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THEORETICAL & EXPERIMENTAL MEDICINE

PSYCHIATRICS & MEDICAL PSYCHOLOGY

ANAMNESTIC RISK FACTORS FOR ASTHMA DEVELOPMENT IN INFANTS

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Abstract

Introduction. Allergic diseases are one of the major problems of the present responsible for almost 700 million cases in the world. Respiratory allergies rank as the most common chronic health condition among them. About 330 million people have bronchial asthma today.
The aim of the study was to identify the risk factors for development of asthma in children with wheezing.

Objective. The purpose of this study was to determine the risk factors for asthma development. The study consisted in assessment of clinical data of 94 children aged 1–7 years with recurrent wheezing. Children were observed for 5 years. The first group included 62 patients with diagnosed acute obstructive bronchitis, and the second group included 32 patients diagnosed with asthma.

Results. Assessment of anamnestic data revealed significant risk factors for asthma development. The study showed a relationship between the risk factor and development of the disease in children with recurrent wheezing.

Conclusions. The obtained data can be used in the algorithm of examination of patients with wheezing. Assessment of risk factors for asthma developent is important for pediatric practice. Thorough history taking and determination of risk factors are important when examining patients with recurrent wheezing.

Key words: *wheezing, asthma, children, anamnestic data, risk factors.*

Allergies rank as the most common chronic health conditions in the contemporary world. Almost 700 million people worldwide suffer from this disorder [1, 2]. Almost 330 million of these patients have respiratory diseases and suffer from asthma [3, 4]. Bearing in mind that chronic inflammation is the main link in the pathogenesis of asthma of the bronchopulmonary system, the disorder development mainly begins in early childhood [5, 6]. Therefore, an important issue today is early recognition of asthma development. The problem of medical society at this stage is the interest in early diagnosis of the disease, because the onset of the disease, namely, manifestations of wheezing are most often observed up to 6 years of age [5–7]. However, late diagnosis entails formation of chronic bronchopulmonary disease in children with recurrent wheezing [5]. The main difficulty in identifying the onset of asthma in children under 6 years of age is that wheezing is accompanied by a large number of nosological forms. Thus, diagnosis and differential diagnosis of these diseases in children cause some difficulties [6, 8]. The prevalence of wheezing in recent years has increased and is 16.1% [9]. About half of young children with acute bronchitis develop symptoms of wheezing. Recurrent wheezing, namely repeated episodes of prolonged exhalation, occur in 32.2% of children [9, 10]. Approximately 30% of first-year children have at least one episode of wheezing, and in 20% of children the symptoms of wheezing persist later on [6, 10, 12]. One in every four children under the age of 6 has bronchial obstruction, most often secondary to acute respiratory diseases. The share of wheezing secondary to acute respiratory conditions is about 50% in children under 6 years, its recurrence is typical for 25% of children [6, 11, 13].

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Differential diagnosis of the disease that cause wheezing and detection of asthma development in the early stages is a pressing issue today.

2. Purposes, subject and methods

2.1. The purpose of the work was to identify anamnestic risk factors for asthma in children with recurrent wheezing.

2.2. Subjects and methods

The study consisted in evaluation of clinical data from 94 children (56 boys and 38 girls) aged 1 to 7 years with recurrent wheezing, undergoing examination and treatment in the city clinical children's hospital. Children were constantly monitored for 6 years to verify asthma development.

All patients underwent a comprehensive examination according to the protocol of treatment of children with wheezing No. 18 dated 13.01.2005 "On approval of the Protocols of providing medical care to children in the specialty "Pediatric Pulmonology", and the protocol of treatment of children with asthma No. 868 dated 08.10.2013 "On approval and introduction of medical-technological documents on standardization of care in bronchial asthma".

Statistical analysis of the data was performed using statistical software "EXCEL FOR WINDOWS" and "STATISTICA 8.0. FOR WINDOWS". Gaussian normal distribution of the samples was determined.. χ^2 Person test was used to determine the characteristics and strength of the relationship between quality indicators. The study implied assessment of relative risk (RR) of event occurrence with the determination of 95% confidence interval. Characteristics and compliance of the obtained values of statistical criteria were evaluated according to Rea & Parker recommendations.

Planned clinical examinations were approved by the local ethics committee. The study was in line with the principles of the Helsinki Declaration. All parents of the children who participated in the study gave written consent to participate.

Conflict of interest. There is no conflict of interests.

3. Results

The first group included 62 patients (n = 62, mean age 2.89±1.47 years), diagnosed with acute obstructive bronchitis, the second group included 32 children (n=32, mean age 4.33±1.57 years), who were diagnosed with asthma. Among the total number of patients, gender distribution showed no statistically significant difference $p>0.05$, namely there were 56 (59.57±5.06%) boys and 38 (40.4 ±5.06%) girls.

Anamnestic data, such as, features of pregnancy, namely threat of miscarriage, TORCH infection and acute diseases or exacerbation of chronic respiratory diseases in the mother during pregnancy, taking into account trimester, preterm birth, delivery and asphyxia in the antenatal period, lower respiratory tract diseases in the neonatal period, IVF in the newborn period, early artificial feeding, frequent acute respiratory and diseases (5 years and over a year) during the first year of life, use of antibacterial therapy in the first 6 months of life, onset of allergic disorders and first episodes of wheezing in the first year of life, presence of concomitant allergic diseases (atopic dermatitis and allergic rhinitis), presence of concomitant diseases, family history of allergic diseases. Social-household history was also evaluated, namely smokers in the family, presence of animals, were carefully studied. Further statistical analysis determined the strength of the relationship between the factors that had significant differences and disease formation. The frequency of risk factors and statistically significant analysis data are presented in *Table 1* and *Table 2*.

4. Discussion. Factors that could be considered as risk factors for asthma in young children were distinguished during the assessment. The so-called critical periods are distinguished in the antenatal period, during which the fetus is extremely sensitive to the effects of various harmful factors, especially the period of implantation of the fertilized egg and the period of placentation. Adverse effects during these periods lead to various consequences [9, 14]. Therefore, the unfavorable course of pregnancy and the mother's acute respiratory diseases, especially in the first trimester of pregnancy, affect the antenatal period and the formation of the baby's body as a whole, and in particular the bronchopulmonary system. The study showed that compromised obstetric history increases the risk of asthma development by 3 times.

The history of frequent acute respiratory diseases, especially in the first year of life, can significantly affect the body's defenses. With this in mind, in the first place, the negative influx will be directed to the immune system and to the further development of chronic diseases of the bronchopulmonary system [11, 14, 15]. At the same time, along with frequent respiratory diseases, development of the immune system is affected by antibacterial therapy. These data were confirmed during the study and statistically significant risk outcomes were obtained. Namely, frequent

Table 1

Frequency of adverse factors in children with recurrent wheezing

Factor	Group 1 (n=62)		Group 2 (n=32)		p
	n	p%±$s_{p\%}$	n	p%±$s_{p\%}$	
Male sex	37	59.7±6.23	19	59.4±8.68	>0.05
Pregnancy complicated by concomitant bronchopulmonary disease of the mother in the first trimester	11	17.74±4.85	20	62.50±8.56	< 0.01
Pregnancy complicated by concomitant bronchopulmonary disease of the mother in the second trimester	6	9.68±3.75	3	9.37±5.15	>0.05
Pregnancy complicated by concomitant bronchopulmonary disease of the mother in the third trimester	2	3.22±2.24	1	3.12±3.07	>0.05
Preterm labor	1	1.61±1.60	2	6.25±4.30	>0.05
Labor by cesarean section	6	9.68±3.75	5	8.06±4.81	>0.05
Diseases of the lower respiratory tract during infancy	3	4.84±2.72	4	12.50±5.85	>0.05
Severe asphyxia during labor	1	1.61±1.60	1	3.12±3.07	>0.05
Artificial lung ventilation during infancy	1	1.61±1.60	2	6.25±4.30	>0.05
Acute respiratory diseases during the first year of life (5 and more episodes a year)	28	45.16±6.32	26	87.50±5.85	< 0.01
Frequent antibacterial therapy at the age under 6 months	24	38.71±6.19	25	78.13±7.31	< 0.01
Early artificial feeding	35	59.45±6.24%	17	53.13±8.82	>0.05
Compromised family history with maternal relatives with asthma	11	17.74±4.85	14	43.75±8.77	< 0.01
Compromised family history with paternal relatives with asthma	6	9.68±3.75	4	12.50±5.85	>0.05
A common form of atopic dermatitis	27	43.55±3.00	25	78.13±7.31	< 0.01
The onset of manifestations of atopic dermatitis during the first year of life	22	35.48±6.08	24	75.00±7.65	< 0.01
Allergic rhinitis	5	8.06±3.46	10	31.25±8.19	< 0.01
First wheezing episode during the first year of life	13	20.97±5.17	14	43.75±8.77	< 0.05
Concomitant otolaryngology disorder	4	6.45±3.12	4	12.50±5.85	>0.05
Smokers in the family (passive smoking)	5	8.06±3.46	9	28.13±5.06	< 0.01
Animals in the house	16	25.81±5.56	15	46.87±8.82	<0.05

Note:

1. Selective share in percent; sp% is the statistical error of the sample fraction, expressed as a percentage;
2. * – % of the total number of patients.

acute respiratory diseases in the first year of life (5 and more times a year) increase the risk of asthma development by 5 times, antibacterial therapy at the age of 6 months increases the risk of asthma development by 3 times. Data that frequent respiratory diseases are a significant adverse factor in asthma development have been described [6, 14, 16]. Whereas asthma is, first and foremost, an allergic disease, and wheezing has an allergic link in its pathogenesis, we carefully studied life and, above all, allergic and family history [1, 3]. Since concomitant allergic diseases or congenital susceptibility to allergic reactions play a significant role in the formation of the disease [1, 2]. The study found that the risk of developing the disease in children with the presence of a common form of atopic dermatitis is 3 times higher, the onset of concomitant allergic diseases, namely first manifestations of atopic dermatitis in the first year of life increases the risk of developing the disease three times, the presence of allergic rhinitis is 2 times higher, compromised family history of allergic diseases increases the risk of asthma development in children by 2 times.

Chronic inflammation is considered to be the basis of its pathogenesis, which is determined by the variable narrowing of the bronchi, which is regulated by various cellular elements and mediators of inflammation [5, 6]. In every chronic process, early onset of the disease is prognostically unfavorable. Contemporary literature describes the importance of early wheezing onset as a predictor of development asthma [16–18]. Therefore, the study paid attention to the early onset of wheezing, namely the presence in the

Table 2

The nature and strength of the association between risk factors and asthma formation in children with recurrent wheezing

Factor	RR	χ^2	C'
Pregnancy complicated by concomitant bronchopulmonary disease of the mother during the first trimester	3.39 [CI 95% 1.91-6.00]*	19.130	0.582
Acute respiratory diseases during the first year of life (5 and more episodes a year)	4.75 [CI 95%1.81-12.45]*	11.245	0.462
Antibacterial therapy at the age under 6 months	3.28 [CI 95% 1.57-6.83]*	13.140	0.495
Atopic dermatitis	2.89 [CI 95% 1.39-6.00]*	10.209	0.443
Onset of atopic dermatitis during the first year of life	3.13 [CI 95% 1.57-6.24]*	13.189	0.496
Allergic rhinitis	2.39 [CI 95% 1.46-3.96]*	8.460	0.406
First episode of wheezing at the first year of life	1.9 [CI 95% 1.13-3.30]**	5.350	0.328
Compromised family history with relatives with asthma	2.15 [CI 95% 1.13-3.30]*	7.313	0.380
Smokers in the family	2.24 [CI 95% 1.33-3.76]*	6.701	3.765
Animals in the house	1.79 [CI 95% 1.04-3.09]**	4.239	0.294

Notes:

1. RR is the relative risk of an event occurring with a 95% confidence interval;
2. χ^2 is the criterion for assessing the significance of differences in results depending on the interaction of the risk factor;
3. C' is the normalized value of the Pearson coefficient;
4. * is the level of statistical significance $p<0.001$;
5. ** is the level of statistical significance $p<0.005$.

history of the first episode of wheezing in the first year of life. The findings revealed the patterns of asthma formation in children under 6 years of age, with recurrent wheezing. Thus, a history of the first episode of wheezing up to 1 year increases the risk of developing the disease twice.

Social and household factors play an important role in the development and formation of functions of the bronchopulmonary system. This is due to the direct influence of exogenous factors on the respiratory tract [12, 18, 19]. Thus exogenous factors include smokers in the family, pollution, presence of animals in the house, the use of various chemicals in everyday life [12, 20, 21]. All of the above relates to inhalation agents, which contributes to the pathological restructuring of the respiratory tract. As for social and household factors, presence of smokers in the family increases the risk of developing the disease by 2 times and the presence of animals in the dwelling by almost 2 times.

The data obtained can be incorporated into an algorithm for the examination of patients with wheezing for objective assessment of risk factors and the possibility of asthma formation, which is extremely important for pediatric practice. Therefore, careful history taking and identification of these factors is of great importance when managing patients with recurrent wheezing.

Conclusions

1. Asthma development can be caused by unfavorable factors occurring during the course of pregnancy, neonatal period, the period of the first year of life, by compromised family and allergic history, social and living conditions.

2. The course of pregnancy complicated by concomitant bronchopulmonary disease of the mother in the first trimester of pregnancy, frequent acute respiratory diseases in the first year of life (5 or more times a year), antibacterial therapy at the age of 6 months, the first episode of wheezing in the first year life, children with common form of atopic dermatitis, onset of concomitant allergic diseases in the first year of life, allergic rhinitis, compromised family allergic history, smokers in the family, animals in the house increase the risk of asthma development in children.

3. Careful history taking and identification of these risk factors should be used to predict asthma development in children under 6 years.

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MARKERS OF ENDOTHELIAL DYSFUNCTION AND THEIR PROGNOSTIC VALUE IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA

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Abstract
Acute lymphoblastic leukemia (ALL) in children is associated with development of a large number of serious complications. Inflammatory and pro-aggregation activation of endothelial cells accompanies the course of leukemia. Endothelial dysfunction (ED) is believed to be an integral part of the pathogenic link of ALL and its complications. **Materials and methods.** The content of nitric oxide in serum with the concentration of its stable metabolites – NO_2, NO_3 – was determined in 73 patients with ALL and 19 healthy children. Determination of nitric oxide was performed at different stages of the presentation of ALL: 49 children were examined during the manifestation of leukemia (group 1A), of whom 36 children (group 1B) were re-examined during the first course of treatment – induction of remission. Also 8 children (group 2) were examined during supportive therapy and 16 children (group 3) who underwent complete chemotherapy treatment. **Results.** The study showed that nitrite increase and nitrate reduction secondary to reduced total nitric oxide content during all treatment periods in children with ALL. In addition, signs of endothelial dysfunction were recorded in patients after treatment. ROC analysis showed that reduction in NO_2 content lower than 12.31 μmol/l and the sum of NO_2 + NO_3 lower than 32.99 μmol/l significantly had a negative effect on the survival of children with ALL. **Conclusions.** ED accompanies the course of ALL in children. Determination of nitric oxide is of diagnostic and prognostic importance in patients with leukemia. The critical fall in the concentration of NO and NO_2 accompanies development of multiple organ failure with fatal outcome in children with ALL. Preservation of signs of ED after the end of the treatment of leukemia indicates the need for cardiac monitoring of patients with ALL in history.
Key words: *acute leukemia, children, endothelial dysfunction, nitric oxide.*

Acute lymphoblastic leukemia (ALL) is associated with a large number of complications due to the disease itself and multicomponent therapy [1–3]. Infectious-toxic complications are particularly severe as they are more often lethal than relapse of leukemia [4]. The survival rate of children diagnosed with ALL has been improved due to anthracycline agents, but their use is accompanied by vascular endothelial damage with subsequent development of endothelial dysfunction (ED) [5, 6]. Moreover, it has been proved that ED is part of ALL phenotype in children [5, 7–9], which also explains the effect of anthracycline agents. Studies have shown that there are functional cross-links between hematologic malignancies and vascular endothelium [10]. Hematopoietic stem cells, including myeloid precursors, can integrate into existing functional vascular endothelium with differentiation into phenotypic and functional epithelial-like cells. It is claimed that vascular endothelium is an unrecognized reservoir for blast cells, which plays an important role in the pathogenesis and recurrence of leukemia [10].

Endothelial dysfunction can be defined as inappropriate (increased or reduced) formation in the endothelium of vessels of various biologically active substances. Reduced bioavailability of

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L-arginine, accelerated catalysis, or decreased concentration of nitric oxide (NO) is one of the foundations of ED [7].

The term "nitric oxide" refers to the reduced form of nitric oxide (NO) with a half-life of 2 to 30 seconds, which complicates the method of determining it in the blood. Indirect markers of nitric oxide concentration in the body are its more stable end products nitrite (NO_2) and nitrate (NO_3) [11]. It is the content of these metabolites that determines the total content of nitric oxide.

To date, it has been proven that ED is a common feature of many early complications of chemotherapy, which are a significant cause of morbidity and mortality, despite optimization of treatment protocols [5]. However, there is insufficient data on the role of ED markers in the treatment and remission of ALL in children [8].

2. Purposes, subjects and methods

2.1. The purpose of the work was to evaluate the profile of endothelial function during diagnosis and different periods of treatment and remission in children, to attest the presence of correlation of endothelial dysfunction markers with early adverse clinical outcome of ALL.

2.2. Subjects and Methods

General

The study was performed between 01 September 2016 and 01 November 2020. 73 children (49 boys and 24 girls) with ALL who were hospitalized at Kharkiv City Clinical Children's Hospital No. 16, were studied. The patients' age ranged from 1 to 17 years. The control group consisted of 19 healthy age- and gender-matched children brought to Kharkiv City Outpatient Hospital No. 16 for routine health control or vaccination.

The diagnosis of ALL was established according to the diagnostic criteria specified in the Acute Lymphoblastic Leukaemia Intensive Chemotherapy Berlin Frankfurt Munich 2009 (ALL IC BFM 2009) [12]. After the diagnosis has been established, the patients began an inductive chemotherapy course under the program ALL IC BFM 2009 [12].

The criteria for inclusion in the study were verified diagnosis of ALL, signed consent from parents and/or patients. The criteria for exclusion in the study were refusal of the parents or/and patients to sign the consent or death of patients.

Determination of nitric oxide metabolites

All 73 patients with ALL were involved in determination of the content of serum nitric oxide by spectrophotometric method with nitrogen oxide in M.O. Kovaleva et al. modification (2007) by concentration of its stable metabolites (oxidation products) of nitric oxide – NO_2, NO_3 [13]. The results of NO_2, NO_3 concentrations were expressed in μmol/l.

Determination of nitric oxide was performed at different stages of ALL presentation: 49 children were examined during the manifestation of leukemia (group 1A), of whom 36 children (group 1B) were re-examined during the first course of treatment – induction of remission. Also, 8 children (group 2) were examined during supportive therapy and 16 children (group 3) who underwent complete chemotherapy treatment and had a bone marrow remission period of 2 to 12 years.

Nineteen children in the control group were tested for nitric oxide once during a routine pediatric examination.

Ethics approval and consent to participate

Each study participant and his/her parents were informed about the nature of the study and signed a consent to participate in the study. The study was approved by Ethics and Bioethics Committee of Kharkiv National Medical University, Ukraine (Protocol No. 8 of 5th October 2016) and was conducted according to Helsinki Declaration (1975).

Statistical analyses

For statistical analyses of data STATISTICA 8 (Tulsa, OK) was used. Shapiro–Vilka test was used for verification of the distribution according to the Gauss law. Non-parametric variables included median (Me), interquartile range [Lq – lower quartile; Uq – upper quartile]. To compare the two dependent samples, the non-parametric Wilcoxon test (T) was used. To compare two independent samples, non-parametric Mann–Whitney U-test has been used. All P-values were two-tailed, and values <0.05 were considered significant. ROC analysis was used to evaluate the specificity and sensitivity of the method.

Conflict of interests

The authors of the article declare no conflict of interest.

3. Results and discussion

The study involved 73 children with ALL. General characteristics of patients are presented in *Table 1*. There was a significant (p = 0.006) prevalence of boys (67.0%) than girls (33.0%). Patients had a median age of 6.7±5.0 years (range 1 to 17 years).

Among the immunophenotypic variants of leukemia, B cell lineage leukemia was significantly more common (p = 0.000) – 60/73 children (82.2%). Only 13/73 (17.8%) children had T cell lineage leukemia.

Table 1

General characteristics of patients in diagnosis

Characteristics	n=73	%
Gender		
male / female	49 / 24	67.0 / 33.0
Age, years		
< 3	15	20.5
3–6	27	37.0
7–10	15	20.5
11–14	5	7.0
15–18	11	15.0
Race		
European / Other	73 / 0	100 / 0
Immunophenotype		
B – lineage / T – lineage	36 / 10	78.3 / 21.7
Risk group		
standard / high	56 / 17	76.7 / 23.3

The level of metabolites of nitric oxide, namely nitrites (NO_2) and nitrates (NO_3) in the serum of children with ALL, was significantly different from the normal parameters of the control group (*Table 2*). Based on the fact that ED is a pathological increase or decrease in biologically active substances in the vascular endothelium [7], the deviations obtained from our control values confirm the manifestations of ED in all patients studied.

NO? content in patients of all groups (1A, 1B, 2, 3) was significantly higher than the nitrite level of the control group children (*Table 2*). NO_2 had the opposite pattern and was significantly lower in patients with ALL than nitrate levels in healthy children. At the same time, despite the high concentration of NO_2, the total amount of NO_3 + NO_3 in children with ALL during all observation periods (manifestation, intensive care, supportive therapy and remission of leukemia) was significantly lower than in the control group children, with a maximum reduction during induction polychemotherapy (group 1B patients).

Adrian Doroszko et al., 2016 [9], Woo Jung Jang, 2013 [6] obtained similar results (reduction of NO content) and confirmed the pathogenic hypothesis of impaired NO synthesis in children with ALL. The low concentration of nitric oxide was explained by intensification of ED secondary

Table 2

Indicators of nitric oxide levels in children with acute lymphoblastic leukemia and controls (Me (Lq; Uq))

Indicator	Group				
	Group 1		Group 2	Group 3	Control group
	1A n=49	1B n=34	n=8	n=16	n=19
NO_2, µmol/l	17.55 11.12 21.28	19.56 13.56 24.15	21.10 20.42 22.13	20.54 17.80 24.44	12.79 12.35 14.00
W Test: p_{1A}-$p_{1Б}$ = 0.0552 MW U Test: p_{1A}-p_2 = **0.0465**; p_{1A}-p_3 = **0.0346**; p_{1A}-$p_к$ = **0.0150**; $p_{1Б}$-p_2 = 0.2682; $p_{1Б}$-p_3 = 0.3911; $p_{1Б}$-$p_к$ = **0.0002**; p_2-p_3 = 0.6406; p_2-$p_к$ = **0.0000**; p_3-$p_к$ = **0.0000**					
NO_3, µmol/l	14.97 11.28 17.34	15.51 12.00 19.34	14.40 12.63 15.27	12.06 11.00 17.09	30.93 28.79 32.05
W Test: p_{1A}-$p_{1Б}$ = **0.0002** MW U Test: p_{1A}-p_2 = 0.7598; p_{1A}-p_3 = 0.3489; p_{1A}-$p_к$ = **0.0000**; $p_{1Б}$-p_2 = 0.5476; $p_{1Б}$-p_3 =0.1044; $p_{1Б}$-$p_к$ = **0.0000**; p_2-p_3 = 0.6931; p_2-$p_к$ = **0.0000**; p_3-$p_к$ = **0.0000**					
NO_2+NO_3, µmol/l	30.64 27.86 35.28	33.55 31.45 39.19	35.83 34.10 37.91	35.54 28.36 42.10	42.27 41.51 45.61
W Test: p_{1A}-$p_{1Б}$ = **0.0056** MW U Test: p_{1A}-p_2 = 0.0796; p_{1A}-p_3 = 0.3284; p_{1A}-$p_к$ = **0.0000**; $p_{1Б}$-p_2 = 0.6339; $p_{1Б}$-p_3 = 0.6398; $p_{1Б}$-$p_к$ = **0.0001**; p_2-p_3 = 0.7468; p_2-$p_к$ = **0.0017**; p_3-$p_к$ = **0.0032**					

Note. W Test – Wilcoxon test, MW U Test – Mann–Whitney test

to cytostatic therapy with anthracycline antibiotics and an increase in the production of NO synthetase inhibitors [6, 9]. For example, asymmetric dimethylarginine (ADMA) is a competitive inhibitor of NO synthase and is increased in patients with ALL. ADMA is a product of tumor cell degradation, oxidative stress, manifestation of liver failure and inflammation of the endothelial wall [5, 8, 9].

It should be noted that there are a number of scientific studies on the levels of nitric oxide in ALL, which have shown the results of increasing total NO [7, 14, 15]. However, studies were conducted on adult patients who likely had concomitant cardiovascular diseases.

When comparing nitrite levels across all groups, it was found that starting high NO_2 content during leukemia manifestation (group 1A) continued to increase significantly ($p<0.05$) at subsequent stages of treatment (induction therapy, supportive therapy, and during remission of ALL).

NO_3 concentration remained at a level lower than normal throughout the study periods (manifestation, induction, supportive therapy and remission). No statistical significance was found between the patients of groups 1A, 2 and 3 ($p>0.05$). Wilcoxon's test between NO_3 groups A and 1B demonstrated a significant ($p = 0.0002$) tendency to an increase in this index during complications of induction therapy compared to the acute phase of the disease.

Thus, at a low level of nitric oxide, an increase in nitrite values accompanied by a decrease in nitrate indicators was observed. A much higher nitrite content (NO_2) than nitrate level (NO_3) indicated severe tissue damage, endogenous intoxication [16] due to the manifestations of leukemia. Perhaps, secondary to a low total NO level, there were compensatory changes in the ratio of nitrogen metabolites in the form of an increase in NO_2 level due to the inverse conversion of NO_3 to NO_2. In some conditions, the reaction of the nitrogen cycle may be dominated by the synthesis of NO from arginine, or its reduction from nitrite ion [16].

In patients of group 1A, direct correlation was found between the levels of metabolites of nitric oxide and indicators of humoral immunity (immunoglobulins A, G), circulating immune complexes, glycoproteins (*Table 3*). Thus, an increase in ED during the increase in the severity of leukemia and immunodeficiency was recorded. In addition, nitrogen metabolites showed a weak indirect correlation between blood pressure levels in patients during the onset of ALL, confirming the vasoactive function of nitric oxide [14].

Group 1B patients were found to have indirect correlations between NO_2, $NO_2 + NO_3$ and uric acid (*Table 3*), demonstrating an increase in ED during blast cell lysis. The metabolites of nitric oxide also had correlation with C-reactive protein, albumin α1-globulins and γ-globulins. The above indicates a close correlation of endothelial function with the intensity and severity of the inflammatory process in ALL.

In patients with remission of leukemia, after completion of treatment (group 3), the content of $NO_2 + NO_3$ tended to increase, i.e. almost

Table 3

Correlation relationships between endothelial dysfunction markers, laboratory and physical indicators

Laboratory value	NO_2, μmol/l	NO_3, μmol/l	NO_2+NO_3, μmol/l
Group 1A (n=49)			
Glycoproteins, U.		+0.536*	
CIC, U.	+0.502*	+0.639*	+0.534*
Immunoglobulin A, g/l	+0.581*		
Immunoglobulin G, g/l	+0.510*		
Splenomegaly, cm	+0.417*		
Blood pressure, mm Hg	-0.347*		-0.411*
Diastolic pressure, mm Hg	-0.355*		-0.418*
Group 1B (n=34)			
Uric acid, mmol/l	-0.898*		-0.898*
C-reactive protein, g/l	+0.759*	-0.604*	
α1-globulins, %	-0.696*		
Albumins, %		-0.642*	
γ-gobulins, %		+0.541*	

reached normal values. This is evidently due to the normalization of a number of NO synthetase inhibitors in children in remission [8] and the absence of pathological factors (remission of leukemia, end of treatment).

It should be emphasized that children who received anthracycline drugs are at risk of cardiovascular disease even after therapy [6, 9, 17]. Persistence of a high NO_2 concentration and a low NO_3 concentration in the serum of patients during remission confirms incomplete endothelial recovery. This substantiates the need for cardiac monitoring of patients with ALL in history.

Influence of nitric oxide metabolite levels on the development of adverse complications

Five (6.8%) patients out of 73 died. Of them, 3 (4.1%) children died during the induction of remission from complications of ALL and chemotherapy. All three children had manifestations of multiple organ failure secondary to toxic ulcerative necrotic enteropathy, mucositis, respiratory failure, myelosuppression (neutropenia), and hemorrhagic syndrome. One child had bilateral pneumonia. The other child had acute renal failure and angioplastic encephalopathy.

The other 2 children died of recurrence of leukemia after the end of our study. To analyze the possible prognostic value of NO_2, NO_3 levels, their sums among patients of group 1B, who died during the development of complications during chemotherapy, ROC analysis was used. The results of the analysis showed that the NO_2 level was lower than 12.31 μmol/l (*Figure 1*), predicts lethality with a sensitivity of 100% (95% CI 29.2 – 100), specificity 92.9% (95% CI 76.5–99.1) (AUC = 0.952 [0.81; 0.99]. These data confirm the contribution of NO_2 to the development of leukemia complications.

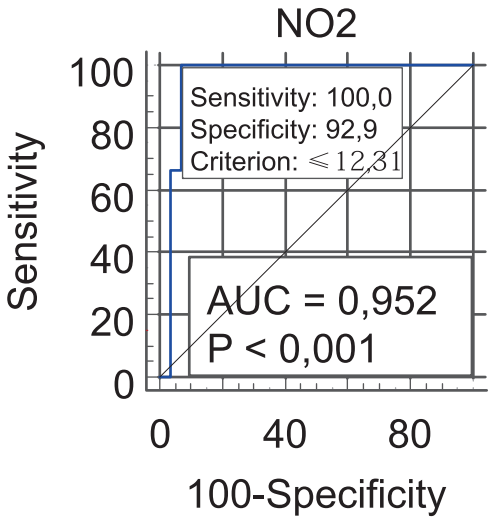

Figure 1. ROC curve predicting the likelihood of lethality according to NO_2 level of patients i n group 1B

Reduction of biological activity of nitric oxide (NO) leads to stimulation of vasoconstriction, inflammation and thrombosis, damage to tissue structures, plays a crucial role in the pathogenesis of ED [5, 8, 16]. The role of nitric oxide in disorders of cardiovascular function under conditions of endotoxic shock has been proved [16]. The timely detection of this process can have therapeutic and prognostic consequences [5, 8]. We observed a maximum decrease in the total amount of nitrates and nitrites during induction polychemotherapy. ROC analysis confirmed (*Figure 2*) that the NO_2 + NO_3 level was lower than 32.99 μmol/l, predicting fatal complications with sensitivity of 100% (95% CI 29.2 –100), specificity 92.9% (95% CI 37.2 – 75.5) (AUC = 0.810 [0.62; 0.92])

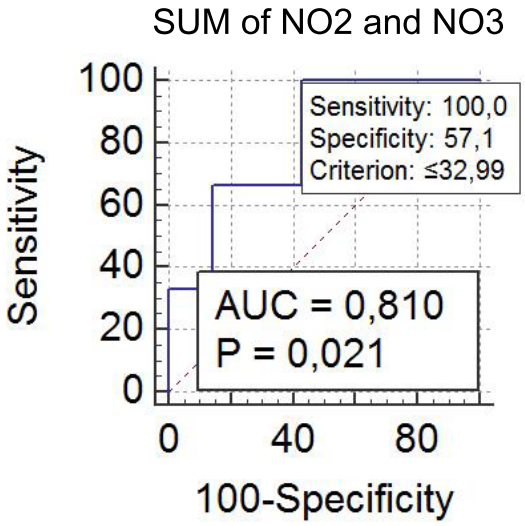

Figure 2. ROC curve predicting the likelihood of death according to the level of the sum of NO_2 and NO_3 patients in group 1B

NO_3 concentrations did not show any correlation between their content and reliable likelihood of severe clinical adverse events (*Table 4*).

Thus, the critical localization of endothelial cells between circulation and other components of the vascular wall places them in the pathophysiological bed of cardiovascular and other consequences of oncohematological diseases [5]. Inflammatory and pro-aggregation activation of the endothelium is common in children with ALL [5, 7–9]. Therefore, ALL should be regarded as a multiple organ disease, and ED as an integral part of the pathogenic link of leukemia [9] and its complications.

Table 4

ROC indicators of NO? levels and lethal complications of induction therapy in acute lymphoblastic leukemia

Parameter	AUC	"cut-off point"	Se, %	95% CI	Sp, %	95% CI	+LR	-LR
NO_3, μmol/l	0.518	⩽7	33.33	0.8-90.6	100	87.7-100	-	0.67

Conclusions

Our study determined significant violations of nitric oxide synthesis in the form of nitrite increase and nitrate reduction secondary to reduced total nitric oxide content. This confirms that ED accompanies the course of ALL in children.

The negative effects on the cardiovascular system of the concentration of nitric oxide and its metabolites in ALL in children are well known. There is a lack of information on the likely role of these markers in the pathogenesis of other (toxic, infectious) complications. The findings of our work demonstrate that the determination of nitric oxide is of diagnostic and prognostic significance in patients with ALL. The critical fall in the concentration of NO and NO_2 accompanies the development of multiple organ failure with fatal outcome in children with ALL.

Due to the preservation of the signs of ED after the end of treatment in children with ALL, it is necessary to monitor the state of functioning of the cardiovascular system in the future.

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PROGNOSIS OF INDIVIDUAL RISK OF HEART RHYTHM DISTURBANCES AND CONDUCTIVITY IN NEWBORNS IN THE EARLY NEONATAL PERIOD

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Abstract

Background. In the majority of cases neonatal arrhythmias have a good prognosis for recovery. However, they can also have an adverse course and lead to development of life-threatening conditions. Therefore, it is important to search for earlier markers of myocardial lesion, diagnostic criteria and predictors of arrhythmias. **Purpose.** Improvement of diagnosis and prediction of the risk of cardiac arrhythmias and conduction in newborns in the early neonatal period by identifying factors that play a role in the prediction of neonatal arrhythmias. **Subjects and Methods.** The study involved 76 newborns. Group 1 included 57 infants with arrhythmias according to Holter monitoring, Group 2 included 19 infants without arrhythmias. The history data, laboratory and instrumental findings, levels of troponin I and copeptin were compared. To predict development of neonatal arrhythmias, logistic regression analysis was performed. The quality of the model was tested using the Percent Concordant (PC). Quality Score was evaluated by R2 Nigelkerke. Model adequacy was estimated using the Hosmer–Lemeshow test. **Results.** The study showed that the factors that can influence development of arrhythmias in the early neonatal period are the level of umbilical cord blood, the levels of troponin I, copeptin, GGT, assessment of Apgar scale in the 1st and 5th minutes, asphyxia at birth, indices of wave R amplitude in V3 and V5 of chest leads, ST segment deviation from the isoline according to standard surface ECG, QTc levels and mean daily maximum, minimum heart rate according to Holter monitoring. **Conclusions.** Predictors of neonatal arrhythmias development are indicators of laboratory-instrumental parameters of cardiovascular system status, troponin I level above 0.29 ng/ml and copeptin level above 0.1 ng/ml.

Key words: *neonatal arrhythmias, risk factors, troponin I, copeptin.*

Introduction

Disorders of the heart rhythm and conduction in newborns in the early neonatal period mostly have a benign course and end with recovery [1]. However, some arrhythmias have an adverse course and can lead to development of conditions that threaten the life of the newborn [2]. The most important causes of arrhythmias in infants include perinatal hypoxia and development of metabolic disorders [3, 4]. These processes result in apoptosis and dystrophy in the cells of the sinus node and other parts of the conduction system, critical changes in the electrical activity of cardiomyocytes, which, in turn, are the cause of development of various types of arrhythmias [5]. In recent years, earlier markers of myocardial damage, diagnostic criteria and predictors of arrhythmias have been searched. Troponin has long been a known marker of myocardial damage [6–8]. However, given that troponin isoforms are released from cardiomyocytes approximately 4 hours after myocardial damage and reach peak values only after 12 hours [9], it becomes clear that its diagnostic value in neonatal practice is limited by the period immediately after birth. Copeptin is a metabolite of arginine-vasopressin, which is also one of the markers of endogenous stress, including hypoxic events. It is synthesized within 5–20 minutes after the development of acute myocardial damage and remains stable for several days [10]. There is evidence of the use of copeptin as a marker of the development of various

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pathological conditions in newborns [11–14], but its role in the prediction of neonatal arrhythmias in newborns together with troponin I has not been studied.

2. Purposes, subjects and methods:

2.1. The purpose of the work was to improve the diagnosis and prediction of the risk of cardiac arrhythmias and conduction in newborns in the early neonatal period by identifying factors that play a role in the prediction of neonatal arrhythmias.

2.2. Subjects and Methods

The study involved 76 infants aged 1–3 days. Group 1 included 57 infants with disorders of cardiac rhythm and conduction established using Holter monitoring, Group 2 included 19 infants without arrhythmias. The study implied comparison of history data, clinical and laboratory-instrumental findings, levels of troponin I by immuno-enzymatic analysis ("Biomerica" (USA) Troponin I) and copeptin ("Phoenix Pharmaceutical" (USA) Copeptin). To find out the factors that should be taken into account making the prognosis of neonatal arrhythmias development, a logistic regression analysis was performed, where development of cardiac arrhythmias and conduction disturbances was "1" and absence of cardiac arrhythmias development was "0". Each qualitative sign was coded as "1" if the newborn was found to have this sign, or "0" if this sign was not determined. The model quality was tested by Percent Concordant (PC). Assessment of the quality of the model was performed by R^2 Nigelkerke. Model adequacy was estimated using the Hosmer–Lemeshow goodness-of-fit test. If the index χ^2 was numerically small and the *p* value was above 0.05 and approaching 1, then the logistic model was considered adequate. Statistical analysis. Statistical analysis was performed using StatSoft STATISTICA Version 10.

Ethical aspects. All parents of participants were informed about the goals, objectives and scope of the study and gave written informed consent. The study was approved by the Ethics Committee of Kharkiv National Medical University (Ethics Committee Protocol No. 8 as of 5.10.2016) and conducted in accordance with the recommendations of the Declaration of Helsinki (1975).

Conflict of interests

The authors of the article declare no conflict of interest.

3. Results and discussion

The construction of a logistic regression model using the method of input of selected prognostic factors for development of cardiac rhythm and conduction disturbances in newborns in the early neonatal period was carried out in 3 stages.

At the first stage, potential independent predictors of neonatal arrhythmias were identified among qualitative indicators of the data of history and instrumental studies of the cardiovascular system by constructing a correlation matrix with respect to the dependent variable.

The study showed that factors that should be taken into account when predicting development of neonatal cardiac rhythm and conduction disturbances include indicators of assessment of the physiological adaptation of the newborn with Apgar score on the 1st and 5th minutes, deviation of ST segment from the isoline as an indicator of changes in ventricular myocardial repolarization according to the standard surface ECG and birth asphyxia (*Table 1*).

Table 1

Matrix of correlation of qualitative indicators under investigation with respect to the presence of arrhythmias

Variable	Coefficient	p
Apgar score on the 1st minute	-0.25	0.03
Apgar score on the 5th minute	-0.24	0.03
ST deviation from the isoline	-0.31	0.01
Asphyxia	0.27	0.02

In the following, the variables obtained were introduced as regressors into the binomial regression model. The quality of the model was tested by Percent Concordant. The efficiency of the obtained model of binomial logistic regression was confirmed by the results of the Hosmer–Lemeshow test: $\chi^2 = 5.343$; degrees of freedom (df) = 8, p = 0.72.

Table 2 presents the final data on the variables included in the logistic regression equation, with impact coefficients, calculated odds ratios, and confidence intervals.

The logistic regression equation, which was obtained at the first stage of construction of the model of neonatal arrhythmias prediction using qualitative indicators of history and data from instrumental studies of the condition of the

Table 2

Variable logistic regression equations

Variable	B	p	CR	95 % CI	
				Lower	Upper
Asphyxia	0.735	0.272	2.086	0.561	7.751
Apgar score on the 1st minute	-0.128	0.829	0.880	0.275	2.816
Apgar score on the 5th minute	-0.214	0.766	0.808	0.198	3.297
ST deviation from the isoline	-1.258	0.030	0.284	0.091	0.888
Constant	3.215	0.240	24.903		

* B – an individual coefficient for each logistic regression equation variable; p – a coefficient of reliability; CR – chance ratio; 95 % CI – confidence interval, lower and upper, respectively.

cardiovascular system, helped to correctly identify 45 cases of cardiac rhythm disturbances out of 57 (78.9%).

At the second stage, potential independent predictors of neonatal arrhythmias were determined among quantitative history and laboratory-instrumental indicators of cardiovascular status.

It was found that the factors to be taken into account in predicting the formation of neonatal disorders of cardiac rhythm and conduction include sodium levels of umbilical cord blood (Na), levels of troponin I, copeptin, gamma-glutamyltransferase (GGT), R amplitude index in the 3rd (V_3) and 5th (V_5) thoracic leads, values of mean minimum (heart rate min) and maximum heart rate (heart rate max) and daily corrected QT interval (QTc) according to the daily ECG monitoring data (*Table 3*).

The quality of the model was tested by PC. The efficiency of the obtained model of binomial logistic regression was confirmed by the results of the Hosmer–Lemeshow test: $\chi^2 = 12.577$; degrees of freedom (df) = 8, p = 0.13.

Table 4 presents the final data on the variables included in the logistic regression equation, with impact coefficients, calculated odds ratios, and confidence intervals.

Logistic regression equation, obtained in the second stage of construction of the model of prediction of neonatal arrhythmias using qualitative indicators of history and data of instrumental studies of the condition of the cardiovascular system, helped to correctly identify 45 cases of cardiac arrhythmias of 57 (78.9%).

The last step in development of the prognostic model was to create a binomial regression equation that included qualitative and quantitative indicators that reflected history, clinical, instrumental, and laboratory data selected from previous logistic and statistical analysis. Thus, it was determined that the factors that can influence the development of arrhythmias in the early neonatal period are the level of umbilical cord blood, the levels of troponin I, copeptin, GGT, Apgar scale in the 1st and 5th minutes, asphyxia at birth, indices of the amplitude of the R wave in the 3rd (V_3) and 5th (V_5) thoracic leads, deviation of the ST segment from the isoline according to the standard surface ECG, the value of the average daily maximum, minimum of heart rate and daily average adjusted QT interval (QTc) according to Holter monitoring.

The quality of the model was tested by PC. The ability of the obtained model of binomial logistic regression was confirmed by the results of the Hosmer–Lemeshow test: $\chi^2 = 1.771$; degrees of freedom (df) = 8, p = 0.987.

Table 5 presents the final data on the variables included in the logistic regression equation, with impact coefficients, calculated odds ratios, and confidence intervals.

Table 3

Matrix of correlation of the quantitative indicators under investigation regarding the presence of arrhythmias

Variable	Coefficient	p
Na	0.26	0.03
Troponin I >0.29 ng/ml	-0.05	0.64
Copeptin>0.1 ng/ml	-0.13	0.09
GGT>151 U/l	-0.21	0.08
R in V_3	0.21	0.07
R in V_5	0.24	0.04
Heart rate min	-0.23	0.05
Heart rate max	0.17	0.14
QTc	0.21	0.07

Table 4

Quantitative variables included in the binomial regression equation

Indicator	B	P	CR	95 % CI	
				Lower	Upper
Asphyxia	1.387	0.128	4.002	0.672	23.836
Apgar score on the 1st minute	-0.369	0.608	0.691	0.169	2.832
Apgar score on the 5th minute	0.085	0.928	1.088	0.173	6.837
ST deviation from the isoline	-1.159	0.154	0.314	0.064	1.546
Na	0.085	0.348	1.089	0.911	1.302
Troponin I >0.29 ng/ml	-0.872	0.072	0.418	0.162	1.080
Copeptin>0.1 ng/ml	-0.499	0.686	0.607	0.054	6.805
GGT>151 U/l	-0.009	0.029	0.991	0.982	0.999
R in V_3	-0.015	0.909	0.985	0.761	1.275
R in V_5	0.088	0.370	1.092	0.901	1.322
Heart rate min	-0.001	0.958	0.999	0.957	1.042
Heart rate max	0.027	0.267	1.027	0.980	1.076
QTc	0.029	0.092	1.030	0.995	1.065
Constant	-24.439	0.088	0.000		

* B – an individual coefficient for each logistic regression equation variable; p – a coefficient of reliability; CR – chance ratio; 95 % CI – confidence interval, lower and upper, respectively.

Table 5

Quantitative variables included in the binomial regression equation

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GGT>151 U/l	-0.009	0.029	0.991	0.982	0.999
R in V_3	-0.015	0.909	0.985	0.761	1.275
R in V_5	0.088	0.370	1.092	0.901	1.322
Heart rate min	-0.001	0.958	0.999	0.957	1.042
Heart rate max	0.027	0.267	1.027	0.980	1.076
QTc	0.029	0.092	1.030	0.995	1.065
Constant	-24.439	0.088	0.000		

* B – an individual coefficient for each logistic regression equation variable; p – a coefficient of reliability; CR – chance ratio; 95 % CI – confidence interval, lower and upper, respectively.

The logistic regression equation obtained at the third stage of the neonatal arrhythmia prediction model construction using qualitative history and instrumental studies of the cardiovascular status also identified 47 cases of cardiac arrhythmias out of 57 (82.9%).

According to the results of the analysis, a prognosis model for the binomial regression equation was created:

$$y = -24.439 + (1.387 \times Asphyxia) - (0.369 \times Apgar\ 1\ min) + (0.085\ ?\ Apgar\ 5\ min) - (1.159 \times ST\ deviation\ from\ the\ isoline) + (0.085 \times umbilical\ cord\ blood\ sodium) - (0.872 \times Troponin\ I) - (0.499 \times copeptin) - (0.009 \times GGT) - (0.015 \times RV3\ amplitude) + (0.088 \times RV5\ amplitude) - (0.001 \times HRmin) + (0.027 \times HRmax) + (0.029 \times QTc)$$

The quality of the obtained prognostic model of the development of neonatal cardiac rhythm and conduction disturbances including history and laboratory-instrumental indicators of the status of the CVS was determined by the following parameters: sensitivity = 94.7%, specificity = 47.4%, positive predictive value (PPV) = 84.4%, negative predictive value (NPV) = 75%.

Therefore, the predictors of development of cardiac rhythm and conduction disturbances in the early neonatal period is the presence of changes in the physiological adaptation of the newborn secondary to asphyxia at birth, which is consistent with the literature data [15, 16].

A number of studies have been conducted to determine the prognosis of various factors of the metabolic or instrumental constituent of the cardiovascular system (CVS) for development

of posthypoxic myocardial injury [17–22]. Apart from the metabolic component the authors thoroughly studied the influence of the state of the prooxidant and antioxidant system of protection of the body under conditions of hypoxia, levels of brain natriuretic peptide, endothelial growth factors, creatine kinase, isoforms of troponin and others [17, 18, 21, 22].

The most important factors in development of posthypoxic cardiac events were determined by the parameters of daily ECG registration and amplitude-integrated electroencephalogram [19, 20]. However, the metabolic and instrumental components of the condition of the CVS have been studied separately, which, in our opinion, does not give a holistic view of the degree of impaired myocardial function and the development of possible complications. In our opinion, it is advisable to objectively assess the degree of damage to the heart muscle and predict the development of cardiac rhythm and conduction disturbances, only in the context of a comprehensive assessment of laboratory-instrumental parameters of the condition of the cardiovascular system and the involvement of specific laboratory indicators (predictors). Such predictors are troponin I levels above 0.29 ng/ml and copeptin levels above 0.1 ng/ml.

Conclusions

1. Clinical history risk factors for cardiac arrhythmias and conduction in the early neonatal period are disorders of physiological adaptation of the newborn (OR = 1.1, $p<0.05$) secondary to asphyxia at birth (OR = 4.0, $p<0.05$).

2. It is possible to predict development of neonatal cardiac arrhythmias with the help of a prognostic model of the binomial regression equation with the use of standard surface and daily electrocardiography data.

3. Predictors of development of cardiac arrhythmias in newborns are troponin I levels above 0.29 ng / ml and copeptin levels above 0.1 ng/ml.

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VARIANTS OF HYPERLIPIDEMIA IN CHILDREN WITH INSULIN RESISTANCE

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Abstract
Obesity in adults and children is characterized by epidemiological prevalence with a tendency to increase. The purpose of the study was to analyze the lipid profile in overweight children, depending on the presence of insulin resistance. 247 overweight and obese children aged 2 to 18 were examined, including 160 boys and 87 girls. Obesity was diagnosed if the BMI exceeded 97 percentile, according to gender and age. Assessment of the lipid profile included measurements of total cholesterol, triglycerides, low-density lipoprotein, and high-density lipoprotein. To evaluate the parameters of the lipid profile, we used the National Cholesterol Education Program (NCEP) according to the latest edition (2006). Lipid values depending on the presence or absence of insulin resistance were analyzed. BMI was also evaluated according to Z-BMI. Insulin resistance was detected in 69.9% of children. Hyperlipidemia was detected in 24.9% of children and dyslipidemia in 83% of the children examined. A change was found in all indicators of the lipid profile, depending on the presence of insulin resistance. A significant increase in Z-BMI was revealed depending on the presence of insulin resistance. Conclusions: the majority of overweight children have insulin resistance and dyslipidemia; the type of dyslipidemia in children with obesity directly depends on the presence of insulin resistance.
Key words: *Hyperlipidemia, insulin resistance, obesity, pediatrics.*

Introduction

The prevalence of obesity in the world has reached an epidemic level [1]. The prevalence of obesity in children is on the special focus [2]. The prevalence of obesity in Ukraine among children aged 0 to 17 years in 2016 was 16.41 cases per 1000 urban children and 11.90 per 1000 rural children [3]. The pediatric obesity epidemic has resulted in population of children with abnormal lipids. Obesity related dyslipidemia includes combination of elevated triglycerides, decreased high density lipoprotein cholesterol, and high normal or slightly elevated low density lipoprotein cholesterol [4].

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Obesity is one of the main causes of insulin resistance (IR) development [5], which plays a major role in the relationship between obesity and dyslipidemia [6].

Overweight and obesity, impaired glucose metabolism, dyslipidemia and hypertension are the basis of metabolic syndrome (MetS). The prevalence of MetS in children and adolescents is increasing, in parallel with the pediatric obesity incline. Obese children have an increased risk of MetS in their adulthood and become prone to type 2 diabetes mellitus and cardiovascular diseases [7].

Thus, it is important to assess the relationship between lipids and insulin resistance as a components of the MetS in obese children with the purpose of better understanding potential cardiovascular risk.

2. Purposes, subjects and methods:

2.1 The purpose of the work was to analyze the lipid profile in overweight children depending on the presence of insulin resistance.

2.2 Subjects and Methods

247 overweight and obese children (160 boys and 87 girls) aged 2 to 18 were examined in the pediatric endocrinology department. The diagnosis was made according to the national standard [8]. THE Patients' height (in m) and weight (in kg) were measured according to standard procedures and followed by the calculation of body mass index (BMI). Obesity was diagnosed when BMI exceeded 97 percentile for gender and age. For further analysis BMI standard deviation (Z-BMI) evaluated by WHO charts were used [9].

Blood samples were collected at fasting state (at least 8-hours after the last meal). Assessment of the lipid profile included measurements of total cholesterol (TC), triglycerides (TG), low-density lipoproteines (LDL), and high-density lipoproteins (HDL). To evaluate the lipid parameters, recommendations of the latest edition of the National Cholesterol Education Program (NCEP) (2006) were used [10].

To assess carbohydrate metabolism, fasting blood glucose and insulin levels were measured followed by HOMA-IR calculation. Insulin resistance was determined if HOMA-IR values exceeded the recommendations of the IDEFICS [11]. The parameters were grouped by the presence (IR+) or absence (IR-) of insulin resistance.

Standard statistics was used for the data analysis. P-values were two-sided and values <0.05 were considered statistically significant.

Declaration of Ethics. The patients were informed about the study, its purpose and possible results. Written informed consent was obtained from the patients and their parents. The study was approved by the Ethics Committee of Kharkiv National Medical University (Ethics Committee Protocol No.8 from 03.10.2018) and conducted in accordance with the recommendations of the ethical committees on biomedical research, the legislation of Ukraine on health, the Helsinki Declaration 2000 and European Society Directive 86/609 on the role of people in biomedical research.

Conflict of interests. There is no conflict of interests.

3. Results

Insulin resistance was detected in 69.9% of children; 72% of the examined girls and in 68% of the examined boys were insulin resistant. Insulin resistant subjects had statistically higher BMI than insulin sensitive ones (P<0.05).

Hyperlipidemia was detected in 24.9% and dyslipidemia in 83% of the surveyed children. Dyslipidemia was found in 96% IR+ children and in 86% of IR- children (P=0.012), which means that insulin resistant overweight were more dyslipidemic.

Average level of TC was moderately elevated in children with insulin resistance, whereas average level of TC was within normal ranges in children without insulin resistance (*table*).

HDL values are borderline reduced in overweight children regardless of the presence of insulin resistance.

TG in children without insulin resistance are borderline elevated in both IR+ and IR- children with excessive body mass.

The average LDL levels were significantly increased in IR+ group (p <0.05).

4. Discussion

Insulin resistance seems to play a key role in the metabolic status in obese subjects. Our findings showed significant difference in LDL-C levels with a relevant tendency for the TC and TG.

The study by Marko Kostovski et al. that included 96 obese children (45 boys, 51 girls) aged 4–17 years showed that insulin resistant children had higher BMI and more increased TG than non-insulin resistant ones [12]. Atabek ME et al. came to the similar conclusions in their study which included 196 obese children aged 7–18 years [13].

Romualdo M.C. et al. investigated 220 children aged 5–14 years and met the same results concerning BMI and TG, but they established that median total cholesterol, and LDL-C were increased and HDL-C were decreased in the presence of IR [14]. It is s somewhat different from the data that we obtained as we did not established significant difference in TC and HDL-C levels between groups.

Lipid profile in children with varying degrees of excess body weight, depending on the presence or absence of insulin resistance

	IR+ N=137	IR- N=59	P
Z-BMI	2.38±0.61	2.11±0.41	<0.05
TC, mmol/l	4.64±1.02	4.32±1.03	0.06
TG, mmol/l	1.65±0.77	1.42±0.56	0.14
LDL, mmol/l	2.40±0.97	1.69±0.81	<0.05
HDL, mmol/l	1.22±0.25	1.20±0.31	0.67

A direct correlation between BMI and IR was also confirmed in the study conducted by Ling J et al. in which BMI statistically significantly positively correlated just with fasting insulin and HOMA-IR, whereas lipids were not predictive for IR in obese children [15].

Our findings demonstrate that IR in obese subjects is associated with cardiovascular risk related dyslipidemia (elevated LDL-C). Meantime, differences when compared with the data of other researchers can be explained by the different degree of adiposity, ethnicities and puberty stages of participants. Furthermore, there is no reason to neglect dyslipidemia as an important risk factor of the cardiovascular risk in children of different age.

It is worth mentioning that the study of the interplay between carbohydrate and lipid metabolism in obese children must be continued as it may well become an important early predictor of clinical events in adulthood.

Conclusions

1. The majority overweight children are insulin resistant.
2. Global data on the increase of Z-BMI in insulin resistant obese children were confirmed.
3. The type of dyslipidemia in obese children depends on the presence of insulin resistance.
4. Increased LDL-C levels in insulin resistant obese children is an alarming predictor of cardiovascular problems in the future.

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FORENSIC ASSESSMENT OF ADVERSE OUTCOMES OF ISOLATED DIAPHYSEAL FEMORAL FRACTURES

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Abstract

Fractures of the femur diaphysis are one of the most frequent mechanical injuries of the skeleton, however, the frequency and causes of development of complicated post-traumatic period are not well understood. The aim of this work was to study the causes of development of adverse outcomes of isolated diaphyseal femural fractures. **Object and methods** of the work. A retrospective analysis of the protocols of clinical and radiological examination of 21 patients with adverse outcomes of a femoral diaphysis fracture, which, according to the results of the initial expert assessment, did not establish the severity of injuries due to the development of complications in the postoperative period was performed. **Results.** The main cause of fractures in this category of patients is road traffic accidents (90.4% of cases); closed fractures of the femoral diaphysis (85.7%) in the middle third (76.1%) in men (76.2%) prevailed. The following causes of the unsatisfactory results of surgical treatment of the femur diaphyseal fractures were revealed: 1) patient-dependent: a combination of overweight and arthrosis of adjacent (hip and knee) joints – 57.1%; violation of the motor regime in the form of physical inactivity (19.0%) and excessive axial load on the operated leg (19.0%); 2) implant-dependent: a mismatch between the dimensions of the device for immersion osteosynthesis and the anatomical sizes of the corresponding segments of the femur in all cases; 3) surgical-dependent causes: unresolved intraoperative displacement of fragments of the femur (23.8%), violation of the technology of radiation diagnosis (14.3%), violation of the terms of postoperative x-ray monitoring (23.8%) and perioperative antibiotic prophylaxis (14.3%). The consequence of this was a violation of the stability of osteosynthesis in 95.2% with a secondary displacement of bone fragments of the femur (47.6%), delayed consolidation of a hip fracture (61.9%), the formation of pseudarthrosis of the femur (38.1%), and post-traumatic femoral deformity bones (71.4%), development of post-traumatic contracture of the knee joint (81.0%), suppuration of the postoperative wound (23.8%), development of post-traumatic osteomyelitis (14.3%), migration of screws from the osseous plate (47.6%), migration of a distal blocked nail from the intramedullary rust (4.8%), intramedullary rod migration (4.8%).

Key words: *diaphyseal femoral fracture, surgical treatment, perioperative risk factors, postoperative complications, unsatisfactory outcomes.*

Introduction

The fractures of the femur that were not complicated by the damage to the main vessels and nerves, are assessed as bodily injuries of moderate severity. However, in the process of the fracture fusion, regardless of the treatment method (immobilization or various types of osteosynthesis), complications can develop. The most common complication is a defect of reparative processes in the fracture zone (delayed consolidation, non-fusion of bone fragments, pseudarthrosis), as well as persistent contractures of the adjacent joints [1, 2]. Development of complications in the post-traumatic period usually aggravates the outcome of the injury and,

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according to the "Rules of Forensic Medical Determination of the Severity of Bodily Injuries", often leads to severe consequences, as a result of harm to the health.

The frequency and features of complications that develop during the treatment of femoral fractures had been already studied. Errors and complications in the treatment of hip fractures in victims with polytrauma [3, 4], in patients with fractures of particular segments of the femur [5, 6] had been studied as well.

2. Purposes, subjects and methods:

2.1. The purpose of the work was to identify the causes of development of adverse outcomes in isolated femoral diaphyseal fractures.

2.2. Subjects and Methods

Of study were retrospective analysis of protocols of clinical and radiological examination of 21 patients with adverse outcomes of isolated femur shaft fractures, which, according to the results of the initial expert assessment, did not establish severity of bodily injuries due to the development of complications in the postoperative period. All patients were observed at M.I. Sitenko Institute of Spine and Joint Pathology (NAMS of Ukraine) in 2014–2019.

Criteria for inclusion in the study were adverse outcomes of the fracture of the femur diaphysis (non-fusion of bone fragments, pseudarthrosis, post-traumatic shortening and/or deformation of the femur, post-traumatic contracture of the knee joint). Criteria for exclusion from the study were hip fractures with fractures of several bones, combined injuries, fractures of the proximal or distal femur epimetaphysis.

All patients underwent surgical treatment using various methods of internal fixation of fragments of the femur using metal structures. When studying the features of the adverse results of femoral diaphysis fractures, an EFORT classification was used, according to which patient-dependent, implant-dependent and surgery-dependent causes and/or risk factors for complications after surgical treatment of orthopedic and traumatic patients were distinguished [7].

The Quetelet body mass index was calculated using the formula 'm/h2',

where m – body weight in kilograms, h – height in meters.

In the statistical processing of the material methods of descriptive statistics were used.

Conflict of interests. There is no conflict of interests.

3. Results

Among the patients included in this study, men predominated (76.2%). All patients were of working age. The main causes of fractures in this category of patients were traffic accidents (90.4% of cases); closed femoral diaphysis fractures (85.7%) in the middle third (76.1%) predominated. Only 2 primary open fractures were revealed (gunshot and due to a fall from a height) and 1 – secondary open as a result of an accident (*Table 1*).

Table 1

Distribution of patients by gender, age and some features of femoral diaphysis fractures

Options	N (%)
Sex	
Male	16 (76.1)
Female	5 (23.8)
Average age 32.12 ± 14.33 years old (18–48 years old)	
Cause of fracture	
Traffic accident	19 (90.4)
Fall from height	1 (4.8)
Gunshot wound	1 (4.8)
Fracture localization	
Top 1/3	3 (14.3)
Middle 1/3	16 (76.1)
Bottom 1/3	2 (9.6)
Type of fracture	
Open	3 (14.3)
Closed	18 (85.7)

It should be noted that osteoporosis or cases of hormone therapy, which are significant risk factors for delayed fracture consolidation, were not detected before the surgery.

Examination of primary radiographs made it possible to establish that only one patient with an open gunshot hip fracture in the middle third, by features of bone-traumatic injuries (defect of the femoral diaphysis up to 4 cm long) initially had a complicated and prolonged postoperative period with delayed fracture consolidation, the need for recovery the anatomical length of the damaged segment, a high risk of developing post-traumatic osteomyelitis. In all other cases, features of fracture did not affect the development of postoperative complications with a worsening treatment outcome.

When studying the causes of unsatisfactory results of surgical treatment of diaphyseal fractures of the femur, the following patient-related, surgery-related and implant-related causes were identified (*Table 2*).

Table 2

The causes of the unsatisfactory results of the surgical treatment of femoral diaphysis fractures

No.	The causes of the unsatisfactory results of the surgical treatment of femoral diaphysis fractures	N (%)
	Patient-related causes	
1	Alcohol intoxication at the time of injury (light degree)	3 (14.3)
2	_ _ _	8 (38.1)
3	History of coxarthrosis (Ist degree)	2 (9.6)
4	History of gonarthrosis (Ist degree)	6 (28.6)
5	Violation of the orthopedic regime in the immediate postoperative period	4 (19.0)
6	Violation of the orthopedic regime during the rehabilitation period	4 (19.0)
	Implant-related causes	
7	Inconsistency of the length of the intramedullary shaft and the length of the bone marrow canal of the femur	1 (4.8)
8	Inconsistency between the diameter of the intramedullary shaft and the width of the bone marrow canal of the femur	1 (4.8)
9	Discrepancy between the length of the distal blocking nail and the anteroposterior diameter of the distal femur metaphysis	2 (9.6)
10	Inconsistency of the length of cortical screws with the diameter of the femoral diaphysis	9 (42.9)
	Surgeon-related causes	
11	Violation of x-ray technology	3 (14.3)
12	Violation of the terms of postoperative x-ray monitoring	5 (23.8)
13	Violation of the terms of postoperative antibiotic therapy	3 (14.3)
14	Untreated intraoperative displacement of femur fragments	5 (23.8)

Among the patient-dependent causes, the most significant risk factors for complications after an open reposition of a femoral diaphysis fracture were a combination of obesity, arthrosis of adjacent (hip and knee) joints in 12 (57.1%) patients. Even with initial changes (Ist degree obesity, Ist degree coxarthrosis, Ist degree gonarthrosis), inadequate motor regime in the form of physical inactivity in the postoperative period potentiated the development of persistent restriction of movements in the knee joint with the formation of extensor contracture, relative (functional) shortening of the lower limb, and violation of the motor stereotype in 4 (19.0%) patients. These patients also had a significant reduction in the dosed load on the damaged lower limb in early stages of primary fibrocartilage callus formation in the fracture zone, which was accompanied by inhibition of reparative regeneration processes with a delayed formation of secondary fibrocartilage callus. Violation of the motor regime with excessive axial load on the operated limb during the rehabilitation period was also accompanied by delayed consolidation in the fracture zone (another 4 (19.0%) observations) - see *tables 2, 3*.

The study of implant-dependent causes showed that in all cases of development of postoperative complications, a mismatch was found between the standard size of the metal structure used for immersion osteosynthesis and the anatomical sizes of fractured femur's fragments. Migration of a short intramedullary rod was observed in one (4.8%) case. In another case (4.8%) of blocked intramedullary osteosynthesis of the hip diaphysis fracture in the lower third, the use of a narrow intramedullary nail and a short distal blocked nail was accompanied by migration of the blocked nail and rod's breakage at the level of the femoral fracture, secondary displacement

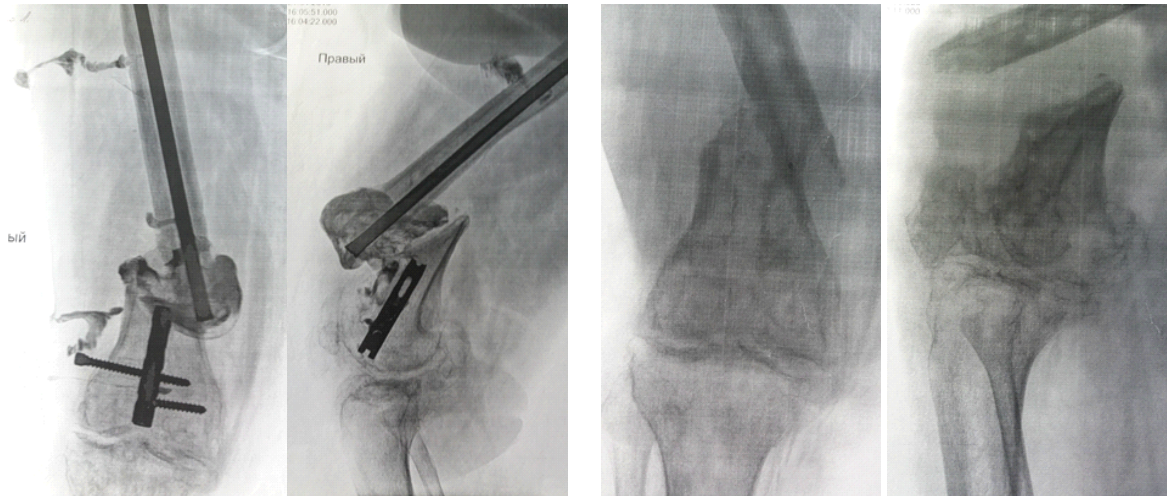
of bone fragments and the absence of fracture consolidation signs. Unstable fracture osteosynthesis in this patient potentiated development of chronic post-traumatic osteomyelitis (*Fig. 1, a, b, tables 2, 3*).

The use of short cortical screws for bone osteosynthesis, especially in conditions of comminuted fracture of the femoral diaphysis, caused migration of screws with impaired stability of bone fragments fixation, delayed fracture consolidation (4 patients; 19.0%), and the formation of a false joint (5 patients; 23.8 %).

Surgically dependent causes that could lead to development of complications of osteosynthesis of diaphyseal hip fractures were noted throughout the perioperative period, but mainly after surgery. Most often, the terms of postoperative x-ray monitoring of the operated segment and unrepaired intraoperative displacement of the fragments of the femur – in 5 (23.8%) of observations were violated. Violation of the technology of x-ray diagnosis by performing x-ray of the femur only in the lateral projection, as well as only in the area of the diaphyseal fracture without adjacent joints, was noted in 2 (9.6%) and 1 (4.8%) cases, respectively.

In one of the clinical cases with osteosynthesis of comminuted hip fracture, violation of the x-ray diagnostic technology (performing only one projection of the damaged segment) did not allow intraoperative visualize if there was a completely insufficient fixation of femur fragments (*Fig. 2a*), which was revealed only after 4 months after open reduction (*Fig. 2, b, c*) and led to delayed consolidation of the fracture with a tendency to false joint formation and post-traumatic deformation of the femoral diaphysis (*Fig. 2, d*).

In 3 (14.3%) patients, antibiotic therapy was started only after development of inflammatory changes in the postoperative wound area.



A *B*

Fig. 1 – *A* – fistulograms of a femoral fracture in the lower third, during surgical treatment, with the development of fistulous type of post-traumatic osteomyelitis: breakage of the intramedullary blocking rod, migration of the upper distal screw, fistulous passages filled with contrast; *B* – the absence of reparative fusion in the fracture area after removal of the intramedullary shaft

Table 3

Types of adverse outcomes of osteosynthesis of diaphyseal fractures of the femur

No.	Types of adverse outcomes of osteosynthesis of diaphyseal fractures of the femur	N (%)
1	Fracture of the intramedullary nail	1 (4.8)
2	Intramedullary rod migration	1 (4.8)
3	Migration of a distal blocked nail from an intramedullary nail	1 (4.8)
4	Migration of screws from the bony plate	10 (47.6)
5	Secondary displacement of bone fragments of the femur	10 (47.6)
6	Suppuration of a postoperative wound	5 (23.8)
7	Development of post-traumatic osteomyelitis	3 (14.3)
8	Development of post-traumatic contracture of the knee	17 (81.0)
9	Slow hip fracture consolidation	13 (61.9)
10	Formation of pseudarthrosis of the femur	8 (38.1)
11	Post-traumatic femoral deformity	15 (71.4)

A *B* *C* *D*

Fig. 2 – radiographs of a comminuted femoral fracture in the middle third during surgical treatment: *A* – (on the day of the injury, anteroposterior view) – insufficient (short) fixation of the proximal fragment, diastasis between the proximal, distal and comminuted fragments; *B* (anteroposterior view), *C* (lateral view) c in 4 months after the operation, the osteoporosis zone is visualized around the distal screws (b), diastasis between the distal fragment and the osseous plate (c); *D* (anteroposterior view) 6 months after the injury – removal of the osseous plate; diastasis between the proximal and distal fragments of the femur is observed

4. Discussion

Femur diaphysis fractures are one of the most common skeletal injuries. The total frequency of this injury, taking into account femoral diaphysis fractures during multiple and combined injuries, has been kept at a practically constant level in recent years: for 1 year, the average number was 20.8 per 100,000 adult population in 2018 [8] and 21 per 100,000 adult population in 2013 [9]. The frequency of isolated fractures of the femoral diaphysis within 1 year reaches an average of 10 per 100,000 adult population [10]. Since the femur is the largest in the skeleton, surrounded by the largest muscle mass, and one of the main supporting bones of the lower limb, isolated hip fractures are accompanied by significant blood loss, the development of traumatic shock, and lead to long-term disability regardless of the level of (high or low) kinetic energy traumatic factor [11].

The mechanism and location of the femoral fracture depend on the age of the victims. At the age of 40–45 years, femoral diaphysis fractures usually occur due to high-energy injuries [12]; the most common type of injury is an accident (up to 75%), a fall from a height (up to 7.3 – 10.0%), gunshot injuries (2.3 – 4.5%) [13, 14]. In older people, the proximal part of the femur is mainly damaged due to low-energy injuries, more often as a result of a fall from a small height [15].

Despite the advantages of surgical treatment of mechanical damage to the skeleton (stable fixation of the damaged segment, early motor activation of patients and early initiation of active rehabilitation treatment), the use of open fixation for fractures of the femur is still a matter of discussion [16, 17]. Such a restrained approach to choosing a surgical method of treatment is associated with a rather high frequency of postoperative complications, especially provided that such complications in patients with a fracture of the femur are more common and more difficult than with fractures of long tubular bones of a different location [18], which not only leads to lengthening of the disability period of the patients, but also – to their disability [19].

It should be noted that the internal fixing metal structures, which are used in the surgical treatment of skeleton bone fractures, during their usage can be damaged by loads exceeding the strength of

both the device structure itself and the bone to which they are fixed [20, 21]. This problem can be: 1) iatrogenic (the result of a violation of preoperative preparation, osteosynthesis technology or postoperative management) [22, 23]; 2) associated with the patient, when, due to violation of limits regime concerning the operated limb loads, these loads exceeded the durability of the implant or bone [24]; 3) due to the manifestation of a latent structural defect, which can lead to the fixator fracture in the absence of obvious external causes and full compliance with both surgical technology and recommendations for the postoperative regime [25, 26].

The most common cause of complications of submersible osteosynthesis of fractures of long tubular bones is violation of the technology of internal fixation of the bone fragments [27–29]. The first step to successful consolidation of the fracture is an open reduction with the restoration of the anatomical relationship in the damaged bone [27, 30, 31]; in the process of open reduction, it is necessary to avoid extensive skeletalization of the fragments [32, 33], which allows to save the maximum possible vascularization of the fracture zone. Important aspects of prevention of complications are preoperative planning with indications for the method of internal fixation, the correct operation with the optimal use of fixing structures [34], long and short screws depending on the fracture type (comminuted, non-fragmented) and the fracture location [35, 36], an early active development of movements in adjacent joints and a dosed load on the operated limb.

In our study, unstable fixation of the diaphyseal femoral fracture, due to various reasons, was detected in 20 (95.2%) cases. This led to a significant restriction of the motor regime in the postoperative period and potentiated development of knee joint contractures in 17 (81.0%) patients and impaired consolidation in all cases with a slower fracture fusion in 13 (61.9%) patients, and the formation of a false joint in 8 (38.1%) patients (*Table 3*).

Such outcomes of diaphyseal fractures of the femur are assessed as a serious injury to health by a forensic medical examination. At the same time, a thorough study of the causes of the unfavorable outcome of this fracture with a differentiated approach and taking into account the influence of patient-related, implant-related and surgery-related factors on given treatment outcome is necessary.

Conclusions

1. Isolated femoral diaphysis fractures are mostly closed (85.7%), localized mainly in the middle third (76.1%), and occur more often in men (76.1%) due to road accidents (90.4%).

2. The main causes for development of postoperative complications are unstable fixation of the fracture area (95.2%) and untreated displacement of femur fragments (71.4%) which lead to development of knee joint contractures in 17 (81.0%) patients with a delayed fracture fusion in 13 (61.9%) and the formation of a false joint in 8 (38.1%) cases.

3. The factors that potentiate development of postoperative complications in patients with hip diaphysis fractures are patient-dependent causes: obesity (38.1%), coxarthrosis (9.6%) and a history of gonarthrosis (28.6%), impaired orthopedic regimen the immediate postoperative period (14.3%) and the rehabilitation period (19.0%).

Research prospects. The revealed causes of complications after surgical treatment of femoral diaphysis fractures allow development of a set of organizational and therapeutic measures aimed at improving the results of open osteosynthesis of these fractures.

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INVESTIGATION OF THE PROPERTIES OF THE ORAL LIQUID AND POLYMORPHISM OF THE MUC5B GENE IN CHILDREN WITH CYSTIC FIBROSIS

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Abstract
The article presents the study of biophysical and biochemical properties of saliva and polymorphism of the MUC5B protein gene in children with cystic fibrosis.
It has been determined that the development of chronic gingivitis in children with cystic fibrosis occurs secondary to an increase in saliva elasticity and a decrease in salivation rate. The presence of the MUC5B gene allele with 9 repeats (59 bp) in the intron 36 in the genotype for cystic fibrosis patients likely indicates a lower tendency to gingivitis, while the patients possessing the allele with 6 repeats in the genotype were found to have a higher percentage of moderate gingivitis. These data could be used to predict the development of chronic gingivitis, depending on the genetic polymorphism of the MUC5B gene and to form risk groups for differentiated prescription of preventive and therapeutic measures.
Key words: *children, cystic fibrosis, gene polymorphism, gingivitis, oral fluid.*

Introduction

To date, many studies have proven a significant prevalence of periodontal tissue diseases among children with a common disorder [1, 2]. Cystic fibrosis (CF) is considered to be one of somatic diseases that have an effect on the course of diseases of the organs of the oral cavity.

CF is a genetic disease caused by mutation of the gene of the cystic fibrosis transmembrane conductance regulator (CFTR). There are three main links in the pathogenesis of CF, namely an impaitment of exocrine glands function, a disruption of electrolyte metabolism and connective tissue damage. These processes condition a release of products with altered physical and chemical properties and the development of pathological changes mainly in the pulmonary, digestive, endocrine and reproductive systems. Diseases of bronchopulmonary organs (airway obstruction, chronic rhinitis, nasal polyps and chronic colonization of the respiratory tract characteristic of the microflora) are the most characteristic manifestations of CF. Violations in the digestive system are manifested by pancreatic insufficiency, gastroesophageal reflux disease, liver disease, and intestinal disorders [3–6].

Secondary pathological changes in many organs also have an impact on the development of dental diseases. Submandibular salivary glands are known to be filled with eosinophilic substrates of high viscosity in patients with CF. The parotid and small salivary glands are not histologically altered, but they produce a secretion with an excess of sodium and chloride ions [7]. Moreover, it is associated with an increase in the concentration of free and bound calcium in the submandibular glands and the variation in the pH, content of inorganic calcium and some enzymes in the oral fluid. Impaired salivation can result in nonspecific parotitis [8, 9]. Properties of mixed saliva, which performs important functions of providing homeostasis, mineralization, protection and cleaning in the oral cavity, undergo considerable changes.

Diabetes is a concomitant disease in these patients. It is known that the early stages of the

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disease are characterized by hypersalivation with atrophic changes in parenchyma and hypofunction of glands at later periods [10, 11].

The content of mucins also determines the properties of oral fluid playing an important role in the protection against pathogens and in physiological microenvironment support for microorganisms [12]. It has been proved that MUC5B mucin production is increased in patients with CF [6]. There is scientific evidence that *R. aeruginosa* and *S. aureus* (infections comorbid with cystic fibrosis) activate cell membrane receptors and stimulate mucin secretion. At the same time, interaction of carbohydrate structures of mucin with these microorganisms results in deterioration of oral fluid quality [13]. Deviation from the normal parameters of the oral fluid contributes to the local pathological processes, such as dental caries and periodontal diseases [12]. Analysis of literature sources also revealed that allelic variants of the MUC5B gene could be assessed as markers of early diagnosis of predisposition to gingivitis [14–16].

Thus, the study of the effect of CF pathogenesis on the properties of oral fluid as one of gingivitis risk factors in children, as well as the search for possible genetic predictors to inflammatory periodontal diseases is of particular scientific interest.

2. Purposes, subjects and methods:

2.1. The purpose of the work was to evaluate mucin level, the level of oral fluid elasticity (viscosity), saliva flow rate and alleles of MUC5B protein gene CNV polymorphism in children with CF, as well as to analyze the effect of determined parameters on the progress of chronic gingivitis in this cohort of patients.

2.2. Subjects and Methods

To achieve the goal, the study involved 30 children with a confirmed diagnosis of CF aged from 2 to 17 years (the main group). The comparison group included 23 children of similar age without somatic diseases.

The assessment of the periodontal condition was carried out using the PMA index (Parma, 1960), which reflects the degree of intensity of gum inflammation from "mild" to "severe".

The rate of the salivation was determined by the method of T. L. Redinova and A. R. Pozdeeva (1994). Graduated test tubes were used to collect unstimulated oral fluids.

Saliva elasticity was determined by the method of P. A. Leus and L.V. Belyasova (1995) which was conducted with the help of a dental pincer. The oral liquid was pulled out with thin threads, and the results were evaluated from "sharply positive" to "sharply negative".

Unstimulated saliva was collected from patients in sterile tubes in the amount of 2 ml to study the level of mucin. The analysis was carried out in a laboratory using Benedict's reagent.

The structure of MUC5B gene involves 48 exons and 47 introns. It contains a large central exon 30 (10713 bp) and a series of straight sequential repeats of 59 bp. (Sequence – cctgtgcggt gagtgggggc ggccccgggc cccccagacc cctcggcctc tctgagtgt) in intron 36 [17]. We chose this CNV polymorphism (the number of copies of indicated sequence) for the analysis.

For genotyping, buccal epithelial cells were used. DNA was isolated from these cells using the Diatom ™ DNA Prep 100 commercial kit (Russian Federation). CNV polymorphism typing in the intron 36 of MUC5B gene was performed using a polymerase chain reaction (PCR) with the detection of amplified fragments in agarose gel [17]. The following primers were used for amplification: MUC5B F – 5'-AGTGTGCAGTGACTGGCGAG-3' and MUC5B R - 5'-CTAGAGTTGCAGGTGGCAGG-3'. For PCR, an automatic thermal cycler "Terzik" (Russian Federation) and commercial reagent kits GenPak ™ PCR Core (0.5 ml) (Russian Federation) were used. Fragment sizes were determined by comparison with the molecular weight marker pUC19 DNA / Mspl (HpaII) Marker (Thermo Fisher Scientific Inc.). All reagents and devices were used according to the manufacturer's instructions.

The results were statistically processed with the standard software Statistica 8.0 Microsoft Excel.

The institutional Committee in Ethics and Bioethics approved the investigation. Written consent from children`s parents were obtained.

Conflict of interests. There is no conflict of interests.

3. Results and discussion

The average index of the PMA showed significant differences in the main and control groups: 47.98±3.47% and 9.17±2.29%, respectively ($p < 0.01$). The estimation of the periodontal status revealed clinical signs of chronic generalized catarrhal gingivitis in all (100%) children of the main group. Thus, 7 (23.3%) children had a mild degree, 15 (50%) children a moderate and 8 (26.7%) children had a severe degree of chronic catarrhal generalized gingivitis.

Analysis of the studied parameters of oral fluid revealed a deterioration of these values and

their difference in children with CF compared with the control group. Instead, the content of mucin in the oral fluid in children of both groups did not show a significant difference between the indicators (*Table 1*).

The study of correlation between periodontal indices and oral fluid properties also showed a difference in the studied groups. Thus, in the main group, the index of elasticity of oral fluid directly affected the value of the PMA index (r=0.55; p < 0.01). This link in the comparison group was missing. Patients with CF were found to have a significant negative correlation between the rate of salivation and the elasticity of oral fluid (R = -0.67; p <0.05), and direct connection between the rate of salivation and the mucin content (R = 0.53; p <0.05).

Based on genotyping results alleles of the MUC5B gene polymorphism were established for 48 patients (*Table 2*). There were 15 different genotype variants in the control group and 16 in the main group. In both groups there were individuals homozygous for a specific allele (39% and 40%, respectively), heterozygous for two alleles (22% and 28%, respectively), as well heterozygous individuals possessing three different alleles (39% and 32%, respectively).

Table 1

Indicators of properties of the oral fluid in the studied groups

Indicator	Total , (M±m)	
	The main group	The control group
Saliva rate, ml / min	0.19±0.01* (n = 24)	0.39±0.01* (n = 23)
Elasticity of oral fluid, unit	1.07±0.17* (n = 30)	-1.13±0.25* (n = 23)
Mucin of oral fluid, g/l	2.16+0.06 (n = 30)	2.37+0.14 (n = 23)

Note: * the difference is statistically significant (p <0.01) between the groups.

For the control group homozygous 2 // 2 (17.4%), heterozygous 3 // 5 // 8 (13%) and homozygous 8 // 8 (13%) genotypes were more common. Some of these variants were also found in the case group: 3 // 5 // 8 (12%) and 8 // 8 (12%), but not 2 // 2 homozygous ones.

The analysis of the periodontal status using PMA index showed that only the carriers of 9 repeats allele in the case group significantly differed from those without such allele. The

Table 2

The genetic diversity of the control and main groups by alleles of the CNV polymorphism in the MUC5B gene

Control group			Case group		
Genotype	(n)	(%)	Genotype	(n)	(%)
2//2	4	17.4	3//5	1	4.0
2//3	1	4.3	3//5//8	3	12.0
2//7	1	4.3	3//6	1	4.0
2//7//9	1	4.3	3//6//8	3	12.0
3//4//7	1	4.3	3//6//9	1	4.0
3//5//8	3	13.0	3//7//8	1	4.0
3//6//8	1	4.3	4//4	1	4.0
4//6//7	1	4.3	4//7	1	4.0
5//5	1	4.3	5//8	1	4.0
5//7//9	1	4.3	5//9	1	4.0
6//6	1	4.3	6//6	2	8.0
6//7//8	1	4.3	6//8	1	4.0
6//9	2	8.7	6//9	1	4.0
7//9	1	4.3	7//7	3	12.0
8//8	3	13.0	8//8	3	12.0
			9//9	1	4.0
Total					
15	23	100	16	25	100

presence of 9 repeats allele in MUC5B gene in genotype ($U = 9.5$, $p < 0.05$, Mann–Whitney test) in CF patients could indicate a lower tendency to chronic gingivitis. Among children with a moderate degree of gingivitis development prevailed ($U = 22.0$, $p < 0.05$) the carriers of 6 repeats allele. At the same time, PMA index in both studied groups did not correlate with the total number of 59 bp repeats in the intron 36 of MUC5B gene.

In the case group the analysis of linkages between the presence of some MUC5B allele in genotype and the index of saliva elasticity revealed some associations (*Table 3*), while none of them were proved statistically. We consider this fact reflects quite a small sample size of the main group. With a larger sample we expect to obtain more significant indices. For now, we can admit the tendency to negative association between the presence of 4 repeats MUC5B allele in genotype and low saliva elasticity. Similar thing is shown for 8 repeats allele carriers. On the contrary, positive association is supposed to be for 9 repeats allele carriers, i.e. almost all the carriers of this allele typically have low saliva elasticity and these patients have a lower level of gingivitis.

Conclusions

The development of chronic gingivitis in children with CF occurs secondary to an increase in saliva elasticity and a decrease in the rate of salivation.

In CF patients the presence of the allele of MUC5B gene with 9 repeats (59 bp) in the intron 36 in the genotype likely indicates a lower tendency to gingivitis, while patients possessing the allele with 6 repeats in the genotype were found to have a higher percentage of moderate gingivitis. These data can be used to predict the development of chronic gingivitis, depending on the genetic polymorphism of the MUC5B gene and to form risk groups for differentiated prescription of preventive and therapeutic measures.

The presence of some MUC5B alleles in the genotype and index of saliva elasticity had some associations, but the relationship has not been proved statistically.

Table 3

The results of analysis of linkages between the presence of some MUC5B allele in genotype and the index of saliva elasticity for cystic fibrosis patients (main group)

Allele *MUC5B*	χ^2 (*p*)	*Q*, coefficient of association	*Y*, coefficient of colligation	*V*, coefficient of contingent	*OR*	(*CI*)	*Z* stat.	*p*
3	0.01 (>0.05)	0.04 (<0.5)	0.02	0.02	1.5	(0.27–8.45)	0.46	0.65
4	1.17 (>0.05)	**-1 (>0.5)**	**-1**	-0.23	0.32	(0.01–7.45)	0.71	0.47
5	0.83 (>0.05)	0.41 (<0.5)	0.22	0.19	2.4	(0.36–16.21)	0.89	0.37
6	0.01 (>0.05)	-0.05 (<0.5)	-0.03	-0.02	0.9	(0.15–5.26)	0.12	0.91
7	0.49 (>0.05)	0.37 (<0.5)	0.19	0.15	2.17	(0.24–19.28)	0.69	0.49
8	1.81 (>0.05)	**-0.54 (>0.5)**	-0.29	-0.28	0.3	(0.05–1.79)	1.32	0.19
9	1.55 (>0.05)	**0.65 (>0.5)**	0.37	0.26	4.67	(0.35–61.83)	1.17	0.24

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DISTRIBUTION OF THE CAUSATIVE AGENTS OF RESPIRATORY TRACT INFECTIONS IN CHILDREN

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Abstract
The study aimed to investigate the prevalence of microorganisms depending on the site of isolation and disease. The study involved 48 children aged 1 to 17 years. The patients were diagnosed with acute bronchitis (54.17%), community-acquired pneumonia (CAP) (33.33%), bronchial asthma (12.50%). 173 strains of microorganisms were isolated. 106 strains (61.3%) of Gram-positive microorganisms, 49 strains (28.3%) of Gram-negative microorganisms, 18 strains (10.4%) of fungi were detected. The study implied assessment of 100 samples from the nose (nasal swabs), pharynx (throat swabs) and sputum. Gram-positive microorganisms were isolated in 83 cases, Gram-negative microorganisms in 36 cases, fungi in 18 cases. The assessment showed that *Staphylococcus aureus* was most often isolated from patients with acute bronchitis; Gram-negative microorganisms were most often detected from throat swabs, comparing with microorganisms detected from nose swabs and sputum.
Key words: *microorganisms, biofilms, respiratory diseases, children.*

Introduction

Lower respiratory tract infections are still one of the leading causes of mortality in young children worldwide, accounting for 1.4 to 1.8 million deaths annually [1]. It has been hypothesized that the composition of the microbiota in the respiratory tract might be altered during respiratory diseases [2]. There is a lack of knowledge regarding the role of respiratory microbiota in susceptibility to pneumonia [3]. By the age of 2 years, almost 95% of children are colonized in the nasopharynx, in the form of a bacterial biofilm, by one of the greater than 90 serotypes of *Streptococcus pneumoniae*. Biofilm formation is a fundamental step in pathogenesis, as biofilms promote bacterial persistence, competence, immune evasion, and resistance to antibiotics, all while serving as reservoirs for local and invasive disease [4–7]. Another common upper respiratory tract opportunistic pathogen is *Staphylococcus aureus*. This microorganism colonizes the anterior nares and nasopharynx of 30 to 80% of individuals, often in biofilms, which serve as a reservoir for local and invasive disease [8–11]. Pneumonia caused by *Klebsiella pneumoniae* is characterized by an exacerbated inflammatory response, associated with excessive neutrophil and macrophage infiltration, high production of pro-inflammatory cytokines and severe lung injury [12, 13]. The opportunistic pathogen *Pseudomonas aeruginosa* is known to be an important human pathogen that produces several virulence factors. Its Quorum sensing (QS) systems are probably the best characterized among Gram-negative bacteria [14]. QS systems and biofilm formation are extemelly important components in the development of acute and chronic infections, particularly for *Pseudomonas aeruginosa* [15, 16]. Nontypeable *Haemophilus influenzae* (NTHI) biofilm formation has been associated with respiratory tract infections such as chronic rhinosinusitis and exacerbations of both chronic obstructive pulmonary disease (COPD) and cystic fibrosis [17]. Bacteria residing within

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biofilms are up to 1,000 times more resistant to antibiotics and innate immune effectors than their planktonic counterparts [18], which contributes to the chronic and recurrent nature of these biofilm-associated diseases. Consequently, there is a great need for novel ways to manage biofilm-associated NTHI infections [19].

2. Purposes, subjects and methods:

2.1. The purpose of the work was to investigate the prevalence of microorganisms depending on the site of isolation and disease.

2.2. Subjects and Methods

The study involved 48 children aged 1 to 17 years with respiratory diseases: community-acquired pneumonia (CAP), acute bronchitis, bronchial asthma, which were treated at Kharkiv Regional Children's Clinical Hospital in the intensive care unit, pulmonary department. There were 29 boys (60.42±7.06%) and 19 girls (39.58±7.06%). (*Fig. 1*).

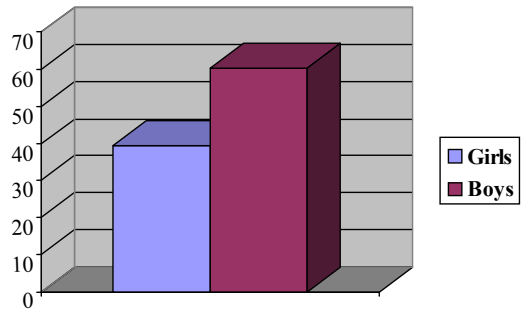

Fig. 1. Distribution of boys and girls in the research (%)

Isolation and identification of microorganisms from the nose (nasal swabs), pharynx (throat swabs), and sputum was performed according to the Order No. 535 of 22 April 1985 "On unification of microbiological methods of the research used in clinical-diagnostic laboratories in hospitals". Sputum production was induced by inhalation of 5.0% hypertonic saline solution, and the sputum sample was obtained by aspirating the nasopharynx through the nostrils or by expectoration if the child was old enough to produce an adequate sputum sample [20, 21].

The data were first entered in the Microsoft Excel and were analysed by SPSS V.19. Differences between places of isolation were assessed by means of χ^2 analysis. Statistical significance was set at 0.05.

Conflict of interests. There is no conflict of interests.

3. Results and discussion

Acute bronchitis developed in 26 cases (54.17±7.19%), CAP in 16 cases (33.33±6.8%), bronchial asthma in 6 cases (12.50±4.77%) (*Fig. 2*).

We investigated 100 samples from the nose (nasal swabs), pharynx (throat swabs) and sputum. Gram-positive microorganisms were isolated in 83 cases, Gram-negative microorganisms in 36 cases, fungi in 18 cases. *Table 1* shows that Gram-negative microorganisms in most cases were isolated from the pharynx as compared with the nose and sputum.

Overall, 173 strains of microorganisms were cultured. We detected 106 strains (61.27±3.70%) of Gram-positive microorganisms, 49 strains (28.32±3.43%) of Gram-negative microorganisms, 18 strains (10.40±2.32%) of fungi (*Fig. 3*).

100 strains were isolated from the pharynx. There were 52 strains (52%) of Gram-positive

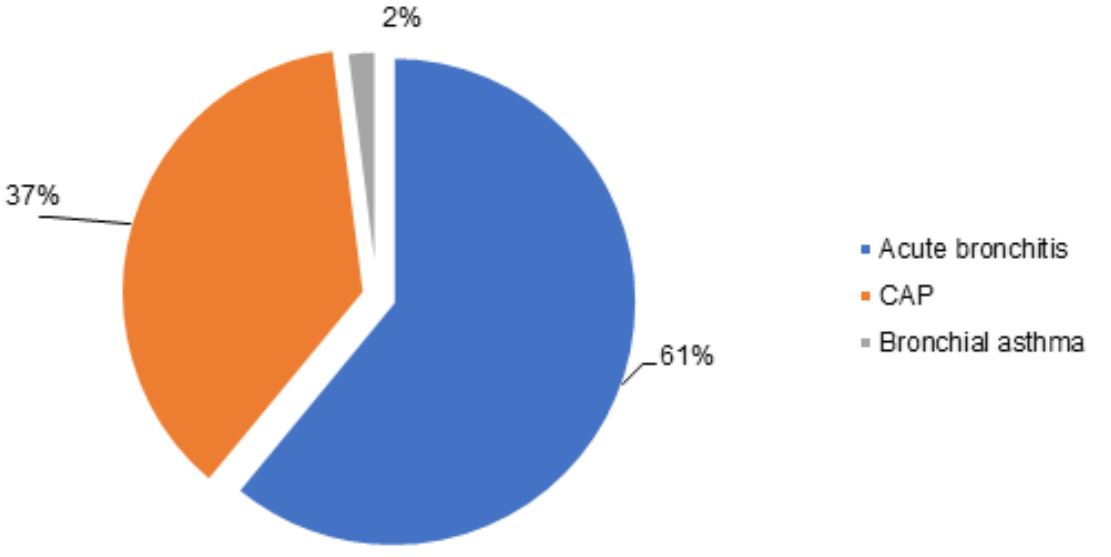

Fig. 2. Distribution of acute bronchitis, pneumonia and bronchial asthma in the research (%)

Table 1

Frequency of Gram-negative organisms distribution depending on the site of detection

	Pharynx	Nose	Sputum	*p*
Gram-negative microorganisms	24 (66.67%)	8 (22.22%)	4 (11.11%)	0.002

Note: differences between the samples with Gram-negative microorganisms from the pharynx, nose, sputum are statistically significant (p<0.05).

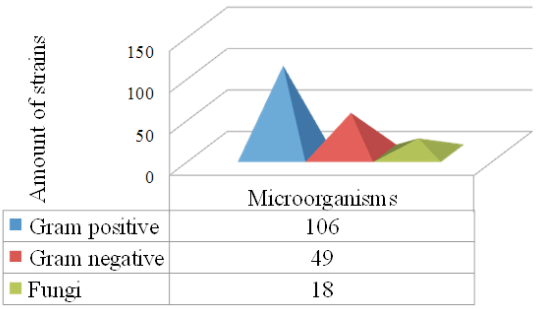

Fig. 3. Groups of microorganisms isolated from the patients

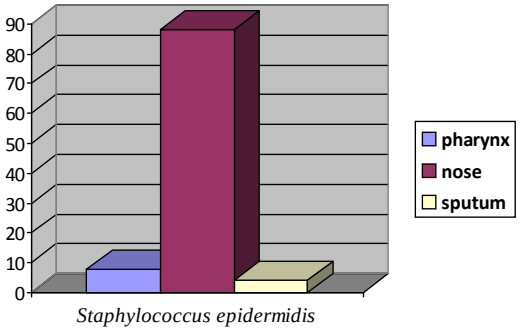

Fig. 4. The distribution of Staphylococcus epidermidis depending on the place of isolation (%)
Note: Amount of probability type 1 error, p=0.000006<0.05

microorganisms: *Streptococcus pneumoniae, viridans streptococci, group A β-hemolytic streptococci, Staphylococcus aureus, Staphylococcus epidermidis, Enterococcus faecalis* (*Table 2*).

Gram-negative microorganisms detected 34 strains (34%): *Klebsiella pneumoniae, Neisseria spp., Haemophilus influenzae, Pseudomonas aeruginosa* and other *Nonfermenting Gram-negative bacilli (NFGNB)* (*Table 3*). 14 strains of fungi were detected (14%): *Candida spp.* and other fungi.

We found that *group A β-hemolytic streptococci, Streptococcus pneumoniae, Klebsiella pneumoniae, Neisseria spp., Candida spp.*, were isolated from throat swabs more often as compared to nasal swabs and sputum (*Table 4*).

Staphylococcus epidermidis was most often isolated from nasal swabs – 22 cases (88%), as compared to throat swabs – 2 cases (8%) and sputum – 1 case (4%) (*Fig 4*).

Staphylococcus aureus predominated in patients with acute bronchitis – 23 cases (63.89%), comparing with CAP – 6 cases (16.67%) and bronchial asthma – 7 cases (19.44%) (*Fig. 5*).

Table 2

The number and percentage of Gram-positive microorganisms isolated from the pharynx (throat swabs)

Serial number	Name of organism	Number of organism (n=52)	Percentage of total organisms
1	*viridans streptococci*	17	32.69±6.50 %
2	*Staphylococcus aureus*	15	28.85±6.28 %
3	*group A β-hemolytic streptococci*	10	19.23±5.47 %
4	*Streptococcus pneumoniae*	7	13.464.73 %
5	*Staphylococcus epidermidis*	2	3.85±2.67 %
6	*Enterococcus faecalis*	1	1.92±1.90 %

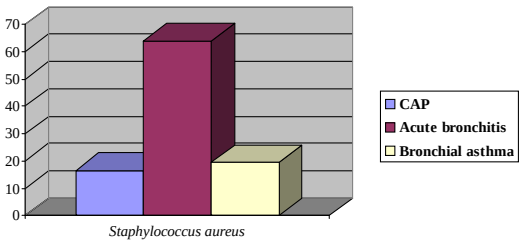

Fig. 5. The distribution of Staphylococcus aureus depending on the diagnosis (%)
Note: Amount of probability type 1 error, p=0.025<0.05

Discussion:

We showed that *Staphylococcus aureus* most often was isolated from patients with acute

Table 3

The number and percentage of Gram-negative organisms isolated from the pharynx (throat swabs)

Serial number	Name of organism	Number of organism (n=34)	Percentage of total organisms
1	*Klebsiella pneumoniae*	12	35.29±8.20%
2	*Neisseria spp.*	12	35.29±8.20%
3	NFGNB	3	8.83±4.86 %
4	*Pseudomonas aeruginosa*	5	14.71±6.07%
5	*Haemophilus influenzae*	1	2.94±2.90 %
6	*Escherichia coli*	1	2.94±2.90 %

Table 4

The frequency of microorganisms distribution depending on the site of detection

	Pharynx	Nose	Sputum	*p*
Streptococcus pneumoniae n=12	7 (58. 34 %)	1 (8. 33%)	4 (33. 33%)	0.0004
viridans streptococci n=20	17 (85. 0 %)	0 (0%)	3 (15.0%)	0.0004
group A β-hemolytic streptococci n=12	10 (83.34%)	1 (8.33%)	1 (8.33%)	0.015
Candida spp. n=16	12 (75.0%)	2 (12.50%)	2 (12.5%)	0.013
Klebsiella pneumoniae n=18	12 (66.66%)	3 (16.67%)	3 (16.67%)	0.017
Neisseria spp. n=13	12 (92.31%)	0 (0%)	1 (7.69%)	0.001

Note: differences between specimens from pharynx, nose, sputum are statistically significant ($p<0.05$).

bronchitis. This microorganism has a lot of virulence factors, but most important in the developing respiratory diseases are toxins (α-toxin, β-toxin, Panton-Valentine leukocidin) and biofilms formation. Theophilus K.Adiku et al. from Ghana [22], who investigated etiology of acute lower respiratory infections among children, noted that *Staphylococcus aureus* was a prevalent bacterial organism isolated from the throat (8%) and from the nasopharynx (16%). On the one hand *Staphylococcus aureus*, which was the most common bacterial organism in the nasopharyngeal aspirate samples, is known to occur as a normal flora in the nasopharynx of most healthy children. On the other hand, their data shows that *Staphylococcus aureus* may be an important cause of septicemia among children in Ghana [22]. In our research we could not prove the exact microorganism prevailing in patients with CAP, but we can suppose that without adequate treatment, bronchitis could transform to pneumonia. El Seify M.Y. et al., from Cairo, Egypt [23] showed that *Staphylococcus aureus* (n = 12; 13.3%) was the most common typical bacterial cause of pneumonia followed by *Streptococcus pneumonia* (n = 7; 7.8%) and *Klebsiella pneumoniae* (n = 7; 7.8%). In their study, *Staphylococcus aureus* was found to be the most common typical respiratory pathogen causing CAP (13.3%) [23]. Bhuyan G.S. et al. from Dhaka, Bangladesh [24], detected microorganisms from nasal swabs in children with acute respiratory infections and demonstrated that the most commonly isolated bacteria were *Streptococcus pneumoniae (39%), Klebsiella pneumoniae (22%), and Haemophilus influenzae (6%)*. Our investigation showed that Gram-negative microorganisms in most cases detected from throat swabs as compared to microorganisms detected from nosal swabs and sputum. It may be the purpose for further investigation of virulence factors of *Klebsiella pneumoniae* (capsule, endotoxin, siderophores, biofilm formation), *Pseudomonas aeruginosa* (pigments, siderophores, elastases, biofilms formation), *Haemophilus influenzae* (capsule, biofilm formation) and their mechanisms of resistance to the action of antibiotics. We found that *Streptococcus pneumoniae* was isolated from the pharynx more often compared with nose and sputum. It is a common commensal that quiescently colonizes the upper respiratory tract, forming biofilms adhering to the epithelium of the nasopharynx, lungs, rather than planctonic cells. [25–30]. Capsule and pneumolysin are the main virulent factors that protect *Streptococcus pneumoniae* against phagocytosis. Honkinen M. [31], and Gentile A. [32] showed that

Streptococcus pneumoniae and *Haemophilus influenzae* predominated in frequency in children with CAP [31, 32].

Conclusions

1. The present study demonstrates that group *A β-hemolytic streptococci, Streptococcus pneumoniae, Klebsiella pneumoniae, Neisseria spp., Candida spp.*, were isolated from the pharynx more frequently as compared to the nose and sputum.

2. The study showed that Gram-negative microorganisms were most often detected in the pharynx, than in the nose and sputum.

3. Our findings suggest that *Staphylococcus aureus* most often was isolated from patients with acute bronchitis.

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PECULIARITIES OF MEDICAL AND PSYCHOLOGICAL REHABILITATION OF PARTICIPANTS OF MILITARY ACTIONS WITH POSTCONCUSSIONAL SYNDROME

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Abstract

Forty-eight families of veterans with post-concussion syndrome were directed for medical and psychological rehabilitation. At the beginning of the rehabilitation course, personal psychological problems (by anonymous survey and testing) were recognized in 42 veterans and 44 veterans' wives. Problems related to family relationships were identified in 39 families. The weekly courses included: the physical component of rehabilitation, psychological (psychotherapeutic) rehabilitation component and measures conditionally related to micro-social rehabilitation.

The applied psychological assistance methods in combination with the physical and social components of the program led to an improvement in the personal psycho-emotional state of 96% of participants. Improvement of family understanding and psychological microclimate was noted by 100% of families. 60% of families expressed a desire to continue working with a psychologist. Based on the experience gained, recommendations have been made to improve mutual understanding in families.

The use of these methods of intra-family psychological self-regulation will contribute to the normalization of relations, reduction of the clinical physical and mental effects of contusion, and improvement of the resocialization of veterans.

Key words: *post-concussion syndrome, medical and psychological rehabilitation, family psychotherapy.*

Introduction

The urgency of the problem: Traumatic brain injuries take a significant place in combat injuries. This is noted by foreign researchers and confirms the experience of our compatriots according to the results of the military actions in the Donbass region [1].

Neurotrauma has its own peculiarities that necessitate a special approach to first-aid dressing, treatment and rehabilitation [2]. Postconcussional syndrome in modern conditions is caused by the high specific gravity of mild traumatic brain injury (according to the NATO terminology – mild traumatic brain injury, mTBI) in the structure of combat trauma among combatants in eastern Ukraine. This is due to the fact that today more than 80% of combat damage is a mine-explosive injury, and a third of injuries are injuries of the head. In its turn, in the structure of head injuries, mTBI is about 83% [3, 4].

Mild traumatic brain injury, including concussion and brain injury of a mild degree according to terminology familiar to the Ukrainian medical community, in the absence of proper treatment and rehabilitation, in about one third of cases, leads to long-term complications in the form of postconcussional syndrome, which manifests itself mainly as disorders of the autonomic nervous system and disorders in the psycho-emotional sphere [5].

Combination of organic pathology with changes in the psycho-emotional sphere, aggravation of somatic and neuro-vegetative

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symptoms by the effects of combat mental trauma, adaptation disorders (including psycho-social) and difficulty of reintegration, complications in the form of various addictions (alcohol, other substances, gambling), require the search and application of comprehensive measures of therapeutic and rehabilitation impact, among which an important place is occupied by medical and psychological rehabilitation [7].

At the same time, the lack of awareness of the peculiarities of the disease course, the attempt to organize medico-psychological rehabilitation of war veterans in homogeneous closed groups does not allow patients to escape from a closed circle, to fully readapt to a normal peaceful life, to resocialize [8]. Each time, during such "rehabilitation" there is a re-traumatization of memories, the restoration of the "heroic" phase, the deepening of intrusive memories, which fits well with the background aggravated by alcohol consumption in the environment of similar veterans [9].

2. Purposes, subject and methods

2.1. The purpose of the work was development and testing of a comprehensive program of medical and psychological rehabilitation of combatants with postconcussional syndrome.

2.2. Subjects and methods

In order to achieve the set goal, 48 families of combatants diagnosed with F 07.2 postconcussional syndrome were surveyed during 2017–2019 complying with bioethics principles.

The following survey methods were used in the study: clinical and psychological; psychodiagnostic using the post-trauma stress disorder symptoms Self-Assessment Scale PCL; Traumatic Impact Scale (IES-R): Methods for Studying Styles of Coping Behaviors (Copying Methods (adapted by T.A. Kryukova, 2002), K. Thomas's Methods for "Determining Conflict Management Methods" edited by N.V. Gryshyna (by D.Y. Raygorodsky, 2002) [10].

The symptoms of the postconcussional syndrome were evaluated by the Cicerone Questionnaire (1995) [11] – assessment of the symptoms of the present time, the study of the state of vegetative regulation was carried out by completing the questionnaire for subjective evaluation of dystonia [12].

The mathematical and statistical processing of the results of the study was carried out using specialized software packages (Statistica 6.0, MS Excel) using the Student's t-test method.

Conflict of interest. There is no conflict of interests.

3. The results and discussion

As shown by the results of the study, in the examined families of veterans with postconcussional syndrome, family dysfunction was characterized by impaired interpersonal interaction between spouses (82.3%), psychosocial maladaptation (64.1%); breach of interpersonal relationships (86.2%); deformation of family interaction (72.3%).

In the course of work, we developed and tested a system of psychosocial rehabilitation, which included: physical component of rehabilitation (hiking and walking, mountain climbing, training on the simulators, sports games, visits to the salt room), psychological (psychotherapeutic) component of rehabilitation (individual and group work with a psychologist, art therapy using design techniques, training in psychological recovery and the acquisition of skills of resilience [8] and measures related to microsocial readaptation.

At the beginning of the rehabilitation course, 42 veterans and 44 veteran wives found personal psychological problems (according to an anonymous survey and testing). In 39 families, problems related to the family were identified.

The methods of psychological assistance used in combination with the physical and social components of the program led to an improvement in the personal psycho-emotional state of 96% of the program participants. Improvement of family understanding and psychological microclimate was noted in 100% of families. 60% of families showed the desire to continue working with a psychologist. It is the teaching of the family to live with the consequences of the past, "bringing peace to war, not war to peace", which became the core of the program of long-term psychological support and psychological self-regulation.

As a result of the work, a program of intrafamilial support for veterans with postconcussional syndrome was developed, which included the following modules:

I. Informational module (Understanding) was aimed at filling the information deficit of knowledge about etiopathogenesis and clinical manifestations of postconcussional syndrome, the need for pharmacotherapy, the importance of psychosocial rehabilitation; reducing the level of stigmatization and self-stigmatization.

II. Family support. Family response should be active at all stages (return, treatment, rehabilitation, etc.), family members should learn

not only weaknesses, but also new resources and strength of the victim (the opportunity to be a volunteer, if not to serve; support of brothers, etc.). All family processes should be present and future oriented, but it must be remembered that there are effective survival strategies that have helped the family in the past and are aimed at creating and developing hope and positive expectations, encouraging and supporting new forms of behavior, encouraging concentration attention and finding a solution, not the problem itself. The family should keep in mind the health orientation and that changes are due to strengthening.

The main compatible goal of the family is to regain the ability to control the situation, focus on quality of life, not pathology.

III. Warning. Unfortunately, in conditions when a person can no longer perform the functions that he performed before the trauma, a feeling of needlessness, neglect, and futility of his own existence develops. It is very important to understand that a constant depressed state, a sense of a shorted future, an inability to "find yourself" is a possible prerequisite for alcohol abuse and suicidal thoughts. Especially in the early stages of adapting to the new state, it is important to observe and share the views of all family members. Avoiding direct confrontation, joint needs identification, family support to restore decision-making ability (to understand the purpose, nature and impact of actions and actions management), development of partnerships in a joint way to recovery, development of health promotion behaviors and achievement of functional independence through various current measures, elimination of simultaneous life factors of stress.

IV. External involvement. Engaging with consent in social support networks – community, veteran, to abstract from the severe and negative manifestations of trauma is the key to a healthy future of a veteran and a family in general.

As the results of dynamic observation showed, stable positive dynamics of psychological state with statistically significant reduction of post-trauma stress disorder symptoms (76.9%), reduction of family conflict (71.4% of families), harmonization of marital relations (66.2% of families), positive transformation of the coping strategy (56.6%) - decision-oriented and social distraction and activation of adaptive forms of coping (70.5% of families) focused on social support, and a political approach to solving problems were noted against the background of the proposed system of medical and psychological rehabilitation in the examined families.

The data obtained of the complex of medical and psychological rehabilitation program of combatants correlate with the data of domestic researchers devoted to the development directions of prevention and algorithms for the formation of personalized programs of therapy and rehabilitation of combatants with traumatic stress disorder with [13, 14], he identification of specific target symptoms, is critically important for the recovering of the mental health, living conditions of combatants, and actually medical rehabilitation intervention complex includes: combination of psychotherapy and psychopharmatherapy. However, the proposed system of personalized medico-psychological rehabilitation of combatants with post-concussion syndrome, is new one, as well as, using in psychosocial rehabilitation complex with a particular focus for intimate family support of veterans.

Conclusions

Thus, the obtained results made it possible to substantiate the feasibility of the proposed system of medical and psychological rehabilitation of combatants with postconcussional syndrome.

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DEPRESSIVE DISORDERS IN PATIENTS WITH MITOCHONDRIAL PATHOLOGY (MECHANISMS OF FORMATION, CLINICAL TYPOLOGY, SYSTEM OF CORRECTION AND PREVENTION)

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Summary
The article introduces a modern understanding of the functioning of mitochondria, the flow of the main biochemical processes that are related to energy metabolism. A generalized characteristic of the mitochondrial genome mutations and types of mitochondrial dysfunctions are presented. The modern ideas about the occurrence of mental disorders in patients with mitochondrial pathology are analyzed. **Material and methods.** In order to study depressive disorders, 171 patients of both sexes with mitochondrial pathology were examined, aged from 18 to 57 years. **Results.** The most common clinical and psychopathological features of the examined patients with MP were analyzed. The most common complication of MP was depressive state. An additional analysis of depressive disorders and their variants were carried out. The study involved screening of 26 families of patients with mitochondrial pathology, including 14 families of patients with MP, accompanied by depressive disorders and 12 families of patients with MP without affective disorders. High incidence of psychosomatic symptoms and depression were detected by mother's side relative probands. According to the results of the study, patients received pharmacotherapy and psychotherapy care, depending on the clinical manifestations of depressive symptoms and the degree of their severity. Psycho-educational work was conducted with patients and their families.
Keywords: *antidepressants, anxiety, depression, mitochondrial diseases, mitochondrial dysfunction, psychoeducation, psychotherapeutic interventions.*

Introduction

Mitochondrial diseases are caused by genetic and structural-biochemical defects of mitochondria, accompanied by a violation of tissue respiration and, as a consequence, a systemic defect in energy metabolism, which results in the most diverse combination of the most energy-dependent tissues and target organs: the brain, skeletal muscles and myocardium (mitochondrial encephalomyopathy), pancreas, organ of vision, kidneys, liver [1, 3, 12].

Clinical disorders in the mentioned organs can develop at any age. At the same time heterogeneity of symptoms complicates the clinical diagnosis of these diseases [2, 4, 9].

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The peculiarity of the functioning of mitochondria is the presence of its own mitochondrial genome, the circinate mitochondrial DNA (mtDNA), which contains 37 genes, whose products are involved in the process of energy generation in the respiratory chain of mitochondria [1, 2, 11].

The mitochondria undergo major biochemical processes related to energy metabolism:

– a cycle of tricarboxylic acids (Krebs cycle),
– beta-oxidation of fatty acids;
– carnitine cycle;
– transportation of electrons in the respiratory chain;
– oxidative phosphorylation.

Any of the mentioned processes may be violated and cause mitochondrial failure. (*Fig. 1*).

The immediate cause of the emergence and development of mitochondrial dysfunction is the defect of the oxalic phosphorylation system, imperfection of the apparatus mechanisms, absence of histones, and presence of free oxygen radicals, by-products of aerobic respiration [1, 7, 10].

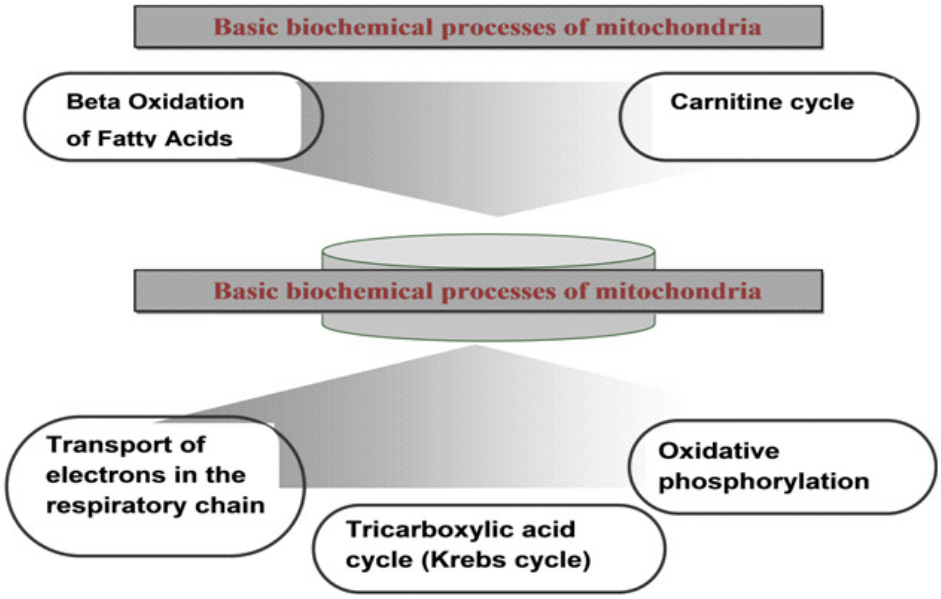

Fig. 1. Basic biochemical process of mitochondria

The main qualities of the mitochondrial genome are cytoplasmic imitation of genes, the lack of recombination (thus, the reorganization of the genetic material through the exchange of individual segments, DNA double helix areas) and the high rate of mutation occurrence.

The mitochondrial genome is different by:

1) Expressed instability

2) High speed of nucleotide substitutions

3) During the life of the individual, there are often somatic mutations.

For mutations in the mitochondrial genome, the phenomenon of heteroplasmia is characterized by the simultaneous presence of many copies of DNA (that is, mitochondria) that carry the normal or mutant allele and varies widely (1–99%).

In the process of cell division, both types of DNA (with normal or mutant alleles) are distributed randomly between the daughter cells [12].

Therefore, in subsequent generations, one part of the cells can only have normal mtDNA, the second part is only mutant, and the third part is the normal and mutant type of mtDNA [12, 13].

The number of mitochondria with mutant mtDNA increases gradually. Due to the "lag of the period", future patients often reach puberty and give offspring that almost always carry the same mutations in mtDNA [1, 2].

When the amount of mutant copies of mtDNA reaches a cell of a certain concentration, the energy metabolism in the cells becomes significantly disturbed and manifests itself as a disease. (The peculiarity of hereditary mitochondrial diseases is often the complete absence of any pathological signs at the beginning of the patient's life) [3, 5].

Since mtDNA in the body has virtually exclusively maternal origin, during the transmission of the mitochondrial mutation, the offspring in the pedigree have a maternal type of imitation – all the "daughters" of the sick "mother" are ill [8, 10].

This is a typical pathological process that does not have etiological and nosological specificity.

It is manifested by pathobiochemical mechanisms of neurodegenerative disorders of a wide spectrum.

The development of mitochondrial dysfunction leads to:

– violation of reuptake of mediators (catecholamine, dopamine, serotonin);

– violation of ion transport, generation and conduction of the pulse, as well as the synthesis of de novo protein;

– violations of broadcasting and transcripts;

– "parasitic" energy-generating reactions that lead to the loss of energy stores of the nerve cell are activated.

Types of mitochondrial dysfunction:

Primary – as a consequence of an innate genetic defect.

Secondary – under the influence of various factors: hypoxia, ischemia, oxidative and nitrosating stress, the expression of proinflammatory cytokines.

Changes in the energy metabolism of the brain violate its work and lead to the development of mental disorders [6, 8].

From 40 to 60% of the energy of ATP in neurons is spent to support the ionic gradient on

their outer envelope and the transfer of the nerve impulse. Violation of the function of the mitochondria leads to a violation of the processes of synaptic transmission of the nerve cell, a violation of cell growth and apoptosis.

Therefore, the violation of the function of the central nervous system in classical "mitochondrial diseases" is of great importance and makes it possible to call the main symptom "mitochondrial encephalomyopathy" [9,14].

Clinically in the foreground there are such brain disorders as dementia, seizures and stroke-like episodes.

The severity of these forms of the disease, combined with severe somatic disorders, can be so great that other violations associated with the person with emotional or personal changes, remain in the shadows [7, 11].

In recent years, the role of the contribution of mitochondrial disorders to the development of mental illness, foremost of the depressive disorder, is widely discussed. Indirect confirmation of the connection between mitochondrial and some mental illness is also a tendency to the focus of their clinical manifestations.

According to previous studies, 20% of patients with mitochondrial diseases had an associated BAR, with 0.38% of BAR having DNA polymerase mutations (y) (DNA polymerase gamma-POLG) that cause the development of mitochondrial diseases. In a new work (2018) conducted by Japanese scientists at the RIKEN Center for Brain Science (RIKEN), it has been established that mitochondrial dysfunction may affect the activity of serotonergic neurons with A1NT1 mutations [20].

2. Purposes, subject and methods

2.1. The purpose. Analyzing the abovementioned information, we conducted our own study in order to study the presence of depressive disorders in patients with mitochondrial pathology and develop a comprehensive approach to their therapy.

2.2. Subjects and methods.

Object of research: on the basis of Kharkiv Specialized Medical Genetic Center, 171 patients with mitochondrial pathology of both genders aged from 18 to 57 years were examined. Screening survey of 26 families of patients with mitochondrial pathology was conducted.

In this work a set of research methods was used: clinical and psychopathological, with the study of presentation, assessment of the mental status of patients, the allocation of the main psychopathological syndromes, their dynamics, as criteria of diagnosis selected criteria MKH-10, patients were further examined by a physician geneticist; Clinical-anamnestic, psychodiagnostic using the clinical Scales of Anxiety and Depression of Hamilton (M. Hamilton, 1967), adapted to MKH-10 (G.P. Pankeleva, 1988), Questionnaire of Neuro-Psychic Stress (T.A. Nemchin, 1984), SCL Scale, catamnestic and methods of mathematical statistics. The results are presented in the form of an average value ± mistake of representativeness at a probability level of $p<0.05$.

Conflict of interests

There is no conflict of interests.

3. Results and discussion

According to the results of the first stage of the study, all patients were divided into two groups. The first group consisted of patients with mitochondrial pathology without signs of mental disorders (32.7% of the surveyed), the second group included 67.3% of patients with mitochondrial pathology, which had signs of depressive disorders (*Fig. 2*).

At the second stage, patients with mitochondrial pathology with signs of depression were analyzed in details. According to the results of the analysis,

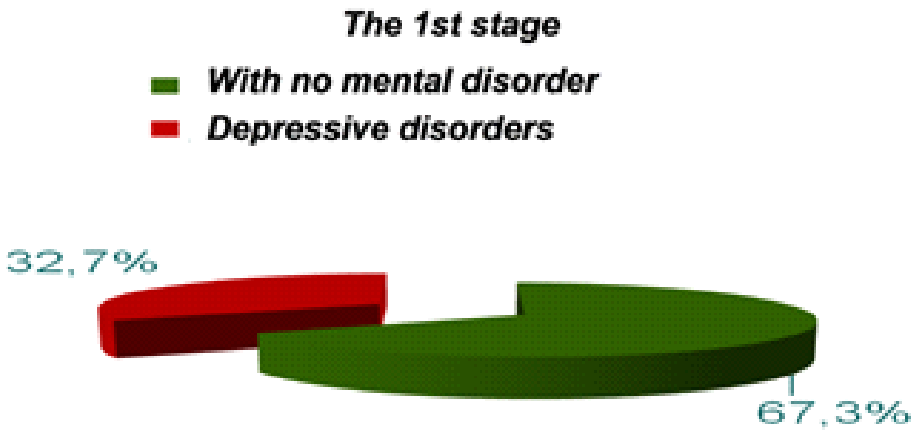

Fig. 2. Results of research of the 1st stage (%)

the patients were divided into three groups: the first group included patients with mild manifestations of depression (17.6%), the second group included patients with moderate depression (72.1%) and the third group were patients with severe manifestations of depression (10.3%) (*Fig. 3*).

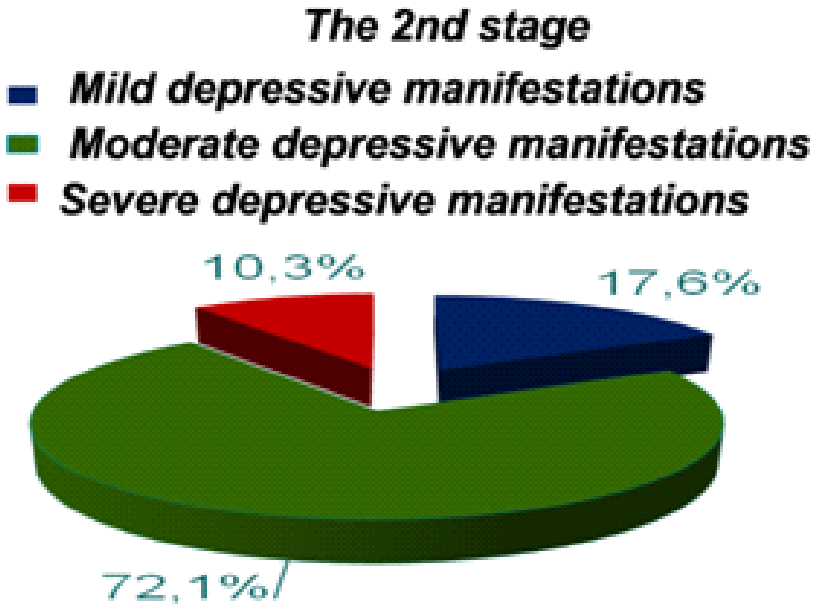

Fig. 3. Results of the second stage study (%; p<0.05)

In the study of the severity of anxiety and depressive manifestations in patients with mild, moderate and severe depression, the data presented in *Figure 4* were obtained.

It is noteworthy that in patients with mitochondrial pathology of mild to moderate depressive manifestations, higher rates of both anxious and depressive symptoms were given to women (p<0.05). In patients with mitochondrial pathology with mild to moderate depression, no significant differences between women and men were recorded, but both anxiety and depression rates are somewhat higher in the female group (p≤0.001). In patients with mitochondrial pathology with mild to moderate depression, signs of anxiety and depression were also higher among women (p≤0.001).

Analyzing the results of evaluation of the expressiveness of the nervous-psychic stress in the examined patients with mitochondrial pathology, we can notice that the level of pediatric neuro-psychic stress is significantly increased in men compared to women (p<0.05). The level of intense neuropsychiatric stresses in men and

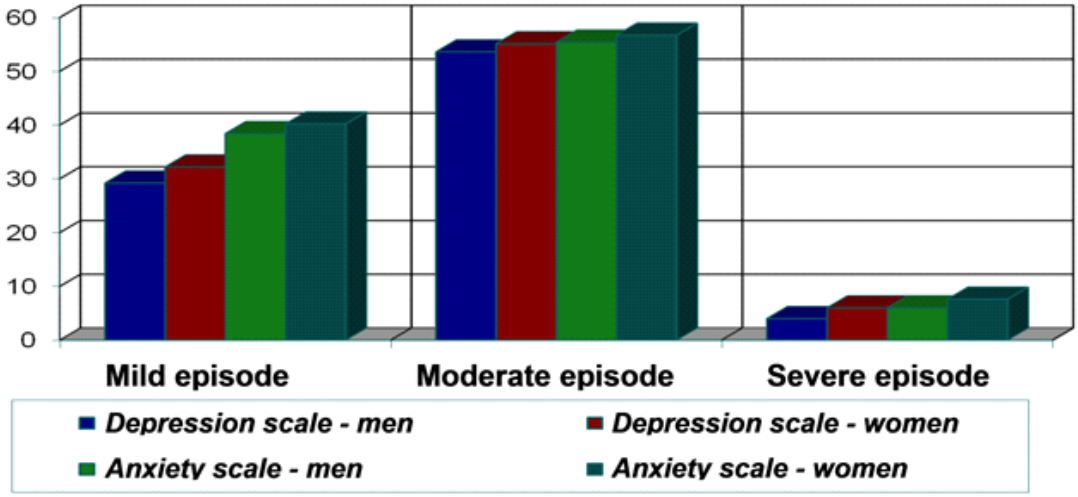

Fig. 4. Level of anxiodepressive manifestations in patients with mitochondrial pathology (p<0.05; p≤0.001)

women has approximately the same rates (p≤0.001). On the contrary, assessing the level of extensive nervous and mental stress, there is a significant increase in this indicator in the female group (p<0.05). *Fig. 5.*

Additional analysis of the variants of depressive disorders was carried out to distinguish anxiety-depressive (50.3%), asthenodepressive (18.5%), dysphoric and depressive-apatitic (15.6% each) variants. *Fig. 6.*

In half of the patients (50.3%) an anxiodepressive variant of the depressive disorder was recorded, which was manifested by the presence of low mood secondary to anxiety, dissatisfaction with oneself, fears without real causes, sleep disturbances, self-excuse, and feeling of lucidity.

Asthenodepressive variant was characterized by the presence of low mood, a sense of social detachment, self-incrimination, feelings of guilt, tears, loss of appetite, indecisiveness, increased fatigue, difficulties at work.

The dysphoric variant in the clinical structure had a combination of a steady decrease in mood with irritability, conflict, dissatisfaction, suicidal thoughts, loss of body weight, sleep disturbance.

The depressive-apathetic variant was characterized by reduced activity on the background of emotional oppression, a sense of agony, loss of sexual desire, the patient had difficulties performing simple self-service functions, they spent a lot of time in bed, were not interested in others, did not feel hunger, refused to eat.

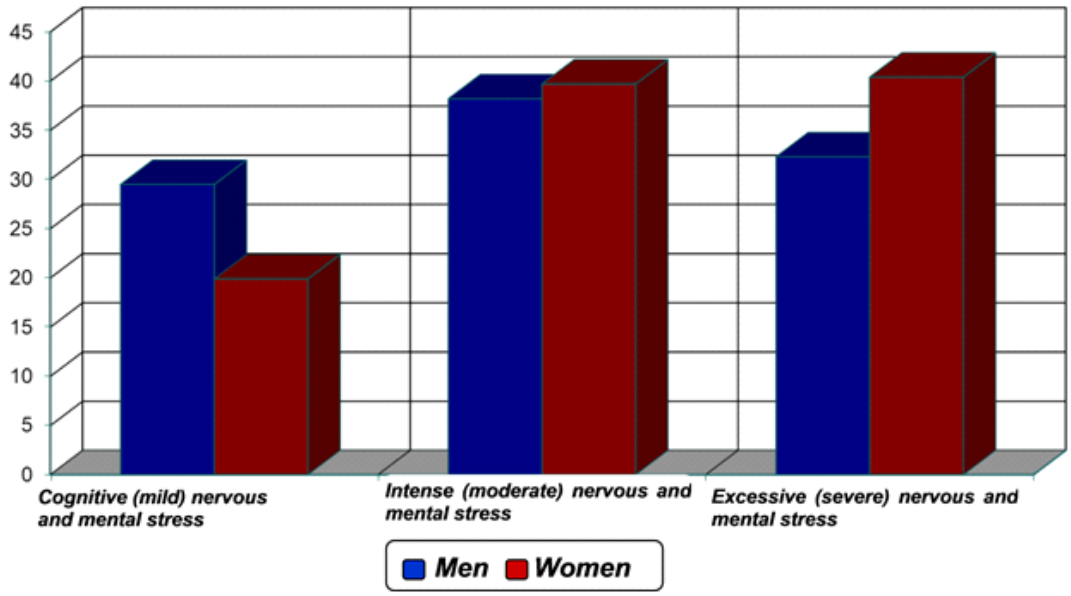

Fig. 5. Level of expressiveness of nervous and mental stress in patients with mitochondrial pathology (p<0.05; p≤0.001)

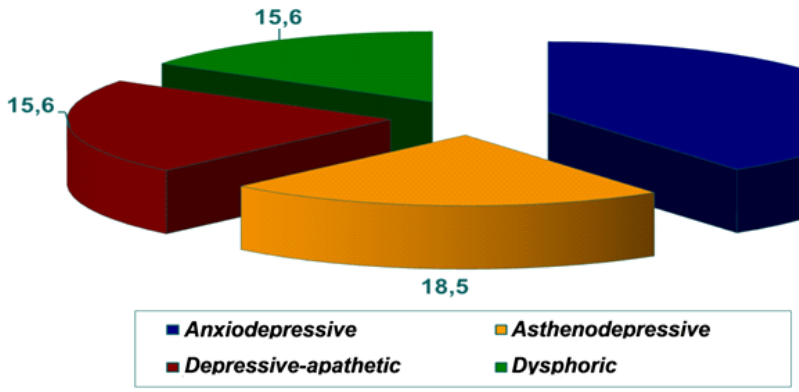

Fig. 6. Variants of depressive disorders in patients with mitochondrial pathology (%; p<0.05)

At the next stage of the study, a screening survey of 14 families of patients with mitochondrial pathology, accompanied by depressive disorders and 12 families of patients with mitochondrial pathology without affective disorders was conducted.

Among the issues, there were those relating to the health of parents of patients and the closest relatives (along the lines of father and mother).

When processing the results, the following data was obtained:

– In the first group of patients with relatives on the mother's side, in contrast to the parent, a higher frequency of psychosomatic symptoms and depression was detected (59.1% and 48.3% respectively).

– In the second group, 15.7% and 10.3% of the respondents met psychosomatic and depressive symptoms.

– On the father's line in both groups, these figures were 7.4% and 9.2% respectively.

On the basis of the obtained data, a comprehensive system of correction and prevention of depression was developed in patients with mitochondrial pathology, which included pharmacotherapy, psychotherapy and psychoeducation.

Patients with mild manifestations of depression provided psychotherapeutic assistance in the form of cognitive-behavioral, family psychotherapy, cognitive training, training for solving interpersonal problems.

Patients with moderate and severe manifestations of depression received both psychotherapeutic care and therapy with antidepressants. Preference was given to selective serotonin reuptake inhibitors (SSRI) at doses corresponding to the severity of depressive disorders.

With all patients, regardless of the severity of depression, psychoeducational work was conducted. The patients' families were also accepted with psycho-educational assistance. Psychoeducation was aimed at various aspects of the disease: biological, psychological and social. Patients explained the purpose of treatment, how to achieve a positive outcome of treatment, the advantages and disadvantages of different therapies. With each patient individually and in group, a crisis plan was worked out, that is what to do in the case of recurrence, whom to address, what drugs to take, etc.

Before the start of therapy, as well as in the second, fourth and sixth weeks of treatment, the dynamics of changes in the state of depression on the Hamilton scale was estimated in patients.

The obtained results are presented in *Fig. 7*.

According to the results of the complex system of treatment, patients managed to achieve reduction of anxiodepressive disorders (73.5% of patients), decrease in motor and ideatric inhibition (65.2%), decrease in the duration and intensity of depressive appetite (72.5%), recovery of interest to activities that previously interested patients (69.5%), improvement of psychosocial adaptation (81.3%), transition of maladaptive types of attitude to the disease into adaptive (66.5%), improvement of the quality of life of patients (83.2%).

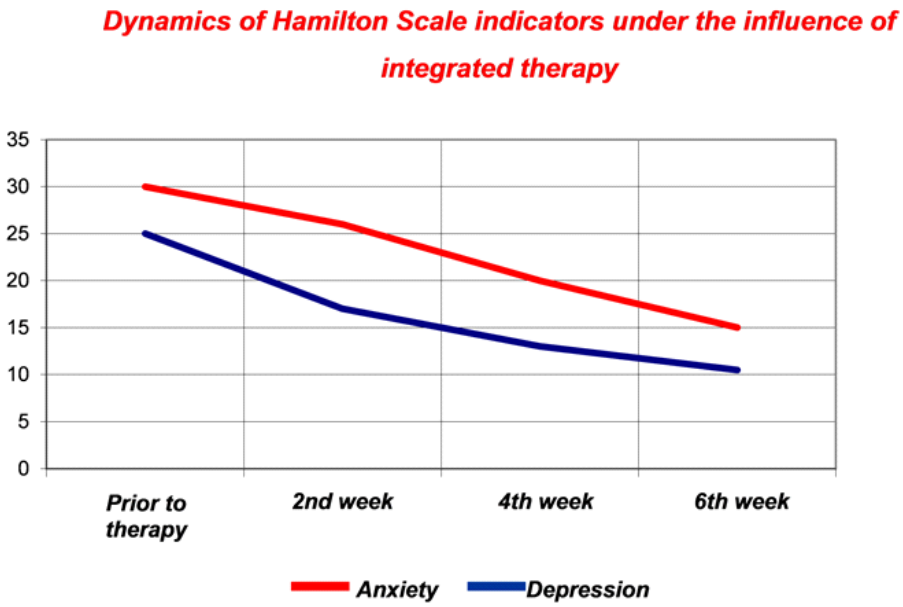

Fig. 7. Dynamics of changes in the state of depression on the Hamilton scale ($p<0.05$)

Over the past 12 months, foreign scientists Burnet B. and co-authors (2005) surveyed patients with mitochondrial diseases and their family members, especially maternal relatives. The results obtained in their work indicate a higher frequency of migraines in combination with irritable bowel syndrome and depression. At the time, our study, which lasted for 3.5 years, not only revealed the presence of depressive disorders in the surveyed contingent, but also identified different variants of depression manifestations. In our work, in maternal relatives of patients, unlike paternal relatives, it was found a higher frequency of psychosomatic symptoms and depression, in contrast to the study by Burnet B. and co-authors [19]. In the study of Babenko V.N. and co-authors (2018), an experiment with laboratory animals (male mice) confirmed that persistent social conflicts cause severe mitochondrial dysfunction in the brain [18]. In our study, patients and their family members who had internal family problems and impaired social functioning were more likely to have clinical manifestations of depressive disorders. Researchers Kato T.M., Kubota-Sakashita M., Fujimory-Tonou N. et al. (2018) have shown that mitochondrial dysfunction influences the activity of serotonin neurons in mice with a mutation of the ANT1 gene and is a predictor of the emergence of neuron-mediated depressive-like episodes [20]. It can be assumed that the presence of such a mutant gene in a person with mitochondrial pathology may be a signal indicator of the emergence of depressive disorders and will allow to extract it as a diagnostic marker. These findings are of scientific interest and are promising for further studies.

The results obtained during the study do not contradict the previous foreign studies, but do have some differences in the number of patients, their age and the duration of the study. In the course of work the variants of depressive disorders were distinguished, certain differences in manifestations of depressive disorders between women and men with mitochondrial pathology were revealed.

Analyzing the information received regarding the approaches to correction of mental disorders in the mitochondrial pathology, it can also be noted that antidepressants can also affect brain metabolism and mitochondrial activity. This effect has differences in different parts of the brain and is regulatory.

Conclusions

Consequently, the several stages of the study made it possible to distinguish the most common clinical and psychopathological features of the examined patients with mitochondrial pathology, which was complicated by depressive disorders. In patients with mitochondrial pathology with signs of depression, moderate levels of depression and intense (moderate) neuro-mental stress dominated. The structure of depressive disorders was dominated by anxiety-depressive symptom complex with a low mood secondary to anxiety, dissatisfaction with oneself, fears without real causes, sleep disturbances, self-prosecution, and feeling of lucidity. In the families of patients with mitochondrial pathology, accompanied by depressive symptoms, relatives on the mother's side, in contrast to the father's side, were found to have a higher incidence of psychosomatic symptoms and depression.

The time course of a depressed state was evaluated during therapy and psychotherapeutic interventions, psychoeducation and was characterized by positive changes.

Pharmacotherapy and psychotherapy were differentiated and depended on clinically psychopathological manifestations of depressive disorders, their severity and general condition of patients. The complex of psychotherapeutic methods included cognitive-behavioral, family psychotherapy, cognitive training and training to solve interpersonal problems. Psychoeducational assistance was provided to the patients and their families.

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