid homeostasis with artificial saliva of the original composition, which bring *in vitro* experiments as close as possible to the real conditions of long-term functioning of teeth in the oral cavity.

The analysis of the stress–strain state in the process of tribological tests is traditionally performed by methods of strain gauging, photoelasticity, and digital dynamic spectrum photography or using mathematical models. The use of the LHI method for these purposes was demonstrated by the novelty of the approach and good results regarding objectivity and reproducibility of the results obtained and clarity of their presentation. Thus, the physical knowledge obtained from the results of equivalent clinical trials enabled for determining the critical periods, "foci," and types of deformations in demineralized enamel before and after various treatment techniques. New data on the patterns of biodegradation of composite material in the composition of demineralized enamel were obtained, which confirmed the correctness of the chosen ultraconservative approach to its treatment based on impregnation of the affected areas of enamel with a flowable composite with prolonged preliminary conditioning stage and subsequent finishing lamination of infiltrated and perifocal enamel with a bioactive glass ionomer.

CONCLUSIONS

1. Equivalent cyclic tests of samples of teeth with enamel caries treated using the classical method of caries infiltration show the clinically high (23.9 %) incidence of complications in the form of secondary caries 1–1.5 years after treatment. This is experimentally confirmed by the appearance of microcracks in 55.5 % of the tested samples at a time period corresponding to 12 months of functioning in the oral cavity.

2. The advantages of a two-stage (impregnation with a composite with a prolonged conditioning stage and subsequent coating with a bioactive glass ionomer) method of treating enamel caries are confirmed by experimental studies (the appearance of the first signs of microdeformations of the enamel surface after 18-month follow-up).

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RISK FACTORS FOR DEVELOPMENT OF TYPE 2 DIABETES MELLITUS IN PATIENTS WITH OBESITY IN LATE POST-COVID PERIOD

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ФАКТОРЫ РИСКА РАЗВИТИЯ САХАРНОГО ДИАБЕТА 2-го ТИПА У ПАЦИЕНТОВ С ОЖИРЕНИЕМ В ОТДАЛЕННОМ ПОСТКОВИДНОМ ПЕРИОДЕ

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Objective. To study the risk factors for developing type 2 diabetes mellitus in patients with obesity after COVID-19.
Materials and methods. 61 case histories and outpatient card abstracts of patients with obesity, who suffered from moderate and severe forms of COVID-19 from 02.2021–04.2022 were analyzed. Demographic, laboratory and clinical parameters were studied during hospitalization and 12 months after discharge from the hospital. All patients initially were divided into 2 groups according to the glycated hemoglobin level. Group 1 consisted of 46 patients with prediabetes and group 2 included 15 patients without carbohydrate disorders.
Results. The median age of all patients was 64 (59–66) years. Median of HbAlc was 6,0 (5,6–6,2) %, BMI – 34 (33–35) kg/m^2. 24 patients from group 1, who took DPP-4-inhibitors in early post-COVID-19 period constituted subgroup 1A and 22 patients, who refused treatment with these drugs, constituted subgroup 1B. Currently, 2 patients from subgroup 1A and 10 patients from subgroup 1B (χ^2=8,2 p=0,004) have been diagnosed with DM2. In patients who developed DM2 in late post-COVID period the levels of HbAlc, fasting plasms glucose and BMI at the time of admission to the hospital were significantly higher (n=12) than in patients with persistent prediabetes (n=34), (p<0,05). Positive correlation between these parameters and the risk of developing DM2 (R=0,5, p<0,05; R=0,74, p<0,05; R=0,54, p<0,05, respectively) was determined. In group 2, DM2 is currently di-

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agnosed in 2 male patients with BMI over 40 kg/m^2. When comparing subgroup 1B and group 2, it was found out that DM2 in the post-COVID period occurs in every second patient with the previous initial carbohydrate disorders: in 10 people of 22 – in subgroup 1B (every 2nd patient) versus 2 patients from group 2 (every 7th patient), (χ^2=4,2, p=0,04). Online calculator from medstatistic. ru was used to determine relative risk (*RR*).
Conclusions. Thus, presence of impaired glucose tolerance increases the risk of development of DM2 in late post-COVID period. In patients with hyperlycemia on hospitalization for COVID-19, who did not receive incretin therapy (subgroup 1B) risk of DM2 was 3,4 times higher (*CI* 95 %=0,87–13,40). Patients, who received incretin (subgroup 1A) had risk of DM2 = 0,6 (*CI* 95 %=0,09–3,97). It should be assumed that incretin therapy prevents development of DM2 in patients with hyperglycemia/impaired glucose tolerance after COVID-19.
Keywords. Obesity, COVID-19, hyperglycemia, impaired glucose tolerance, type 2 diabetes mellitus, dipeptydilpeptidase-4-inhibitors.

Цель. Изучить факторы риска развития сахарного диабета 2-го типа (СД2) у лиц с ожирением, перенесших COVID-19.
Материалы и методы. Проанализирована 61 история болезни и выписки амбулаторных карт пациентов с ожирением, перенесших COVID-19 средней и тяжелой степени в период 02.2021–04.2022. Изучены демографические, лабораторные и клинические параметры в период госпитализации и спустя 12 месяцев с момента выписки из стационара. По уровню гликированного гемоглобина (HbAlc, %) больные исходно были разделены на две группы: группу 1 составили 46 пациентов с нарушением толерантности к глюкозе и группу 2 – 15 человек без углеводных нарушений. Количественные данные представлены в виде медианы.
Результаты. Медиана возраста 61 больного составила 64 (59–66) года. Медиана HbAlc 6,0 (5,6–6,2) %, индекса массы тела (ИМТ, кг/м^2) – 34 (33–35) кг/м^2. После выписки из стационара 24 пациента из 1-й группы, принимавшие ингибиторы дипептидилпептидазы 4-го типа (иДПП-4), сформировали подгруппу 1А, в подгруппу 1Б вошли 22 пациента, не принимавшие терапию по причине отказа. К настоящему времени СД2 диагностирован у 2 пациентов из подгруппы 1А и у 10 человек из подгруппы 1Б (χ^2 = 8,2; p = 0,004). Значения HbAlc, глюкозы плазмы натощак и ИМТ в период госпитализации по причине COVID-19 были достоверно выше у пациентов с развившимся в позднем постковидном периоде СД2 (n = 12) по сравнению с пациентами без СД2 (n = 34) (p < 0,05). Выявлена положительная корреляция между риском развития СД2 и HbAlc, глюкозой плазмы натощак, ИМТ (R = 0,5, p < 0,05; R = 0,74, p < 0,05; R = 0,54, p < 0,05, соответственно). В группе 2 в настоящее время диагноз СД2 установлен у 2 мужчин с ИМТ более 40 кг/м^2. При сравнении подгруппы 1Б и группы 2 выявлено, что СД2 в постковидном периоде встречается при наличии предшествующих углеводных нарушений у каждого второго пациента: у 10 человек из 22 в подгруппе 1Б против 2 человек из группы 2 (каждый 7-й пациент) (χ^2 = 4,2; p = 0,04). Для определения относительного риска (*RR*) взят online-калькулятор с medstatistic.ru.
Выводы. Таким образом, наличие гипергликемии (нарушение толерантности к глюкозе) увеличивает риск развития СД2 в отдаленном постковидном периоде. У пациентов с гипергликемией во время госпитализации с COVID-19, не получавших инкретиновую терапию (подгруппа 1Б), риск развития СД2 увеличивался в 3,4 раза (ДИ 95 % = 0,87–13,40). У пациентов, получавших инкретин (подгруппа 1А), риск развития СД2 увеличивался в 0,6 раза (ДИ 95 % = 0,09–3,97). Следует предположить, что инкретиновая терапия профилактирует развитие СД2 у пациентов с гипергликемией после перенесенного COVID-19.
Ключевые слова. Ожирение, COVID-19, гипергликемия, нарушение толерантности к глюкозе, сахарный диабет 2-го типа, ингибиторы дипептидилпептидазы 4-го типа.

INTRODUCTION

The current decade brought together two pandemics, obesity and COVID-19, which have aggravated pathogenetic mechanisms. Studies have shown that obese patients constituted the main cohort of patients hospitalized with COVID-19 [1; 2]. Notably, obesity leads to a more severe course of COVID-19 and increases the risk of poor outcomes of this viral infection [3]. In 2020, mortality from COVID-19 was 10

times higher in countries where majority of the adult population is overweight [4]. According to Chinese researchers, obesity occurs in 41.7 % of patients with COVID-19, second to arterial hypertension [5]. Owing to its pathogenetic commonality, obesity is often combined with carbohydrate disorders, including among patients with COVID-19 [6]. According to the Russian studies "ACTIV" and "ACTIV 2," hyperglycemia was detected in 28.9 % of COVID-19 patients [7]. In such patients, newly diagnosed hyperglycemia may indicate the presence of diabetes mellitus (DM), prediabetes, or transient steroid-induced hyperglycemia [8]. To verify the type of carbohydrate disorder, glycated hemoglobin was determined. Not all patients, especially those without an established DM diagnosis, have normal glycemic levels during the period of convalescence after COVID-19. The history of COVID-19 infection contributes to the deterioration of control of existing DM and progression of prediabetes to diabetes, which aggravates the course of postCOVID syndrome in these patients [9; 10]. Researchers have focused on new cases of type 2 DM (T2DM) following COVID-19. The risk of developing T2DM after COVID-19 is 4.9 % higher than the average in the population [11]. Special attention should be paid to patients with prediabetes, as they have a greater risk of developing T2DM. DM was found to aggravate the course of COVID-19, and therefore, the search for drugs that reduce hyperglycemia and improve the prognosis continued throughout the epidemic. The effects of the main groups of glucose-lowering drugs (GLDs) on the course of the acute period of COVID-19 in T2DM patients have been analyzed. Dipeptydilpeptidase-4 (DPP-4) inhibitors and sodium-glucose co-transporter 2 inhibitors are promising and priority groups of GLDs in the treatment of postCOVID hyperglycemia; however, no data was recorded on their use in patients with prediabetes (*of label* prescription) and hyperglycemia [10]. Studies that analyzed the outcomes of newly diagnosed hyperglycemia against COVID-19 in patients with prediabetes in the late postCOVID period are few.

Thus, this study aimed to assess the significance of risk factors for the development of type 2 DM in obese individuals with a history of COVID-19.

MATERIALS AND METHODS

A retrospective analysis of 61 medical histories and outpatient records of obese patients who had moderate COVID-19 between February 2021 and April 2022 was performed. Demographic, laboratory, and clinical parameters during hospitalization and 12 months after discharge from the hospital were studied. Laboratory data include determination of levels of hemoglobin, ESR, blood plasma glucose, glycated hemoglobin (HbAlc, %), D-dimer, and procalcitonin, which were determined on hospitalization day two. Data were obtained from electronic health records. Inclusion criteria were age >50 years, COVID-19 diagnosis confirmed by chest CT and PCR, BMI >30 kg/m^2, and HbAlc level (%)

upon admission <6.5 %. All patients received glucocorticosteroid therapy during the acute phase of COVID-19. According to the level of HbAlc (%) determined upon hospital admission, patients were distributed into two groups: group 1, 46 patients with prediabetes (HbAlc < 6.5 %–> 5.8 %), and group 2, 15 people without carbohydrate disorders. Statistical processing of the results was performed using the Statistica 6 package. Quantitative characteristics are presented in the form of a median. The significance of differences between the groups was assessed using nonparametric comparison methods based on qualitative and quantitative criteria, namely, the Mann–Whitney U test and χ^2 criterion. Spearman's test (*R*) was used for correlation analysis. To determine the relative risk, an online calculator from medstatistic.ru was utilized.

RESULTS AND DISCUSSION

The median age of the 61 patients was 64 (59–66) years; of the total number of patients, 34 were men (56 %) and 27 (44 %) were women. The median glycated hemoglobin and BMI was 6.0 (5.6–6.2)% and 34 (33–35) kg/m^2, respectively. A comparative analysis of all parameters was performed between the groups of patients at baseline. The data is presented in Table 1.

After hospital discharge, patients in group 1 were recommended to have a combination of metformin and DPP-4-inhibitors for 3 months, with further referral to an endocrinologist.

The use of DPP-4 inhibitors in the group with impaired glucose tolerance (IGT) should be clarified. At the time of hospital discharge, all patients from group 1 (IGT) were diagnosed with T2DM, which was established based on additional glycemic tests performed during hospitalization, despite having an initial HbAlc level of <6.5 %. Low availability of endocrinologists on an outpatient basis at that time was considered, which required hospital endocrinologists to prescribe glucose-lowering therapy upon discharge. Therefore, the use

Table 1

Comparative characteristics of the main parameters in groups 1 and 2 (median)

Parameter	Group 1, *n* = 46	Group 2, *n* = 15	*P*
Age, years	64 (59–67)	63 (58–65)	n.a.
BMI, kg/m^2	34.5 (33.5–35.0)	35.0 (34.0–39.0)	n.a.
Hemoglobin, g/l	125 (120–128)	127 (120–138)	n.a.
ESR, mm/h	62 (55–67)	49.5 (32–60)	n.a.
Glucose, mmol/l	8.1 (7.9–9.1)	6.5 (5.9–7.3)	0.000
Glycated hemoglobin, %	6.0 (6.0–6.2)	5.3 (5.2–5.5)	0.000
Procalcitonin, ng/ml	1.2 (0.22–2.1)	0.8 (0.2–1.6)	n.a.
CRP, mg/l	53.5 (24–83.5)	43 (12–79)	n.a.

Note: n.a., not available.

Table 2

Comparative characteristics of the main parameters in subgroups 1A and 1B (median)

Parameter	Subgroup 1A, $n = 24$	Subgroup 1B, $n = 22$	P
Age, years	63.5 (60.5–67)	62 (56–69)	n.a.
BMI, kg/m^2	33.0 (32.5–35.0)	34.0 (34.0–36.0)	n.a.
Hemoglobin, g/l	126 (121–128)	122 (120–135)	n.a.
ESR, mm/h	60 (57–61)	59.5 (42–63)	n.a.
Glucose, mmol/l	8.0 (7.7–8.8)	8.2 (7.9–8.9)	n.a.
Glycated hemoglobin, %	6.0 (5.9–6.2)	6.1 (5.8–6.3)	n.a.
Procalcitonin, ng/ml	1.4 (0.25–2.0)	0.9 (0.4–1.8)	n.a.
CRP, mg/l	43.5 (24–76.5)	48 (32–80)	n.a.

of DPP-4 inhibitors in this situation was indicated. Moreover, during that time, information about the beneficial effects of incretin therapy on the course and outcomes of DM in COVID-19 was provided [12; 13]. Thus, these patients had a diagnosis of T2DM; however, from the perspective of current concepts, we continued to interpret their condition as IGT.

Among 46 patients, 24, who made up the subgroup 1A, took these drugs for at least 3 months, and 22, who made up the subgroup 1B, refused treatment. No significant differences were noted between the main parameters in the subgroups during hospitalization (Table 2).

By the time of outpatient consultation with an endocrinologist, 4–6 months after hospitalization, the patients were not taking DPP-4 inhibitor therapy or metformin. After additional analysis, the patients' condition was reclassified. By this time, T2DM was diagnosed in two patients from subgroup 1A and 10 from subgroup 1B ($\chi^2 = 8.2$; $p = 0.004$). In patients with currently confirmed T2DM ($n = 12$), during hospitalization, the values of HbAlc, fasting plasma glucose, and BMI were significantly higher compared to the corresponding data for those who maintained the prediabetes state ($n = 34$; $p < 0.05$) (Figs. 1–3). In patients with persistent prediabetes, the median HbAlc values were 6.0 (5.8–6.1)%, fasting plasma glucose values were 8.0 (7.7–8.2) mmol/l, BMI were 34 (32–35) kg/m^2, versus 6.35 (6.2–6.4)%, 10.25 (9.4–11.1) mmol/l, and 35.5 (34.5–37) kg/m^2 ($p < 0.05$), respectively (Figs. 1–3).

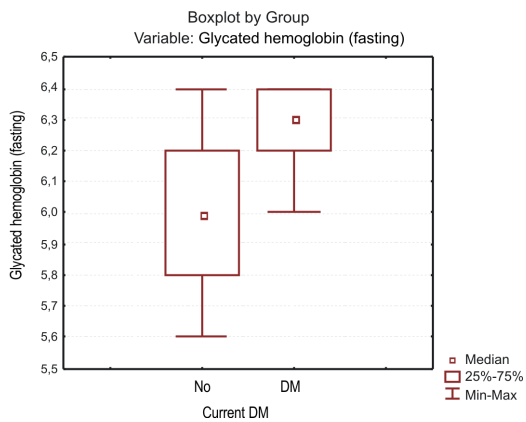

Fig. 1. HbAlc values during hospitalization in patients with currently established T2DM diagnosis and persistent prediabetes

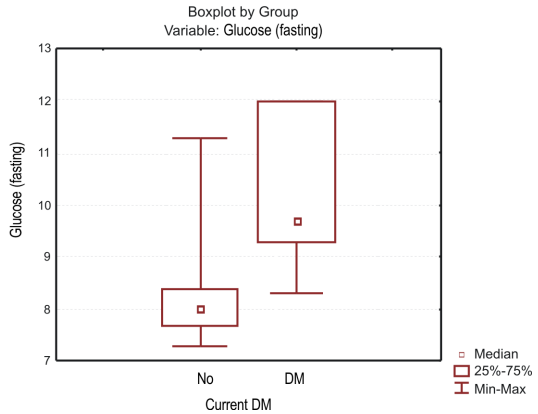

Fig. 2. Maximum plasma glucose values during hospitalization in patients with a current T2DM diagnosis and persistent prediabetes

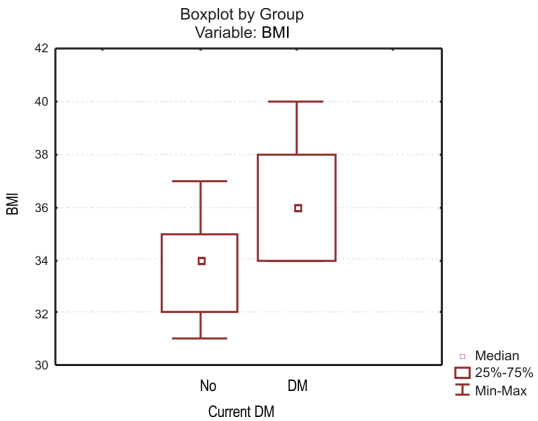

Fig. 3. BMI values during hospitalization in patients with currently established T2DM diagnosis and persistent prediabetes

A positive correlation was revealed between the maximum values of fasting blood plasma glycemia, glycated hemoglobin, BMI, and the risk of developing T2DM ($R = 0.5$, $p < 0.05$; $R = 0.74$, $p < 0.05$; $R = 0.54$, $p < 0.05$, respectively). No significant differences were noted in age, levels of acute phase proteins, and the degree of lung tissue damage during hospitalization between the groups of patients with new-onset T2DM and persistent prediabetes.

In group 2, T2DM was diagnosed in two male patients with morbid obesity (BMI > 40 kg/m^2). When comparing data between subgroup 1B and group 2, it was revealed that T2DM in the postCOVID period occurs in the presence of previous carbohydrate disorders in every second patient who did not use treatment, that is, in 10 of 22 patients in subgroup 1B versus 2 patients from group 2 (every seventh patient) ($\chi^2 = 4.2$; $p = 0.04$). When comparing data from subgroup 1A and group 2, it was determined that T2DM in the postCOVID period occurs in every 11th patient with hyperglycemia who received DPP-4 inhibitors and in every 6th–7th patient without carbohydrate disorders at hospitalization ($p > 0.05$). In patients who did not receive incretin therapy (subgroup 1B), the risk of T2DM occurrence increased by 3.4 times (95 % CI = 0.87–13.40). In patients receiving incretin (subgroup 1A), the risk of T2DM increased by only 0.6 times (95 % CI = 0.09–3.97). Thus, patients with hyperglycemia who received DPP-4 inhibitors in the early postCOVID period and those without carbohydrate disorders have similar incidence of T2DM in the long-term period. It should be assumed that DPP-4 inhibitors reduce the risks of T2DM in patients with hyperglycemia after COVID-19. The protective role of DPP-4 inhibitors in COVID-19 is presented in several studies and justifies the use of this group of drugs in T2DM patients with a history of COVID infection. Studies have reported that patients taking DPP-4 inhibi-

tors had a milder course of this viral infection [14; 15]. The mechanism by which DPP-4 inhibitors protect the body from severe COVID-19 has been studied and is due to inhibition of the DPP-4 enzyme. Moreover, a high level of this enzyme increases the body's susceptibility to the SARS-CoV-2 virus, reduces the effect of incretins and thus causing hyperglycemia, and increases the production of proinflammatory factors [16]. T2DM and obese patients have shown increased DPP-4 activity, which affects the course of the acute phase of COVID-19 and postCOVID period and the risk of T2DM occurrence.

Conclusions

Previous carbohydrate disorders (prediabetes) and high BMI values are crucial in the development of T2DM in obese patients who have had moderate and severe COVID-19. The presence of hyperglycemia (IGT) increases the risk of developing T2DM in the late postCOVID period. Therapy with metformin and DPP-4 inhibitors may play a protective role in this process. The absence of incretin therapy in patients with hyperglycemia after COVID-19 leads to the development of T2DM 3.5 times more often than in patients taking DPP-4 inhibitors. It should be assumed that incretin therapy prevents T2DM development in patients with hyperglycemia (IGT) after COVID-19, and the possibility of prescribing this group of drugs to all patients with obesity and hyperglycemia should be considered. In obese patients without carbohydrate disorders, weight loss should be recommended to reduce the risk of developing T2DM.

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LITERATURE REVIEW

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CLINICAL AND MORPHOLOGICAL FEATURES AND UNSOLVED ISSUES IN DIAGNOSIS OF AGGRESSIVE FORMS OF PAPILLARY THYROID CARCINOMA

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КЛИНИКО-МОРФОЛОГИЧЕСКИЕ ОСОБЕННОСТИ И НЕРАЗРЕШЕННЫЕ ПРОБЛЕМЫ ДИАГНОСТИКИ АГРЕССИВНЫХ ВАРИАНТОВ ПАПИЛЛЯРНОГО РАКА ЩИТОВИДНОЙ ЖЕЛЕЗЫ

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Differentiated thyroid cancer (DTC) is a disease with a favorable clinical course and high survival rate compared to other epithelial tumors. The majority of DTC (up to 85 %) is represented by various subtypes of papillary thyroid cancer (PTC). Aggressive forms of PTC characterized by early lymphogenous and hematogenous metastasis, low avidity to radioiodine therapy (RIT), low relapse-free survival rates and high mortality rate compared to other types of PTC occur among them. Preoperative diagnosis of PTC is based on the results of ultrasound (US) examination and fine-needle aspiration biopsy (FNA) with cytological examination of the as-

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pirate. At the same time, the capabilities of cytological examination in determining the histological type of PTC are limited and it does not allow to predict its aggressiveness and plan adequate treatment. Molecular genetic tests of the tumor cytological and morphological material are effective in prognosis of aggressiveness of PTC due to the determination of specific mutations in the BRAF, TERT, RAS genes and the quantitative expression of oncogenic and tumor suppressive microRNAs. Some of these indicators are already used in the morphological classification of tumors of the endocrine system.
At the same time, there are contradictory data concerning the connection of the molecular genetic portrait of PTC, the clinical manifestations of its aggressiveness (extrathyroidal invasion, early metastasis, and radioiodine resistance) and its pathomorphological structure. We tried to summarize and analyze the literature data regarding the diagnosis of aggressive variants of PTC.
Keywords. Aggressive variants, papillary thyroid carcinoma, molecular genetic test, microRNA, BRAF mutation.

Дифференцированный рак щитовидной железы (ДРЩЖ) является заболеванием, благоприятным по клиническому течению и выживаемости, в сравнении с другими опухолями человека. Большая часть ДРЩЖ (до 85 %) представлена различными вариантами папиллярного рака щитовидной железы (ПРЩЖ), среди них встречаются агрессивные формы ПРЩЖ, которые характеризуются ранним лимфогенным и гематогенным метастазированием, резистентностью к радиойодтерапии (РЙТ), а также низкими показателями безрецидивной выживаемости с высокими показателями летальности по сравнению с другими вариантами ПРЩЖ. Дооперационная диагностика ПРЩЖ основана на результатах ультразвукового исследования (УЗИ) и тонкоигольной аспирационной биопсии (ТАБ) с цитологическим исследованием аспирата. При этом возможности цитологического исследования в определении гистологического типа ПРЩЖ ограничены, что не позволяет спрогнозировать его агрессивность и адекватно планировать лечебную тактику. Одним из перспективных методов, показавших свою эффективность в прогнозировании агрессивности ПРЩЖ, является молекулярно-генетическое исследование клеточного материала опухоли с определением специфических мутаций в генах BRAF, TERT, RAS и количественная оценка онкогенных и онкосупрессорных микроРНК. Некоторые из этих показателей уже используются в морфологической классификации опухолей эндокринной системы. При этом в литературе имеются противоречивые данные о взаимосвязи молекулярно-генетического портрета ПРЩЖ с клиническими проявлениями его агрессивности (экстратиреоидный рост опухоли, раннее метастазирование и радиойодрезистентность) и его патоморфологической структурой. Проводится анализ литературных данных, касающихся диагностики агрессивных вариантов ПРЩЖ.
Ключевые слова. Агрессивные варианты, папиллярный рак щитовидной железы, молекулярно-генетическое исследование, микроРНК, мутация BRAF.

INTRODUCTION

This review used scientific publications from PubMed and eLibrary. Filters were used in the search procedure, namely, date of publication from 2013 to the present; keywords papillary thyroid cancer, aggressive variants, molecular genetic studies, microRNA, mRNA, papillary thyroid microcarcinoma, papillary thyroid carcinoma, hobnail variant, diffuse sclerosing variant, tall cell variant, columnar cell variant, solid variant, BRAF, RET, RET/PTC, KRAS, NRAS, HRAS, and TERT; search queries for diagnostics of aggressive variants of papillary thyroid cancer; molecular testing of thyroid nodules; and diagnosis and management of thyroid nodules.

Differentiated thyroid cancer (DTC) is a heterogeneous group of epithelial tumors with a favorable course and prognosis and high survival rates [1].

Papillary thyroid carcinoma (PTC) is the most common thyroid cancer characterized by relatively low aggressiveness with a high 10-year disease-free survival rate [2]. Most PTC patients experience a slow course of the tumor process and most often treatment ends with recovery after surgery, even in the presence of metastatic lesions of the lymph nodes [3; 4]. Survival rates in DTC patients are high, which is associated with distant metastases in a small proportion of patients and local recurrence with invasive tumor growth. The proportion of patients exceeding the 10-year survival threshold remains at 85 % [4–6].

In patients with a confirmed cytological diagnosis of DTC, surgical treatment of the primary tumor is indicated to the extent determined by preoperative and intraoperative examination (hemithyroidectomy, thyroidectomy, cervical lymphadenectomy of levels 2–6). Radioactive iodine therapy (RIT) is prescribed after surgery based on postoperative stratification of the risk of tumor persistence and recurrence. If radioiodine refractoriness develops, targeted therapy is advised. Moreover, external beam radiation therapy is recommended as palliative treatment in patients with unresectable metastases and metastases that do not accumulate radioactive iodine [7].

Standard preoperative diagnostic methods, such as ultrasound (US) and fine-needle aspiration biopsy (FNAB) of thyroid nodules with cytological examination of the biopsy material, cannot determine the presence of an aggressive subtype of PTC in the patient examined. Cytological diagnostics has significant limitations and is inappropriate for stratifying the risk of malignancy and predicting whether a patient has an aggressive subtype of PTC [8–10]. Despite clear pathological criteria for diagnosing these tumors, there is currently no consensus among pathologists who can accurately determine the aggressiveness of a tumor based on its morphological features. Moreover, the use of immunohistochemical research methods (IHC) does not provide clear answers in diagnostics, as no specific immunohistochemical markers have been identified that would reliably classify the tumor as an aggressive subtype of PTC [11; 12].

The 5th edition of the classification of thyroid tumors, published by the World Health Organization (WHO) in 2022, describes 13 subtypes (variants) of PTC. Five of these variants are designated as aggressive: diffuse sclerosing variant (PTC DSV), tall cell variant (PTC TCV), hobnail variant (PTC HV), columnar-celled variant (PTC CSV), and solid variant (PTC SV). The special status of these tumors is due to more frequent lymphogenous and hematogenous metastasis, increased frequency of radioiodine resistance, high relapse rates, and low survival rates compared to other PTC subtypes [13–15]. Papillary thyroid microcarcinomas (tumors smaller than 1.0 cm) are no longer considered as histological subtypes of PTC. These tumors remain debatable, whether they are the least

aggressive clinically, and whether they require active surveillance or surgical intervention in a short time [16; 17]. The various variants of papillary cancer, related to tumors with aggressive and non-aggressive clinical course (oxyphilic cell, PTC DSV, and PTC SV), namely, their pathomorphological characteristics and data on the clinical course, remains unclear [18–21].

The polymorphism of thyroid tumors is caused by various molecular disorders at the cellular level, which are presently well studied. Despite the large number of studies on the genetic basis of the tumor process in PTC, several questions remain, and thus, studies on the influence of molecular genetic disorders on the tumor process and its development and course are of interest [22–26]. The aggressive course of PTC was associated with a mutation in the $BRAF^{V600E}$ gene; however, no association was found between the expression of the $BRAF^{V600E}$ gene and aggressive course of PTC [27–29]. After studying the molecular genetic composition of PTC, a set of markers was identified, consisting of oncogenic and tumor-suppressing microRNAs. These markers are linked with a specific type of thyroid malignancy, particularly papillary and medullary thyroid cancer (MTC) [30–34]. However, to date, specific markers that can be determined in patients with aggressive PTC (both in cytological and morphological material) have not been determined. Thus, identifying these indicators would help in diagnosing aggressive PTC variants, provide a personalized approach when choosing treatment methods, and reduce the incidence of relapses and adverse outcomes in these patients.

This review describes the problems related to diagnostics of aggressive variants of thyroid cancer and outlines the immediate prospects for improving preoperative diagnosis.

AGGRESSIVE PTC VARIANTS

Diffuse sclerosing variant. PTC DSV occurs in approximately 6 % of PTC cases and was first described in 1985 [2; 15; 19]. Tumor growth is characterized by diffuse proliferation of tissue without the formation of a delimited formation. In most cases, tumor growth is manifested by diffuse damage to one lobe or the entire thyroid gland without the formation of nodes [3; 18; 19]. Compared to the classic variant of PTC (cPTC), PTC DSV has features and is characterized by a more frequent development against Hashimoto's thyroiditis, a younger age of patients, and a more frequent occurrence in women [35]. The mean age of patients is 30 years, with a median of 28 years and a range of 6–78 years. The women-to-men ratio is 4.3:1 [15; 19].

Moreover, this PTC variant has often been detected after exposure to high levels of ionizing radiation. In a study, 10 % of PTC after an accident at a Chernobyl nuclear power plant were represented by PTC DSV. This variant is characterized by a higher risk of extrathyroidal invasion and distant metastasis compared to cPTC (72.2 % vs. 56.3 %,

7.3 % vs. 4.3 %, respectively, $p < 0.001$) [2; 15]. Despite this aggressive behavior, the overall survival rate of this variant is similar to that of the classic type and is about 93 %. Cancer relapse and mortality were registered in 14 % and 3 % of cases, respectively, whereas distant metastases were detected in approximately 5 % of cases [11; 13]. Survival rate with this variant is 6 % lower compared to cPTC. Good overall survival rates in this group of patients are associated with a more radical surgical intervention and radioiodine therapy.

PTC DSV exhibits more aggressive clinical and pathological behavior and has a higher incidence of vascular invasion, extrathyroidal extension, lymphatic invasion and lymph node and distant metastases. Therefore, PTC DSV patients have a higher probability of relapse and worse overall survival [15].

The main histological characteristic is diffuse damage to one or both lobes of the thyroid gland with severe fibrosis/sclerosis, pronounced multifocal lymphocytic infiltration, extensive lymphovascular invasion, a large number of psammoma bodies, and the presence of squamous metaplasia [3; 11; 19]. Cytological diagnostics of this variant can be difficult. The presence of squamous cell differentiation in cytological material can lead away from the diagnosis of PTC to anaplastic carcinoma with squamous cell signs. Furthermore, Hashimoto's thyroiditis is noted in 85 % of cases, which complicates differential diagnostics with benign processes in the thyroid gland [8; 11; 30]. The studied immunohistochemical characteristics in PTC DSV are characterized by p63 (28.6 % of cases), p53 (42.9 %), Galectin-3 (83.7 %), and antigen-epithelial membranes (EMA) (40.8 %) expressions [11; 15].

The molecular profile varies and is characterized by the presence of RET/PTC and RET/PTC3 translocation with the $BRAF^{V600E}$ mutation. Changes in the RET gene are determined in patients at an advanced stage of the disease, characterized by poor clinical outcome, and often detected at a younger age. Additionally, BRAF mutation has been noted in PTC DSV, but with a lower incidence than in classic PTC [29]. This can be due to the accumulation of mutations and increased expression of the BRAF gene with age [33]. Thus, PTC DSV has different histopathological and molecular genetic profiles compared to cPTC.

Tall cell variant. PTC TCV was described in 1976; however, the morphological changes characteristic of this PTC variant have been mentioned in the literature since 1948. This variant is diagnosed in 3 %–19 % of cases. WHO [2; 11; 13] defines PTC TCV as a tumor that contains "cells that are 2–3 times as tall as they are wide" and have abundant eosinophilic (oncocyte-like) cytoplasm. Since such morphological changes are often present in conventional papillary carcinomas, tall cells should constitute ≥30 % of all tumor cells for PTC TCV to be diagnosed. Some pathologists recommend indicating the tall cell focus in the histological report, regardless of the percentage.

This PTC variant occurs in women 2.9 times more often and in approximately 1 %–19 % of cases of all PTC tumors. Furthermore, PTC TCV tumors are commonly large [13; 15]. The average age of patients with PTC TCV is usually higher than that of cPTC patients and ranges from 41 to 66 years [15; 35; 36]. Among the features, the higher relapse rate and lower survival rate than with cPTC (22 % and 8 %; 79 and 93 %, respectively) are noteworthy. This may be because of the fact that this subtype is registered in older patients and has a larger tumor size, and therefore, extrathyroidal invasion is more often diagnosed. When analyzing survival and relapse rates, it was revealed that PTC TCV and cPTC have similar results when performing total thyroidectomy and radioiodine therapy [37]. Moreover, if the tumor contains even 10 % of the highly cellular component, it is associated with a poor clinical outcome. Factors associated with poor clinical prognosis were significantly more common in cPTC patients with focal tall cell changes in the tumor and in patients with PTC TCV diagnosed based on WHO diagnostic criteria. Additionally, according to some studies, only tumors with more than 50 % of the tall cell component have a more aggressive course [11; 14; 29].

The proportion of the tall cell component that is clinically relevant remains controversial. In addition, it is crucial to emphasize that there is significant subjectivity and lack of agreement in identifying PTC TCV among pathologists [11; 15; 37], and therefore, whether PTC TCV is one of the stages of development of the tumor process or a more aggressive variant of PTC remains unclear.

In addition to the histologic presentation abovementioned, PTC TCV has a parallel arrangement of cells lining papillary and elongated follicular structures in histologic sections and produces a tram-track sign that, at low magnification, resembles a trabecular architecture. Most PTC TCV tumors are not encapsulated, but encapsulated tumors are rare. Other histologic criteria include eosinophilic cytoplasm, clear cell boundaries, and well-defined nuclear features of PTC. Some studies have defined the cytological features of PTC TCV that differentiate it from cPTC. The tall cell variant is characterized by elongated cells with oncocytic cytoplasm with clear boundaries; multiple intranuclear inclusions, which make them soap-bubble-shaped, prominent nucleoli; and higher lymphocytic infiltration [8; 9; 11].

Regarding the molecular genetic features of the tumor, $BRAF^{V600E}$ mutations occur in 80 %–100 % of all PTC TCV cases. The incidence of BRAF mutations is higher when the tumor contains more than 50 % of the tall cell component. Further, there is a secondary mutation (usually in the TERT promoter gene) and possible RET/PTC and RET/PTC3 translocation, which is two times more common compared to cPTC [28; 31–32; 38]. This variant of PTC is also characterized by miR-21 expression [39].

Columnar-celled variant. PTC CSV is a rare DTC subtype, which accounts for 0.15 %–0.2 % of all PTC [15; 35]. A characteristic clinical sign of this variant is the rapid rate of tumor growth associated with extrathyroidal invasion and early development of lymphogenous metastasis and a high relapse rate [40]. This aggressive variant may not respond to $_{131}$I RIT [42]. The WHO classification described PTC CSV as "a typically multicellular neoplasm with papillary or glandular spaces lined with pseudostratified epithelium, with the presence of vacuolization or even cytoplasm without inclusions in the cells" [13; 14]. The immunohistochemical presentation in this variant has been relatively well-studied. This variant has increased mitotic activity and the Ki67 index is ≥20 %; indicators such as TTF1, thyroglobulin, cyclin D1, bcl-2, and membrane β-catenin are positive, and the expression of estrogen and progesterone is increased, regardless of the patient's gender, and may also be CDX2 positive (up to 55 % of cases) [8; 11; 15; 41]. PTC CSV often presents as a nodular formation or as a massive nonmoving tumor. Examination often reveals a tumor formation in the neck without any clinical manifestations [35; 42]. The average age of patients ranges from 34 to 49 years.

When diagnosing PTC CSV, extrathyroidal tumor spread and lymphogenous and hematogenous metastasis are commonly detected, and lower overall survival rate is registered compared to cPTC. The average survival rate is lower, and the mortality rate is eight times higher compared to cPTC. In some studies, tumors of patients of this subtype were classified into encapsulated minimally invasive and extensively invasive. Encapsulated tumors were identified predominantly in young women, and extensively invasive tumors were determined in older patients with an almost equal sex ratio. Encapsulated tumors were less aggressive, whereas extensively invasive tumors had poor outcomes. Literature data show that the columnar-celled variant, in the presence of a capsule and absence of extrathyroidal invasion, has results similar to cPTC [43]. In this regard, for risk stratification and choice of treatment approach, it is crucial to identify these morphological structure features of the tumor.

The histological characteristics of PTC CSV are determined by the presence of a significant number of columnar cells with pseudostratified nuclei. Nuclear changes, such as pseudoinclusions and grooves, characteristic of cPTC tumor cells, are not as well-developed in PTC TCV. The morphological presentation of PTC CSV can mimic adenocarcinoma of the gastrointestinal tract and endometrium. There is currently no consensus on the percentage of columnar cells required to make a diagnosis. However, according to various authors, it ranges from 30 % to 80 % [10; 11; 15; 28].

There are several cytomorphological features common to all PTC CSV tumors, namely, high cellularity, the presence of papillary structures, medium to large cell size, single cells, and pseudostratified nuclei

(nuclei condensation). Other characteristics noted in >50 % of cases include the presence of colloid, elongated cells, dark or densely packed chromatin, absence or presence of mild nuclear atypia, inconspicuous nucleoli, and absence of intranuclear inclusions. Based on these data, cytological signs that may be diagnostic have been identified, namely, the presence of a hypercellular smear consisting of papillary structures and scattered single cells without necrosis; presence of crowded cells and pseudostratified nuclei with dark chromatin without atypia, nucleoli, and mitosis; rare/absent nuclear pseudoinclusions, and predominantly rare/absent nuclear grooves [8; 9].

Using IHC, these tumors show variable expression of thyroglobulin but consistent TTF1 expression [41].

In the differential diagnostics of TCV and PTC CSV, the latter is characterized by the absence of light eosinophilic cytoplasm and thin cell boundaries and intranuclear pseudoinclusions and nuclear grooves [15; 41]. Encapsulated PTC CSV should be differentiated from the cribriform-morular variant of DTC, as the latter is associated with the presence of familial adenomatous polyposis. The columnar-celled variant usually lacks the flat morulae typical of the cribriform-morular variant and does not show nuclear expression of β-catenin [14; 15].

The incidence of $BRAF^{V600E}$ gene mutation in patients with PTC CSV is 33 %. The molecular genetic structure of this tumor variant has not been sufficiently studied owing to its rare occurrence and small number of studies [2; 15; 44].

Solid (solid-trabecular) variant. Described in 1985, PTC SV represents 3 % of all PTC tumors [2; 11]. This variant was initially associated with young age, RET/PTC3 translocation, and radiation exposure. However, because of its low specificity, exposure to ionizing radiation cannot be considered a defining diagnostic criterion [15; 22].

PTC SV is associated with a higher risk of metastasis and a worse prognosis than cPTC [13; 18] and is characterized by areas of solid and/or trabecular growth [11; 12; 14]. However, the term "solid variant" should only be used when all or almost all of the tumor has a solid, trabecular, or nidulant (insular) appearance. This tumor variant is often differentiated from follicular cancer, which has a solid component [21] and poorly differentiated thyroid carcinoma (PDTC). One-third of patients have vascular invasion and extrathyroidal invasion [4; 47]. Treatment outcomes and mortality and risk of relapse rates in patients with PTC SV are comparable to those in cPTC patients. These data and the rare occurrence and insufficient knowledge of this subtype indicate uncertainty about the aggressiveness of this variant.

A problem when trying to diagnose PTC SV is the lack of consensus among pathologists regarding what percentage with a solid component of the tumor as a diagnostic criterion is critical to diagnose PTC SV [11; 12; 15]. It is crucial to differ-

entiate PTC SV from PDTC, because the latter has a much lower survival rate. Although they share a common growth pattern, the solid variant is characterized by morphological changes in cell nuclei characteristic of cPTC and lacks the high mitotic activity and signs of tumor necrosis characteristic of PDTC.

In this PTC variant, RET/PTC3 translocation is usually noted. Moreover, a new BRAF mutation has been identified, namely, a triplet deletion leading to the replacement of valine and lysine with glutamate ($BRAF^{V600E}$+K601) [15; 27].

Hobnail variant. PTC HV was described as a moderately differentiated variant of PTC with aggressive behavior and higher mortality rates compared to cPTC. Further, PTC with such a morphological structure is associated with an increased risk of relapse of thyroid cancer [2; 15; 45]. This variant is characterized by cells with apically located tumor nuclei protruding above the epithelial surface, a micropapillary growth pattern, and a high nuclear/cytoplasmic ratio. According to the WHO classification of endocrine tumors, for a tumor to be classified as "hobnail" type, cells with the appropriate morphological structure should make up at least 30 % of the tumor [8; 11; 12]. According to several studies, the average age of patients with this variant is 57 years. The disease is typically manifested by a space-occupying lesion in the neck and enlargement of the cervical lymph nodes [6; 15]. Vascular invasion was detected in 70.8 % of cases, extrathyroidal spread in 58.3 %, and metastases to the lymph nodes in up to 75 % [15; 28; 45]. Additionally, Hobnail-type cells are noted in PDTC, which may be a sign of evolutionary transformation of cPTC into a tumor with more malignant potential. PTC HV is associated with more aggressive behavior and refractoriness to radioiodine therapy, rapid disease progression, and a higher mortality rate compared to classic PTC [15; 35]. PTC HV responded to therapy with $_{131}$I radioactive iodine in 33.3 % of cases.

The presence of cells with a high nuclear/cytoplasmic ratio and apical nuclei, sometimes with grooves that form a bulge on the surface, explains the term "hobnail" and is a pathological criterion for establishing the diagnosis. Hobnail cells can vary in size and shape, from small lymphocyte-like cells to larger cuboidal cells and tall/columnar cells. This is associated with loss of cell polarity and indicates epithelial–mesenchymal transformation as a possible mechanism of metastasis [9; 11; 45].

When using IHC, PTC HV actively expresses thyroglobulin, TTF1, and EMA. Overexpression of p53 protein was noted in 77 % of cases. Moreover, PTC HV contains cytokeratin 7, cytokeratin 19, and HBME-1, and the average index of proliferative activity Ki67 is approximately 10 %, which indicates a high rate of mitosis [2; 46]. Classic PTC may have hobnail-type cells in a significant portion of the tumor and remain slowly progressive. However, these cells differ from PTC HV in the absence of other aggressive histological signs, such as high

mitotic rates and extrathyroidal invasion. Thus, the aggressiveness of this variant and whether it is caused by a high rate of mitosis and the presence of widespread invasion or the presence of typical hobnail-type cells remains unclear [12; 14; 15].

The PTC HV molecular genetic structure is characterized by a mutation in the BRAF gene in approximately 57.1 % of cases. In a molecular genetic study, 80 % of tumors are positive for $BRAF^{V600E}$ mutation, whereas 20 % are associated with the RET/PTC1 mutation [13; 15; 30]. Furthermore, the most common genetic changes in these tumors are found to be BRAF and TP53 mutations (72.2 % and 55.6 %, respectively), followed in frequency by hTERT (44.4 %), PIK3CA (27.8 %), CTNNB1 (16.7 %), EGFR (11.1 %), AKT1 (5.5 %), and NOTCH1 (5.5 %). The structure of mutations remains unchanged in the primary tumor and metastases [32; 40; 46].

Treatment

According to clinical guidelines for DTC treatment, the treatment of all histological PTC variants is standardized and includes hemithyroidectomy for single solitary tumors T1-T2 and total thyroidectomy with cervical lymph node dissection for confirmed metastatic lesions of the cervical lymph nodes or prophylactic central lymph node dissection for tumors T3 and T4. If the patient is in a moderate or high-risk group or a low-risk group with an uncertain tumor status, radioiodine therapy is indicated [7].

PTC DSV, which is more common in children and young patients, is prone to increased $_{131}I$ accumulation, and even patients with distant metastases have a good prognosis. In contrast, other variants, which mainly affect elderly patients and have signs of extrathyroidal invasion and a high rate of local recurrence, are refractory to RIT and have a poor prognosis [15; 35].

In PTC TCV, approximately 20 % of these tumors should be considered refractory to radioiodine therapy. There is evidence that the morphological features of PTC TCV indicate a higher incidence of extrathyroidal invasion and distant metastases, which correlates with an increased frequency of relapses and a worse prognosis of PTC TCV compared with cPTC. Thus, more radical treatment is recommended, especially in the early stages of the disease. Moreover, 20 % of PTC TCV cases are represented by tumors with extensive extrathyroidal extension into adjacent fibroadipose tissue and/or skeletal muscle. However, with all the known data, there is no consensus regarding the volume of surgical intervention for PTC TCV, which is considered optimal as regards surgical aggression and radical according to oncological principles. This also concerns patients whose tall cell component does not reach the diagnostic threshold during histological examination [2; 15; 48].

Regarding PTC CSV, it is crucial to identify encapsulated cases as they have a better outcome. In relation to PTC SV, some authors recommend that treatment should

be more radical with active postoperative follow-up, because this tumor type has a higher rate of vascular invasion and relapse than cPTC [47].

Based on the literature and biological behavior of all PTC variants abovementioned, higher stage tumors with extensive extrathyroidal invasion or lymphatic and/or distant metastases are characterized by greater aggressiveness and worse outcome. However, the decision on the extent of thyroid resection is based on the tumor size at the time of admission, and not on the histological PTC type [26; 31; 34; 49].

RESULTS AND DISCUSSION

Classic PTC is characterized by a slow clinical course with a 10-year survival rate of > 93 %–95 % [2]. Contemporary views place aggressive PTC variants on a spectrum between low-grade PTC and PDTC/anaplastic thyroid carcinoma. The aggressive clinical course of these tumors is usually associated with large tumor sizes that have extrathyroidal invasion or lymphogenous or distant metastases. Such aggressive behavior often occurs in the absence of extrathyroidal tumor invasion and in the small tumor node located within the thyroid tissue. The rarity of these tumors and poor understanding of their biology and molecular genetic structure can lead to inadequate treatment, even when standard protocols are followed. The determining markers of aggressiveness at the stage of preoperative diagnostics of these tumors will help provide a differentiated choice of treatment approach and improve the prognosis for these patients.

The diagnosis of aggressive PTC can be confirmed using FNAB in extremely rare cases. However, these cytological reports should be interpreted with caution, as the FNAB sample may not be representative of the entire lesion because the diagnostic criteria for some variants should reach certain threshold values of the percentage of tumor morphological structure [8]. In almost all cases, aggressive PTC variants are diagnosed after surgery. Therefore, pathologists and surgeons should be aware of the effects of these variants on risk stratification of PTC patients.

Certainly, each of these aggressive PTC variants has a worse outcome than classic PTC. The prognostic value of these variants when stratified by stage or other aggressive histological features, such as mitotic rate and degree of invasion, should be clarified. This has been elucidated for the rarest aggressive variant – PTC CSV. The encapsulated form of PTC CSV is characterized by an indolent course, whereas the widely invasive form is very aggressive [15; 43]. Thus, it is encapsulation, and not the presence of columnar cells, that is a feature of PTC CSV that determines the treatment outcome and prognosis for this tumor variant. Unfortunately, there is no clear approach to the treatment of other aggressive variants, especially PTC TCV. However, there is evidence that in the absence of invasive signs, TCV, CSV, and PTC DSV have overall survival

rates similar to that of cPTC, and some studies have shown that the presence of tall cells does not worsen prognosis [47].

Given this uncertainty regarding the aggressiveness of PTC variants, it is critical to consider validated prognostic features such as tumor size, extent of extrathyroidal invasion, and presence of vascular invasion when deciding on the extent of surgery or the need for adjuvant radioiodine therapy. Unfortunately, recently there are frequent situations when extrathyroidal invasion is detected microscopically based on the results of postoperative histological examination. These results do not comply with the fundamental oncological principles of complete excision of the tumor with no growth at the resection margin, which should remain the gold standard for surgical treatment of thyroid cancer.

Thanks to the study of the molecular genetic structure of DTC, new opportunities appear in the early diagnostics of aggressive PTC variants [25–27], which will personalize the approach to treating patients at the preoperative stage and will reduce the risk of relapse and reduced survival rate.

Identification of new molecular markers will improve preoperative diagnostics and develop new treatment algorithms to determine the extent of surgical intervention that will be radical and provide a sufficient level of quality of life for PTC patients. To date, a small number of trials focused on studying this problem [24; 29–32]; however, the results of these studies in the future may improve the prognostic accuracy of diagnostics and the detection of aggressive PTC variants.

Conclusions

Diagnosing aggressive PTC variants is challenging for both clinicians and experts in related specialties (cytologists and pathologists). The problems of diagnosing this spectrum of tumors remain relevant, because when treating these tumors, the result and prognosis are worse than with the classic variant of PTC. Unfortunately, even with pathomorphological examination, we are faced with challenges in determining the criteria for aggressiveness in these tumors, which poses a number of questions for specialists. The molecular genetic structure of DTC is based on determining the presence of mutations in the BRAF, TERT, and RAS genes. The presence of these mutations underlies the modern classification of thyroid tumors. The ambiguous results of studies comparing the presence of these mutations and aggressiveness of thyroid tumors do not allow them to be accepted as a diagnostic criterion for the aggressiveness of a PTC. Diagnostics uses methods for determining the level of micoRNA expression, which enable the diagnosis of PTC and MTC and are highly specific and accurate. Moreover, markers characteristic of aggressive PTC variants have not been sufficiently studied, and their presence requires clarification.

Thus, the volume of required surgical treatment and radioiodine therapy in PTC

patients should be determined individually, considering all the data, using molecular genetic diagnostic methods if possible.

According to clinical guidelines for DTC treatment, at present, the preoperative diagnostics of thyroid tumors does not include molecular genetic testing of cytological material. In the future, to improve preoperative diagnostics and determine the treatment approach, the use of molecular genetic panels to identify mutations associated with the development of DTC should be investigated. Accumulated evidence suggests the presence of mutations and increased expression of several microRNAs characteristic of PTC tumors. Existing data on molecular genetic test systems do not provide information on markers of aggressive subtypes of PTC. Research in this direction may provide new opportunities for targeted treatment methods for aggressive PTC variants. Moreover, this will help improve the preoperative diagnostics of aggressive PTC variants, the results of which will allow individual planning and improvement of treatment outcomes for PTC patients.

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A METHOD OF DIAGNOSING NON-ALCOHOLIC FATTY LIVER DISEASE WITH THE CALCULATION OF THE STEATOSIS INDEX

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СПОСОБ ДИАГНОСТИКИ НЕАЛКОГОЛЬНОЙ ЖИРОВОЙ БОЛЕЗНИ ПЕЧЕНИ С РАСЧЕТОМ ИНДЕКСА СТЕАТОЗА

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Objective. To work out the index for diagnosing non-alcoholic fatty liver disease (NAFLD) in women with metabolic syndrome (MS) in postmenopause using generally available markers.

Materials and methods. 62 females with NAFLD and MS in early postreproductive period took part in the study. They were compared to 24 relatively healthy females not suffering from obesity in postmenopause. The average age of the patients was 49,9 ± 1,1. Hepatic steatosis was diagnosed by ultrasound examination. The

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mathematic model of hepatic steatosis index (HSI) calculation included: body mass index (BMI), waist size (WS), triglycerides and low-density lipoproteins (LDL).

Results. BMI and WS in patients with MS and NAFLD in postmenopause were considerably more than in relatively healthy women. Hypertriglyceridemia and an increase of LDL in blood serum were noted in patients with steatosis. Biometric and laboratory data were included in the mathematical formula which allows to calculate HSI. With HSI 0,5 and more NAFLD is diagnosed in women in post menopause, when HSI is lower than 0,5 it is not diagnosed. Indicators of sensitivity and specificity of the method were 98,4 % and 95,85 % respectively.

Conclusions. The minimally invasive method suggested above allows to reveal hepatic steatosis in females with MS in early postreproductive period. High diagnostic characteristics of the method are worth mentioning, as well as the possibility to be widely used thanks to simple biometric and laboratory data employed.

Keywords. Non-alcoholic fatty liver disease, hepatic steatosis, steatosis index, menopause.

Цель. Разработать индекс для диагностики неалкогольной жировой болезни печени (НАЖБП) у женщин с метаболическим синдромом (МС) в постменопаузе с использованием общедоступных маркеров.

Материалы и методы. В исследовании приняли участие 62 пациентки с НАЖБП и МС в раннем пострепродуктивном периоде, средний возраст 49,9 ± 1,1 года, и 24 относительно здоровые женщин без ожирения в постменопаузе, которые составили группу сравнения. Диагноз стеатоза печени был поставлен методом ультразвукового исследования. В математическую модель расчета индекса стеатоза печени (ИСП) печени были включены следующие параметры: индекс массы тела (ИМТ), объем талии (ОТ), триглицериды (ТГ) и липопротеиды низкой плотности (ЛПНП).

Результаты. ИМТ и ОТ у пациенток с МС и НАЖБП в постменопаузе были значимо выше, чем в группе сравнения ($p = 0{,}001$ и $p = 0{,}001$ соответственно). Также у женщин со стеатозом регистрировалась гипертриглицеридемия и повышение ЛПНП в сыворотке крови. Исследуемые биометрические и лабораторные показатели были включены в математическую формулу, позволяющую рассчитать ИСП. При значении ИСП, равном 0,5 и более, диагностируют наличие НАЖБП у женщин в постменопаузе, при ИСП менее 0,5 – отсутствие. Показатели чувствительности и специфичности метода составили 98,4 и 95,8 % соответственно.

Выводы. Предложенный малоинвазивный метод позволяет выявить стеатоз печени у женщин с МС в раннем пострепродуктивном периоде. Стоит отметить высокие диагностические характеристики предлагаемого метода и его доступность для широкого применения, так как используются простые биометрические и лабораторные показатели.

Ключевые слова. Неалкогольная жировая болезнь печени, стеатоз печени, индекс стеатоза, менопауза.

INTRODUCTION

Metabolic syndrome (MS) and associated nonalcoholic fatty liver disease (NAFLD) present pathologic manifestations and diagnosis that are in the area of interest of almost every therapeutic specialist. The presence of MS and/or obesity signs in a patient indicates a high NAFLD risk, and therefore, routine screening examination of this group of patients is recommended to identify NAFLD. Postmenopausal women, especially those not taking hormone replacement therapy, are at risk for NAFLD. Hypoestrogenism leads to obesity, which is recorded in more than half of

postmenopausal women [1–3]. Liver steatosis is detected in 60 %–75 % of patients aged 40–50 years with metabolic disorders. Some reports showed that postmenopausal women develop liver fibrosis faster than premenopausal women and men [4].

According to the clinical guidelines for the management of NAFLD patients, ultrasound is used for detecting liver steatosis [5; 6]. Ultrasound was found to detect steatosis in only 12 %–20 %, and its accuracy may decrease in morbidly obese patients [7]. Moreover, liver biopsy with a morphological study of the specimen may be conducted to diagnose steatosis. However, the use of this method in widespread clinical practice is limited owing to its invasiveness and risk of complications. Currently, noninvasive and minimally invasive diagnostic methods of NAFLD using laboratory markers are preferred [5; 8].

The development of new accessible diagnostic methods for NAFLD continues, which should facilitate screening for liver steatosis in patients primarily from risk groups including women of post-reproductive age.

The study aimed to develop an index for diagnosing NAFLD in postmenopausal women with MS using publicly available markers.

MATERIALS AND METHODS

The study included 62 post-reproductive patients with NAFLD and MS, with a mean age of 49.9 ± 1.1 years, and 24 relatively healthy nonobese postmenopausal women who comprised the comparison group. Written informed voluntary consent was obtained from all study participants. Liver steatosis was diagnosed by ultrasound.

The mathematical model for calculating the hepatic steatosis index (HSI) included the following parameters: body mass index (BMI), waist circumference (WC), triglyceride (TG) levels, and low-density lipoproteins (LDL). The blood serum concentrations of TG and LDL were determined using a Landwind LW C200i analyzer (Shenzhen Landwind Industry Co., Ltd., China) with kits from Vector-Best (Novosibirsk, Russia).

The constant and coefficients for this equation were calculated using the multiple regression method. The indicator of the presence of liver steatosis according to liver ultrasound was used as the dependent variable.

RESULTS AND DISCUSSION

BMI and WC in postmenopausal patients with MS and NAFLD were significantly higher than those in the comparison group ($p = 0.001$ and $p = 0.001$, respectively). Hypertriglyceridemia and increased LDL in the blood serum were recorded in women with steatosis. Various studies have reported dyslipidemia in 50 %–80 % of NAFLD patients [7; 9].

The studied biometric and laboratory indicators were included in the following equation used to calculate HSI[1]:

$$HSI = -1.4672 + 0.0096 \cdot BMI + \\ + 0.0151 \cdot WC + 0.0157 \cdot TG + 0.1406 \cdot LDL.$$

NAFLD is diagnosed in postmenopausal women when the HSI value is ≥0.5, whereas an HSI of <0.5 indicates that NAFLD is absent. The predictive value of each model parameter was assessed using the area under the ROC curve (AUC) scale (Figure).

The sensitivity and specificity of the method were 98.4 % and 95.8 %, respectively.

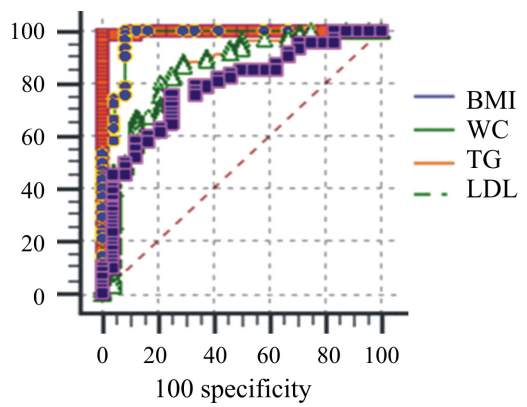

Fig. ROC curve for liver steatosis index scores

In recent decades, several tests (indices) for diagnosing NAFLD have been proposed, and biochemical, clinical, and biometric indicators are used for their calculation. For example, HSI is calculated using sex, presence/absence of diabetes, BMI, and transaminase ratio. The sensitivity and specificity of HSI are 93.1 % and 92.4 %, respectively. Another example is the fatty liver index (FLI), which includes TG and gamma glutamine transferase concentrations, BMI, and WC. If FLI is < 30, nonalcoholic hepatic steatosis is ruled out; an FLI between 30 and 60 indicates possible nonalcoholic hepatic steatosis; and an FLI >60 confirms nonalcoholic hepatic steatosis. The method has good diagnostic sensitivity (87 %), but low specificity (64 %). Furthermore, the SteatoScreen test has good diagnostic characteristics, and its calculation equation includes 10 blood parameters and biometric data, which limits its use in clinical practice [5; 6; 10]

The threshold values of the tests included in the mathematical model were determined to rule out NAFLD: 25.8 for BMI, 79 cm for WC, 0.95 mmol/l for TG, and 3.15 mmol/l for LDL. The proposed minimally invasive method can be used to detect liver steatosis in women of early post-reproductive age with MS. The high diagnostic characteristics and availability for widespread use of the proposed method should be noted, since simple biometric and laboratory indicators are used.

Conclusions

1. An index has been developed for diagnosing NAFLD in postmenopausal

[1] I.A. Bulatova, A.A. Sobol, I.L. Gulyaeva, and V.S. Sheludko, Patent of the Russian Federation No. 2785905, IPC G01N 33/573 (2022.08), A method for Diagnosing Non-Alcoholic Liver Steatosis in Menopausal Women, applicant and patent holder E.A. Wagner State Medical University of the Ministry of Health of the Russian Federation; application No. 2022125229; appl. 09/26/2022; publ. 12/14/2022.

women using publicly available markers (BMI, WC, TG, and LDL) and showed high diagnostic characteristics. The presence of NAFLD is confirmed in postmenopausal women when the HSI is ≥ 0.5 and its absence with HSI < 0.5.

2. The threshold values of tests included in the mathematical model were calculated to rule out NAFLD in this category of patients.

3. The use of the proposed minimally invasive and accessible method will ensure early detection of fatty changes in the liver in this risk group, enabling prompt treatment.

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PROSPECTS FOR INDUCTION OF LABOR WITH OXYTOCIN WITHIN 12 HOURS (PERINATAL CENTER EXPERIENCE)

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ПЕРСПЕКТИВЫ ПРОВЕДЕНИЯ ИНДУКЦИИ РОДОВ ОКСИТОЦИНОМ В ТЕЧЕНИЕ 12 ЧАСОВ (ОПЫТ ПЕРИНАТАЛЬНОГО ЦЕНТРА)

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Objective. Demonstrate the expediency and effectiveness of labor induction for more than 6 hours (up to 12 hours of the latent phase).

Materials and methods. A cohort, descriptive, single-center study was conducted. The effectiveness of labor induction with oxytocin for more than 6 hours was estimated. The birth medical histories of 3265 patients of SBHI of Sverdlovsk Region "Yekaterinburg Regional Perinatal Center" from January 2020 to December 2022, who underwent pre-induction and induction of labor, were analyzed.

Results. The group of patients, who underwent induction of labor with oxytocin, was 2261 cases. In 1269 (56.13 %) patients, vaginal delivery occurred after less than 6 hours of labor induction with oxytocin. In 992 patients (43.87 %) induction of labor with oxytocin was continued for more than 6 hours. Only 320 labors resulted in abdominal delivery due to ineffective labor induction, which amounted to 32.3 % (of the number of labor inductions with oxytocin for more than 6 hours) and 14.2 % (of the total number of labor inductions with oxytocin).

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Conclusions. Induction of labor with oxytocin for more than 6 hours (up to 12 hours of the latent phase) is reasonable and effective. It allows to avoid abdominal delivery in up to 30 % of patients of the study group.
Keywords. Induction of labor, oxytocin, latent phase.

Цель. Показать целесообразность и эффективность проведения индукции родов более 6 ч (до 12 ч латентной фазы).
Материалы и методы. Проведено когортное описательное одноцентровое исследование. Оценивалась эффективность осуществления индукции родов окситоцином более 6 ч. Были проанализированы истории родов 3265 пациенток ГБУЗ СО «ЕКПЦ» с января 2020 г. по декабрь 2022 г., которым проводилась преиндукция и индукция родов.
Результаты. Группа пациенток, подлежащих индукции родов окситоцином, составила 2261 случай. У 1269 (56,13 %) пациенток роды через естественные родовые пути произошли менее чем через 6 ч индукции родов окситоцином. 992 пациентки (43,87 %) продолжили индукцию родов окситоцином более 6 ч. Только 320 родов закончились абдоминальным родоразрешением в связи с неэффективной индукцией, что составило 32,3 % (от числа индукций родов окситоцином более 6 ч) и 14,2 % (от общего числа индукций родов окситоцином).
Выводы. Индукция родов окситоцином более 6 ч (до 12 ч латентной фазы) целесообразна и эффективна. Это позволяет избежать абдоминального родоразрешения до 30 % в исследуемой группе.
Ключевые слова. Индукция родов, окситоцин, латентная фаза.

INTRODUCTION

Induction of labor is a commonly used procedure in obstetrics. Induction of labor is typically performed for medical reasons when prolongation of pregnancy due to maternal illness or complications poses a high risk of adverse maternal and perinatal outcomes. In some cases, elective induction is performed (induction of labor at term without maternal or fetal medical indications, but with the aim to improve outcomes by selecting the optimal time and conditions for the best care)[1].

The use of labor induction is increasing worldwide, with a 6.8 %–35.5 % incidence rate in different countries. On the one hand, the increase is caused by the increasing number of women with high obstetric and perinatal risk, including somatic diseases (diabetes mellitus, obesity). On the other hand, the increase in the frequency of induction is due to improved outcomes for the mother and fetus, a beneficial effect on reducing the incidence of complications of pregnancy and cesarean section owing to the introduction of new technologies for cervical preparation and labor induction[2].

Induction of labor is widely used in obstetrics as a method of elective vaginal delivery in case of indications for delivery and the absence of spontaneous labor. However, induction of labor (compared to physiological labor) increases the risk of pathology both in the mother (cesarean section, bleeding, chorioamnionitis) and newborn (fetal distress during labor, asphyxia). Induction of labor is typically performed for medical reasons when prolongation of pregnancy due to maternal illness or complications poses a high risk of adverse maternal or perinatal outcomes [1].

The efficiency of induction and incidence of complications are determined by several factors:

[1] Failed Attempt at Labor Stimulation (cervical preparation and labor induction): clinical recommendations, Moscow (2021).

[2] Ibid.

1) Timing and indications for induction
2) Assessment of cervical maturity
3) Choice of induction method
4) Adequate dosage of drugs used for induction
5) Ineffectiveness of induction and timely change of delivery method
6) Intrapartum monitoring of the fetal condition [2]

Currently, there is no regulatory document that provides a clear definition of the time for induction of labor with oxytocin. In the Failed Attempt at Labor Stimulation clinical practice guideline (2021), the recommended timing of oxytocin administration is 5–15 hours until active labor is achieved. These frameworks are unclear and remain the prerogative of the medical institution.

The study aimed to demonstrate the feasibility and efficiency of induction of labor for more than 6 hours (up to 12 hours of latent phase).

MATERIALS AND METHODS

A cohort descriptive single-center study was conducted. We assessed the efficiency of induction of labor with oxytocin for more than 6 hours.

The birth histories of 3,265 patients of the Yekaterinburg Regional Perinatal Center who underwent pre-induction and induction of labor between January 2020 and December 2022 were analyzed. The studied cases were divided into two groups: group 1 included patients who underwent pre-induction of labor (cervix at the time of examination on the Bishop scale, < 8 points) and group 2 included patients who did not need preliminary preparation of the cervix (cervix at the time of examination on the Bishop scale, ≥8 points). In groups 1 and 2, 1,991 (61 %) and 1,274 (39 %) cases were analyzed, respectively (Fig. 1).

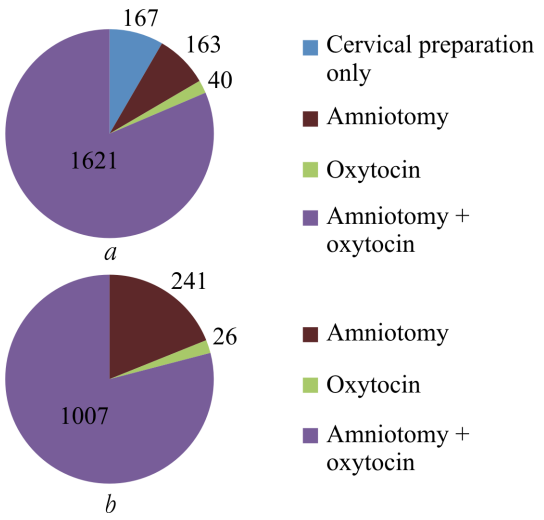

Fig. 1. Induction methods: a – group 1; b – group 2

Most patients received induction with oxytocin. In the Yekaterinburg Regional Perinatal Center, the induction of labor with oxytocin (low-dose infusion) was designed for 12 hours, subject to the satisfactory condition of the mother and fetus (continuous cardiotocography monitoring for all patients subject to induction of labor with oxytocin; continuous blood pressure monitoring for certain groups of patients and observing for hypertensive disorders and the presence of a scar on the uterus; continuous use of a uterine cardiotocograph sensor to assess the contractile activity of the uterus to prevent tachysystole).

If within 12 hours of induction of labor with oxytocin the patient reaches the active phase of labor (5 cm or more), induction is continued; oxytocin is not terminated in this situation. If after 12 hours the patient

does not reach the active phase of labor, induction is regarded as ineffective, and the labor management plan is revised in favor of abdominal delivery (cesarean section).

RESULTS AND DISCUSSION

We considered a group of female patients subject to labor induction with oxytocin, which enabled assessing more clearly the effectiveness of administering oxytocin for more than 6 hours. In this group, the birth histories of 2,261 patients (including 71 (3.1 %) with a uterine scar after cesarean section) were analyzed. The remaining 1,004 (among 3,265) patients had labor without the use of oxytocin (entered labor independently during pre-induction of labor or after induction of labor by amniotomy and those who required abdominal delivery before induction of labor with oxytocin).

In 1,269 (56.13 %) patients, vaginal delivery occurred after <6 hours of labor induction with oxytocin. If induction of labor with oxytocin had been stopped after 6 hours, the remaining 992 patients (43.87 %) would have required abdominal delivery according to the indication of labor induction with oxytocin without effect. In the present study, this group of patients continued induction of labor with oxytocin for > 6 hours.

Only 320 births ended in abdominal delivery because of ineffective induction of labor, accounting for 32.3 % of the number of inductions of labor with oxytocin for > 6 hours and 14.2 % of the total number of inductions of labor with oxytocin (Fig. 2).

Therefore, induction of labor with oxytocin for >6 hours helped to avoid abdominal delivery in 672 cases, which accounted for 29.7 % of the total number of inductions of labor with oxytocin, including in patients with a uterine scar.

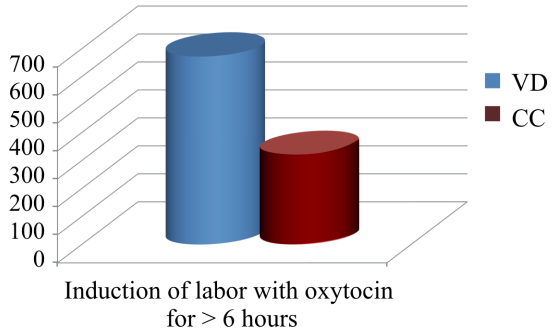

Fig. 2. Results of delivery after induction of labor with oxytocin for >6 hours (n = 992). Note: VD, vaginal delivery; CS, cesarean section

We conducted a comparative analysis of complications of outcomes of vaginal delivery in two groups of patients, in which group 1 received induction of labor with oxytocin for < 6 hours and group 2 received it for > 6 hours. The results are presented in Table 1.

Based on the analysis results, we can conclude that induction of labor for > 6 hours is accompanied by a significant increase in trauma to the birth canal, purulent-septic complications, and operative vaginal delivery. However, considering the percentage of vaginal delivery (67.7 %) in this group of patients, we recommend inducing labor with oxytocin for > 6 hours.

The use of labor induction is increasing worldwide, with a 6.8 %–35.5 % incidence rate in different countries[3]. For example, in the United States, the rate of labor induction

[1] Failed Attempt at Labor Stimulation (cervical preparation and labor induction): clinical recommendations, Moscow (2021).

Table 1

Comparative analysis of complications of vaginal delivery outcomes

Complications		Control group (n = 1,269) Oxytocin less than 6 hours n (%)	Study group (n = 672) Oxytocin more than 6 hours n (%)	p
Hemorrhage more than 1,000 ml (n)		103 (8.1)	84 (12.5)	0.002*
Severe neonatal asphyxia (n)		5 (0.4)	4 (0.6)	0.788
Shoulder dystocia (n)		11 (0.9)	6 (0.9)	0.844
Injuries (n) including by type:		645 (50.8)	465 (69.2)	< 0.001*
	Hysterocervicorrhesis (n)	103 (16.0)	86 (18.4)	0,307
	Perineal rupture degree 1–2 (n)	408 (63.3)	268 (57.7)	0,068
	Perineal rupture degree 3 (n)	6 (0.9)	4 (0.9)	0,760
	Perineal rupture degree 4 (n)	0 (0)	0 (0)	1,0
	Deep vaginal laceration (n)	50 (7.8)	31 (6.7)	0,570
	Episiotomy (n)	78 (12.0)	76 (16.3)	0,054
Purulent-septic complications (n) including by type:		7 (0.6)	16 (2.4)	< 0.001*
	Chorioamnionitis (n)	7 (100)	16 (100)	1,0
	Endometritis (n)	0 (0)	0 (0)	1,0
Operative vaginal delivery (n)		126 (9.9)	107 (15.9)	< 0.001*

Note: *, differences are significant.

increased from 9.6 % in 1990 to 25.7 % in 2018 [3]. This is due to the expansion of the list of indications for programmed childbirth and the latest research data that elective induction of labor at week 39 compared with expectant management (until a gestational age of more than 41 weeks) significantly reduced the risk of delivery by cesarean section (13.9 % vs 35.9 %), maternal morbidity (16.5 % vs 21.2 %), stillbirth (0 % vs 0.13 %), risk of maternal infection (2.8 % vs 5.2 %), and risk of transfer of newborns to the intensive care unit (3.5 % vs 5.5 %) [4–5]. Currently, the UK clinical guidelines state that requests for induction of labor can be considered only after discussing the benefits and risks with the woman, taking into account her circumstances and preferences [6].

Considering all the abovementioned, crucial questions about the duration of labor induction with oxytocin and at what stage it should be considered ineffective arise. According to the "Failed Attempt at Labor Stimulation" clinical guidelines (2021), the lack of effect of oxytocin administration is manifested by the absence of labor and dynamics of cervical dilatation within 3–5 hours or the inability to achieve the active phase of labor within 5–15 hours. The definition is relatively vague and the time frame is extremely broad, especially because the latent phase of labor is significantly longer in induced labor compared to spontaneous labor, and the diagnosis of "no effect up to 6 cm" in women undergoing induction should be made with caution (Table 2). During the active phase of stage 1 of labor, no significant difference was detected [7].

Table 2

Comparative study results

Parameter	Value							
Dilatation, cm	4–10	3–4	4–5	5–6	6–7	7–8	8–9	9–10
Induction	5.5	1.4	1.3	0.6	0.4	0.2	0.2	0.3
Spontaneous delivery	3.8	0.4	0.5	0.4	0.3	0.3	0.2	0.3

Based on the study results, we believe that it is reasonable not to consider induction of labor ineffective in the latent phase until oxytocin is administered at least 12 hours after membrane rupture [1].

Conclusions

Induction of labor with oxytocin for > 6 hours (up to 12 hours of the latent phase) is appropriate and effective. It was beneficial for avoiding abdominal delivery in up to 30 % of the study patients, which, in turn, prevented a further increase in the number of patients with a uterine scar after cesarean section.

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ANALYSIS OF HARDWARE METHODS OF TREATMENT OF PATIENTS WITH TEMPOROMANDIBULAR JOINT DYSFUNCTION

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АНАЛИЗ АППАРАТНЫХ МЕТОДОВ ЛЕЧЕНИЯ ПАЦИЕНТОВ С ДИСФУНКЦИЕЙ ВИСОЧНО-НИЖНЕЧЕЛЮСТНОГО СУСТАВА

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Objective. To conduct a comparative analysis of effectiveness of treatment of patients with dysfunction of temporomandibular joint (DTMJ) using hardware methods of treatment with occlusive mouthguards of various types.
Materials and methods. 99 patients (88 females and 11 males aged 41,2 ± 10,7) with different combinations of revealed and confirmed signs of DTMJ were examined from 2013 to 2023. According to the personal clinical picture, complaints revealed, aetiology and pathogenesis of the disease all the patients of the comparison group and 2 main groups (99 patients) were administered individual complex treatment for DTMJ. The treatment was aimed at the elimination of pain, relief of masticatory muscles spasm, improvement of the extent of opening of mouth, correction of the lower jaw position, occlusive interference elimination and restoration of height of the lower third of the face.
While carrying out the hardware treatment, occlusive mouthguards of 3 types were made based on the jaw model of the patients. The models were got from the silicone imprints of the jaw.
Results. MR images of temporomandibular joints of the 99 patients showed that ventral dislocation of articular disk occurred most frequently (in 88 patients). Less frequently (4 % of cases) it was combined with dislocation of the disk laterally. Rarely distal dislocation of the articular disk occurred – 1,9 % of cases (2 patients). Study of the results of the appliance of different types of occlusive mouthguards demonstrates the necessity to work out the types of occlusive devices allowing decompression of temporomandibular joint elements, centering lower jaw position and orthodontic elimination of dentofacial abnormalities and deformities which lead to occlusive interference.
Conclusions. Analysis of occlusive devices used in the diagnosis and treatment of patients with DTMJ shows that the most effective devices are those that successfully combine elements of splints with a narrower purpose.
Keywords. Dysfunction of temporomandibular joint, occlusal splint, occlusive mouthguards, splints, orthotics, occlusal abnormalities.

Цель. Провести сравнительный анализ эффективности лечения пациентов с дисфункцией височно-нижнечелюстного сустава (ДВНЧС) с применением аппаратурных методов лечения окклюзионными каппами различных видов. Высокая распространенность среди стоматологических заболеваний дисфункций височно-нижнечелюстного сустава (ДВНЧС) обусловливает необходимость совершенствования имеющихся и создание инновационных методов лечения. Высокая корреляция ДВНЧС с нарушениями смыкания зубных рядов обусловлена высокой встречаемостью аномалий, деформаций зубных рядов, а также частичной потери зубов, дефектов твердых тканей среди пациентов разных возрастных

групп и гендерной принадлежности. Среди консервативных методов, входящих в комплексный подход лечения пациентов, страдающих ДВНЧС, особое место занимают аппаратурные методы лечения.
Специалистами предложены различные виды ортопедических конструкций: сплинты, окклюзионные шины, окклюзионные каппы, ортотики и другие. Все виды лечебно-диагностических аппаратов имеют конструктивные сходства и отличия, выполняются из различных стоматологических материалов, могут быть отнесены к одному из видов: разобщающие, центрирующие (репозиционные), релаксационные, стабилизирующие шины.
Материалы и методы. В период с 2013 по 2023 г. обследовано 99 пациентов с различными комбинациями выявленных и подтвержденных признаков ДВНЧС, с распределением по гендерному признаку – 88 женщин, 11 мужчин (средний возраст 41,2 ± 10,7 года). В соответствии с индивидуальной клинической картиной, выявленными жалобами, этиологией и патогенезом заболевания всем пациентам группы сравнения и двух основных групп ($n = 99$) назначалась индивидуальная тактика комплексного лечения ДВНЧС. Лечение было направлено на устранение болевого синдрома, снятие спазма жевательных мышц, нормализацию объема открывания рта, нормализацию положения нижней челюсти относительно верхней, устранение окклюзионных интерференций, восстановление высоты нижней трети лица.
При проведении аппаратурного лечения всем пациентам по моделям челюстей, полученным по силиконовым оттискам, по показаниям изготавливали окклюзионные каппы трех видов.
Результаты. Данные анализа МРТ ВНЧС 99 обследованных показали, что наиболее часто (у 88 обследованных из 99) встречается вентральная дислокация суставного диска – в 88,8 % случаев, и реже в комбинации со смещением диска латерально 4 %. Редко встречается дистальный сдвиг суставного диска – 1,9 % (2 пациента из 99). Изучение результатов применения различных видов окклюзионных капп позволяет сделать вывод о необходимости разработки видов окклюзионных аппаратов, сочетающих в себе возможности декомпрессии элементов ВНЧС, центрирования положения нижней челюсти относительно верхней и ортдонтического устранения зубочелюстных аномалий и деформаций, являющихся причинами окклюзионных интерференций.
Выводы. Анализ окклюзионных аппаратов, применяемых при диагностике и лечении пациентов с ДВНЧС показывает, что наиболее эффективными являются аппараты, удачно сочетающие в себе элементы шин с более узким назначением.
Ключевые слова. Дисфункция височно-нижнечелюстного сустава, окклюзионные шины, окклюзионные каппы, сплинты, ортотики, окклюзионные нарушения.

INTRODUCTION

Orthopedic structures that provide a disconnecting, centering (repositioning occlusal splints), relaxing, and stabilizing effect are commonly used in the complex treatment of dysfunction of the temporomandibular joint (DTMJ) [1–8]. Among the types of occlusal splints, importance is given to orthotics, which are devices commonly developed for the lower jaw, having on their surface imprints of the chewing surface of antagonist teeth made of silicone. Such an occlusal device allows for separation of the dentition, thereby achieving decompression in the temporomandibular joint (TMJ) and masticatory muscle relaxation and centering the position of the lower jaw relative to the skull. However, this design of the occlusal apparatus does not allow normalizing the shape of the dental arches and the position of the teeth in the dental arch and does not eliminate occlusal interference [9–16].

In the practice of orthodontists, along with the use of edgewise techniques, an in-

novative method of treating anomalies, dental deformities, and occlusal interferences has become widespread, namely, the use of removable aligners. Several specialists consider occlusal interference as a cause of DTMJ, which, in turn, is caused by anomalies and deformities of the dentition [17–20]. Hence, orthodontic treatment is considered for preventing and treating DTMJ. However, with a narrow approach to correcting deformities and anomalies of the dentition, without proper comprehensive functional diagnostics of the state of the TMJ and masticatory muscles, normalization of the dentition occlusion do not always lead to spontaneous compensation of the morphological, functional, and combined changes in the TMJ and masticatory muscles [21–24].

This study aimed to conduct a comparative analysis of the efficiency of DTMJ treatment using instrumental treatment methods.

Materials and methods

In 2013 to 2023, we examined 99 patients with various combinations of identified and confirmed signs of DTMJ, distributed by sex (88 women, 11 men; average age: 41.2 ± 10.7 years).

The inclusion criteria were complaints of pain, crunching, jamming, clicking, and stiffness in the TMJ. In accordance with ICD-10, patients were diagnosed with temporomandibular pain-dysfunction syndrome (Costen's syndrome; K07.60), recurrent dislocation or subluxation of the lower jaw (K07.62), arthrosis of the TMJ (M19.0X), and clicking jaw (K07.61).

Exclusion criteria were lack of patient consent to participate in the study, cancer, acute stage or exacerbation of a chronic general somatic disease, history of trauma to the skull and maxillofacial area, pregnancy, and lactation.

Using free randomization, patients were distributed to a comparison group with 33 people (28 women, 5 men) and two main groups with 33 patients each.

All patients included in the study underwent comprehensive and clinical examinations, an interview, obtaining diagnostic casts and models of the jaws, and MRI of the TMJ.

Magnetic resonance imaging (MRI) of the TMJ was performed in MRI laboratories in Stavropol, with an MRI scanner power of at least 1.5 Tesla. The study was performed with the patient's mouth closed and open (with fixation with an individual mouthguard), in T1, T2, and PD modes and in oblique sagittal, frontal, and horizontal views.

In accordance with the individual clinical presentation, identified complaints, etiology, and pathogenesis of the disease, individual approach for complex DTMJ treatment was prescribed to all patients in the comparison group and two working groups (n = 99). Treatment aimed to eliminate pain, relieve masticatory muscle spasms, normalize the volume of mouth opening, normalize the position of the lower jaw relative to the upper jaw, eliminate occlusal

interference, and restore the height of the lower third of the face.

For this purpose, drug treatment was used (nonsteroidal anti-inflammatory drugs, painkillers, chondrotropic drugs), and massage of the masticatory muscles, myogymnastics, and instrumental treatment were prescribed. When performing hardware treatment, occlusive mouthguards were made for all patients based on jaw models obtained from silicone imprints.

The main group 1 included 33 patients (3 men, 30 women) whose complex treatment included using an orthotic device. An orthotic for the lower jaw was created from dental silicone for mouthguards and had imprints of the teeth of the upper jaw, allowing the lower jaw to be directed to a centric position when closing the jaws when closing the mouth, which corresponded to the centric relation (CR) of the jaws, determined by analyzing the TMJ MRI of each patient.

The orthotic was made by vacuum pressing according to the model of the lower jaw. The amount of separation of the dentition (thickness of the orthotic) was determined by analyzing the TMJ MRI based on the articular disc thickness criterion. The orthotic was used daily, for 2–3 hours during the day and all night.

The main group 2 included 33 patients (3 men, 30 women), whose complex treatment included orthodontic aligners to eliminate occlusal interference associated with dentition anomalies and deformities. When planning orthodontic treatment, special locking elements (pads) were introduced into the design of the aligners in the software, allowing the centering and stabilization of the lower jaw position relative to the upper jaw in accordance with individual data obtained from TMJ MRI analysis.

The planning, design, and production of aligners were performed in specialized laboratories using silicone imprints of the patients' upper and lower jaws. The CR of the jaws was determined using an anatomical and functional method, and registration was performed using occlusal silicone.

The aligners were used for 20–22 hours per day, with breaks for meals and hygiene procedures; each pair of aligners was replaced with the next set every 2 weeks.

Group 3 (comparison group) included 33 patients (28 women, 5 men) whose complex treatment of DTMJ, in addition to drug treatment according to clinical indications, selective grinding of occlusal interferences, normalization of the volume of mouth opening, myogymnastics, and massage of the masticatory muscles, involved using a 0.2-mm-thick dental mouthguard, made by vacuum thermopressing according to the model of the lower jaw to provide a placebo effect. The occlusal surface of the mouthguard wherein the antagonist teeth occlude was perforated to eliminate barriers and distortions of the usual occlusal contacts to ensure greater efficiency of the placebo effect.

All participants were asked to read and sign an informed consent to participate in a scientific study, on a mutually free basis,

providing information about the possible use of the placebo effect in the treatment plan through random randomization. Patients who refused to sign the informed consent under the specified conditions were excluded, and the results of the examination and treatment of these patients were not used in data processing.

Statistical processing of the data obtained as a result of the study was performed using Statistica 8.0 software. To assess the type of distribution of characteristics, the Shapiro – Wilks criterion was used. Values were presented as $M \pm SE$, where M is the sample mean and SE is the standard error of the mean. The significant differences in the average values of independent samples were assessed using the nonparametric Mann–Whitney U-test. In the case of normal distribution, the paired Student's T-test was utilized to compare the samples. When comparing several groups with each other, the Bonferroni correction was applied. In statistical analysis, the achieved level of significance (p) was considered, and the critical level of significance was equal to 0.05.

RESULTS AND DISCUSSION

During a clinical examination of patients and analysis of diagnostic models of the jaws of all 99 patients included in this study, various anomalies and deformities of the dentition and dental occlusion were identified in 29.3 % of cases (29 patients).

Dental anomalies and deformities were detected in 30.3 % of cases in group 1 (10 patients), in 27.2 % of cases in group 2 (9), and in 30.3 % of patients in the control group (10).

The most common dental anomalies were distal occlusion in 42.4 % of cases (43 of 99 patients) and anomalies in the transversal plane (cross occlusion in 26.2 % of cases; 26 of 99 patients). Combined, associated forms of anomalies accounted for 97.1 % (67 of 69 patients with identified maxillofacial anomalies) of all identified cases.

MRI of the TMJ of 99 examined patients showed that ventral dislocation of the articular disc occurs most commonly (in 88 of 99 patients examined; 88.8 % of cases) and less often in combination with lateral displacement of the disc (4 %). Distal displacement of the articular disc is rare (1.9 %; 2 of 99 patients).

When analyzing TMJ MRI in DTMJ patients, the average dimensions of the joint space on the right were determined as 2.7 ± 0.5 mm in the anterior section, 1.9 ± 1.2 mm in the superior section, and 1.9 ± 1.0 mm in the posterior section. The average dimensions of the joint space in DTMJ patients on the left were 2.2 ± 1.3 mm in the anterior section, 2.1 ± 1.1 mm in the superior section, and 2.0 ± 0.3 mm in the posterior section ($p = 0.003$).

During the examination before DTMJ treatment, possible complete reduction of the articular disc from the ventral dislocation on one side, without reduction on the contralateral side, during functional tests of mouth opening/closing, was determined in 45 of 99 patients (45.5 %).

The results of complex treatment of DTMJ using orthotics, aligners with pads, and mouthguards with an open occlusal surface were assessed 14 and 30 days and 6 months after the start of treatment.

The disappearance of symptoms, such as pain and crunching in the TMJ when opening/closing the mouth as a result of complex treatment, and adaptation to occlusive mouthguards occurred 2 weeks later in 53 of 99 patients (52.5 %). Distribution of the degree of reduction of pain, discomfort, and crunching in the TMJ when opening/closing of the mouth as a result of complex treatment in groups, according to the signs of treatment with various devices, occurred unevenly.

Among patients who used an orthotic as an occlusal guard in the complex treatment of DTMJ, 24 patients noted a decrease in pain 2 weeks after the start of treatment.

Among the patients whose complex treatment was performed using orthodontic aligners with pads, 15 patients noted a decrease in the manifestations of DTMJ after 2 weeks.

In the comparison group, where a mouthguard was used to achieve a placebo effect, 14 patients noted a decrease in pain 2 weeks after the start of complex treatment for DTMJ.

In the group of patients whose complex treatment of DTMJ included the use of an orthotic, during a control examination 1 month after the start of treatment, the disappearance of noises and clicks when opening/closing the mouth and the absence of pain on palpation in the TMJ area and masticatory muscles were observed. Less than a quarter of patients report minor discomfort in the morning after removing the orthotic, which was associated with a change in the closure of the dentition and disappears shortly (within 30 minutes). The ongoing adaptation leads at the initial stage of treatment to the possibility in patients of this group to set the jaw in two positions, namely, habitual and reconstructive, determined by orthotic.

After 6 months, possible spontaneous retention of the lower jaw was noted in patients of this group in the reconstructive position due to the masticatory muscles, which was not accompanied by a feeling of discomfort. Moreover, in 11 of 33 patients in this group (33.3 %), a lack of close contact between the dentition of the upper and lower jaws in the lateral sections, simultaneous advancement of the lower jaw and its displacement downward, and a decrease in overbite were noted.

In this case, patients were offered to complete the treatment using a prosthetic method, namely, production of occlusal permanent overlays made of zirconium dioxide composite or pressed ceramics on the occlusal surface of separated teeth in the lateral parts of the jaws. Otherwise, patients were instructed to continue using the orthotic all night and 2–3 hours during the day.

A control analysis of TMJ MRI of patients in this group, performed 6 months after the start of complex treatment of

DTMJ, determined the elimination of ventral dislocation of the articular disc, elimination of joint space narrowing in the distal part, and symmetrical arrangement of the heads of the lower jaw in the analysis of tomograms in frontal view in 18 patients (54.5 %).

When examining patients whose hardware treatment was performed using aligners modified by pads, an improvement in the lower jaw biomechanics was observed in 29 patients (87.9 %) after 14 days, as well as elimination of zigzag movements of the lower jaw when opening/closing the mouth and a decrease or smoothing of clicks in the TMJ when opening the mouth

Six months after the start of complex treatment of DTMJ using aligners, 29 patients (87.9 %) noted the absence of pain and discomfort when opening the mouth, the absence of clicking and crunching, and an increase in the smoothness of opening/closing the mouth. Depending on the severity of dentoalveolar anomalies and deformities, patients using aligners in the treatment process experience varying degrees of improvement in the occlusal relationships of the dentition of the upper and lower jaws. In patients who, according to orthodontic indications, received more than 30 pairs of aligners and in whom treatment lasted more than 1.5 years, the elimination of the main manifestations of DTMJ occurred earlier than the end of the orthodontic correction phase.

MRI analysis of the TMJ in DTMJ patients, whose treatment involved the use of aligners, performed 6 months after the start of treatment, revealed an improvement in the morphofunctional signs of the TMJ, such as a more pronounced centricity of the location of the head of the mandible in the articular fossa, uniformity of the width of the joint space on the right and left, symmetry of the location of the heads of the lower jaw relative to the skull when analyzing the frontal projection, and absence of ventral dislocation of the articular disc (in 13 of 33 patients, 39.3%). In other cases, no reliable MRI data characterizing favorable changes in the structure and function of the TMJ ($p = 0.412$) were obtained. However, analysis of the intermediate or, in some cases, the final stage of orthodontic treatment showed the high efficiency of aligners in eliminating dentofacial anomalies and deformities. In turn, considering the role of dentition anomalies and deformities and associated occlusal disorders in the etiology and pathogenesis of DTMJ, we can assume that such a restructuring of the dentition, aimed at eliminating occlusal interference, can have a favorable effect on the morphofunctional state of the TMJ and may be considered as a measure to prevent the worsening of pathological processes in the TMJ and masticatory muscles.

The use of aligners in the treatment of DTMJ in 84% of patients enabled improvement in the shape and relationship of the dentition, reducing the formation of static and dynamic occlusal interferences. Separation and centering of the dentition using special pads in aligners increased the

efficiency of DTMJ treatment to 98% in the period from 4 months to 8 months ($p = 0.003$).

However, the use of thin orthodontic aligners does not allow effective control of the height correction of the lower third of the face; the disconnection effect when using this type of hardware treatment is achieved to a lesser extent than when using orthotics.

In the comparison group (33 DTMJ patients), the use of a placebo occlusive mouthguard led to a decrease in pain 2 weeks after the start of complex treatment of DTMJ in 14 patients (42.4%) ($p = 0.003$). This proves the efficiency of complex treatment of DTMJ and confirms the lower efficiency and significance of hardware treatment as an individual treatment.

The absence of positive dynamics of morphofunctional changes was confirmed by MRI results of the TMJ obtained 6 months after the start of treatment. In patients of this group, the TMJ MRI analysis revealed ventral dislocation of the articular disc, uneven width of the joint space on the right and left, and shift of the mandible, that is, asymmetrical position of the heads of the mandible relative to the skull, as in the primary analysis, before treatment. Upon completion of participation in the study, the patients in this group were advised to continue treatment using hardware treatment according to indications using an orthotic or aligners. Among 33 patients in this group, 28 continued treatment and follow-up according to the proposed regimen. Having noted a decrease in discomfort and pain in the TMJ area when closing/opening the mouth, five patients decided to discontinue further treatment.

This has led to the conclusion that complex treatment of DTMJ using occlusive mouthguards with an open chewing surface of the dentition as a placebo can reduce the subjective manifestations of DTMJ, which confirms the significant role of the psychoemotional component in the development of this pathology and determines the role of factors such as the height of the lower third of the face and severity of occlusal interference in the pathogenesis of DTMJ based on the results of a TMJ MRI analysis over time, over a 6-month follow-up.

The results of using various types of occlusive mouthguards indicate that it is crucial to develop occlusal devices that combine the capabilities of decompressing the TMJ elements, centering the position of the lower jaw relative to the upper jaw, and orthodontic elimination of dentofacial anomalies and deformities that are the causes of occlusal interference.

CONCLUSIONS

1. The use of occlusive mouthguards in the treatment of DTMJ in the shortest possible time, within 2 weeks, leads to a reduction in pain and discomfort caused by decompression associated with the separation of the dentition and TMJ elements and the efficiency of drug analgesic and anti-inflammatory treatment. The as-

sociated absence of compression of the bilaminar zone prevents degenerative changes in the TMJ structures and deformity of the mandibular head and the articular disc that occur with chronic trauma to the articular surfaces.

2. Analysis of TMJ MRI in DTMJ patients showed that ventral disc dislocation occurs most often (in 88.8 % of cases) in patients in this group.

3. Elimination of ventral dislocation of the articular disc in the complex treatment of DTMJ is most effectively achieved by using occlusive aligners (orthotics), which allow decompression of the TMJ and restoration of the height of the lower third of the face.

4. The use of aligners with pads in DTMJ patients improves anomalies and deformities of the dentition and eliminate occlusal interference, which, in turn, makes it possible to create conditions for preventing the worsening of DTMJ signs, confirmed by dynamic the TMJ MRI results.

5. The use of orthotics and aligners as therapeutic devices in DTMJ patients has certain indications and varying efficiency. The general positive effect of the use of these devices in the treatment of DTMJ is the separation of the dentition and associated decompression in the TMJ, centering the position of the lower jaw relative to the upper jaw. However, completion of treatment with an orthotic requires a prosthetic treatment protocol in 23 % of cases to ensure a stable result in correcting ventral articular disc dislocation.

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THE ORGANIZATION OF EMERGENCY MEDICAL CARE FOR THE CHILDREN'S POPULATION DURING THE COVID-19 PANDEMIC

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ОБ ОРГАНИЗАЦИИ НЕОТЛОЖНОЙ МЕДИЦИНСКОЙ ПОМОЩИ ДЕТСКОМУ НАСЕЛЕНИЮ В ПЕРИОД ПАНДЕМИИ COVID-19

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Objective. To analyze the work of the emergency medical care department (EMCD) of the largest children's polyclinic of the city in 2020-2022 and to evaluate the organization of the EMC for the attached children during the Covid-19 pandemic.
Materials and methods. Mathematical and statistical analysis of quantitative and qualitative indicators of EMCD activity based on a continuous sample of primary accounting and reporting medical documentation.

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Results. A significant increase in the number of EMCD visits was demonstrated. In 2022, the growth was 40,96 % and 28,12 % compared to 2020 and 2021. It was found out that in 97,4–98,0 % of all cases provided with EMC, were the children with respiratory diseases. Their number in 2022 increased 1,3 times compared to 2021, and 1,43 times compared to 2020. In this group of patients, the number of children with pneumonia increased by 63.5 % in 2021 compared to 2020.

Conclusions. The proper organization of the work of the EMCD made it possible to provide children with available, timely and rapid primary medical social and emergency medical care during an increased workload in the context of the COVID-19 pandemic.

Keywords. Children, emergency medical care, pandemic, COVID-19.

Цель. Провести анализ работы отделения неотложной медицинской помощи (ОНМП) самой крупной детской поликлиники города в период 2020–2022 гг. и оценить организацию НМП прикрепленному детскому населению в пандемию COVID-19.

Материалы и методы. Математический и статистический анализ количественных и качественных показателей деятельности ОНМП на основе сплошной выборки первичной учетно-отчетной медицинской документации.

Результаты. Продемонстрировано значительное увеличение количества, выполненных ОНМП, выездов. В 2022 г. прирост составил 40,96 % и 28,12 % в сравнении с 2020 и 2021 гг. Отмечен рост общего числа вызовов, переадресованных из Центра медицины катастроф и скорой медицинской помощи (ЦМК и СМП) в ОНМП (в 1,2 раза по сравнению с 2020 г.). Установлено, что в 97,4–98,0 % случаев дети, которым была оказана НМП, это больные с заболеваниями органов дыхания. Их количество в 2022 г., по сравнению с данными 2021 г., стало больше в 1,3 раза, а по сравнению с 2020 г. – в 1,43 раза. В этой группе больных в 2021 г. в сравнении с 2020 г. выросло число детей с пневмонией на 63,5 %.

Выводы. Правильная организация работы ОНМП позволила обеспечить доступность, своевременность и оперативность оказания детям первичной медико-социальной и скорой медицинской помощи в неотложной форме при возросшей нагрузке в условиях пандемии COVID-19.

Ключевые слова. Дети, неотложная медицинская помощь, пандемия, COVID-19.

INTRODUCTION

One of the significant reserves for optimizing emergency medical care in Russia is improving of emergency medical care departments (EMCD) for adults and children in territorial polyclinics and increasing their efficiency[1].

The creation of EMCD network in primary health care institutions made it possible to optimize the work of Disaster Medicine Center (DMC) and Ambulance: to reduce the number of calls and reduce the number of Ambulance visits during polyclinic opening hours, to reduce the number of deaths before the Ambulance arrival, reduce the ambulance team daily workload, increase the level of accessibility and provision of emergency medical care to the population [1; 2].

The organization of the EMCD activities currently provides for continuity in work with the DMC and Ambulance and the transfer of the emergency calls flow to medical organizations providing pri-

[1] On approval of the Regulations on the procedure for providing primary medical and social care to children: Order of the Ministry of Health of the Russian Federation No. 92n dated March 7, 2017, M. 2017; 64, available at: http://publication.pravo.gov.ru/Document/View/0001201804180005

mary health care to the population on a territorial basis through unified dispatch services[2].

According to the Ministry of Health of the Russian Federation, in 2022, the number of Ambulance calls decreased by 2.9 million. The share of Ambulance visits with a time of arrival to the patient of less than 20 minutes from the moment of the call increased from 83.27 % of cases in 2021 to 85.91 % in 2022. At the end of last year, an improvement in the time of arrival at the scene of a traffic accident to 20 minutes was recorded at 95.03 % instead of 94.38 % in 2021[3].

Proper organization of the EMCD activities prevents the development of exacerbations of chronic diseases and the development of acute conditions in which there is a need to provide urgent medical care (UMC) [3; 4]. The true need for UMC in the structure of all calls does not exceed 10.3–20.5 %. In other cases, more than 80 % require emergency medical care[4].

It is noted that the largest number of emergency calls to children is observed due to illness (up to 67.52 %). In the age category up to one-year-old, the prevalence of calls for active observation is more than 2 times higher than calls for all other reasons [5].

The need for EMC is steadily increasing due to the increase in the prevalence of diseases in childhood – by 5 % annually. It is also known that with timely EMC provision, the prognosis for children's lives improves [6–8]. Therefore, to this day, the issues of organizing and improving primary health care and emergency medical care, including for the children's population, remain relevant. One of the conditions for the Ambulance reorganization and optimization is the effective organization of the EMCD activities in medical organizations providing outpatient care, which helps to reduce the number of Ambulance teams visits and increase Ambulance availability through the redistribution of calls [9; 10].

MATERIALS AND METHODS

A comprehensive study was carried out, methods of mathematical, statistical, content analysis were applied for primary accounting and reporting medical documents of the emergency department for children of Children's City Polyclinic No. 3: forms No. 112/u, Ambulance calls cards (form No. 120/u), signal sheets, logs for accounting of calls/visits for 2020–2022.

[2] On the organization of reception and transmission of ambulance and emergency medical care calls in the Astrakhan region: order of the Ministry of Health of the Astrakhan region dated June 07, 2019 No. 607r., available at: https://old.minzdravao.ru/sites/default/files/2019/2/rasporyazhenie_no_607r.pdf

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RESULTS AND DISCUSSION

Currently, 16 emergency care points are organized in Astrakhan at 15 territorial polyclinics. Close interaction with outpatient health care institutions in the Astrakhan region is ensured through the organization of emergency dispatch based on a single call center.

The unified dispatch service (UDS), created on the basis of the operational department of the State Budgetary Health Institution of the Astrakhan Region "Disaster Medicine Center and Ambulance", daily transmits from 60 to 100 calls to polyclinics for servicing by EMC stations teams.

All calls received by the UDS are divided into two categories: urgent and emergency. The main criteria are the presence or absence of a threat to the patient's life, the urgency of providing medical care.

The choice of Children's City Polyclinic No. 3 (CCP No. 3) as the object of study is due to the fact that it is the largest in terms of the number of served and attached population: it accounts for 14.88 % of the total child population of the region and about a third of the urban child population (–29.43 %) of the number of children living in Astrakhan (112,659 people from 0 to 18 years old). Of the total number of pediatric calls transferred by the DMC and Ambulance to the territorial polyclinics of the city, every 3–4th case is served by the EMCD of this medical institutions. Thus, the organization of work to provide EMC in this polyclinic can significantly influence the availability and quality of not only emergency primary health care, but also Ambulance, including emergency specialized medical care in the region as a whole.

Emergency medical care in CCP No. 3 is provided at home to children from 0 to 17 years 11 months 29 days old on a territorial basis in accordance with the Regulations on EMCD and all regulatory legal documents. 12 hour operating mode (from 10:00 a.m. to 10:00 p.m.), daily, seven days a week.

The EMCD staffing and equipment comply with current standards. The department employs four pediatricians and two nurses with appropriate training in EMC. Staffing is 100 %.

The EMCD is provided with a specialized sanitary and medical vehicle of B category, which is fully equipped in accordance with the equipment standard. There is a plan (scheme) of the activity area with a clear designation of streets, houses, medical institutions, police stations, as well as the necessary instructional and methodological material.

The EMCD is provided with fixed and mobile telephone communications for interaction with the structural units of the polyclinic and with the dispatchers of the DMC and Ambulance. Reception of calls requiring EMC is carried out by a medical registrar. After each home visit, information about each sick child is transferred to the local pediatrician for further medical supervision and treatment.

The size and characteristics of the population served are presented in Table 1.

Table 1

Characteristics of the pediatric population served by the Emergency Medical Care Department for 2020–2022

Name	Total children			Children 0–14 years 11 months 29 days old			Children 15–17 years 11 months 29 days old		
	2020	2021	2022	2020	2021	2022	2020	2021	2022
Total people	33.372	32.950	33.159	28.524	28.348	28.476	4.848	4.602	4.683

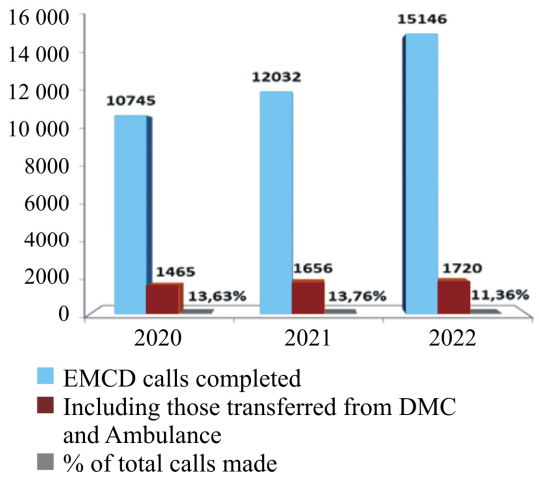

Fig. 1. Number of calls served by the EMCD of the CCP No. 3 for 2020–2022.

As can be seen from the data in Table 1, in 2022, compared to 2020, there is a slight decrease in the population by 213 children (0.64 %), the majority (85.9 %) are children under 14 years 11 months 29 days old, amounting in 2022 to 28,476 people (2020 – 28,524 children, 2021 – 28,348) and 4,683 adolescents (2020 – 4,848, 2021 – 4,602). It should be noted that frequently ill children under 5 years old make up 24.53 % of the population served, of which 1,497, or 4.51 % are children of the first year of life, and 20.02 % – from one to 5 years old.

During the researched period, EMCD specialists served 37,923 calls (Fig. 1).

The results obtained demonstrate a significant increase in the number of completed visits: in 2022 there were 1.26 times more, or by 28.12 % (3,114 visits), compared to 2021, by 1.41 times, or by 40.96 % (4,401), compared to 2020.

The presented data shows that the number of calls transferred from the DMC and Ambulance in 2022, compared to 2021, also increased by 1.03 times and amounted to 1,720, which is 64 cases more than in 2021; in 2021, compared to 2020, increased by 1.2 times (13.03 %) and amounted to 1,656, which is 191 cases more than in 2020. The were no cases returned back to the Ambulance and calls “to themselves" during this period.

The main indicator of timeliness, availability and efficiency of provision is the time of the visit service. The analysis showed that the share of visits serviced within 2 hours from the moment of the call received was 100 %, of which 98 % of the total were serviced within 40 minutes or less. It follows that even during the pandemic, with an increased load on the EMCD, emergency care was provided to children in a timely and efficient manner.

An analysis of the age structure of children who received medical treatment in 2020–2022 was carried out (Fig. 2).

An analysis of the age structure of children who received EMC showed that in 86.9 % of cases in 2020 and in 85 % in 2022,

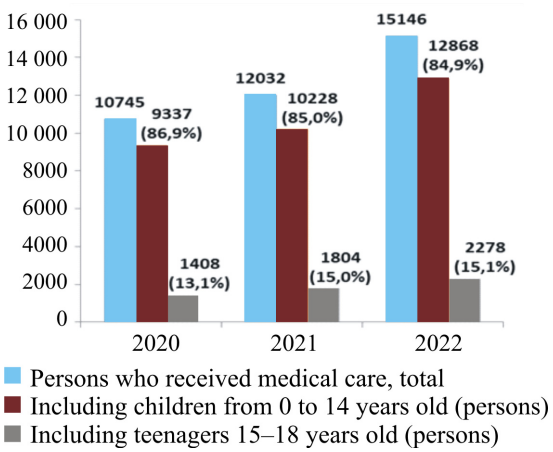

Fig. 2. Age structure of patients who received emergency medical care in 2020–2022

there were children aged 0 to 14 years old. The share of adolescent children accounts for from 13.1 % in 2020 to 15.1 % in 2022. In 2021, the proportion of adolescent children increased, which is associated with a higher incidence of Covid in this particular age category.

The structure of calls to children by disease class is shown in Table. 2.

Thus, the main part of the children who received EMC in these years were patients with respiratory diseases – 98.0 % (in 2021 – 97.4 %, 2020 – 97.3 %). Compared to 2021, visits to those patients increased by 1.3 times (by 3,120), and from 2020 – by 1.4 times (by 4,388).

It should be noted that in 2021 there was an increase in registered cases of pneumonia by 1.6 times. The increase relative to 2020 was 63.5 % (157 cases – 1.3 % in 2021, 96 cases – 0.9 % in 2020) of the total number of calls made. This is explained by the current epidemiological situation: the increase in the incidence of ARI and the COVID-19 pandemic.

In 2022, compared to 2021, there was a decrease in the number of registered cases of pneumonia by 2.8 times, or by 55 cases (0.4 %) of the total number of calls made, which is explained by a decrease in cases of new coronavirus infection among children.

Table 2

Structure of calls served by the Emergency Medical Care Department by nosology for 2020–2022

Class of diseases	Parameter					
	2020		2021		2022	
	abs.	%	abs.	%	abs.	%
Nervous system diseases	60	0.6	63	0.5	64	0.4
Respiratory diseases, including:	10.457	97.3	11.725	97.4	14.845	98.0
ARVI	10.361	96.4	11.568	96.1	14.790	97.6
pneumonia	96	0.9	157	1.3	55	0.4
Skin diseases	28	0.3	32	0.35	29	0.19
Digestive diseases	15	0.17	23	0.24	28	0.18
Diseases of the genitourinary system	–	–	6	0.04	2	0.01
Diseases of the ENT organs	3	0.03	9	0.07	12	0.07
Infectious diseases	182	1.6	174	1.4	166	1.15

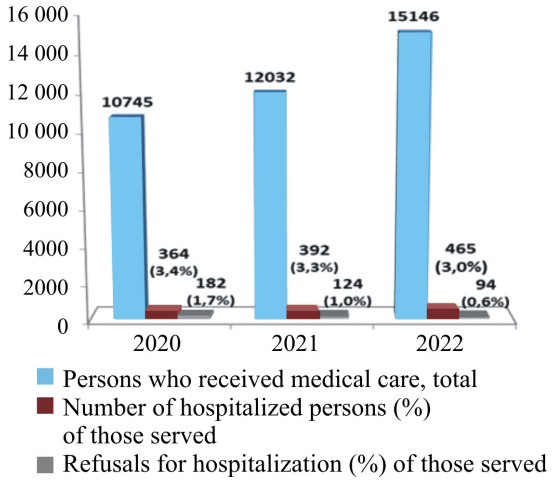

Fig. 3. Number of hospitalizations and refusals of hospitalization for 2020–2022

Infectious diseases are in the second place among the reasons for calling an EMCD doctor, just like in previous years. In 2022, their number decreased by 1.04 times compared to 2021 and amounted to 166 cases (1.15 % of the total number of calls served); in 2021 – 174 (1.4 %), in 2020 – 182 (1.6 %) of the total number of visits.

The third ranking place was taken by diseases of the nervous system – 0.4 %, compared to 2021 (0.5 %), there was a decrease by 1.25 times.

Thus, in the period 2020–2022 there is a trend towards a decrease in the proportion of infectious diseases and diseases of the nervous system, and all other classes of diseases in the aggregate account for no more than 0.6 %.

An analysis was carried out of cases that resulted in patients being hospitalized to in-patient clinic, and refusals of hospitalization (Fig. 3).

The data we obtained demonstrate positive trends towards a decrease in refusals from hospitalization (1.7; 1.0; 0.6 %, respectively, over the years). In 2022, their number decreased by 2.8 times compared to 2020, amounting to 94 cases versus 182. There were no cases of untimely hospitalization that led to a deterioration in the patient's condition. During the researched period, one case of discrepancy in diagnosis upon admission to the hospital was recorded, which did not lead to the development of severe complications or death of the patient.

Due to the epidemiological situation that has developed over the past three years, the share of diseases requiring medical care in a hospital setting has increased and amounted to an average of 3.3 % of the total number of patients served. The most common reasons for hospitalization were broncho-obstructive syndrome with respiratory failure, severe acute respiratory infections, and acute intestinal infections.

Over the past few years, there has been a tendency to increase the share of Ambulance teams calls to children due to the severe course of the new coronavirus infection, respiratory viral infections with a significant increase in body temperature and other complications. During the COVID-19 pandemic in 2020–2022 the load on institutions providing primary health care and emergency care, including emergency specialized medical care, has increased significantly. Effective ways to optimize their work were the creation of a unified dispatch center, a clear differentiation of all calls received from the popula-

tion into urgent and emergency ones, and redirection of calls requiring emergency medical care to primary health care institutions. In this situation, EMCD played a significant role in providing the population with primary health care and emergency medical care.

Over the past year, the number of requests for emergency medical services in the Astrakhan region decreased by 11.1 %; over the last three years, this figure decreased by 16.6 %.

In 2022, the rate of provision of the population with emergency medical care in the area of responsibility of the DMC and Ambulance amounted to 312.9 calls per 1000 persons, with a standard indicator of 290 per 1000 persons (in 2020 – 364.5; in 2021 – 347.6).

Thus, the intensive and effective work of polyclinics' EMCD makes it possible to bring the work of medical organizations providing emergency medical care to the standards of availability established by the Federal Compulsory Medical Insurance Fund, to increase the availability of emergency medical care for the population, including children.

Conclusions

The organization of the EMCD activities in territorial polyclinics in close connection with all departments of the medical organization and continuity with the DMC and Ambulance is aimed at maximum coverage of people in need of emergency medical care. During the pandemic, under conditions of increased workload, EMCDs demonstrated their effectiveness, consistency and great demand for ensuring accessibility, efficiency and timeliness of providing not only primary health care, but also emergency medical care.

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DOI: 10.17816/pmj411141-147

INFLUENCE OF VACCINE PREVENTION ON THE SPREAD AND SEROTYPE COMPOSITION OF *STREPTOCOCCUS PNEUMONIAE* IN MILITARY COLLECTIVES

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ВЛИЯНИЕ ВАКЦИНОПРОФИЛАКТИКИ НА РАСПРОСТРАНЕННОСТЬ И СЕРОТИПОВОЙ СОСТАВ *STREPTOCOCCUS PNEUMONIAE* В ВОИНСКИХ КОЛЛЕКТИВАХ

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Objective. To study the effect of pneumococcal infection vaccination on the spread and serotype composition of *S. pneumoniae* in military personnel.

Materials and methods. The study was conducted in a military unit of Sverdlovsk region, 369 military men took part in it. Determination of the frequency and serotype of *S. pneumoniae* was carried out on admission and 1.5 months after stay in the military collective using the multiplex PCR method.

Results. After 1.5 months of stay in the military collective, a significant activation of pneumococcal circulation was established ($\chi^2_{McNemar} = 24.038$; $p < 0.001$). The risk of *S. pneumoniae* infection in unvaccinated military personnel was 1.39 times higher than in the vaccinated ones (*RR* = 1.39; 95 % *CI* 1.209–1.596). In the

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group of unvaccinated military personnel, the frequency of occurrence of vaccine serotypes was 2.6 times higher than in vaccinated ones ($\chi^2 = 6.25$; $p = 0.01$).
Conclusions. The proactive influence of immunization against pneumococcal infection on the spread and serotype composition of *S. pneumoniae* has been determined: a low spread of vaccine serotypes and a predominance of non-typeable serotypes have been revealed among vaccinated individuals.
Keywords. Military personnel, carriage, *S. pneumoniae*, serotype landscape, vaccinated and unvaccinated.

Цель. Изучение влияния вакцинации против пневмококковой инфекции на распространенность и серотиповой состав *S. pneumoniae* у военнослужащих.
Материалы и методы. Исследование проведено среди 369 военнослужащих воинской части Свердловской области. Определение частоты и серотипа *S. pneumoniae* проведено при поступлении и через 1,5 месяца пребывания в воинском коллективе с использованием метода мультиплексной ПЦР.
Результаты. Через 1,5 месяца пребывания военнослужащих в воинском коллективе установлена достоверная активизация циркуляции пневмококка ($\chi^2_{McNemar} = 24{,}038$; $p < 0{,}001$). Риск инфицирования невакцинированных военнослужащих *S. pneumoniae* был в 1,39 раза выше по сравнению с вакцинированными (*RR* = 1,39; 95 % ДИ 1,209–1,596). В группе непривитых военнослужащих частота встречаемости вакцинных серотипов была в 2,6 раза выше, чем у привитых ($\chi^2 = 6{,}25$; $p = 0{,}01$).
Выводы. Установлено упреждающее влияние иммунизации против пневмококковой инфекции на распространенность и серотиповой пейзаж *S. pneumoniae*: Выявлена низкая распространенность среди привитых вакцинных серотипов и преобладание нетипируемых.
Ключевые слова. Военнослужащие, носительство, *S. pneumoniae*, серотиповой пейзаж, привитые и непривитые.

INTRODUCTION

Respiratory diseases (RD) have long remained a pressing problem for the medical service of the Russian Armed Forces, annually occupying priority positions in the structure of morbidity among military personnel serving both conscript and contract [1–3].

Conscripted military personnel are at the highest risk of developing diseases, which is due to a change in their usual lifestyle in connection with conscription and the need to adapt to new working, living and nutritional conditions [4; 5].

In 2022, the incidence of RD among conscripted military personnel, compared to 2021, increased by 36.8 % (in 2022 – 698 ‰, in 2021 – 510 ‰). In the RD structure the share of acute respiratory infections was 74 % (432 ‰), acute bronchitis – 7.4 % (51.2 ‰), acute tonsillitis – 8 % (45.3 ‰), community-acquired pneumonia – 9.8 % (38.8 ‰), influenza – 0.1 % (0.8 ‰) [6].

The main reasons for the RD emergence and spread are: the introduction of infection into military groups with young recruits from various territories of the Russian Federation; seasonal increase in incidence in the autumn-winter and spring-summer periods, associated with the "mixing" of military personnel during the reception of young recruits and the recirculation of the pathogen among renewed military teams; staffing training military units in a short time with a significant number of personnel [3; 7].

At the same time, it should be noted that recruits arriving in the troops must have immunity against infections relevant to the troops [8], which is provided for by current regulatory documents. Persons sub-

ject to conscription for military service are vaccinated against influenza as part of the National Immunisation Schedule, as well as immunization against varicella, meningococcal and pneumococcal diseases as part of the Preventive Vaccinations Schedule for Epidemic Indications[1].

Pneumococcal Disease (PD), as is known, is represented not only by manifest, but also by non-manifest forms of infection. The impact of immunization of military personnel on the incidence of community-acquired pneumonia is widely discussed in domestic and foreign literature; the importance of military personnel immunization in reducing the incidence of respiratory diseases has been proven. Meanwhile, studies on the effect of vaccination on the prevalence of *S. pneumoniae* in military groups are few, and the results are very contradictory.

In light of the above, *the purpose of the study* is to research the effect of vaccination against pneumococcal disease on the prevalence and serotype landscape of *S. pneumoniae* in military personnel.

MATERIALS AND METHODS

The study was conducted from 2021 to 2023 in the military unit of the Sverdlovsk region. 369 military men aged 18–21 years were examined, including 38.5 % (142) vaccinated with the 23-valent pneumococcal polysaccharide vaccine (PPV23) and 61.5 % (227) of those not vaccinated before conscription.

The study of *S. pneumoniae* carriage included two stages. Biomaterial was collected from the mucous membranes of the nasal cavity and nasopharynx from conscripts upon arrival at the military unit and again after 1.5 months of being in the military collective in order to detect *S. pneumoniae* and determine its serotype[2].

Serotyping was carried out using multiplex PCR using 21 leading serotypes/serogroups of *S. pneumoniae*, including 18 serotypes of the PPV23 vaccine.

Statistical processing of the results was carried out by comparing data for related samples using the nonparametric McNemar test, and for unrelated samples using the Pearson χ^2 criterion. Differences were considered significant at $p < 0{,}05$. Based on the results of the cohort study, the relative risk (*RR*) was determined using the generally accepted method [9].

Statistical processing of the obtained data was carried out using Microsoft Excel 2010, Past 4.14, and an online calculator (https://medstatistic.ru/calculators.html).

RESULTS AND DISCUSSION

Upon arrival at the military unit, carriage of pneumococcus was detected in 12.5 % (46) of the examined conscripts.

[1] On approval of the National Immunisation Schedule, the Preventive Vaccinations Schedule for Epidemic Indications and the Procedure for Carrying out Preventive Vaccinations: Order of the Ministry of Health of the Russian Federation dated December 6, 2021 No. 1122n. 2021; 15.

[2] Laboratory diagnosis of community-acquired pneumonia: guidelines. M.: Federal Center for Hygiene and Epidemiology of Rospotrebnadzor 2014; 39.

After 1.5 months of the conscript's stay in the military team, an increase in the circulation of pneumococcus was detected, as a result of which the number of carriers among the examined military personnel increased by 2 times and amounted to 26.0 % (96) ($\chi^2_{McNemar}$ = 24.038; p < 0.001). At the same time, in 20.8 % of those examined (77), *S. pneumoniae* was isolated for the first time.

A comparative assessment of the hidden component of the epidemic process of pneumococcal disease in dynamics among vaccinated and unvaccinated military personnel revealed significant differences. Among unvaccinated military personnel, after 1.5 months, the number of *S. pneumoniae* carriers increased 3 times (from 17 to 52; $\chi^2_{McNemar}$ = 26.064; p < 0.0001), and amounted to 36.6 % (Fig. 1). In vaccinated military personnel, the number of carriers increased only 1.5 times (from 29 to 44; $\chi^2_{McNemar}$ = 3.947; p = 0.047), amounting to 19.4 %, which indicates the proactive effect of immunization on the risk of military personnel infection with *S. pneumoniae*.

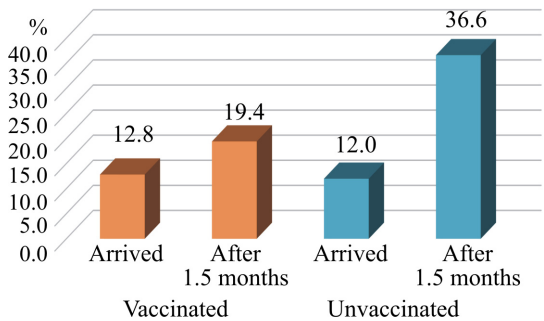

Fig. 1. Frequency of S. pneumoniae carriage in vaccinated and unvaccinated military personnel, %

The risk of *S. pneumoniae* infection in the group of persons not vaccinated against PD was 1.39 times higher compared with vaccinated persons (*RR* = 1.39; 95 % CI 1.209–1.596).

Upon arrival at the military unit, the serotype landscape of pneumococci was represented by four vaccine isolates 1, 3, 4, 18A/B/C/F (1.9 %) and non-typeable serotypes (10.6 %).

After 1.5 months of stay in the military collective, the serotype composition of the isolated pneumococci changed significantly: the leading role belonged to non-typeable serotypes (20.1 %), the proportion of vaccine isolates of pneumococcus significantly increased to 5.4 % ($\chi^2_{McNemar}$ = 6.76; p = 0.01), with a predominance of serotypes 3 and 12F/A/B/44/46, the latter was not detected during the initial examination. The long stay of military personnel in a closed military collective led to the emergence of new serotypes: three 9AV, 9LN, 6A/B/C/D, which are part of PPV23, and a nonvaccine serotype – 16F. Four examined persons (1.08 %) had mixed carriage after 1.5 months – a combination of two serotypes (Fig. 2).

When assessing the serotype landscape of *S. pneumoniae* in vaccinated and unvaccinated military personnel, it was found that upon arrival at the military collective, the frequency of occurrence of non-typeable serotypes was the same in both groups and amounted to 10.6 %, vaccine serotypes were represented in three (1, 3, 4 – 2.2 %) in vaccinated and one (18A/B/C/F – 1.4 %) in unvaccinated military personnel (table).

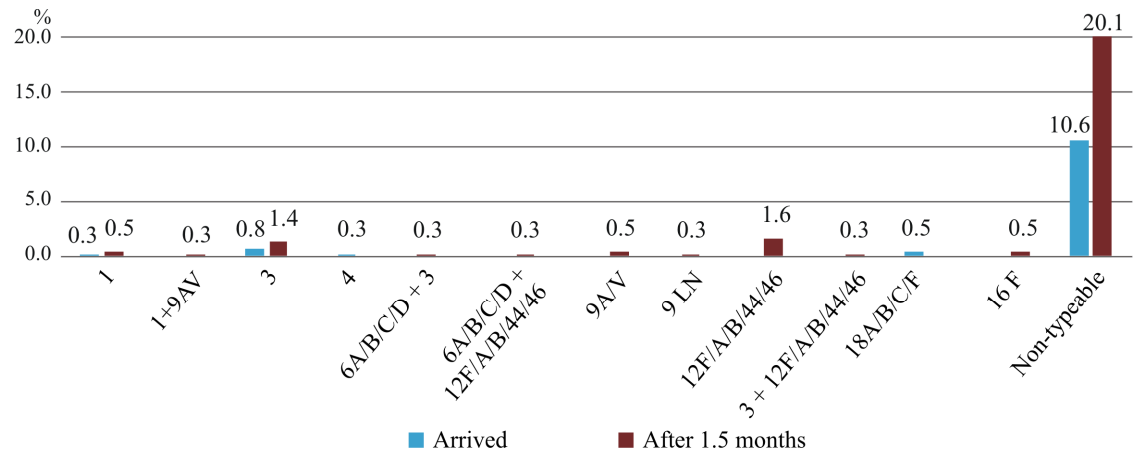

Fig. 2. Serotype composition of isolated S. pneumoniae, %

Structure of *S. pneumoniae* serotypes in vaccinated and unvaccinated military personnel

Serotype (serogroup)	Vaccinated, *n* = 227				Unvaccinated, *n* = 142			
	Arrived		After 1.5 months		Arrived		After 1.5 months	
	number of strains	per 100 examined persons [95 % CI]	number of strains	per 100 examined persons [95 % CI]	number of strains	per 100 examined persons [95 % CI]	number of strains	per 100 examined persons [95 % CI]
6A/B/C/D							2	1.4 [0.4–5.0]
9A/V			1	0.4 [0.1–2.5]			2	1.4 [0.4–5.0]
9LN							1	0.7 [0.1–3.9]
3	3	1.3 [0.5–3.8]	3	1.3 [0.5–3.8]			4	2.8 [1.1–7.0]
12F/A/B/44/46			3	1.3 [0.5–3.8]			5	3.5 [1.5–8.0]
18A/B/C/F					2	1.4 [0.4–5.0]		
1	1	0.4 [0.1–2.5]	2	0.9 [0.2–3.2]			1	0.7 [0.1–3.9]
4	1	0.4 [0.1–2.5]						
16F			1	0.4 [0.1–2.5]			1	0.7 [0.1–3.9]
Non-typeable	24	10.6 [7.2–15.2]	36	15.9 [11.7–21.2]	15	10.6 [6.5–16.7]	38	26.8 [20.2–34.6]

After 1.5 months, both in the vaccinated and unvaccinated groups, predominantly non-typeable isolates were identified: in the unvaccinated – 27.5 %, in the vaccinated – 16.3 %.

In the unvaccinated group, vaccine serotypes were distributed in greater diversity and quantity (6A/B/C/D, 9A/V, 9LN, 3, 12F/A/B/44/46, 1), which was significantly 2.6 times higher that the number of vaccine serotypes in vaccinated persons (9A/V, 3, 12F/A/B/44/46, 1) ($\chi^2 = 6.25$; $p = 0.01$).

Among those vaccinated against pneumococcal disease, a low prevalence of vac-

cine serotypes and a high frequency of non-typeable, including nonvaccine serotypes were revealed, which is consistent with data from similar works by other authors, according to which, against the background of military personnel mass immunization, vaccine strains of *S. pneumoniae* are replaced by serotypes not included in the composition of the vaccines used.

Thus, the conditions of stay of military personnel in military units, characterized by multi-territoriality of arriving conscripts, crowding and the factor of the team "mixing", significantly activate the mechanism of PD epidemic process development in military groups, causing not only an increase of the *S. pneumoniae* carriage frequency, but also the diversity of its serotype landscape, increasing the risk of infection among military personnel. Despite the continued circulation of pneumococcal serotypes in military personnel, the proactive effect of vaccination on the infection process has been proven.

In order to reduce the intensity of pneumococcal circulation and mitigate the risk of infection of military personnel in military units, health authorities of the constituent entities of the Russian Federation need to increase vaccination coverage against PD of persons, subject to conscription for military service.

Conclusions

1. Immunization of military personnel against pneumococcal disease reduces the frequency and diversity of the serotype landscape of circulating *S. pneumoniae*. Among vaccinated persons, a predominance of non-typeable strains was established; vaccine serotypes were found much less frequently than among unvaccinated persons.

2. The proactive impact of vaccination on the prevalence of *S. pneumoniae* among military personnel has been established.

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CLINICAL CASE

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ANALYSIS OF A CLINICAL CASE OF MANAGING A PATIENT WITH INTRAOPERATIVE BLADDER INJURY

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АНАЛИЗ КЛИНИЧЕСКОГО СЛУЧАЯ ВЕДЕНИЯ ПАЦИЕНТКИ С ИНТРАОПЕРАЦИОННОЙ ТРАВМОЙ МОЧЕВОГО ПУЗЫРЯ

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To analyze a clinical case of successful treatment of a patient with intraoperative bladder injury.
The results of examination and treatment of patient S., 39 years old, were studied. The reasons for iatrogenic bladder injury in this clinical case were two surgeries on the bladder in childhood, a pronounced adhesive process of the small pelvis, and the inability to foresee the atypical localization of the bladder welded to the anterior abdominal wall. A pronounced adhesive process might also be caused by endometriosis, which had not been diagnosed and treated in this patient.

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Timely referral of the patient to a gynecologist for a check-up with ultrasound examination of the pelvic organs would have allowed to reveal endometriosis, timely treat it and avoid the formation of a large endometrioid cyst, which required surgical treatment. In this clinical case attention is drawn to the lack of an interdisciplinary approach to the management of this patient, defects in dispensary supervision. Despite the complication occurred, performing the stages of operation together with an urologist, adequately prescribed postoperative therapy for the prevention of recurrence of endometriosis, rehabilitation in the urology department made a favorable course of the postoperative period possible, bladder function was restored and clinical manifestations of endometriosis were controlled.
Keywords. Endometriosis, iatrogenic bladder injury.

Осуществлен анализ клинического случая ведения пациентки с интраоперационной травмой мочевого пузыря. Изучены результаты обследования и лечения больной С., 39 лет. Причинами ятрогенной травмы мочевого пузыря в данном клиническом случае послужили следующие: наличие двух операций на мочевом пузыре в детстве, выраженный спаечный процесс малого таза, невозможность предусмотреть атипичную локализацию мочевого пузыря, спаянного с передней брюшной стенкой. Выраженный спаечный процесс, кроме перенесенных в анамнезе оперативных вмешательств, мог быть обусловлен также и эндометриозом, не диагностированном и не пролеченным у данной больной.
Своевременное направление пациентки на консультацию к гинекологу с проведением УЗИ органов малого таза позволило бы выявить у нее наличие эндометриоза, провести его своевременное лечение и избежать формирования эндометриоидной кисты больших размеров, потребовавшей проведения оперативного лечения. В данном клиническом случае обращает на себя внимание отсутствие междисциплинарного подхода к ведению данной пациентки, дефекты диспансерного наблюдения. Несмотря на возникшее осложнение, проведение этапов операции совместно с урологом, адекватно назначенная в послеоперационном периоде терапия по поводу профилактики рецидива эндометриоза, реабилитация в условиях отделения урологии позволили добиться удовлетворительного течения послеоперационного периода, восстановления функции мочевого пузыря и купирования клинических проявлений эндометриоза.
Ключевые слова. Эндометриоз, ятрогенная травма мочевого пузыря.

INTRODUCTION

Any case of iatrogenic injury is always analyzed both by the doctor himself, in whose work this complication arose, and by his colleagues in order to prevent such injuries in the future, taking into account all the possible reasons that could lead to it. Iatrogenic bladder injury is one of the common causes of intraoperative injuries, and this complication significantly changes the patient's management tactics and requires special postoperative rehabilitation. Therefore, it is important to pay attention to all the features of the patient's medical history, identifying in advance the reasons that may lead to deviations from the typical performance of the operation. One of these reasons may be the presence of adhesive disease of the pelvis in the patient, due to both the presence of previous surgical interventions on the pelvic organs and endometriosis. Endometriosis is traditionally considered as a persistent, often relapsing disease that requires long-term treatment. Management of patients with this pathology can be very complex due to various clinical forms, as well as the degree of their severity, which makes timely diagnosis difficult. The clinical manifestation of endometrioid disease is

possible in the form of the formation of ovarian cysts, which can reach large sizes [1; 2]. In this case, attention should be payed to the typical clinical manifestations of this pathology, such as abnormal uterine bleeding and dysmenorrhea. Undiagnosed and untreated endometriosis can cause disruption of pelvic microcirculation, development and progression of adhesions, creating difficulties during surgical measures [1; 2]. An interdisciplinary approach to the management of patients, knowledge of the clinical symptoms of the pathology, its timely detection and prescription of effective therapy are important. It is advisable to prescribe hormonal therapy in order to prevent relapse of the disease in patients with endometrioid ovarian cysts after their surgical removal and the exclusion of malignancy at the postoperative stage of management. First-line drugs for the treatment of endometriosis include progestogens, which have important advantages in the treatment of the disease both due to their ability to reduce estrogens to average physiological values, and due to their direct effect on the endometriotic lesions themselves, causing their atrophy [1–4]. The progestagen dienogest, in accordance with its chemical structure and pharmacological properties, can be used in a dosage of 2 mg per day, continuously for a long time, stopping the clinical manifestations of endometriosis and also minimizing the risk of surgical measures [3; 5–7]. In the absence of timely diagnosis and treatment of endometriosis, especially in patients with a history of operations affecting the pelvic organs, the risk of negative consequences of this disease increases. Moreover, surgical measures in such patients may be associated with a high risk of both intra- and postoperative complications.

The purpose of the study is to analyze a clinical case of managing a patient with intraoperative bladder injury**.**

MATERIALS AND METHODS

The results of treatment of patient S., 39 years old, were studied. The patient was in inpatient treatment at the Perinatal center of the State Budgetary Healthcare Institution V.D. Seredavin Samara Regional clinical Hospital, she was admitted for planned surgical treatment to the gynecology department. Upon admission – complaints of nagging pain in the lower abdomen, intensifying during the previous menstrual period, heavy, prolonged menstruation. History: abnormal uterine bleeding, dysmenorrhea (menarche since 14 years). Regular supervision by a gynecologist was not carried out. The last visit to the gynecologist with an ultrasound of the pelvic organs was a year ago. Diagnosis: chronic salpingo-oothecitis. Adhesive disease of the pelvic organs. Antibiotic therapy was prescribed and observation by a urologist was recommended. There were no pregnancies, the patient was unmarried, and used mechanical contraception. A mass in the pelvis was discovered during an ultrasound of the abdominal organs, prescribed by a urologist, to whom the patient consulted about nagging

pain in the lower abdomen. Before this, the patient periodically consulted a urologist with similar complaints, and she was diagnosed with chronic pyelonephritis of a single kidney. Nephrolithiasis. Adhesive process of the small pelvis. Courses of antibiotic therapy and uroseptics were prescribed, achieving a slight reduction in pain intensity. The patient had not sought medical help for a year before this incident, and there was no regular urologist supervision. The patient independently took painkillers and uroseptics. According to the patient, in childhood she underwent surgery for defects of the urinary system, which was accompanied by bladder traumatic injury, which required surgical treatment; the postoperative period was accompanied by a septic condition; subsequently, a bladder fistula formed, requiring repeated surgery. Medical reports on these surgical interventions were lost. Subsequently, due to impaired renal function, a left nephrectomy was performed (five years before the present admission to the hospital). The patient also did not provide an extract from the medical history. S. was admitted for surgical treatment as an outpatient, fully examined; no contraindications to surgical treatment were identified.

RESULTS AND DISCUSSION

Referral diagnosis: large formation of the right ovary (endometrioid cyst?). Condition after left nephrectomy. Chronic pyelonephritis of a single kidney in remission. CRI0. Nephrolithiasis. Adhesive process of the small pelvis. Condition after two bladder surgeries in childhood. Estimated scope of surgical measures: laparotomy, adhesiolysis, cystotubovariectomy on the right with a express biopsy of the removed macroscopic specimen intraoperatively with a possible expansion of the surgical intervention scope. During a lower midline incision, an iatrogenic injury occurred to the bladder, which was intimately connected by adhesions to the pelvic peritoneum, aponeurosis, subcutaneous fat for 1.5 cm, and the right ovary. The right ovary is represented by an endometrioid cyst with a diameter of 15 cm, the fallopian tube on the right with signs of chronic inflammation, endometriosis of the uterus. The appendages on the right have been removed. Conclusion of express biopsy: endometrioid ovarian cyst. A urologist was called into the operating room. He clarified the localization of the site of iatrogenic bladder injury, it occurred in the area of its anterior wall, and a pronounced adhesive process of the small pelvis was identified. The anterior wall of the bladder is adherent to the anterior abdominal wall. The urologist performed an operation – suturing an iatrogenic bladder injury. An epicystostomy was performed. The postoperative period was satisfactory. The patient received antibacterial, anti-inflammatory, and infusion therapy, and the urologist's recommendations for the management of the postoperative period were followed. General blood and urine tests, biochemical blood test at discharge – without pathology. The result of a histological examination of the specimen: endometrioid

cyst of the right ovary, chronic right-sided salpingitis. 15 days after the operation, the patient was discharged from the gynecology department and transferred to the urology department of the V.D. Seredavin Samara Regional Clinical Hospital for further treatment, a month later the epicystostomy was closed. The patient was given recommendations for the treatment of endometriosis and pelvic adhesive disease (hirudotherapy, Bovhyaluronidaze azoximer 3000 IU, 10 rectal suppositories at an interval of two days). It is recommended to take dienogest at a dosage of 2 mg continuously for six months, followed by consultation and a decision on further management tactics. During the follow-up examination and general surveying of the patient, carried out six months later, including an ultrasound scan of the pelvic organs, the following conclusion was obtained: the ovary on the left is without pathology, single hypoechoic inclusions in the myometrium (foci of endometriosis). When conducting a biochemical blood test, a general analysis of urine and urine according to Nechiporenko, and a study of bladder function, no pathology was detected. It is recommended to continue taking dienogest at the same dosage, followed by a dynamic examination after six months and subsequent follow-up with a gynecologist and urologist. Clinically, the patient showed a marked improvement in her condition, there was no bleeding or pain. The consequences of iatrogenic bladder injury were also completely eliminated. Bladder function is not impaired.

CONCLUSIONS

The causes of iatrogenic bladder injury in this clinical case were the following: the presence of two surgeries on the bladder in childhood followed by nephrectomy, a pronounced adhesive process of the small pelvis, due to both the presence of surgeries on the pelvic organs and undiagnosed and untreated endometriosis. When analyzing this clinical case, it should be noted that timely referral of the patient for a consultation with a gynecologist with an ultrasound of the pelvic organs would have revealed endometriosis, which could have been suspected, given the typical clinical manifestations. This would make it possible to carry out timely treatment and avoid the formation of a large endometrioid cyst, which required surgical treatment. Observation of the patient with pathology of the urinary system by relevant professionals was not regular and did not include recommendations on the need to consult a gynecologist and conduct clarifying diagnostics. In this clinical case, attention is drawn to the lack of an interdisciplinary approach to the management of this patient and defects in dispensary supervision. Despite the complication occurred, performing the stages of operation together with an urologist, adequately prescribed postoperative therapy for the prevention of recurrence of endometriosis, rehabilitation in the urology department made a favorable course of the postoperative period possible, bladder function was restored and clinical manifestations of endometriosis were controlled.

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Tezikov Yu.V. – research concept.

Lipatov I.S. – collection and processing of materials.

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STUDY OF ANTIARRHYTHMIC ACTIVITY 2-(N-BUTYLPYRROLIDINE)-N-(2-BROMOPHENYL)CARBOXAMIDE HYDROCHLORIDE

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ИЗУЧЕНИЕ АНТИАРИТМИЧЕСКОЙ АКТИВНОСТИ 2-(Н-БУТИЛПИРРОЛИДИН)-N-(2-БРОМФЕНИЛ)КАРБОКСАМИДА ГИДРОХЛОРИДА

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Objective. To study the efficacy of a new derivative 2-(alkylpyrrolidine)-N-(aryl)carboxamide with high antiarrhythmic activity.

Materials and methods. To study the antiarrhythmic activity of the compound, the experiment was carried out on models of arrhythmia caused by intravenous administration of aconitine and adrenaline. The effect was estimated by its ability to prevent the onset of arrhythmia, prolong the survival time of the animals or by the duration of an arrhythmia attack. In addition, the electrocardiogram of awake rats was analyzed. The studied compound and the comparison drug (lidocaine) were injected to the animals intravenously in effective antiarrhythmic doses.

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Results. In aconitine arrhythmia 2-(n-butylpyrrolidine)-N-(2-bromophenyl) carboxamide hydrochloride provides statistically significant limitation of the duration of arrhythmia attacks in experimental animals (1.7 times) in comparison with the control and also reduction of arrhythmia duration in comparison with lidocaine (2.5 times); besides, this compound guarantees animals' survival in 100 % of cases. When causing arrhythmia by adrenaline administration, the compound does not prevent the occurrence of cardiac rhythm disorder. The electrocardiogram readings of animals do not change significantly.
Conclusions. 2-(n-butylpyrrolidine)-N-(2-bromophenyl) carboxamide hydrochloride (compound K-23) shows visible activity in models of arrhythmia caused by administration of aconitine and calcium chloride, which may indicate its ability to impede the sodium flow through the cell membrane by slowing depolarization of cardiomyocytes.
Since the compound studied, demonstrates high antiarrhythmic activity without changing the ECG readings, the drug created on its basis may be effective.
Keywords. Arrhythmia, lidocaine, aconitine model of arrhythmia, adrenaline model of arrhythmia, electrocardiogram, effective antiarrhythmic dose.

Цель. Изучение эффективности нового производного 2-(алкилпирролидин)-N-(арил)карбоксамида, обладающего высокой антиаритмической активностью.
Материалы и методы. Для исследования антиаритмической активности соединения эксперимент проводился на моделях аритмии, вызванной внутривенным введением аконитина и адреналина. Эффект оценивали по его способности предупреждать возникновение аритмии, удлинять время выживания животных или по длительности приступа аритмии. Кроме того, был проведен анализ электрокардиограммы бодрствующих крыс. Исследуемое соединение и препарат сравнения (лидокаин) вводили животным внутривенно в эффективных антиаритмических дозах.
Результаты. 2-(н-бутилпирролидин)-N-(2-бромфенил)карбоксамида гидрохлорид в условиях аконитиновой аритмии обеспечивает статистически значимое по сравнению с контролем ограничение длительности приступов аритмии у экспериментальных животных в 1,7 раза и также снижение продолжительности аритмии по сравнению с лидокаином в 2,5 раза; кроме того, данное соединение гарантирует защиту животных от гибели в 100 % случаев. При создании аритмии, вызванной введением адреналина, соединение не предупреждает появление расстройства ритма сердца. Кроме того, показатели электрокардиограммы животных практически не меняются.
Выводы. 2-(н-бутилпирролидин)-N-(2-бромфенил)карбоксамида гидрохлорид (соединение К-23) проявляет выраженную активность на моделях аритмии, вызванной введением аконитина и хлорида кальция, что может свидетельствовать о его способности затруднять натриевый ток через клеточную мембрану, замедляя деполяризацию кардиомиоцитов.
Поскольку исследованное соединение демонстрирует высокую антиаритмическую активность, не изменяя при этом показатели ЭКГ, лекарственный препарат, созданный на его основе, может оказаться эффективным.
Ключевые слова. Аритмия, лидокаин, аконитиновая модель аритмии, адреналиновая модель аритмии, электрокардиограмма, эффективная антиаритмическая доза.

INTRODUCTION

For many decades, diseases of the cardiovascular system have occupied first place in the structure of morbidity and mortality in the world. Heart rhythm disturbances are one of the most common manifestations of these diseases. Among the factors influencing the increase in their prevalence, the increase in life expectancy of the population and the increase in morbidity of the circulatory system can be highlighted [1; 2]. The main methods of treating heart rhythm disor-

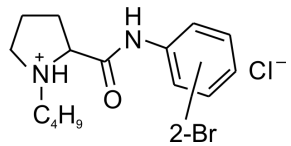
ders are surgical interventions and drug therapy with antiarrhythmic drugs. At the same time, pharmacotherapy remains the predominant method of treating patients with arrhythmias [3]. Consequently, the problem of choosing adequate antiarrhythmic therapy remains relevant and is of considerable interest.

The purpose of the research is to study the action of a new derivative 2-(alkylpyrrolidine)-N-(aryl)carboxamide, which has high antiarrhythmic activity and was synthesized at the Department of General and Organic Chemistry of the Perm State Pharmaceutical Academy (D.V. Kalinin – PhD in Chemistry, Head of the research group at the Institute of Pharmaceutical and Medicinal Chemistry, University of MŸnster, Germany).

MATERIALS AND METHODS

The biological activity of the compounds was assessed in experiments on animals obtained from the Andreevka nursery in the Moscow region. The animals management complied with the Good Laboratory Practice (GLP) and Order of the Ministry of Health of the Russian Federation No. 199n dated April 01, 2016 “Rules of Good Laboratory Practice”, as well as the provisions of the manual for conducting preclinical studies of medicines edited by. A.N. Mironov [4]. The studies were approved by the Bioethics Commission (protocol No. 8 dated January 20, 2023).

A compound was selected for the study, for which the antiarrhythmic index (AI) was initially calculated, depending on the values of the mean lethal and effective therapeutic doses 2-(n-butylpyro-lidine)-N-(2-bromophenyl)carboxamide hydrochloride (compound K-23).



Lidocaine produced by JSC BIOCHIMIC (Russia) was used as a reference drug.

To study antiarrhythmic activity, experiments were carried out on models of cardiac arrhythmia in awake animals caused by intravenous administration of chemicals; in particular, aconitine and adrenaline models of arrhythmia were used.

The study of the antiarrhythmic activity of the compound in a model of arrhythmia caused by the administration of aconitine was carried out on non-pedigree rats of both sexes, sexually mature, weighing 180–250 g. During the experiment, an electrocardiogram (ECG) was recorded in the animals in standard lead II using a single-channel electrocardiograph EK1T-1/3-07 "AXION" (manufactured by "Concern "Axion" LLC, Russia). The dose of aconitine that stimulated the formation of atrioventicular extrasystole was selected experimentally. Aconitine produced by Sigma-Aldrich (USA) was administered into the tail vein. As a result, a dose of 20 mcg/kg was chosen. The death rate of animals was

60 %[1], which made it possible to study the effect of the compound on the duration of arrhythmia in animals. Heart rhythm disturbances appeared 1–2 minutes after administration of aconitine. ECG was recorded for 20 minutes. The test compound was administered intravenously at an effective antiarrhythmic dose 2 minutes before the administration of aconitine. The activity of a substance was assessed by its ability to prevent the development of arrhythmia or increase the survival time of animals [4].

A study of the antiarrhythmic activity of the compound in a model of arrhythmia caused by the administration of adrenaline was carried out on awake chinchilla rabbits weighing 4.5–5 kg. Adrenaline was administered into the marginal vein of the ear at a dose of 15 mcg/kg to assess the heart reaction, which was recorded using an ECG in standard lead II using a single-channel electrocardiograph EK1T-1/3-07 "AXION" (manufactured by "Concern "Axion" LLC, Russia). After restoration of the heart rhythm, the test compound was administered intravenously at a dose equal to ED_{50}, and after 3 minutes, adrenaline was administered again [4]. The antiarrhythmic effect was assessed by the duration of the arrhythmia attack.

When studying the effectiveness of a new antiarrhythmic drug, it makes sense to evaluate its effect on heart function, since some antiarrhythmic drugs can cause changes in its physiological properties. Therefore, an analysis of the electrocardiogram of awake rats was carried out. The test compound, as well as the reference drug, were administered intravenously to animals in effective antiarrhythmic doses.

Statistical processing of the study results was performed using the Statistica 8.0 software package. The results were processed using variation statistics according to the Fisher–Student method [5].

RESULTS AND DISCUSSION

When studying the antiarrhythmic activity of 2-(alkylpyrrolidine)-N-(aryl)carboxamide derivatives, it was found that compounds of this group exhibit fairly high antiarrhythmic activity.

Among these compounds, a substance with maximum antiarrhythmic activity was found – K-23, the ED_{50} of which is 3.2 mg/kg, and the antiarrhythmic index is 13.6.

Thus, the relative activity of 2-(n-butylpyrrolidine)-N-(2-bromophenyl) carboxamide hydrochloride is 2.7 times higher than the activity of lidocaine[2]. In addition, the task was set to determine the degree of the heart protection from fibrillation that appears after disruption of blood flow in the coronary artery using this compound. Analysis of the ECG recorded during a study on a model of acute coronary occlusion in awake rats

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revealed that the K-23 compound prevents the formation of ventricular fibrillation. This fact significantly distinguishes its effectiveness not only from control results, but also from the activity of lidocaine [6].

To be able to suggest a probable mechanism of the new compound action, its antiarrhythmic activity was studied in models using chemicals whose arrhythmogenic effect is associated with an effect on the cardiomyocyte membrane. Studies were carried out on a model of arrhythmia initiated by aconitine, which can interact with voltage-gated sodium ion channels of cardiomycytes, which leads to long-term depolarization. At the same time, the permeability of the membranes for potassium ions that leaves the cell, as well as for calcium ions, increases. An increase in calcium concentration in the cell stimulates the release of acetylcholine, which reacts with cholinergic receptors of postsynaptic membranes, opening sodium channels here and creating a new action potential, which leads to electrophysiological disturbances in myocardial cells [7; 8]. The results of the study are presented in Table 1.

The time of arrhythmia after administration of aconitine to control animals was 626.5 ± 95.2 s. Preliminary administration of the reference drug lidocaine not only does not reduce the duration of the arrhythmia, but also increases this time by 1.5 times in comparison with the control result. At the same time, compound K-23 works quite actively under conditions of aconitine arrhythmia, under its influence there is a statistically significant, compared with the control, limitation of the arrhythmia attacks duration in test animals to 366.7 s, as well as a significant decrease in the duration of arrhythmia, compared with the lidocaine effect (2.5 times). In addition, this compound guarantees protection of animals from death in 100 % of cases, while the use of lidocaine does not prevent their death. Mortality due to an arrhythmia attack when using lidocaine was 40 %, which differs little from the result in the control.

In addition, an arrhythmia model was used using adrenaline, which creates an arrhythmogenic effect caused by an increase in the activity of the sympathetic nervous system and the content of catecholamines, causing activation of slow transmembrane calcium channels mediated by the excitation of β-adrenergic receptors, which provokes the formation of ectopic activity of cardiac pacemakers [9; 10]. From the results of the experiment it follows that when creating arrhythmia caused by the administration of adrenaline, the substance K-23 does not prevent the appearance of cardiac arrhythmia in awake rabbits. In addition, when using this compound, the time of adrenaline arrhythmia increases slightly compared to control data. The results are shown in Table 2.

Table 1

Effect of compound K-23 on the course of aconitine arrhythmia in rats, $M \pm m$

Compound / drug	Number of animals in the experiment	Dose (ED_{50}), mg/kg	Duration of arrhythmia, s	Death, %
Control	10	–	626.5 ± 95.2	70
K-23	10	3.2	366.7 ± 30.4 $p = 0.023$ $p' = 0.047$	0 $p = 0.0002$ $p' = 0.024$
Lidocaine	10	7.7	929.7 ± 263.5 $p = 0.248$	40 $p = 0.196$

N o t e: p is level of statistical significance of differences in comparison with control data; p' is level of statistical significance of differences in comparison with lidocaine.

Table 2

Effect of compound K-23 on the development of adrenaline arrhythmia in rabbits, $M \pm m$

Compound / drug	Dose, mg/kg	Number of animals	Prevention of arrhythmia, % of the number of experiments			Duration of arrhythmia, s	
			full	partial	total	control	experience
K-23	6.4	5	0	0	0	242.0 ± 17.4	297.0 ± 17.4 $p = 0.365$ $p' = 0.121$
Lidocaine	7.7	5	0	0	0	190.0 ± 14.5	152.0 ± 13.5 $p = 0.091$

N o t e: p is level of statistical significance of differences in comparison with control data; p' is level of statistical significance of differences in comparison with lidocaine.

Thus, the test compound exhibits a significant antiarrhythmic effect in the aconitine arrhythmia model. In accordance with the data obtained, the activity of K-23 in aconitine arrhythmia may characterize its ability to block sodium current, slowing down the depolarization of cardiomyocyte membranes.

When taking antiarrhythmic drugs of various groups, characteristic changes often appear on the ECG. In this regard, changes in the heart function of experimental animals were assessed based on an analysis of the ECG of rats after intravenous administration of the K-23 compound in an effective antiarrhythmic dose. The results are presented in Table. 3.

As a result of the analysis of the ECG of awake rats, it was found that intravenous administration of a comparison drug to animals in an effective antiarrhythmic dose leads to individual ECG changes. Lidocaine has a negative chronotropic effect. It reduces the heart rate by 1.2 times. This change is statistically significant compared

Table 3

Effect of compound K-23 on ECG parameters in rats, $M \pm m$

Compound / drug	Number of animals in group	ECG indicator				
		heart rate per minute	PQ interval duration, s	QRS duration, s	QT interval duration, s	amplitude R, mV
Control (0.9 % NaCl solution)	10	442.8 ± 17.05	0.04 ± 0.004	0.03 ± 0.003	0.06 ± 0.003	0.26 ± 0.04
K-23	10	445.3 ± 7.0 $p = 0.614$	0.06 ± 0.005 $p = 0.018$	0.03 ± 0.01 $p = 0.849$	0.07 ± 0.01 $p = 0.614$	0.32 ± 0.04 $p = 0.347$
Lidocaine	10	373.8 ± 11.3 $p = 0.003$	0.05 ± 0.005 $p = 0.220$	0.02 ± 0.002 $p = 0.664$	0.06 ± 0.004 $p = 0.480$	0.49 ± 0.04 $p = 0.018$

N o t e: p is level of statistical significance of differences in comparison with control data.

to the control result. There is also a slight increase in the amplitude of the R wave. Deviations in ECG parameters with the introduction of K-23 compound are insignificant, in particular, there is a slight prolongation of the PQ interval compared to the control result, which indicates a slowdown in the conduction of excitation from the sinus node to the atrioventricular node, heart rate, QRS interval time, QT, wave height R do not differ from these indicators in the control series of experiments.

Conclusions

1. 2-(n-butylpyrrolidine)-N-(2-bromophenyl)carboxamide hydrochloride (compound K-23) shows visible activity in models of arrhythmia caused by administration of aconitine and calcium chloride, which may indicate its ability to impede the sodium flow through the cell membrane by slowing depolarization of cardiomyocytes.

2. Since the compound studied, demonstrates high antiarrhythmic activity without changing the ECG readings, the drug created on its basis may be effective.

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