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THE REMODELING OF LEFT ATRIUM AND VASODILATION FACTORS CHANGES IN ISCHEMIC HEART FAILURE

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Background. *According to the national registries of European countries and epidemiological studies, the prevalence of chronic heart failure (CHF) among adults is 2,0–5,0%, and increases due to age, in people aged over 70 years old it is 10,0–20,0%.*

Objective. *To find out the specific features of remodeling of the left atrium and change of vasodilation factors in ischemic heart failure with reduced and preserved left ventricular ejection fraction and to establish correlation relationships.*

Methods. *A full clinical examination of 153 patients with CHF (105 men and 48 women) was conducted to achieve this objective. The surveyed patients underwent clinical examinations; spectrophotometric parameters: quantification of markers of vasodilation, metabolites of monoxide nitrogen — nitrates and nitrites with Gris reagent; content of endothelial nitric oxide synthase (eNOS) in serum — ELISA for the set of Nitric Oxide Synthase 3, Endothelial (NOS3) Human ELISA Kit (Cloud-Clone Corp, USA). Electrocardiographic (ECG) examination was conducted in 12 standard conventional leads on electrocardiograph by the Hungarian production Heart Screen 112 D.*

Results. *The 1st group of the examined patients with reduced LV EF prevails III (significant) degree LA dilatation in 33 (70.21%) cases, II (moderate) degree of LA dilatation was determined in 14 (29.78%), and I (initial) degree was not defined at all. In the 2nd group of the patients with preserved LV EF mainly the II degree of LA dilatation was determined in 44 (44.51%) cases, and decreased LA dilation in 39 (36.79%) cases ($p<0.01$), and III degree of LA dilation was defined in 23 (21.69%) cases ($p<0.01$). In patients with stable coronary heart disease, complicated by heart failure with reduced LV EF and II degree of LA dilatation, eNOS levels in the serum was 449.00±39.91 pg/ml, whereas in patients with stable coronary heart disease, complicated by heart failure with preserved LV EF and II stage of LA dilatation — 673.56±50.98 pg/ml ($p<0.01$). At III stage of LA dilatation in patients of the 1st group level eNOS was 344.20±51.98 pg/ml in the patients of the 2nd group — 616.90±36.49 pg/ml ($p<0.01$). At the same degree and with LA dilation in the patients of the 2nd group eNOS was 750.27±99.85 pg/ml.*

Conclusions. *The structural and functional changes of the left atrium and changing factors of vasodilation in patients with stable coronary artery disease of II-III functional classes complicated by heart failure of I–III functional classes are studied. It is established that in the examined patients with stable coronary heart disease complicated by heart failure with reduced left ventricular ejection fraction mainly III (significant) degree of dilatation of the left atrium was determined, while in patients with stable coronary heart disease, complicated by heart failure with preserved left ventricular ejection fraction mainly II (moderate) degree of dilation of the left atrium was determined. In comparison with the results of research among the patients with stable coronary heart disease, complicated by heart failure with reduced left ventricular ejection fraction, and a group of patients with preserved left ventricular ejection fraction, it was determined a significant decrease in eNOS, nitrites, total amount of nitrites and nitrates.*

KEY WORDS: **heart remodeling factors of vasodilation, heart failure.**

Introduction

According to the national registries of European countries and epidemiological studies, prevalence of chronic heart failure (CHF) among adults is 2.0–5.0%, and increases in proportion to age, people aged over 70 years it is from 10.0 to 20.0% [16]. Considering demographic trends, in Ukraine as well, to increase the number of population of older age groups, the provision of medical care to patients with CHF is becoming a topical issue [2, 14].

Distinguish systolic and diastolic options of CHF. In version of systolic CHF the presence of clinical signs in patients with ejection fraction (EF) of LV≤45.0%, at diastolic version with LV EF>45.0% is observed [4]. It is known that left ventricular hypertrophy (LVH) precedes the development of CHF and is formed together

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with or even is caused by this disease. Analysis of prognostic value of LVH in patients with CHF with preserved ejection fraction proves that the high risk of lethal outcome is observed in patients with left ventricular myocardial mass that exceeds 400 g [3]. It is established that at the progression of diastolic dysfunction an increased risk of sudden cardiac death in 80.0% occurs [4, 15]. Endothelial dysfunction is very important in development of CHF. The cause of nitric oxide (NO) synthesis inhibition by endothelium as a major component of its dysfunction at CHF is free radical stress [11]. The main cause of CHF is negative impact of free radical factors that leads to lower ventricular myocardial contractile function followed by entire body hypoxia, endothelial dysfunction with the appropriate activation of cellular factors [17]. These processes can lead to increasing of blood coagulation capacity, which is one of the causes of thrombotic complications in patients with CHF [12]. Biosynthesis of NO from L-arginine occurs in three major isoforms of NO-synthase, two constitutive: neuronal (nNOS) and endothelial (eNOS), and inducible one (iNOS) [6].

The objective of the study is to find out the specific features of left atrium remodeling and vasodilation factors changes in case of ischemic heart failure with reduced and preserved left ventricular ejection fraction and to establish correlation relationships.

Material and Methods

To achieve this objective a full clinical examination of 153 patients with CHF (105 men and 48 women) was conducted; they were treated in the Department of Cardiology for patients with arrhythmias of Vinnitsa Regional Centre of Cardiovascular Disorders (Ukraine). All patients were divided into 2 groups: group 1 consisted of patients with stable coronary heart disease complicated by heart failure with reduced LV EF (n=47); group 2 — patients with stable coronary heart disease complicated by heart failure with preserved LV EF (n=106). The control group consisted of 30 healthy individuals. The diagnoses of CH and FC was made due to clinical, laboratory and instrumental criteria, as recommended by the European Society of Cardiology (2012) and the Association of Cardiologists of Ukraine (2012) [16]. Diagnosis and treatment of clinical forms of coronary artery disease were made by order of the Ministry of Health of Ukraine № 152 from 03.02.2016 [9]; functional class (FC) CH — by NYHA classification.

The criteria for inclusion. Patients with stable coronary artery disease II-III FC followed by CHF II-III FC on informed consent to participate in the study. *Exclusion criteria*: CHF FC IV by the NYHA; period up to 3 months from the beginning of acute myocardial infarction, or stroke; implanted artificial pacemaker or need of implantation; severe diseases of respiratory system, kidneys, liver with hepatic insufficiency, anaemic state of haemoglobin 90,0 g/dL and below; the formation of malignant disorders and severe neuro-psychiatric disorders.

Among the patients who participated in the study mail prevailed. The average age of patients was 68.80±0.90 years. Hypertension was defined in 132 (86.27%) patients. Systolic blood pressure (BP) was 142.40±5.01 mm Hg, diastolic blood pressure — 88.30±2.95 mmHg

The examined patients underwent clinical (complaints, case history, physical examination); laboratory tests (general clinical blood and urine tests, blood glucose, bilirubin, cholesterol, β-lipoprotein, creatinine, urea, coagulation parameters); spectrophotometric parameters: quantification of vasodilation markers metabolites of monoxide nitrogen: nitrates and nitrites with Gris reagent; content of endothelial nitric oxide synthase (eNOS) in serum — ELISA for the set of Nitric Oxide Synthase 3, Endothelial (NOS3) Human ELISA Kit (Cloud-Clone Corp, USA). Electrocardiographic (ECG) examination was conducted in 12 standard conventional leads on electrocardiograph Heart Screen 112 D made in Hungary. Statistical analysis was performed with standard statistical package STATISTICA 6.0. For primary analysis of tables and intermediate calculations Microsoft Excel package was used.

Results and Discussion

The decrease in left atrium (LA) dilation degree in patients with stable coronary heart disease complicated by heart failure with reduced and preserved LV EF is presented in Table 1.

In Table 1 the 1st group of the examined patients with reduced LV EF prevails III (significant) degree of LA dilatation in 33 (70.21%) cases, II (moderate) degree of LA dilatation in 14 (29.78%), I (initial) degree is not defined at all. In the 2nd group of patients with preserved LV EF the degree of dilatation II LA was determined in 44 (44.51%) cases, and slightly decreased degree of LA dilation in 39 (36.79%) cases ($p<0,01$), III degree of LA dilation in 23 (21,69%) cases ($p<0,01$).

Table 1. Remodeling of left atrium in case of stable coronary heart disease complicated by heart failure with reduced and preserved left ventricular ejection fraction

The degree o f dilation LA	Patients with heart failure with reduced LV EF group 1, n=47	Patients with heart failure with preserved LV EF group 2, n=106	p
I (initial) LA from 40.0 to 45.0 mm	0	39 (36.79 %)	p<0,01
II (moderate) LA from 45.1 mm to 50.0 mm	14 (29.78%)	44 (41.51%)*	p<0,01
III (significant) LA from 50.1 mm and more	33 (70.21%)*	23 (21.69%)	p<0,01

Notes: LA — the size of the left atrium; * p<0.01 — reliable change index.

Remodeling of myocardium, including hypertrophy and dilation of left ventricle with a changes of its geometry and a contraction violation precedes clinical skim CHF manifestations [7]. Thus, remodeling is a harbinger of heard activity decompensation [7, 13]. The features of myocardial remodeling are studied the most in patients after myocardial infarction with heart failure development [7, 8, 14].

These results are consistent with the data that myocardial ischemia may be accompanied by its dysfunction [7].

The study of factors of vasodilation levels and degrees of LA dilatation in patients with stable coronary heart disease complicated by heart failure with reduced and preserved LV EF is presented in Table 2.

In patients with stable coronary heart disease complicated by heart failure with reduced LV EF and II degree of LA dilatation, eNOS levels in the serum is 449.00±39.91 pg/ml, whereas in patients with stable coronary heart disease complicated by heart failure with preserved LV EF and II stage of LA dilatation — 673.56±50.98 pg/ml (p<0.01). At III stage of LA dilatation in patients of group 1 the eNOS level is 344.20±51.98 pg/ml in patients of groups 2 — 616.90±36.49 pg/ml (p<0,01). At the same degree and with LA dilation in patients of group 2 eNOS is 750.27±99.85 pg/ml. Thus, in the examined patients with the progression of heart failure the endothelial factors decrease, e.g. eNOS.

In the study of levels of metabolites of nitrogen monoxide — nitrites and nitrates in blood serum the following data were obtained: in patients with stable coronary heart disease, complicated by heart failure with reduced LV EF and II and III stage of LA dilatation, the total level of nitrates and nitrites in serum was significantly lower than in patients with stable coronary heart disease, complicated by heart failure with preserved LVEF and II and III stage of LA dilatation (p<0.01).

Among the patients of group 1with reduced LV EF with the progression of LA dilation degree from II (moderate) stage to III (large) one, eNOS levels in blood serum decreased by 23.34%.

Table 2. Vazodilating factors at different stages of left atrium dilation in case of stable coronary heart disease complicated by heart failure with reduced and preserved left ventricular ejection fraction (M±m)

Indices	Patients with heart failure with reduced LV EF group 1, n=47		Patients with heart failure with preserved LV EF group 2, n=106		
	II degree of LA dilatation n=14	III degree of LA dilatation n=33	I degree of LA dilation n=39	II degree of LA dilatation n=44	III degree of LA dilatation n=23
eNOS, pg/ml	449.00±39.91	344.20±51.98	750.27±99.85	673.56±50.98*	616.90±36.49#
Nitrite+nitrate, mmol/l	16.75±0.75	16.05±0.94	19.27±0.67	19.22±0.77*	19.05±1.10#
Nitrite, mmol/l	4.41±0.21	3.95±0.32	5.15±0.20	5.06±0.16*	5.30±0.37#
Nitrate, mmol/l	12.34±0.59	12.09±1.13	14.11±0.61	14.16±0.71*	13.74±0.85

Notes:
* — comparison of patients with stable coronary heart disease complicated by heart failure with reduced LV EF and preserved LV EF and II degree of left atrium dilatation (p<0,01);
# — comparison of patients with stable coronary heart disease complicated by heart failure with reduced LV EF and preserved LV EF and III degree of left atrium dilatation (p<0,01).

Among the patients of group 2 with preserved LV EF with the progression of LA dilatation from I (initial) stage to II one, the level of eNOS in blood serum decreased by 12.89%, and with the progression of the II stage to III one LA dilation reduced by 8.41%.

In the examined patients with stable coronary artery disease complicated by heart failure with reduced left ventricular ejection fraction, the correlation analysis between indicators of linear dimensions of LA and eNOS levels in the blood serum a direct correlation of medium strength (r=0.38) (p<0,01) was established. Carrying out a similar correlation analysis for patients with stable coronary heart disease complicated by heart failure with preserved LV EF also allowed to establish a direct correlation of weak force (r=0.21) (p<0.01).

Progressive hypertrophy and dilatation of heart was followed by subsequent first diastolic disruption and then systolic ventricular dysfunction, increased myocardial oxygen demand, changes in subendocardial blood flow, myocardial bioenergy disorders and increased risk of life-threatening ventricular arrhythmias [1].

Recent research proved instrumental and morphological parallels, so we expand on the remodeling of heart as a single clinical and morphological syndrome that is important for making prognosis in patients with coronary heart disease [18].

The processes of LA remodeling are defined by the degree of new myocardial damage and the presence of viable myocardium.

Conclusions

In patients with stable coronary heart disease complicated by heart failure with reduced left ventricular ejection fraction III (significant) degree of left atrium dilatation was mainly determined, while in patients with stable coronary heart disease complicated by heart failure with preserved left ventricular ejection fraction II (moderate) degree of left atrium dilation was mainly determined.

In the patients with stable coronary heart disease complicated by heart failure with reduced left ventricular ejection fraction in comparison with the group of patients with preserved left ventricular ejection fraction, the significant reduction of eNOS, nitrites, total of nitrites and nitrates was defined during the study.

The research allowed to establish the direct correlation of medium strength (r=0.38) between parameters of linear left atrial size and eNOS levels in blood serum of the patients with stable coronary artery disease complicated by heart failure with reduced left ventricular ejection fraction (p<0.01) and the direct correlation of weak force (r=0.21) in the examined patients with stable coronary artery disease complicated by heart failure with preserved left ventricular ejection fraction (p<0,01).

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VITAMIN D AND DIABETES MELLITUS TYPE 2

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Background. *As it is established that diabetes mellitus causes metabolic disturbances of all types (first of all because of carbohydrate metabolism), affection of blood vessels, nerves, different organs and tissues. However, the influence of DM on the level of microelements is still not investigated enough. Despite the sufficient variety of medicaments, compensation of diabetes mellitus is achieved in less than 30% of patients. For this reason, the search of new ways of treatment which are aimed at the improvement of carbohydrate metabolism is an urgent issue.*

Objective. *The research was aimed to reveal the* deficiency of 25-hydroxyvitamin D3 [25(OH)D3] and its *correlation with carbohydrate metabolism.*

Methods. Thirty five patients with diabetes mellitus Type 2 aged 55–74 with illness duration *2–4 years* were examined. *The control group included 35 healthy people of the same age and sex. Levels of 25-hydroxyvitamin D3 [25(OH)D3] were tested by means of radioimmunoassay. The level of glycated haemoglobin was tested by means of liquid chromatography.*

Results. *Correlation of the level of vitamin D with the degree of diabetes mellitus Type 2 compensation has been detected.*

Conclusions. *A clear tendency to the improvement of diabetes mellitus Type 2 compensation after medication correction with vitamin D was evidenced.*

KEY WORDS: **diabetes mellitus, vitamin D, glycated haemoglobin, glycemic control.**

Introduction

Diabetes mellitus (DM) — is a non-infectious epidemic of XXI century. According to World statistics data, the amount of people with DM doubles every 13-15 years. Today there are 415 million patients with DM in the world, and their amount will increase to 642 million in 2040. New drugs do not contribute to the disease suppression, and the intensification of diagnostic criteria causes rapid increase in the number of patients. Mainly the increase of patients mainly with DM type 2.

Today, in Ukraine the DM compensation is unsatisfactory because the average rate of glycated haemoglobin (HbA1c) within DM type 1 is about 9.0%, and within DM type 2 – about 8.5% [2]. In our day, it is ultimately important to search for pathogenetic links for efficiency of patients' with DM treatment improvement.

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So, much attention is paid to the study of vitamin D influence on different organs and systems in case of different diseases, DM as well. Recently scientists began to study the influence of vitamin D on the course of DM type 2 [4,5,6,7]. Some authors suggest, that vitamin D insufficiency is a risk factor of DM type 2 [6], other — that vitamin D supports glucose homeostasis and is inversely proportional to the level of glycated haemoglobin in DM type 2 [8]. Also there is a suggestion, that an adequate vitamin D intake may be connected with lower risk of a gestational diabetes mellitus development [9].

Physiological effects of vitamin D is polyhedral, it is difficult to select some less significant factors. Vitamin D influences the phosphorus-calcium exchange: calcium and other microelements cannot be absorbed in its deficiency. Activity of vitamin D is directly connected with many physiological processes in organism, in particular with vitamin E admission, liver function and function of intestine, lipid metabolism. Vitamin D is necessary for providing

normal functioning of immune system, reproductive and sexual health, and hematopoietic system. That is why, cholecalciferol and its active metabolites is applied for treatment of immunodeficiency, anaemia, various pathological conditions of liver, gastrointestinal tract, diabetes, cardiovascular system, tuberculosis, cancers of breast and bowel, prostate, locally for the treatment of psoriasis.

According to statistical data [3], among all residents of Ukraine, only 4.6% of the examined people had normal level of vitamin D, 13.6% of the population was diagnosed with its failure, and in 81.8% of residents the deficit was pronounced.

However, how the level of vitamin D can change, and whether its change can influence on indicators of compensation for diabetes remains poorly examined.

According to the literature [10], objective criterion of vitamin D security, is the determination of 25-hydroxy vitamin D3 [25 (OH) D3] contents.

The study is **aimed** to reveal the deficiency of 25-hydroxy vitamin D3 and its cross-correlation with glycated haemoglobin of patients with diabetes type 2.

Material and Methods

35 patients with diabetes mellitus type 2 were examined, the individuals were aged from 55 to 74, illness duration was 2–4 years. The control group included 35 healthy people of the same age and sex. The patients, who suffered from DM type 2, took oral hypoglycaemic drugs of biguanides in therapeutic doses (1000-2500 mg daily). Additionally patients of both group received the drug Alpha D3 Teva, dose 1 mkg daily during 3 month. Levels of 25-hydroxyvitamin D3 [25(OH)D3] were tested by means of radioimmunoassay. The level of glycated haemoglobin (HbA1c) was tested by means of liquid chromatography.

The results of 25(OH)D3 and HbA1c were determined before the start of Vitamin D taking and after 3 months of treatment with it.

Statistical evaluation of results was realized by methods of variation statistics with calculation of frequency performance indicators (P) and averages (the arithmetic mean X). Statistical significance of the result if compared the patient groups with the control one was evaluated using Student t-test. Regression analysis was used to determine the relationship between HbA1c levels and 25(OH)D3.

Results

After examination of patients with DM type 2, all of them were identified either with the deficiency or insufficiency of vitamin D.

In accordance to classification (Holick MF et al) at the level of 25(OH)D3 50 nmol/L (20 ng/ml) to 75 nmol/L (30 ng/ml) D-vitamin failure is diagnosed, and decreased level of 25(OH)D3 below 50 nmol/L (20 ng/ml) — vitamin D3 deficiency.

We have found out that among 35 patients with DM type 2 nobody has normal level of vitamin D. 62.9% of patients were diagnosed with the deficiency of vitamin D ($p<0.01$), moreover in 22.7% of them good compensation was observed ($p<0.01$), in 31.8 % of the patients compensation was satisfactory ($p<0.01$), and in 45.5% of the patients — unsatisfactory ($p<0.01$). Inverse correlation connection between the decrease level of vitamin D and increase level of glycated haemoglobin was detected ($r=-0.40$, $p<0.05$). We considered that compensation of DM was good when the level of glycated haemoglobin was <7.5%, satisfactory — at the level of glycated haemoglobin 7.5-8.5%, unsatisfactory — when the level of glycated haemoglobin was >8.5%. We justified the increased rate of glycated haemoglobin compensation because we examined patients of older age who were recommended to maintain higher indicators of $HbA1_c$ to avoid hypoglycaemia and brain hypoxia.

37,1% of the patients with DM type 2 ($p<0,01$) suffered from vitamin D failure, moreover 61.5% of them had satisfactory compensation of diabetes (the level of glycated hemoglobin was 7.5-8.5%) ($p<0,01$), and 38.5% – unsatisfactory (the level of glycated haemoglobin > 8.5%) ($p<0,01$). Inverse correlation between the decrease of vitamin D and increase of glycated haemoglobin was detected ($r=-0.39$, $p<0.05$).

In the control group (without diabetes mellitus) there were only 25.7% of patients with a good level of vitamin D ($p<0.01$), 54.2% of people in the same group suffered from vitamin D insufficiency ($p<0.01$), and 20.1% of people were diagnosed with vitamin D deficiency ($p<0.01$).

The low implementation of vitamin D for the older patients without diabetes may be associated with alimentary factor and age features of the body.

In the control groups the level of glycated haemoglobin was 5.1±0.05% ($p<0.01$).

The patients of both groups (except those with no deficiency) were provided with a drug Alpha D3 Teva – 1 mkg daily for three month to correct vitamin deficiency. After that, the levels of 25(OH)D3 and HbA1c were tested again.

The improvement of diabetes mellitus compensation is clearly stated for patients: decrease the number of patients with unsatisfactory compensation and increase of number of patients with good and satisfactory compensation. 51.4% ($p<0.01$) of the patients were diagnosed with optimal levels of vitamin D and 11.5% ($p<0.01$) had its failure. Inverse correlation connection between the increased level of vitamin D and decrease level of glycated haemoglobin was detected ($r=-0.38$, $p<0.05$).

We have established that a number of the patients with unsatisfactory compensation decreased, a number of the patients with satisfactory compensation increased, and

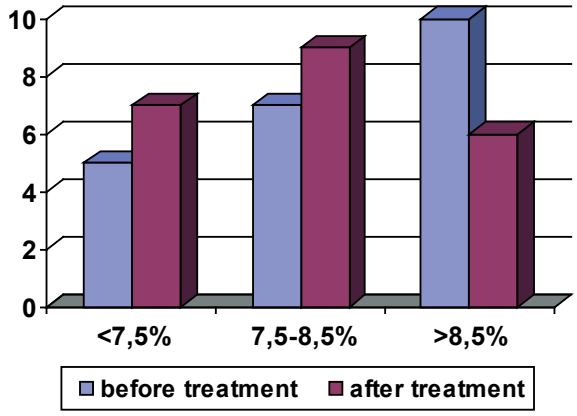

Figure 1. The levels of glycated haemoglobin of the patients with diabetes mellitus type 2, who were diagnosed with Vitamin D deficiency (before and after treatment with Alpha D3 Teva).

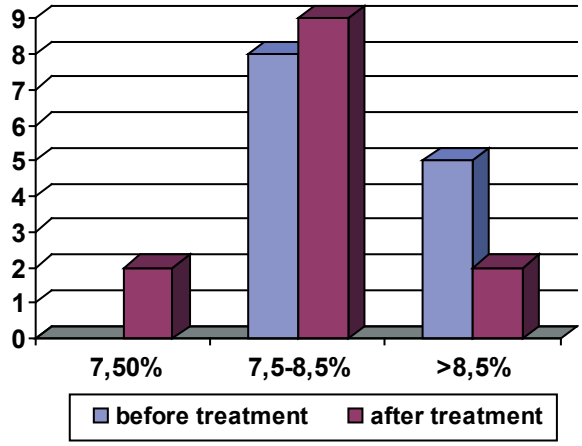

Figure 2. The levels of glycated haemoglobin of the patients with diabetes mellitus type 2, who were diagnosed with vitamin D failure (before and after treatment with Alpha D3 Teva).

patients with high compensation of diabetes was in evidence. After treatment with Alpha D3 Teva, 34.2% out of 37.1% of the patients with vitamin D failure had normal vitamin D level ($p<0.01$), and 2.9% still had its insufficiency ($p<0.01$). Inverse correlation between the increased level of vitamin D and decreased level of glycated haemoglobin has been detected ($r=-0.39$, $p<0.05$).

In the control group, after three months of treatment with Alpha D3 Teva, 74.2% of the patients had normal vitamin D level ($p<0.01$), 20% had its failure ($p<0.01$), 5.8% were diagnosed with the of vitamin D deficiency ($p<0.01$).

Discussion

Many authors consider the matter of vitamin D deficiency, which is a predictor of various diseases and also promotes decompensation of chronic diseases [11]. It is a topical issue in whole world and in Ukraine as well [12]. So, its deficiency correction should not seem less important. The drugs of cholecalciferoli are applied the most for its treatment [13], which, according to research literature, are first-line drugs that include ergocalciferol and structural analogue of vitamin D3-dyhidrotahisterol. The second-line drugs are calcitriol and alfacalcidol. As first-line drugs are exposed to 25-hydroxylation in liver, afterwards are converted into active metabolites in kidneys, therefore, the metabolism of these drugs is decreased in elderly patients with different types of primary and secondary osteoporosis and then don't have positive effect on bone system.

These defects are absent in the second-line drugs [14]. We suggest the drug alphacalcidol for vitamin D deficiency correction, because in our research we examined elderly patients.

Also, many patients suffer from osteoporosis; in such case this drug is more efficient.

The efficiency of alphacalcidol (1 mkg per day) compared to the combination of vitamin D (880 MO per day) and calcium carbonate (1000 mg per day) for patients with postmenopausal osteoporosis and absence of vitamin D deficiency in blood plasma is presented in research literature.

In 12 months in the females, who took alfacalcidol, mineral density of lumbar bone was increased by 2.33% (from base level) and in 18 months by 2.87% ($p<0.001$), and in the group of patients, who received vitamin D and Ca — only by 0.7% [15].

There are various conclusions on calcitriol efficacy. Some researches proved significant

effect of vitamin D on mineral density of bone for patients with postmenopausal osteoporosis [16, 17], others stated that such outcome was not obtained that may be associated with low doses [18].

Thus, we can argue that the drug alphacalcidol can be recommended for patients with vitamin D deficiency, diabetes type 2 and age-related osteoporosis.

Conclusions

A clear tendency to the improvement of diabetes mellitus type 2 compensation after medication correction with vitamin D was evidenced.

So, by correcting vitamin D deficiency the compensation of diabetes is improved.

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NAIL CHANGES IN CASE OF ENTEROVIRAL INFECTIONS IN WESTERN UKRAINE: A REPORT OF 34 CASES

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Background. *Enteroviral infections are common infections, mostly affect children. Nail changes of enteroviral infection including transverse ridging (Beau's lines) and nail shedding (onychomadesis) were described in many countries worldwide.*

Objective. *The aim of the research was to investigate the clinical features of the infection that was followed by nail changes during its outbreak in summer–autumn 2016 in Ternopil region, Ukraine.*

Methods. *A case report of 34 children with nail changes that were observed in October 2016 in Ternopil region, Ukraine is presented. All patients were from one kindergarten. Nail trauma, periungual dermatitis, significant medication intake history, systemic diseases were excluded. The survey and clinical examination of cases was performed. Faecal samples were obtained from 13 children with onychomadesis.*

Results. *The median age of the exemined children was 3.97±0.78 years. Interval between onsets of enteroviral infection to nail changes varied from 4 to 12 weeks. Clinical signs that preceded nail changes are fever (64.7%), herpangina (32.6%), pharyngitis (17.6%), gastrointestinal symptoms (44.1%), cutaneous lesions (82.4%) as maculopapular, vesicular rash (44.2%) or/and skin desquamation (41.2%). These clinical data indicate preceded enterovirus infection. In 21 (61.8%) patients hand-foot-mouth disease was suggested.*

Nail changes were presented by Beau's lines and onychomadesis (nail shedding). The number of affected nails varied from 1 to 16, the median number was 4.88±4.09.

Conclusions. *Our study proved association between the outbreak of onychomadesis (nail shedding) and enteroviral infection, mainly hand-foot-mouth disease, during summer–autumn 2016 in Western Ukraine (Ternopil region). Enteroviral infection was followed by onychomadesis in 4–12 weeks. Clinical features of enteroviral infection were very variable, with prevalence of cutaneous lesions.*

More studies are necessary to determine the serotype of the virus that causes onychomadesis.

KEY WORDS: **enteroviral infections, hand-foot-mouth disease, onychomadesis, children, Ukraine.**

Introduction

Enteroviral infections are commonly encountered infections, caused by group of RNA viruses, especially affect infants and children. Enteroviruses cause more than half of all infectious diseases in children, are accompanied by fever [1–2]. Clinical features of enterovirus infection are very variable; from mild benign fever to life-threatening encephalitis, myocarditis, or sepsis [1].

The enteroviral group includes coxsackievirus, echovirus, and poliovirus. Enteroviral infections may be caused by 23 coxsackievirus A, 6 coxsackievirus B, 28 echovirus, and 5 unclassified enteroviruses.

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Hand-foot-mouth disease (HFMD) is one of the forms of enteroviral infections. HFMD is characterized by sores in mouth (vesicular, erosive stomatitis) or on mouth and on hands, feet and sometimes buttocks and legs (maculopapular, vesicular lesions). The most common cause of HFMD is Coxsackievirus A6, A10, A16 and enterovirus 71 [2, 3]. First cases of nail changes after HFMD were reported in 2000 in 5 children in Chicago, USA [4]. In Europe first four cases of nail changes following HFMD were described in 2001 [5].

Nail changes of enteroviral infection are described as nail matrix arrest, including transverse ridging (Beau's lines) and nail shedding (onychomadesis) [4, 6]. Short-term slowing down of nail formation leads to Beau's lines, while long-term stop of nail growth causes onychomadesis [7].

The aim of the research was to investigate the clinical features of the infection that was associated with nail changes during an outbreak in summer-autumn 2016 in Ternopil region, Ukraine.

Material and Methods

We present a case report of 34 children with nail changes that were observed in October 2016 in Ternopil region, Ukraine. All patients were from one kindergarten.

Nail trauma, periungual dermatitis, significant medication intake history, systemic diseases were excluded.

The study of cases was performed. It included a questionnaire containing the following parameters: age, gender, personal and family history of the children. A questionnaire was completed by parents.

The patients were clinically examined, and their paediatric and dermatological records were studied to confirm precedent enteroviral infection. Direct microscopic examination and cultures for fungi were performed in the first 3 cases.

Faecal samples were obtained from 16 children with onychomadesis for enterovirus testing.

Parents of all patients provided written informed consent before any study-related procedure. The study conformed to the principles outlined in the declaration of Helsinki.

The results have been analysed using standard procedures with Statistica StatSoft 6.0 software package. Values are expressed as mean ± standard deviation (SD) for continuous variables and n (percentages) for categorical variables.

Results

The median age of the exemined children was 3.97±0.78 years (range, 2–5 years). 19 (55.9%) were male.

All patients were presented with nail changes.

Due to the history of present illnesses it was revealed that interval between onsets of enteroviral infection to nail changes varied from 4 to 12 weeks. Clinical features of enteroviral infection were very variable (Table 1).

Table 1. Clinical features of patients with nail changes after enteroviral infection

Clinical features	n	%
Fever	22	64.7
Respiratory symptoms	16	47.1
herpangina	11	32.6
pharyngitis	6	17.6
Gastrointestinal symptoms	15	44.1
Cutaneous lesions	28	82.4
maculopapular, vesicular rash	15	44.1
skin desquamation	14	41.2
Conjunctivitis	2	5.9

The average duration of fever was 1.87±0.92 days. Maximum temperature varied from 37.6 to 40.0 °C. Gastrointestinal symptoms were presented by nausea, vomiting, diarrhea without blood and mucus. In 7 (20.6%) cases maculopapular, vesicular rash was on hands, feet and around mouth. Skin rash was followed by skin desquamation in 3 (8.8%) patients in 2-6 weeks. In other cases skin desquamation occurred in children without history of skin rash. Skin desquamation was both on the palms and soles in 6 (17.6%) cases, only on palms in 3 (8.8%) and only on soles of feet in 5 (14.7%) cases.

The first manifestation of the disease occurred in the middle of summer (July — 8/23.5% cases), in August (10/29.4% cases) and in autumn (September — 13/38.2% cases). In 3 cases parents did not indicate previous problems or any symptoms of enterovirus infection, so we can suggest asymptomatic duration of the infection.

Faecal samples from onychomadesis patients were positive for enterovirus in one case.

Nail changes were presented by Beau's lines and onychomadesis (nail shedding) (Figure 1–4).

The number of the affected nails varied from 1 to 16. The mean number of the affected nails was 4.88±4.09. Fingernails were involved more often than toenails (3.79±2.61, range 1 to 10; 3.31±2.20, range 1 to 9, respectively). In two children we observed changes of toenails colour (Figure 5).

Fig. 1. Beau's lines on the right fingernails after enteroviral infection.

Fig. 2. Different stages of fingernail shedding (onychomadesis) in a 4-year-old child after enteroviral infection.

Fig. 3. Onychomadesis on both great fingernails after enteroviral infection.

Fig. 4. Beau's lines and onychomadesis on both great toenails after enteroviral infection.

Fig. 5. The deformation of toenail bed, toenail plate thickening and change of its colour.

Discussion

Since 2000 a lot of enteroviral infection outbreaks associated with nail matrix arrest have been reported throughout the world [8–12]. Hand-foot-mouth disease was mostly followed by nail changes. However, in Ukraine such cases have been not yet described.

Formerly nail changes were associated with nail trauma, periungual dermatitis, significant medication intake history, autoimmune disorders, etc. Now onychomadesis are described as late complication of enteroviral infection [6, 8].

Onychomadesis are caused by a temporary arrest in nail plate formation [2, 8], but the enteroviruses influence on this process remains unclear today.

Frequently onychomadesis was associated with coxsackievirus (C) A6 serotype of enterovirus [10, 11]. However identification of other enteroviral strains (CA10, CA16 and enterovirus 71) was reported too [9, 10, 12]. Enterovirus serotypes CA10 and coxsackievirus B1 were mainly detected as a monoinfection or co-infection in Valencia, Spain, where an onychomadesis outbreak occurred [12].

Osterback R. et al. from Finland [9] detected CVA6 also in a fragment of shed nail. They suggest that virus replication damages nail matrix and results in temporary nail dystrophy.

The study from Taiwan proves that in 51% CA6 patients desquamation of palms and soles occurred after the infection episode and in 37% CA6 patients onychomadesis developed, which only occurred in 7 (5%) of 145 cases with non-CA6 enterovirus infection [11]. The authors confirmed association of CA6 infection with developing nail abnormalities (p<0.001).

Unfortunately, we were unable to determine the serotype of the virus in our cases. Enterivirus was identified only in one case (6.3%), probably because of a long period from the first symptoms of the disease. Study in Spain [12] reported about 59% positive samples collected from 29 onychomadesis case-patients (23 with HFMD) and 9 exposed persons (1 with HFMD).

In our study typical preceding clinical signs indicated enteroviral infection. In the most cases (21/61.8%) cutaneous lesions (maculopapular, vesicular rash and skin desquamation) were located on hands, feet and around the mouth, which indicated HFMD.

Enteroviral diseases in countries with temperate climate are characterized by summer-autumn seasonality. Unlike, in tropical countries, where the virus is circulating throughout the year seasonal outbreaks are not typical. The first manifestation of the disease in our cases began in the middle of summer (July) and lasted till autumn (September). An outbreak of HFMD with onychomadesis was reported in Finland in 2008 starting in August and continuing at least until the end of the year [10]. In Valencia, Spain HFMD, followed by onychomadesis occurred during summer and autumn 2008 [12]. But in northern Greece, in the region of Thessaloniki an onychomadesis outbreak related to the outbreak of HFMD occurred during autumn-winter 2012–2013 [6].

Conclusions

Our study proved association between onychomadesis (nail shedding) outbreak and outbreak of enteroviral infection, mainly hand-foot-mouth disease, during summer-autumn 2016 in Western Ukraine (Ternopil region). Enteroviral infection was followed by onychomadesis in 4–12 weeks. Clinical features of enteroviral infection were very variable, with prevalence of cutaneous lesions.

More studies are necessary to determine the serotype of the virus causing onychomadesis.

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EMBOSSED AUTODERMOTRANSPLANTS: THEORETICAL SUBSTANTIATION AND PRACTICAL USAGE

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Background. *It is established that granulation wound has uneven bumpy surface and modern electric dermatomes cut skin with a smooth internal surface. Therefore there is no tightly contact between the smooth surface of a graft and granulation tissue.*

Objective. *The method of harvesting by split-skin grafts with variable thickness (embossed) is proposed.*

Methods. *An embossed skin graft with sinusoidal surface of inner side was obtained by modified dermatome with special technological plate. The embossed autografting of skin was applied in 16 patients (21–60 years old) with deep burns of 7–9% of body. For clinical comparison the part of granulating wounds in each patient was covered with embossed transplants and the other part with smooth split-thickness grafts.*

Results. *During ultrasound Doppler examination of grafts on the fifth postop day the active blood flow in embossed transplants with 4–6 blood outbreaks and only 2–3 flashes in smooth graft were found. Clinically the embossed transplants were engraftment on 6–7th day. On 18–21st day the roughness of grafts surface disappeared. In two months the graft together with subcutaneous fatty tissue was taken in crease. Donor wound: on 14–15th day its surface was like "slate" but on 25–30th day the undulation of the re-epithelisation skin disappeared.*

Conclusions. *The improvement of dermatome design with technological plate allows cutting scalloped split-skin grafts of varied thickness (embossed).*

Split-thickness embossed grafts combine anatomic and physiological features of skin cover granulation wound to adhere tightly because of the enlargement of area of their internal surface without retraction and/or secondary shrinkage.

Donor wounds, after the harvesting of embossed skin grafts, contain the morphological elements of dermis which contribute to their complete spontaneous healing.

KEY WORDS: **embossed split-thickness skin graft, granulating wound.**

Introduction

Skin transplantation is a method of choice in the treatment of patients with deep burns. In order to do this procedure, split-thickness skin grafts are harvested from donor areas. The donor wound regrows epithelium within two weeks [1–3, 5]. Sometimes there are complications with transplant skin engraftment to a granulating wound, sometimes areas of donor wounds suppurate [4, 6, 7, 11].

Important aspect of skin graft healing is wound contraction that may present serious functional and cosmetic concerns, depending on the location and severity. Myofibroblasts may cause contraction. The ability of a skin graft to resist contraction is related to the thickness of deep dermal component included in the graft, not just the absolute thickness of the graft. This deep dermal component is able to suppress myofibroblast function. The severity of wound contraction from the least to the most is as follows: full-thickness skin graft cause least contraction, then thick and thin split-thickness skin graft, and finally the open wound has the most contraction [14].

Modern electric dermatomes cut skin with a smooth internal surface, in the meantime granulation wound has uneven bumpy surface [1, 12]. In certain cases there is no close contact between the smooth surface of a skin graft and granulation tissue; there are gaps and voids, where discharge from the wound collects, colonies of microorganisms form, which may cause hematomas or seromas and local inflammatory complications [4, 8, 9, 11].

Thus, for successful healing by a skin graft without contraction of the wound, it is necessary to have the surface adapted to the granulating

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wound surface and contained with the elements of dermis.

The question we are aiming to address is to find out if it is possible to cut skin graft with an uneven embossed surface, similar to the relief of granulations.

The study is **aimed** to introduce a method of harvesting the split-skin grafts of variable thickness.

Material and Methods

Theoretical consideration. The upper boundary of granulation tissue on the tentative vertical cut of the wound is a sinusoid with smaller or larger range, depending on which granulations (small-, medium-, or large-grained) fill the wound. Theoretically these granulations should be covered with analogic sinusoidal surface (Fig. 1). Obviously, the larger is the surface of granulations contact with the graft, the faster graft retention will be. Optimal configuration of internal surface of skin graft with spacing between ledges of π/2, π/4, π/8 mm (equals to 1.57 mm, 0.78 mm and 0.39 mm) is proved mathematically.

Technical solution. To obtain split-skin grafts with variable thickness (embossed) it is suggested to add a so called technological plate with embossed surface [10]. A technological plate is made of plastic mass or metal and consists of a base and shaped elements on it which have ledges of rectangular or other geometric figure shapes (Fig. 2).

Method of embossed skin graft cutting. Instruments and donor area do not differ from regular grafting. The technological plate is fixed to the base of the dermatome. The embossed surface of the plate should adjoin the skin of donor area tightly. Technological plate should be chosen according to the size of granulation on the wound: small-, medium- and large-grained. Fig. 3 shows embossed skin graft harvest of the variable thickness and donor wound with sinusoidal surface.

An embossed skin graft has sinusoidal surface from the inner side, which considerably enlarges area of its contact with granulations on the wound (Fig. 4). E.g., the inner surface is embossed skin graft with 0.78 mm space between ledges is larger by 29.3% if compared to a smooth graft.

Full-scale adhesion to wound surface contributes to a quick engraftment of dermal transplant and minimizes the local postoperative complications. The epithelisation of donor wound is not affected because all skin appendages are preserved.

Clinical application. The embossed autografting of skin was applied for 16 patients with deep burns of 7–9% of the body. The patients aged 21–60 years, 13 mail (81.0%), and 3 female (19.0%). Part of granulating wounds in each patient was covered with embossed transplants, and part of them with smooth split-thickness grafts. Thus it was possible to conduct a clinical comparison of survival of two types of grafts. Next day after the surgery the surface of the embossed skin grafts was of different colours — pale and cyanotic lines along the grafts. On the second or third day cyanosis

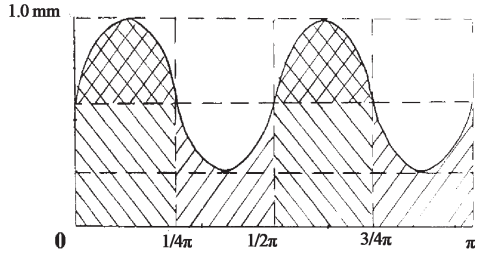

Fig. 1. Scheme of sinusoidal surface of granulation wound with the sinusoid length of π/2 mm (medium grained granulation tissue).

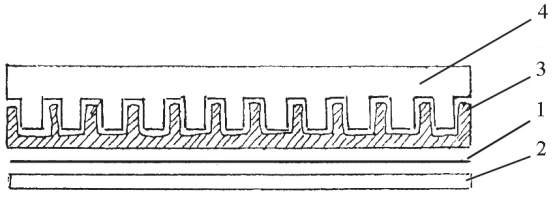

Fig. 2. The scheme of cutting edge of the dermatome: 1 — blade, 2 — protective plate of the dermatome, 3 — split-thickness embossed skin graft, 4 — technological plate.

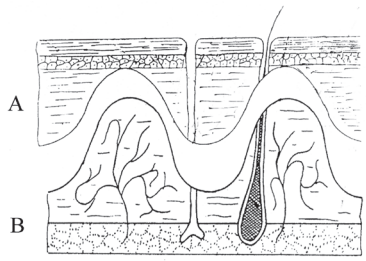

Fig. 3. Scheme of split-thickness embossed skin graft cutting: A — embossed skin graft, B — donor wound.

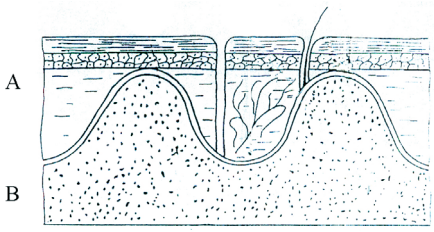

Fig. 4. Scheme of covering the granulation wound with embossed skin graft (A — skin graft, B — granulation wound).

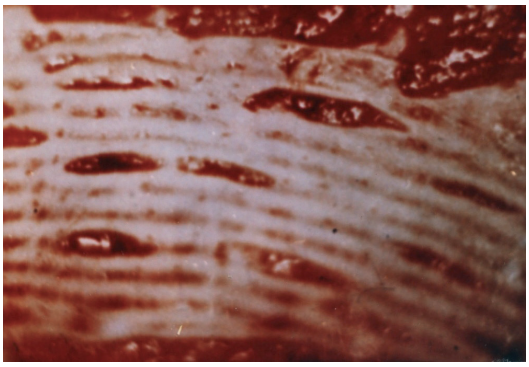

Fig. 5. Embossed split-thickness skin graft (clinical example).

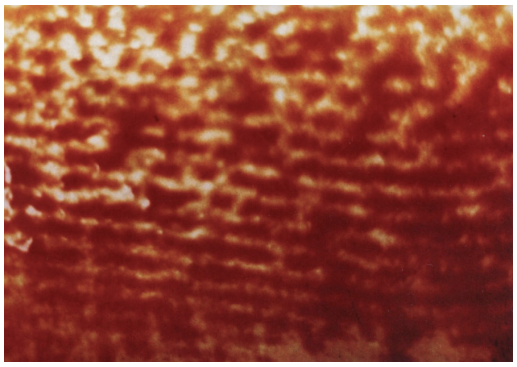

Fig. 6. Fragment of sinusoidal surface of donor wound.

decreased, and on 4–5th days the colour of the embossed grafts became even. Clinically the embossed transplants were engrafted on 6–7th day. On 18–21st day the roughness of grafts surface disappeared. In two months the graft together with subcutaneous fatty tissue was taken in the crease. Donor wound: on 14–15th day its surface was like "slate", but on 25–30th day the undulation of the re-epithelized skin disappeared.

Ultrasound Doppler (Siemens G60S) examination of grafts was performed on the 5th day. Active blood flow was found in some parts of the embossed transplants, there were on average 4–6 blood outbreaks on the length of the linear sensor (L10-5; 7.5–10.0 MHz), but in smooth graft, which was still swelling, there were only 2–3 flashes. Therefore, variable thickness skin graft integrated with granulation tissue faster. Thus, our clinical study proved the use of embossed skin grafts in closing the granulating wounds.

Results and Discussion

The embossed epidermo-dermal transplants with variable thickness contain morphological elements of dermis which the thin smooth split-thickness skin grafts do not have. The thin part of the sinusoidal graft is represented by the epidermis; the thick part contains papillary and reticular dermis. So there are all structures of the skin: loose and dense connective tissue containing blood and lymphatic capillaries and vessels, elastic and collagen fibres, reticular layer with fibroblasts, nerve endings, and epidermal appendages in the gel-like ground substance. The same components are also in donor sites and play a crucial role in its re-epithelialization. That is why after placement of sinusoidal graft, its initial adherence to the wound bed via a fibrin binding is favourable if compared to the straight skin graft.

It is established that the smooth split skin grafts shrink along fibres on cleavage lines and in transverse directions after cutting. Moreover, shrinkage along the cleavage lines is almost twice as much as transverse shrinkage. Convolution of split-thickness skin grafts to internal surfaces is its characteristic feature [1, 6].

The shrinkage of skin grafts increases with the increase of its thickness. Thin transplants of 0.1–0.4 mm shrink minimally, transplants of medium thickness (0.5–0.6 mm) shrink by 15–25%, thick (0.7–1.0 mm) — up to 50%, full-layer (>1.1 mm) — even more. Moreover, longitudinal and lateral components of shrinkage differ in 1.3–1.6 times [1, 3, 4, 12, 14].

Because of the embossed surface the shrinkage of epidermo-dermal transplant decreases considerably and the preserved morphological structure of dermis contributes to fast healing of burn wound as well as regeneration of functions of the transplanted skin.

The choice of skin grafts thickness is very important for successful autodermoplastic surgeries. Autodermoplastics with thin grafts have several advantages: the transplant survives for an average of 3–4 days, and the donor wound epithelizes for 12–14 days. However, this method has its deficiencies as well. Secondary shrinkage of the transplant occurs with usage of thin split grafts for wound covering; besides fatty tissue does not grow under this type of transplant [6, 8, 13].

Usage of full-thickness skin grafts has distinct advantages. They withstand mechanical loads, full fatty tissue grows under them, thanks to the renewed skin integument, which receives the functional and dynamic features. Long term retraction and retention of full-layer grafts are significant disadvantages. They suppurate and are rejected more often. The usage of such grafts creates problems for the treatment of a donor wound, which has to be covered with local tissues or autodermotransplants [1, 7, 9, 12, 14].

Epidermis of various topographical anatomical body areas is different in terms of relief, color and thickness (0.5–0.2 mm). Human skin thickness varies from 0.4 up to 4.0 mm. Thickness of the skin's dermal layer varies from 0.35 up to 4.0 mm and is a significant resource for harvesting split flaps [1, 14].

Opposite to the smooth split grafts, embossed dermotransplants with variable thickness retain anatomical and physiological features of skin in monofunctional structural integrity; herewith there is no substantial transplant shrinkage. The area of contact of transplant and wound increases because of the positioning of surface granulations, which hasten the process of its engraftment.

Conclusions

The improvement of dermatome design with technological plate allows cutting scalloped split-skin grafts of varied thickness (embossed).

Split-thickness embossed grafts combine anatomic and physiological features of skin cover granulation wound to adhere tightly because of the enlargement of area of their internal surface without retraction and/or secondary shrinkage.

Donor wounds, after the harvesting with the embossed skin grafts, contain the morphological elements of dermis which contributes to their complete spontaneous healing.

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THE INFLUENCE OF DIFFERENT VENTILATION PATTERNS ON TREATMENT OF PATIENTS WITH SEVERE TRAUMATIC BRAIN INJURY

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Background. *Respiratory support is a vital method for temporary compensation of external breathing function in patients with severe traumatic brain injury. However, it is not always possible to deal with severe respiratory dysfunction even with the usage of up-to-date respiratory technologies. This work is aimed to find an answer how different pattern of mechanical ventilation influence on a treatment of patients with severe traumatic brain injury.*

Objective.*The influence of respiratory support, as a main method for temporary compensation of external breathing function, on treatment result for patients with severe traumatic brain injury.*

Methods. *Treatment results of 253 patients with severe traumatic brain injury of Ternopil University Hospital were evaluated due to the type of respiratory support used. The results were separately evaluated in alive and dead patients.*

Results. *Mortality rate of patients depended on the type of mechanical ventilation that was used. The highest mortality (58.69 %) was in the group, when a patient was transferred to forced ventilation a volume control. The mortality rate was decreasing by 51.78% in case of adding PEEP. The strategy of using accessory lung ventilation patterns CPAP and BiPAP caused significant (in 1.48 times) decrease of mortality in this group of patients.*

Conclusions. *The survival of patients with severe traumatic brain injury, who were ventilated by the method of consistent combination of forced ventilation with pressure control (CPV) and 2 patterns of accessory lung ventilation: Constant Positive Airway Pressure (CPAP) and Biphasic positive airway pressure (BiPAP), is reliably higher than in the case of forced ventilation with volume control with Positive end-expiratory pressure.*

KEY WORDS: **severe traumatic brain injury, lung ventilation, treatment, intensive care.**

Introduction

The process of treating the patients with severe traumatic brain injury (STBI) is a topical issue of contemporary medicine [1]. It is established that the mortality rate in case of STBI is 80%, it is caused by the development of multiple organ failure [2].

STBI is one of the causes of mortality and disability all over the world. It is also one of the main reasons of human mortality until the age of 40 [3]. In Great Britain STBI frequency is 1 500 people per every 100 000 population. 9 patients out of 100 000 population die because of traumatic brain injury [1, 2]. The problem of STBI treatment is today not only medical, but social as well [2-5].

Among the complications of STBI the prominent role is given to lung affection which develops in 70–80% of patients and becomes one of the contributory factors for the worsening of the patient state with the further negative treatment results [4]. Morphological changes of lungs with underlying STBI are detected in 95–97.7% of cases [4]. The most frequent cause of patients mortality on the 3–5th day of STBI is acute respiratory distress syndrome (ARDS) [2]. It happens in almost every STBI patient with multiple organ failure [6, 7].

Respiratory support is a main method for the temporary compensation of external breathing function [8]. However, it is not always possible to deal with severe respiratory dysfunction even using up-to-date respiratory technologies.

The question is: how will it influence the treatment result? This study is aimed to find an answer.

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Material and Methods

Treatment results of 253 patients with STBI of Ternopil University Hospital were evaluated due to the type of respiratory support used.

The results were separately evaluated in alive and dead patients. As the criteria of treatment effectiveness we used the period of patients stay on mechanical ventilation.

4 different patterns of breathing were used: independent or spontaneous breathing, forced ventilation with volume control (CMV) and forced ventilation with pressure control (CPV). We also used 2 patterns of accessory lung ventilation: Constant Positive Airway Pressure (CPAP) and Biphasic positive airway pressure (BiPAP), a type of ventilation with 2 phases of positive pressure in airway. For ventilation with volume control we used a mechanical ventilation device "Bryz". For ventilation in CPAP, BiPAP and CPV modes we used a mechanical ventilator Carina Drager.

In 2012–2015 years in Ternopil University Hospital there were 253 patients with STBI. Mechanical ventilation was delivered for all these patients. They were divided into 3 groups. In the 1st group patients were ventilated in CMV (5–6 ml/kg). The strategy in 2nd group was the same except during CMV mechanical ventilation we used positive pressure in the end of expiration (PEEP), which didn't exceed 6–9 mbar. In the 3rd group we used the accessory ventilation. If the saturation of these patients in case of independent breathing decreased more than by 92%, the accessory ventilation through endotracheal tube in CPAP pattern or alternatively tracheostomy was implemented. If saturation was lower than 90%, patients were transferred to BiPAP pattern. In case of full absence of independent breathing, the patient of this group was ventilated in CPV pattern. At this strategy PEEP didn't exceed 6–9 mbar, and inspiratory pressure — 20–22 mbar.

Patients usually died because of multiple organ failure, which was followed by ARDS. ARDS was the most frequent cause of patients' deaths during the first 5 days after the surgery. We used Guidelines for the Management of Severe Traumatic Brain Injury of American Association of Neurosurgeons (2007) as the treatment strategy which was modified according to our conditions [4].

In all groups of patients during spontaneous breathing and mechanical ventilation as well, we tried not only to keep the partial oxygen pressure but also to prevent hypocapnia, so the hyperventilation didn't occur.

Infusion therapy was implemented in normovolemic mode in order to avoid hypotension episodes. Additionally, we tried to keep central venous pressure on the level of 8–10 mm Hg and hemodynamic support with the usage of vasopressors was also performed. We also tried to prevent the decrease of average blood pressure (BP) lower than 80 mm Hg. dopamine and adrenaline were the most frequently used among the vasopressors. The highest point of systolic BP was 160 mm Hg. As for the infusion therapy we preferred Normal Saline Solution (NSS) or Ringer's solution.

Analgosedation was used for eradicating hyperventilation and synchronization with ventilator. The ventilation was implemented with $PaCO_2$ 35–40 mm Hg. PEEP was 5–8 mm Hg (not higher than 15 mm Hg). According to the treatment strategy we also cured hyperthermia and anemia, prevented convulsions with carbamazepine and gabantin, used low molecular weight heparins at the absence of hemorrhage. For prevention of stress ulcers was conducted and nutrition therapy was applied. We preferred the fastest administration of enteric nutrition by means of standard food mixes and obligatory glycemia control. Corticosteroids (CS) were used only in case of ARDS development. If intracranial pressure was rising we also used barbiturates. Antibiotic therapy was chosen due to the type of microflora. The drugs of choice were meropenem, colomycin, amikacin.

The mortality rate and ventilation period in each studied group were counted. The ventilation period was counted separately: for those who stayed alive and died.

The permission for research implementation was issued by the Commission on Bioethics of I. Horbachevsky Ternopil State Medical University (protocol №29 from May 20, 2015).

Results

Mortality rate of patients depended on the type of mechanical ventilation that was used. The highest mortality (58.69%) was in the 1st group, when the patient was transferred to CMV pattern in terms of insufficient independent breathing. The mortality rate decreased by 51.78% when PEEP was added. The strategy of using accessory lung ventilation patterns CPAP and BiPAP caused significant (in 1.48 times) mortality decrease in this group of patients.

The usage of different ventilation patterns caused the changes in the period of patients' stay on mechanical ventilation. In the 1st group (CMV), dead patients were on accessory ventilation for very short period and died quickly. Patients, who stayed alive, adversely, stayed on mechanical ventilation for longer period. The accession of PEEP improved ventilation results:

dead patients lived longer and those, who stayed alive, were taken of ventilator earlier. The best results were in groups with accessory ventilation patterns CPAP and BiPAP. Mortality in this group was lower than in 1st and 2nd groups. Among the alive patients with such mechanical ventilation support, the process of excluding patients from mechanical ventilation was much easier, though the mortality in this group was in average for a few days longer if compared to 1st and 2nd group.

Discussion

To our mind, the obtained results depend on peculiarities of ventilation pattern and analgosedation. Anesthesiologists always see that a patient feels pain because vital signs, above all pulse, BP and respiratory rate increase. However, we tried not to use the excessive dosages of analgesics. If there are no signs of pain, we could give no analgesics to a patient at all. In case of even small signs of pain we prescribed narcotic analgesics. Frequently, even not high dosage of analgesics caused the impairment of patients' state. Patient started breathing worse and mechanical ventilation was necessary. The same situation was on in case of hyperventilation, which promoted hypercapnia development. Even small dosages of analgesics caused the decrease in patients' state according to GCS level, partial oxygen pressure in arterial blood less than 60 mm Hg and promoted the necessity of mechanical ventilation.

The main differences and advantages of accessory ventilation are: in CPAP pattern the ventilator helps the patient to inhale even if there are 40 inhales per 1 minute. The ventilator will support the positive pressure in the end of exhale on the determined level, which equals 6-9 cm of water. Also, it helps to make every effort of inhale according to determined measurement of inspiratory support (20 mBar). In BiPAP pattern the respiratory rate is maintained by a ventilator. Inhalation startS by the patients' effort to inhale, and then the inspiratory support level is achieved. The important thing is that patient can breathe independently and breathe between respiratory cycles, which are provided by mechanical ventilation. In order to achieve the synchronization with it, we need smaller dosages of analgesics. We observed the smaller interruption of consciousness and breathing due to GCS

During the last year we succeed in mortality decrease almost in 4 times: before the rate was 9 to 100 000 and now it is 9 up to 200 000. The main causes of this are 2 main factors: the earlier described strategy of respiratory support and the CS in treatment of ARDS.

The most significant problem of intensive care unit is increase of bacteria resistance to carbapenem. In terms of insufficient financial support it is hard to solve.

Table 1. Treatment results of patients with STBI followed by severe sepsis and ARDS depending on type of respiratory support and CS usage

Ventilation mode		Amount of patients	Average age	Ventilation period in days	Survival, %
SV+ IMV	Alive	19	37.52	19.47	58.69
	Dead	27	52.96	5.22	
SV+IMV+PEEP	Alive	27	41.0	17.14	51.78
	Dead	29	52.1	6.76	
CPAP+BiPAP+CPV	Alive	91	40.85	13.93	39.73
	Dead	60	50.0	14.1	

Dead patients

	SV+IMV	SV+IMV+PEEP	CPAP+BiPAP+CPV
SV+IMV	–	0.0734	0.000050
SV+IMV+PEEP	0.0734	–	0.000099
CPAP+BiPAP+CPV	0.000050	0.000099	–

Alive patients

	SV+IMV	SV+IMV+PEEP	CPAP+BiPAP+CPV
SV+IMV	–	0.8194	0.1769
SV+IMV+PEEP	0.8194	–	0.4688
CPAP+BiPAP+CPV	0.1769	0.4688	–

Conclusions

The survival of patients with STBI which were ventilated by consistent combination of forced ventilation with the pressure control (CPV) and 2 patterns of accessory lung ventilation: Constant Positive Airway Pressure (CPAP) and Biphasic positive airway pressure (BiPAP) is significantly higher than in the case of forced ventilation with the volume control with Positive end-expiratory pressure.

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QUALITATIVE AND QUANTITATIVE CHANGES IN THE CELLULAR COMPOSITION OF GUMS IN PATIETS WITH PERIODONTAL DISEASES

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Background. *The results of a comprehensive study of cytological features of epithelial differentiation processes in gums in patients with generalized periodontitis are presented in the article.*

Objective. *The objects of clinical observations were patients with generalized periodontitis. The disease duration was 3–5 years.*

Methods. *Material for the study was gums epithelium taken from a marginal area of gums in young adults, with generalized periodontitis. The epithelium was removed with a spatula, with subsequent transfer to a glass plate and drying in the open air for 3–5 minutes. The colouring of the material was done according to Romanovskyy-Himza, with a next microscopic and morphological analysis, taking into account the percentage of different forms of epithelial cells in normal and due to an age aspect.*

Results. *One of cytological signs of periodontal pathology in young people is epithelial cells with signs of irritation. It should be noted that these cellular elements belong to the intermediate layer of cells that meet histological sections and ribbed surface layers are characterized by homogenization and vacuolization of cytoplasm as a manifestation of prolonged irritation by bacterial cell aggression.*

Conclusions. *Analysis of cytograms proved general periodontitis in the examined patients, in absence of adequate therapy as automotive continuous process. Its components may regress under the influence of effective treatment and re-activate during exacerbation of the inflammatory process under the influence of pathogenic factors such as microbial, hygienic and immune.*

KEY WORDS: **gingiva, epithelium, cells, nucleus, cytoplasm.**

Introduction

As a part of the mucous membrane, buccal epithelium is active within irritating factors that influence it from external and internal environment. It allows to use it for studying the physiology and reactivity of mucous membranes, and in particular as an indicator of local and general disorders of homeostasis [1, 2].

Each anatomical area of gums has a zonal type of keratinization and on histological sections is characterized by basal, ribbed, granular and stratum layers; keratinization occurs in it due to ortokeratoz — a phased process, and as well due to apoptotic changes in the upper layer of epithelium [3].

An only exception is gum sulcus epithelium that is not keratinized and reduces its barrier function and is a target organ at the stage of periodontal pathology development [4].

Taken into account the spread of general periodontitis among young people [5] and the prevailing current points of view about the disease pathogenesis [6, 7, 8], the processes of differentiation of gums epithelial cells in the course of general periodontitis is an urgent matter, as well as diagnosis objectification, determination of severity degree and involvement of adjacent connective tissue to predict course of the disease, exacerbation rate and relapse [9, 10, 11, 12, 13].

Our study was aimed to determine the characteristic features of cellular structure of gums cytogram in patients with general periodontitis.

Material and Methods

Material for the study was gums epithelium, taken from a marginal area of gums in young

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adults, with generalized periodontitis. The disease duration was 3–5 years.

The epithelium was removed with a spatula, with subsequent transfer to a glass plate and drying in the open air access for 3–5 minutes. The colour of the material was done according to Romanovskyy-Himza, with a next microscopic and morphological analysis, taking into account the percentage of different forms of epithelial cells in norm and due to an age aspect.

In order to standardize the epithelium layer and a deeper studying, in our research we used cytological classification; according to it basal, parabasal, intermediate, superficial cells were in the epithelium of gum in the area of keratinized epithelium of gums, horny scales.

Statistical analysis of the results was done at the Department of Statistical Research of I. Horbachevsky Ternopil State Medical University. Parametric methods were used for the indicators, their distribution met general requirements.

To evaluate the nature of distribution the coefficients of skewness and kurtosis were determined. The test was performed with normal asymmetry test by Shapiro-Wilkie. Probable differences of the results for different groups were determined using Student t-test.

The difference was considered likely in common in the medical and research error probability $p<0.05$. The probability of error was evaluated by the Student t-test tables due to the size of the experimental groups. The rate of distribution was statistically significantly different from the expected normal nonparametric criterion (U) Mann-Whitney nonparametric analogue of a Student t-test.

Results

It is established that a major component of the cellular composition of the gums' scraping cytogram in the course of generalized periodontitis are the stratified squamous epithelial cells. They are present in cytograms under normal conditions and during pathology. It should be emphasised that the squamous cells are heterogeneous, reflecting the heterogeneity of epithelial cells gums' cover. In the case of generalized periodontitis a cytological character of scrap changes significantly both in epithelial and connective tissue constituents.

Detailed statistical analysis of cytogram, made it possible to determine the percentage change in gingival epithelial cells of people examined due to the different severity of generalized periodontitis.

This ratio is (2,20±0,10) : (9,30±0,16) : (41,6±0,36) : (37,0±0,31) : (9,9±0,16) (Table 1).

Discussion

The qualitative and quantitative cellular composition of cytogram in periodontitis depends on the clinical course and intensity of inflammatory and degenerative processes in periodontal tissues. The received data was significantly different from the stereotypical percentage of epithelial differentiation of stratified squamous epithelium of gums [3], and ratio that was determined for the examined patients and is characterized by violation of differentiation: disorder of keratinization as evidenced by the presence of all components of different epithelial cells.

This makes it possible to assert that during generalised periodontitis the mechanisms of epithelial desquamation disruption take place causing barrier function of gums at generalized periodontitis; that is the outcome of different epithelial cells differentiation caused by inflammatory infiltrate of lamina propria.

The presence of parabasal cells in cytograms was explained by activation of compensatory mechanisms of epithelium, taken into account high mitotic activity of gum epithelium and increased glycogen-containing epithelial cells.

Table 1. Percentage change in different cells classes of stratified squamous gums epithelium in the course of generalized periodontitis

Indicator	Cells of smears				
	Basic	Parabasal	Intermediate	Superficial	Horny scales
Norm (V.L. Bykov)	0	0	60	8	32
Norm for young person	0	0	59,20±1,15*	7,60±0,34*	33,2±0,65*
Generalized periodontitis	2,20±0,10**	9,30±0,16**	41,60±0,36**	37,0±0,31**	9,90±0,16**

Note: * — $p<0,05$ compared with the control group; ** — $p<0,05$ if compared with the experimental group.

At the same time, reflecting the severity, course and intensity of general periodontitis and intensity of inflammatory and degenerative processes in periodontal tissues, cellular composition of gums in cytograms was divided into four types. The first type is characterized by presence of intermediate and parabasal cells stained by Romanovskyy-Himza, with smaller size compared to intermediate. These epithelial layers correspond to deep ribbed cells in histological sections. They are larger in size if compared to basic cells and have an elongated shape. The core is large, well-countered, vesicular, with small diffuse located chromatin inclusions and relatively wide rim of basophilic cytoplasm. The lack of hematogenous cells row is noted. It is obvious that this cellular composition obtained in cytograms proves evidence of proliferation, i.e. reproduction of parabasal cells and specific prognostic criterion of generalized periodontitis of initial stage.

In the second type of cytogram there were individual representatives of rod flora with advantage of basophilic intermediate (azur positive) epithelial cells from cytological smears of gums. They have cubic, rectangular or polygonal form; cytoplasm contains azure-positive granules. The core is round, sometimes oval. The trend towards clusters of cells location of this class is noted. Intermediate cells in histological sections correspond to ribbed parts of surface layer. These intermediate cells are found in normal and mostly without elements of cytopathology. We argue that this type of cytograms proves chronic generalized periodontitis.

In the third type of cytogram there were cytograms of intermediate cells staining by Romanovskyy-Himza. They have azure-positive cytoplasm nucleus centric located. The cytoplasm is elongated. The cells are usually located in clusters. The change in the microbial composition is determined that in this type of cytogram is represented mainly by cocci that adhere on the surface of epithelial cells.

In addition to the above mentioned epithelial cells in cytogram of the third type of the examined people there are segmented leukocytes, which are split into 3–4 segments core also there are isolated mononuclear leukocytes or young white blood cells it suggests a redistribution pool of myeloid cells in response to the presence of foci of active inflammatory process that causes the process of differentiation of leukocytes available. Present cytological pattern characterizes pointer of general periodontitis in the examined people.

In the fourth type of cytograms there were single basal epithelial cells. During staining by Romanovskyy-Himza, if compared with basal cells of periodontal pockets they have relatively smaller and more rounded shape of cytoplasm. The core is round-shaped and has a narrow rim of cytoplasm. Basal epithelial cells are surrounded by segmented leukocytes.

The predominant cells in the fourth type of cytograms are the cells of intermediate layer, their cytological organization corresponds a lot to the class affiliation and level of differentiation, a pathogenic microflora is evidenced as well as the cells of inflammatory response. Superficial cells are also verified.

A powerful microbial composition initiates further necrobiotic processes in epitheliocytes and in segmented leukocytes. Due to phagocytosis, the destruction of the cytoplasm of segmented leukocytes occur, it is 'incomplete phagocytosis'.

Coccal microflora adheres not only on the surface of epithelial cells, but also on the surface of segmented leukocytes. Along with haematogenous cells in cytograms predominantly coccoid flora and single thread of pseudomycelia of Candida fungi are evidenced.

So cytograms of the fourth type present the increase of phagocytic reaction of segmented leukocytes, and as a result of incomplete phagocytosis formation of 'pus cells' occurs. During phagocytosis of leukocytes specific changes in the form of restructuring of its nuclear takes place, while in epitheliocytes of gums a necrobiotic process occurs primarily in the cytoplasm and in the nucleus.

The described type of cytogram corresponds to the clinical manifestations of general periodontitis with abscess formation.

The emergence of epithelial cells with signs of irritation in cytograms is one of cytological signs of periodontal pathology in young people. These cellular elements belong to the intermediate layer of cells that on histological sections correspond to ribbed surface layers and are characterized by homogenization and vacuolization of the cytoplasm as a manifestation of prolonged bacterial irritation. The vacuolization of epithelial cells cytoplasm in general periodontitis gingival complex proves disorders of water and electrolyte and protein metabolism, causing the changes in colloid-osmotic pressure in the cell. The violation of cell membrane permeability, accompanied by their collapse is also significant. It leads to lysosomal membranes labilization with activation of their hydrolytic

enzymes that break intramolecular connection with water. In fact, these changes are a manifestation of early cell focal necrosis [5].

At the same time the increase in the volume of epithelial cells was evidenced, the cytoplasm is full of vacuoles, containing a clear liquid. The core is moved to the periphery of the cell, sometimes are vacuoles or core shrinks. Later disintegration of ultra-structural elements of cells occurs and it overflows with water. These changes are the features of the intermediate stage of cellular metabolism disorders caused by inflammation and preceding cell cytotransformation into balloons filled with fluid. Such changes characterize balloon cell degeneration.

Analysing the cytogram we can reach the conclusion that unlike previous smear from epithelial gums it has the greater degree of destruction accompanied by cariopiknosis and kariorexis of nucleus and cytoplasm homogenization. Horny scales in cytograms decreased in comparison to their quantitative composition in cytograms of people with health periodontium. They are mainly eosinophilic, of polygonal shape, characterized by no clear contours.

Conclusions

The changes in the gums in the course of generalized periodontitis are subject to rather wide limits, which depend on the clinical course and exogenous factors. Analysis of cytograms gave the opportunity for consideration of general periodontitis in the examined patients due to the absence of adequate therapy as automotive continuous process. Its components (inflammatory infiltrative and destructive) may decrease after the effective treatment and reactivate during the exacerbation of inflammatory process under the influence of pathogenic factors such as microbial, hygienic and immune.

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CLINICAL EFFECTIVENESS OF TREATMENT THE PATIENTS WITH CHRONIC APICAL PERIODONTITIS

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Background. *The success of endodontic treatment is provided by a thorough instrumental and antiseptic treatment of infected root canals, and it depends on the composition of filling material, the degree of adhesion to dentin, hermetic obturation of apical foramen, solubility of sealer.*

Objective. *The study was aimed to evaluate the effectiveness of root canal obturation with BioRoot™ RCS sealer in the treatment of patients with chronic apical periodontitis.*

Methods. *Endodontic treatment of 23 teeth in 20 patients with chronic apical periodontitis by method of lateral compaction of gutta-percha was carried out. In the main group root canals were obturated with BioRoot™ RCS, in the control group the canals were filled with Apexit Plus. The percentage of efficient or non-efficient cases was evaluated on the basis of radiographic comparison of treated chronic apical periodontitis immediately after obturation, in three, six months and one year. Radiographic conditions were defined as existing state, improvement and worsening.*

Results. *In a year of dynamic evaluation the final results were: in the main group — 54.55% of the patients had complete bone healing, in 27.27% of cases the focus of bone destruction was decreased by 1/2 or more of the initial sizes, 18.18% — resorption lesion was decreased by less than1/2; in the control group — 33.33% of improvement, 25.0% of existing state and 41.66% of worsening.*

Conclusions. *BioRoot™ RCS using for root canals obturation in the treatment of chronic apical periodontitis we proved the high effectiveness of the treatment undertaken: complete healing or improvement of radiographic conditions of periapical bone destruction with X-ray signs of bone regeneration.*

KEY WORDS: **apical periodontitis, endodontics, sealers, filling, root canals.**

Introduction

Treatment of patients with chronic apical periodontitis is complex and is an important task of modern dentistry due to their high prevalence, frequent complications after traditional treatment and absence of consistent results [1, 2]. According to numerous researches, the basic principles of effective treatment of destructive forms of apical periodontitis are the thorough instrumental and antiseptic treatment of infected root canals with their subsequent filling with pastes based on calcium hydroxide [3, 4]. It prevents microbial contamination and its influence on surrounding tissues, acceleration the processes of apexification and bone regeneration [5, 6]. The success of endodontic treatment is also impossible without quality hermetic obturation of root-canal system for forming of reliable barrier between tooth cavity and periodontal ligament [1, 2, 6].

Among all modern endodontic sealers a mineral-based root canal sealer BioRoot™ RCS "Septodont" has the above-mentioned features. This material is bioactive by stimulating bone physiological process and mineralization of dentinal structure [7]. It creates a favourable environment for periapical healing and bioactive features including biocompatibility, hydroxyapatite formation, mineralization of dentinal structure, alkaline pH and sealing qualities. Due to the use of Active BioSilicate Technology which is monomer free, there is no shrinkage of BioRoot™ RCS during setting for a tight seal of root canal [8, 9].

Therefore, this study was aimed to evaluate the effectiveness of root canal obturation with BioRoot™ RCS sealer in the treatment of patients with chronic apical periodontitis.

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Material and Methods

Endodontic treatment of 23 teeth from frontal and distal groups with chronic apical periodontitis (by an equal distribution between chronic granulating and granulomatous periodontitis) in 20 patients aged 25–45 years without accompanying pathology was carried out. Disinfection and irrigation procedures of root canals were made by standard methods: the canals were shaped and underwent an appropriate tapered preparation, disinfected with a 3% sodium hypochlorite solution activated with mechanical agitation and rinsed with 17% EDTA. Permanent obturation of root canals was carried out by method of lateral compaction of gutta-percha. In the main group the root canals of 11 teeth were obturated with BioRoot™ RCS "Septodont" as a sealer, in the control group the root canals of 12 teeth were filled with Apexit Plus "Ivoclar Vivadent".

The percentage of efficient or non-efficient cases was evaluated by radiographic comparison of treated chronic apical periodontitis immediately after obturation, and in three, six and twelve months. Radiographic conditions were defined as existing state, improvement and worsening. Dynamics of periapical tissue healing were also controlled by a periapical index PAI [10].

Descriptive statistics was compiled by Microsoft Excel and Student's t-test. Mann-Whitney U-test (nonparametric criteria) was used to evaluate differences between groups at baseline (immediate postoperative) and at the 12-month follow-up evaluation. Changes in PAI score for each group from baseline to 12-month follow-up evaluation were tested with Wilcoxon signed rank test. The secondary outcome measures, proportion of teeth in each group that could be considered as improved (PAI score decreased) or healed (PAI<2) were assessed with Chi-square test [3]. Clinical symptoms and abnormal findings at the follow-up examination were recorded but not subjected to statistical analysis.

Results

By using BioRoot™ RCS for nonsurgical treatment of destructive forms of chronic apical periodontitis in the patients of the main group we did not observe any complications after contact with this material. In three months after the treatment was completed the dynamics of clinical and radiographic changes were practically identical in both groups of patients, although complete healing of periapical bone area was not evidenced in any clinical cases. The average values of PAI before and after 3 months of the treatment did not differ significantly in the study groups of the patients ($p>0.05$).

In 6 months the patients of the main group were absolutely asymptomatic. Clinical symptoms or abnormal findings (spontaneous pain, swelling, mobility, or sensitivity to percussion or palpation) in the main group of patients were absent. In 2 patients of the control group the exacerbation of chronic apical periodontitis was diagnosed and the teeth were re-treated. Intergroup significant differences of PAI were determined after 6 months of dynamic observation: in the main group — 2.09±0.26 points, in the control group — 2.92±0.31 points ($p<0.05$).

In six months on the X-ray images of treated teeth in the main group of patients the absence of periapical zone destruction was determined in 36.36% of cases (4 teeth) and reduction of focus of apical bone resorption by ½ or more was established in 45.45% (5),the existing state was in 18.18% (2). In the control group in six months after root canal obturation the widening of periapical destruction zone was revealed in 25.0% (3) of cases, the focus of bone destruction was not changed in 33.33% (4), it was decreased by less than 1/2 in 25.0% (3), and it was decreased by 1/2 or more from the initial size in 16.67% (2).

12-month follow-up examinations proved that the patients of the main group did not have any complaints. During intraoral examination the gingiva in the area of tooth apexes was of normal colour without visible pathological changes, except scar formation from gingival fistula after the treatment of chronic granulating periodontitis. Percussion reaction was painless, regional lymph nodes were not palpable. The teeth performed full functional load. Mean PAI score in the main group was 3.27±0.43 at baseline and decreased up to 1.55±0.35 in 1 year of observation, in the control group it was 3.25±0.39 and 2.75±0.38, respectively. The decrease in PAI in the main group was up to 1.72, in the control group — up to 0.5. The decrease in PAI was statistically significant in both groups of the patients ($p<0.001$).

In a year of dynamic assessment of the main group the improvement of radiographic state was evidenced: 54.55% (6) of the patients had complete bone healing, 27.27% (3) of cases the focus of bone destruction was decreased by 1/2 or more of the initial sizes, 18.18% (2) — the resorption lesion was decreased by less than

1/2. Final results in the control group of patients were: 33.33% (4) of improvement, 25.0% (3) of existing state and 41.66% (5) of worsening. The absence of positive dynamics during 12 months of observation of the main group patients was in 18.18% (2) of cases; the percentage of non-efficient cases in the control group was 41.66% (5).

Discussion

Repair of periradicular tissues consisted of a complex regeneration involving bone, periodontal ligament, and cementum. Although clinical as well as radiographic data were used to monitor cases, the relative absence of clinical symptoms in chronic apical periodontitis made the evaluation primarily a radiographic one [10]. Due to the use of BioRootTM RCS in the main group of patients the positive dynamics of clinical manifestations was confirmed radiographically during periodic follow-up examinations. Three months after the treatment was completed we revealed the lesions size reduction, increasing of the number of bone trabecules and decreasing of intertrabecular spaces. In six months of BioRootTM RCS exposition in root canals the expansion of area destruction in periapical tissues and the appearance of new rarefaction or the increase in initial rarefaction were not determined in any clinical case. On X-ray images bone thickening was observed, periodontal contours were not widened. Moreover, in six months the radiographic evidence of trabecular pattern of bone was determined at the area the periradicular lesion was present before. In a year of dynamic assessment a significant thickening of bone and regeneration of periapical tissues were evidenced. The contour, width, and structure of periodontal margin were normal. The area of mineral loss gradually was filled with bone and the radiographic density was increased in most clinical cases.

Composition of BioRootTM RCS (bioactive tricalcium silicate setting system with the absence of any aluminate and calcium sulphate), high degree of adhesion to the dentin and gutta-percha points, zero shrinkage as monomer-free sealer and great flowability created a favourable environment for periapical healing, the acceleration of apexification and regeneration processes of the bone, and provided a hermetic obturation of apical foramen [9]. There were no complications after the conducted treatment.

Comparing the clinical and radiographic results of both groups it should be noted that in the patients of the main group BioRoot™ RCS usage resulted in the improvement of clinical symptoms of the disease. Repair of periapical bone structures and periodontal tissues, increasing of mineral saturation of bone were accelerated by this sealer, especially it was a characteristic feature of pathological processes with size of destruction up to 3 mm in diameter. Regeneration in these clinical cases occurred faster than in the cases of larger sizes of destructive lesions. Although the action of BioRoot™ RCS was also effective in the patients with the large periapical lesions, but in the control group the healing of such lesions was not evidenced.

Conclusions

BioRoot™ RCS using for root canal obturation in the treatment of chronic apical periodontitis we proved the effectiveness of the treatment undertaken: complete healing or improvement of radiographic states of periapical bone destruction with X-ray signs of bone regeneration. These changes were confirmed by the higher indicators of radiographic dynamics of periapical index in the main group of patients than in the other.

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MEDICAL ERRORS IN CLINICAL PRACTICE OF PHYSICIANS IN TERNOPIL REGION (UKRAINE)

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Background. *The professional occupation of a doctor quite often meets different imperfections, which have negative outcome for patients.*

Objective. *The study was aimed to investigate the expert characteristics of improper performance of the professional duties by medical staff on the example of a particular region of Ukraine.*

Methods. *In the study the archival materials (commission forensic medical examinations) held in Ternopil Regional Bureau of Forensic Medical Examination in 2007–2014 years were analysed. The research results are summarized and processed with the use of general statistical methods.*

Results. *It is defined that during this period 112 examinations concerning medical malpractice were implemented (9.05% of all commission examinations).*

Conclusions. *Medical errors were combined, specifically during the diagnostics, treatment and in medical records. The majority of cases (82.1%) of medical malpractice were caused by the objective reasons.*

KEY WORDS: **medical malpractice, medical errors, forensic medical examinations, dereliction of duty by medical personnel, negligence.**

Introduction

The professional occupation of a doctor quite often meets different imperfections, which have negative outcome for patients. After the implementation of Criminal Code of Ukraine in 2001, especially Article 140 "Unjustifiable dereliction of duty by the medical or pharmaceutical practitioner", the number of criminal proceedings on the so-called "medical cases" significantly increased. This article became very important for forensic medical examination and for law in general, because of unintended events during medical care delivery and their legal correct qualification were foreseen in Ukrainian law for the first time. The experts of the World Health Organization consider that near 6–7 patients die every day in Ukraine as a result of medical errors. The Journal of Patient Safety released a new study in 2013 that estimated number of preventable medical errors above 440,000 annually [1].

Every case of inadequate medical care becomes an object of investigation which can't be implemented without the conclusion of the commission on forensic medical examination. This problem is not studied enough in contemporary Ukrainian forensic medicine. That is why the study of structure, occurrence and peculiarities of medical malpractice is the aim of the research.

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Material and Methods

In the study archival materials (commission forensic medical examinations) handled in Ternopil Regional Bureau of Forensic Medical Examination (TRBFME) in 2007–2014 years were analysed. The research results are summarized and processed by general statistical methods.

Results and Discussion

During the studied period 112 examinations were conducted in TRBFME for "medical matters" (9.05% of all commission examinations). Improper medical care was revealed in majority of the cases (92 (82.1%)) when dereliction of duty by medical personnel occurred. The notion improper medical care or medical malpractice covers all unintended and wilful failures, omissions and defects of medical care delivery, treatment and intervention. Due to Anglo-Saxon system of law all failures in the doctors

conduct are divided into two types: medical negligence and professional misconduct [2]. From the point of Ukrainian legislation all cases of unintentional medical intervention or lack of such can by classified into three main groups: medical maloccurrence, professional crimes of medical practitioners and medical errors. The term maloccurrence is the same both Anglo-Saxon and Slavonic law systems: inspite of good medical attention and care, an individual fails to respond properly or may suffer from adverse reactions of the drug [3]. The definition "professional crimes of medical staff" means inadequate medical care or medical intervention that foresees criminal liability of defendant. Gross breach of the standards of medical care occurs in those cases the severe consequences develop for a patient. This is similar to the term "criminal negligence" in Anglo-Saxon law system. Other cases of unsuccessful medical care are usually qualified in Ukrainian law as "medical errors" (similar to "civil negligence").

Totally, 19 cases (20.6%) met unjustifiable medical care and were determined as crimes in medical sphere. Medical errors (civil negligence) were recognized by the forensic medical expert commissions in 73 cases (79.4% of all). The majority of such deviations were committed by hospital doctors and were performed during emergency medical care delivery [4]. The proportion of physicians facing an unintentional medical intervention or lack of such [5] ranged from 39.3% in anaesthesiology, 21.4% in internal medicine, 18.7% in obstetrics and gynaecology, 17.8% in paediatrics, 14.3% in general surgery and 4.5% in family medicine, ophthalmology, neurosurgery, 3.6% in neurology, 2.7% in oncology and 1.8% in psychiatry (Table 1).

The following mistakes were diagnosed by forensic medical examination: misjudgement of diagnostics (61.1% of cases), mistakes in medical records (46.4%); mistakes during invasive procedures or medical treatment including medication errors (40.2%), institutional defects (27.7%), failures in the doctors conduct which impinge upon their professional relationships (deontological) were determined in 21.4% of cases. As a rule, forensic medical expert commissions established the presence of two different types of the mistakes (23.2%) simultaneously, if for example misjudgement of clinical symptoms was combined with medication errors. The presence of 4 different types of mistakes were ascertained in 16.7% of "medical cases"; 3 defects (14.3%); all kinds of defects (3.6%). The following failures in the diagnostics were normally revealed: insufficient or incomplete general-clinical, laboratory and instrumental examinations; misjudgement of clinical symptoms; inadequacy of the clinical diagnosis; unskilful implementation of conventional special diagnostic methods and procedures. Mistakes during medical treatment including medication errors took place: the excess doses of medication, insufficient or excess amount of infusion, unjustified prescription of many the-

Table. 1. The prevalence of malpractice among medical practitioners

Specialty of a physician	The number of cases in which deficiencies of paramedics of certain specialties were established (n=112)	The percentage on total amount of research cases (%)
Anaesthesiologists	44	39,3%
Therapists	24	21,4%
Obstetricians	21	18,7%
Paediatricians	20	17,8%
Surgeons	16	14,3%
Traumatologists	15	13,4%
Infectiologists	14	12,2%
Emergency doctors	10	8,9%
Family doctors	5	4,5%
Ophthalmologists	5	4,5%
Neurosurgeons	5	4,5%
Radiologists and clinicians ultrasound	5	4,5%
Neurologists	4	3,6%
Oncologists	3	2,7%
Psychiatrists	2	1,8%

Note: digital values exceed the total number of cases due to (in one case) deficiency assumptions on medical professionals of different specialties.

rapeutic agents concurrently. Besides of these, the breach of care delivery standards, lack of monitoring data about a patient, omission of patient's condition, unforeseen complications etc. were detected too. The following institutional defects seemed more frequent: equipment malfunctions, absence of consultations; incorrect admission of a patient; inadequate monitoring of hospital administration about medical care. Numerous mistakes in medical records were found: the absence of hemodynamic parameters; superficial and incorrect description of objective state, lack of informed consent of a patient, inadequate clinical diagnosis, incomplete epicrisis etc. All deontological errors were commonly conducted with a poor qualification of medical staff, negative relationships between a patient and doctor, doctor and patient's relatives [6]. Approximately 30% of all studied cases faced the breach of medical care standards.

Forensic medical examination confirmed the objective reasons for medical malpractice in almost 80% of cases. They were: the severity of patient's condition or the presence of comorbidity (32.1% of cases); rapid duration of pathological process (21.4%); difficulties in diagnostics or atypical manifestations of disease (13.4%). The subjective reasons (i.e., due to poor qualification of medical personnel) were detected in 9.8% of cases. All these cases were qualified as criminal negligence. Dereliction of duty by medical staff as a rule was accompanied by severe consequences for patients (fatality, grievous degree of severity, prolongation of illness, moderate degree of severity).

Conclusions

Dereliction of duty by medical personnel is confirmed more than 80% of "medical cases" in the conclusions of the commission forensic medical examinations.

The occurrence of unintentional medical intervention or lack of such in anaesthesiology, therapeutic, obstetrical/gynaecological, paediatric and surgical cases was more frequent. Unintended medical incidents were associated with and occurred during diagnostics, treatment, medical care, medication and in medical records. Considered inadequate medical care was usually insufficient and combined with breach of care standards.

The majority of medical errors happened due to different objective reasons. Criminal negligence was identified in about 20% of medical malpractice cases.

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PHYSICAL ACTIVITY LEVELS IN FEMALE STUDENTS OF TERNOPIL STATE MEDICAL UNIVERSITY

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Background. *Lifestyle is one of the major factors in the optimal development of systems and functions of human organism and health. The appropriate level of physical activity is the integral component of a healthy lifestyle.*

Objective. *The study was aimed to investigate the level of physical activity of the female students of Medical University.*

Methods. *We applied the International Physical Activity Questionnaire (IPAQ) in the long version as a research method.*

Results. *The study was conducted among 333 female students of Medical University of the first, second and third year of study. Detailed International Physical Activity Questionnaire evaluating physical activity, such as work, housework, sport, and physical activity during leisure time, was completed.*

Conclusions. *The examined female students of medical university are characterized by high level of total physical activity. Place of residence during the studies does not differentiate significantly the level of physical activity of female students.*

KEY WORDS: **physical activity, the International Physical Activity Questionnaire, female students, medical university.**

Introduction

Lifestyle is one of the major factors in the optimal development of systems and functions of human organism and health. Healthy lifestyle is a way of living aimed at preserving and improving state of health. The appropriate level of physical activity is the integral component of a healthy lifestyle (a natural biological need defined by the set of movements performed by a person) [1, 2]. Many scientific studies proved the value of physical activity for the human body [3–8].

Physical activity is an integral part of man's life which largely affects the cardiovascular and muscular-bone systems of the body, and has a significant impact on body metabolism. In current living conditions people focus less on physical activity due to well-developed transporting infrastructure, sedentary work and passive rest at home. Such way of live may lead to the development of various health risks. At lack of physical activity and excessive neuro-emotional stress the functional state of the central nervous system, as a mediator between the muscles and internal organs, is damaged. This causes the dysfunction of individual organs and systems of the body and contributes to the development of various diseases.

The influence of physical activity on human health is widely recognized and studied among international researchers [9–13]. The issues of physical activity of students both in bio-physiological and cultural terms are more and more commonly discussed on different continents: South Asian [14], South Africa [15, 16], Europe [17–19] and in other different countries [6, 7, 20–22], including medical universities students [23–26].

The research studies [6, 7] show the tendency of the decrease of physical activity among students of higher educational institutions. Therefore, the subject of our research was to

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investigate the level of physical activity of the female students of Ternopil State Medical University.

Material and Methods

The study was conducted among 333 female students of Ternopil State Medical University during October 2015. The participants were the first-, second- and third-year-students aged 17-20 years old. The total of 333 female students included: 106 first-year-students (31.83%), 116 second-year-students (34.83%), and 111 third-year-students (33.33%). They were asked to complete questionnaires on demographic and general physical activity. We applied the International Physical Activity Questionnaire (IPAQ) in the long version [27] as a research method. Detailed International Physical Activity Questionnaire (IPAQ) evaluating physical activity, such as work, housework, sport, and physical activity during leisure time was completed. Dependence analyses were carried out using t-test and Pearson correlation coefficient. The level of statistical significance was adopted at $p<0.05$.

Results

The results of the research have shown significantly higher level of total physical activity of female students 5590.2 MET min/week than in Poland [28] and Turkey [20]. On the basis of the International Physical Activity Questionnaire (IPAQ) it was evidenced that the activity associated with work amounted 1428.9 MET min/week. In case of the activity associated with movement it amounted 1443.7 MET min/week. For physical activity associated with housework the results were recorded at 1162.2 MET min/week. Physical activity in sport amounted 1555.4 MET min/week. Thus, the highest level of physical activity was identified in female students during sports workouts and the lowest during housework, but these results did not differentiate significantly (Fig. 1).

Based on the data obtained from IPAQ questionnaire on the course of study, we analysed the dynamics of physical activity during in the learning process. We established that the highest level of total physical activity of 6955.8 MET prevails in sophomores, which is by 24.42% higher than the total-level of PA, while the lowest level of total physical activity of 3855.5 MET was found in the third-year female stu_dents, which is by 31.0% less than the total level of PA. Such high rate of physical activity index in the second-year-students was observed due to high levels of physical activity at work/study, which was in 2812.7 MET (40.43%), when the index of physical activity at home was 1212.2 MET (17.42%), in the sport — 1519.4 MET (21.84%), and the rest — 1411.6 MET (20.29%). However, in the third-year female students compared to the students of the first and second years of study the lowest level of physical activity during work/training of 463.3 MET (12.01%), with activity around the house of 939.1 MET (24.35%), in the sport — 1380.7 MET (35.81%), and the rest — 1072.4 MET (27.81%) was evidenced. When comparing different types of activities of the female students, excluding physical activity during work / study, it was found that freshman have higher physical activity among students at home — 1341.3 MET, in sport — 1777.7 MET, and the rest — 1867.6 MET,

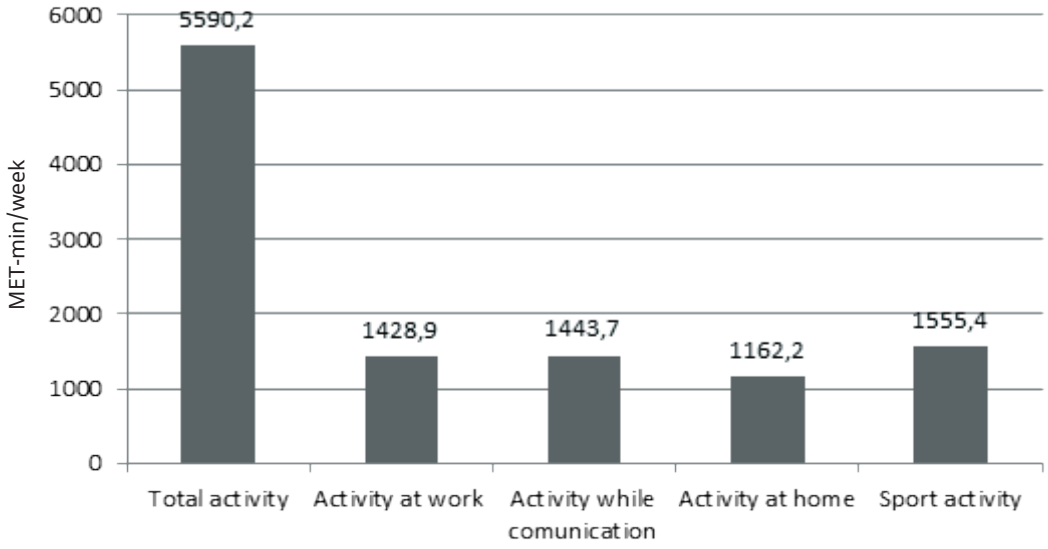

Figure 1. The level and areas of physical activity of the female students

than the second- and third-year female students, which have the lowest physical activity in all types of physical activity (Fig. 2, Table 1).

We found it interesting to discover whether the place of living of the recipients influence upon the level of their physical activity. Three places of residence were used for the analysis: dormitory, family home and parents' apartment. Also this combination showed no significant differences in the total physical activity of the female students depending on the place of daily residence (Fig. 3, Table 2).

Discussion

As a result of our research we have discovered a high level of overall physical activity in female students of Medical University (55902 MET min/week). After analysing the dynamics of physical activity of female students depending on the year of study we have found out that the highest overall physical activity (6955.8 MET) prevailed in the second-year students. Such high rate of physical activity in sophomores was due to high level of physical activity at work/study (it was 40.43% of the total recipients of physical activity). This fact can be explained by the location of university departments and buildings, where classes for the second-year-students took place and by the lack of direct transportation between those locations. The average level of physical activity at the work/school is 463.3 MET in the third-year-students and it may explained by the increased duration of classes. In all other areas of physical activity (work, at home, leisure or sport) students showed high level of the researched activity, but it tended to slight decrease from the first to the third year of study. As for the residence of students, no direct connection between the place of residence (dormitory, apartment or parents' house) and the physical activity was found [17].

Thus, we can assume that regardless of the year of study and the place of residence the main factor of physical activity type among the female students of medical university is their correct (common sense) understanding of the role of this factor in a healthy lifestyle.

However, is should be emphasised that a questionnaire is the cheapest method of collec-

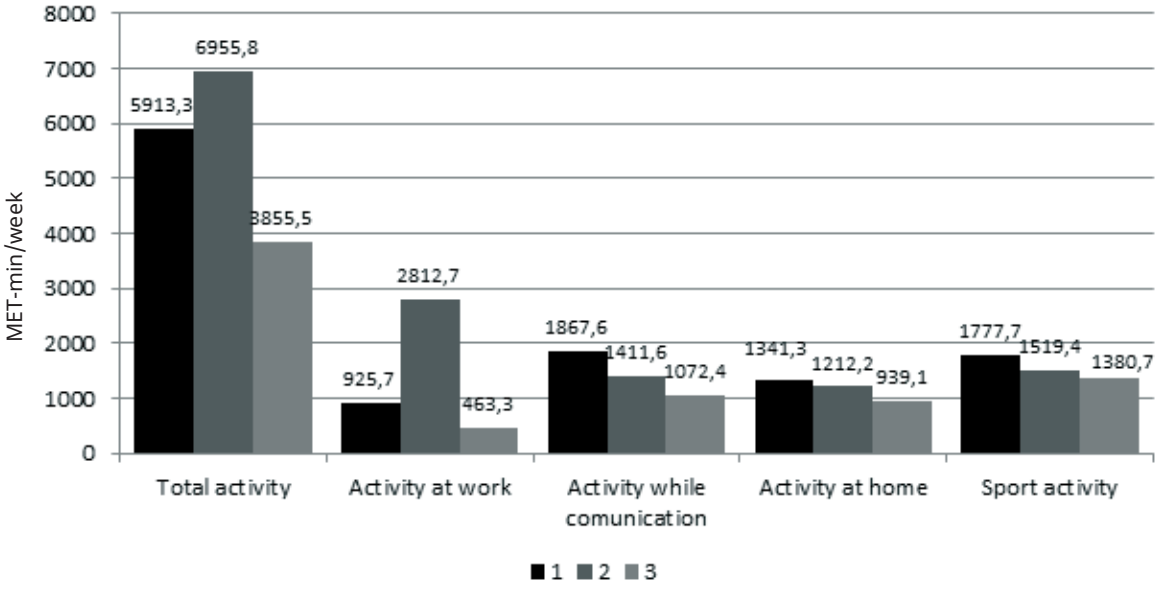

Figure 2. The level and areas of physical activity of the female students with regard to the year of study

Table 1. The differentiation of the level and areas of physical activity of the female students with regard to the year of study

Kruskal-Wallis Test			
Physical Activity Type	H	p	Differentiation
Total activity	29,47	0,0001*	1-3; 2-3**
Activity at work	38,21	0,0001*	1-2,3; 2-3**
Activity while commuting	14,30	0,0008*	1-3**
Activity at home	7,84	0,0198*	1-3**
Sport activity	8,47	0,0145*	1-3**

Note. * — significant differences at p<0,05; ** — years of study between which there is a statistically significant variation.

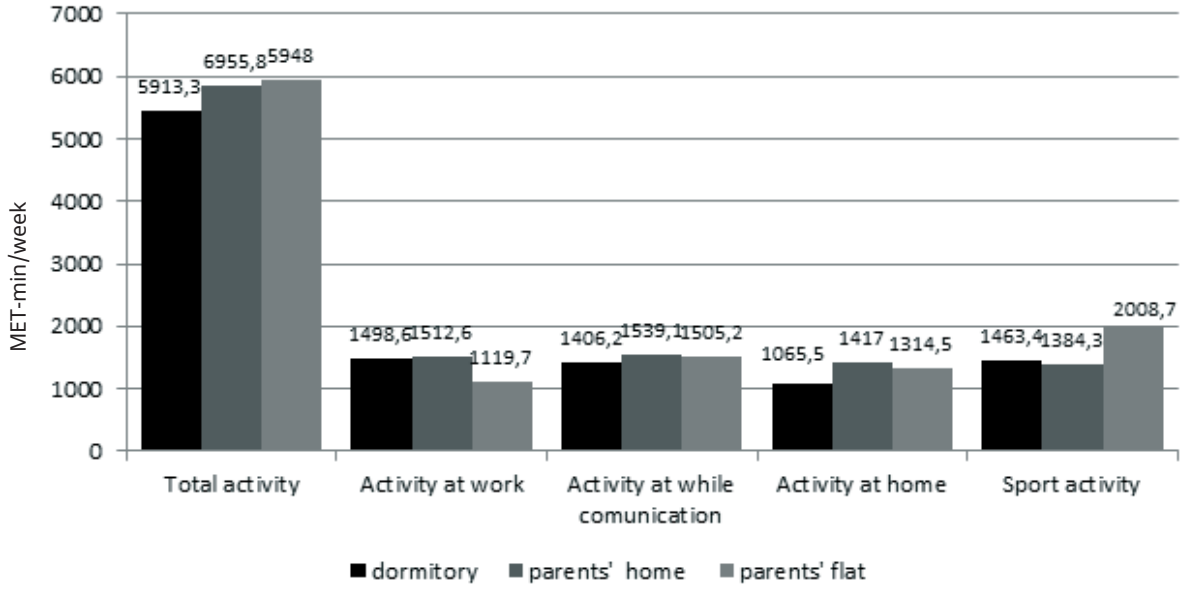

Figure 3. The level and areas of physical activity of the female students with regard to the place of residence

Table 2. The differentiation of the level and areas of physical activity of the female students with regard to the place of residence

Kruskal-Wallis Test			
Physical activity Type	H	p	Differentiation
Total activity	0,32	0,8505	-
Activity at work	0,58	0,7477	-
Activity while commuting	0,11	0,9484	-
Activity at home	2,03	0,3616	-
Sport activity	5,14	0,0767	-

ting data on a large number of respondents (national and international research) regardless of their sex, age or health [29].

Conclusions

The female students of medical university are characterized by high level of total physical activity compared with the male students.

The highest level of total physical activity prevails in the second-year female students.

The place of residence during the studies (dormitory, family house or apartment) does not differentiate significantly the level of physical activity of the female students.

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BIOCHEMICAL PARAMETERS OF LIPID METABOLISM IN ANIMALS AFFECTED BY HEAVY METAL SALTS AND TREATED WITH CARNITINE CHLORIDE AND SODIUM ALGINATE

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Background. *Lipid metabolism disorders in the organism affected by environmental pollutants, including poisoning with cadmium and lead salts are of topical matter nowadays.*

Objective. *The study was aimed to examine biochemical features of lipid metabolism in rats subjected to toxic damage by lead and cadmium salts and treated with carnitine chloride and Algigel.*

Methods. *Experiments were carried out on white mature outbred male rats weighing 180-200 g. To cause the toxic damage the animals were administered with aqueous solution of cadmium chloride and lead acetate daily for the period of 30 days using intra-gastric lavage. The indices of lipid metabolism were detected by biochemical methods.*

Results. *In animals treated with cadmium chloride and lead acetate the following changes were observed: HDL-cholesterol concentrations significantly decreased, resulting in 87% of the levels in the intact animals on the third day, 84% on the fifth and 80% on the seventh day. Conversely, concentrations of HDL-cholesterol and VLDL-cholesterol significantly increased during the experiment. Respectively, the ratios for HDL-cholesterol are 240%, 352%, and 388%; and for VLDL-cholesterol 108%, 116%, and 132%.*

Conclusions. *Lipids profile of the rats displayed changes in the levels of cholesterol, triglycerides and lipoproteins of low, high and very low density.*

KEY WORDS: **blood lipid profile, heavy metal toxicity.**

Introduction

The lipid metabolism disorders in the organism affected by environmental pollutants including poisoning with cadmium and lead salts were extensively studied. Salts of these metals are assigned Class II of toxicity in the European Union's classification system, which is regulated by Directive 67/548/EEC. They have a large migration ability (in the soil to plant, and animal to person systems), and also high cumulativeness [4, 14]. This creates a threat of their increased uptake if an organism is exposed to several contaminated sources at once: inhaling the air (car exhaust), soil particles along the roadways, industrial dust, and ingesting fertilizer residue with vegetables and produce [8, 9]. Due to their association with industrial waste, these compounds often occur in combination. However, although there is a large amount of the literature describing mechanisms of action of either lead or cadmium salts on the body, the number of studies of the combined effect of these two toxins is limited [16, 17].

This study demonstrates that in rats lipid metabolism disorders caused by the combined toxic effect of Cadmium (Cd) and Plumbum (Pb) ions were effectively corrected after carnitine chloride and sodium alginate administration. This conclusion is proved by changes in the concentrations of lipid metabolism biomarkers in blood plasma.

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Material and Methods

Experiments were carried out on white mature outbred male rats weighing 180-200 g, which were kept on a standard diet and housed in the animal facility of Ternopil State Medical University. The animal maintenance, treatment and euthanasia were conducted in accordance with the internal safety and ethical regulations, as well as European convention for the protec-

tion of vertebrate animals used for experimental and other scientific purposes [15], and Applied recommendations for the maintenance and work with laboratory animals [10].

To cause the toxic damage, animals were administered with aqueous solution of cadmium chloride, dose 3.3 mg/kg (0.05 LD_{50}), and lead acetate, dose 11 mg/kg (0.05 LD_{50}), daily for the period of 30 days using intra-gastric lavage [2].

The treatment used for toxic damage correction consisted of 2% solution of carnitine, dose 50 mg/kg, and enterosorbent (chelator) Algigel, dose 400 mg/kg [5, 12]. Both of these substances were administered daily using intragastric lavage.

All experimental animals were divided into the following groups: Group 1, intact rats; Group 2 (control), animals treated with cadmium chloride and lead acetate; Group 3, animals treated with cadmium chloride, lead acetate as well as carnitine and Algigel enterosorbent.

After finishing the heavy metals and corrective agents administration, we tested the lipid metabolism rates on the 3rd, 5th and 7th days. To determine lipid metabolism the following methods were used: triacylglycerols (TG) were extracted from blood serum using isopropanol with simultaneous removal of phospholipids mix from the reaction by precipitation with aluminum oxide [3]; the presence of cholesterol was determined using a colour reaction whereby in the presence of acetate anhydride and a mixture of acetic and sulphuric acid a green compound was formed [1]. Levels of cholesterol were evaluated by the intensity of the colour using colorimetry. Free cholesterol was detected using digitonin precipitation [3]. Cholesterol-digitonin complex was subsequently dissolved in chloroform, evaluated by Liebermann–Burchard test. The level of high-density lipoprotein (HDL) cholesterol was determined in soluble fraction after low density (LDL) and very low-density (VLDL) cholesterols in blood plasma were precipitated using heparin in the presence of manganese ions [4]. Levels of LDL and VLDL cholesterols were evaluated by mathematical models.

The results of the study were analyzed by the Department of Statistical Research of Ternopil State Medical University using STATISTICA software (StatSoft). Arithmetic mean of the sample (m), its variance and mean squared error (MSE) were calculated for all biochemical indices. Significance of the difference between independent quantitative values was determined using Student's t-test.

Results and Discussion

Cadmium and lead belong to a group of heavy metals; their ions have the ability to accumulate in the body. Since in this study these substances were administered for 30 consecutive days in subthreshold doses, we looked for general toxic effect in addition to the parameters of lipid metabolism. Thus, for the duration of the experiment, we observed the influence of cadmium chloride and lead acetate on the overall condition of the animals, change in body mass, peripheral blood composition and certain other hematological parameters [7, 8, 11, 13].

The early symptoms of poisoning with heavy metal are: rejection of food by the animal, general depression, and weight loss. Our observations indicate that on the 5th day of the experiment, the control group of animals sometimes refused their food or ate smaller quantities compared to the intact animals. We also observed lethargy, reduced movements, and changes in fur cover. To prove these observations, we measured body mass of the intact and control animals, which allowed us to determine its changes.

We determined the levels of total cholesterol, triacylglycerols (TG), HDL, LDL and VLDL cholesterol in plasma of the experimental and control animals [6]. The data presented in Table 1 proves that in plasma of the animals injected with heavy metals, the concentrations of TG significantly increased compared to intact animals, for instance on the third day the it was 186%, on the fifth — 179%, and on the seventh — 166% of the levels of the intact animals.

We consider that the increase of triacylglycerol levels in response to prolonged cadmium chloride and lead acetate intoxication occurs according to a specific mechanism. The intoxication by salts of lead and cadmium causes disruption of energy processes at the mitochondria. This evidences the activation of tissue lipases and mobilizing of fatty acids from fat depots, which are rapidly delivered to the liver synthesizing the surplus amounts of triacylglycerols.

Cholesterol is an important indicator allowing lipid metabolism evaluation. Under physiological conditions, the levels of cholesterol in the body are in a state of equilibrium between HDL and LDL cholesterols: the amount of cholesterol incoming with food and synthesized *de novo* corresponds to what is excreted as bile

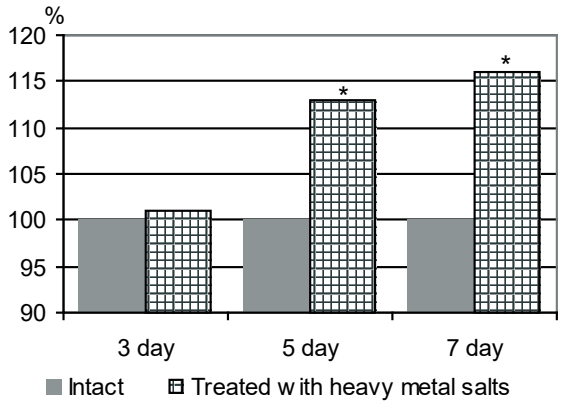

Figure 1. Total cholesterol content in blood plasma of the animals.
Notes: * — significant difference if compared to the control animals.

acids and free cholesterol. In this study, the concentration of total cholesterol in blood of the intact animals was 2.18±0.09 mmol/L (Table 1), but it was different in the group of the animals that received heavy metal salts. The maximal differences between the two groups were recorded on the seventh day after the beginning of toxicant administration, when cholesterol concentration increased by 16% in the treated animals compared to the intact ones.

Cholesterol is present in blood as its free form and its esters of fatty acids. Each of these forms has specific physiological functions and diagnostic value for understanding of lipid metabolism disorders. Free cholesterol is exchanged between tissues, so its concentration in serum reflects the total content of cholesterol in the body. The results of this study prove that there are significant differences in the rates of different form of cholesterol between the intact and control groups.

Thus, toxic action of heavy metal ions disrupts esterification of cholesterol decreasing concentrations of its esters in blood serum.

Lipids are transported in blood as complex supramolecular complexes, lipoproteins. Therefore, in this study we determined concentrations of different lipoprotein classes. In the animals treated with cadmium chloride and lead acetate the following changes were observed: HDL-cholesterol concentrations significantly decreased, resulting 87% of the levels in the intact animals on the third day, 84% on the fifth day and 80% on the seventh day. Conversely, the concentrations of HDL-cholesterol and VLDL-cholesterol significantly increased during the experiment. Respectively, the rates for HDL-cholesterol were 240%, 352%, and 388%; and for VLDL-cholesterol — 108%, 116%, and 132% (Table 1).

Treatment of exogenous intoxication involves the use of antioxidants, metabolic factors,

Table 1. Concentrations of total cholesterol, triacylglycerols, HDL, LDL and VLDL cholesterol in rats' blood serum during 30-day administration of cadmium chloride and lead acetate (n=6)

Intact animals, group 1	Animals treated with cadmium chloride and lead acetate, group 2		
	3rd day	5th day	7th day
0.86±0.06	1.60±0.09 p_1<0.01	1.54±0.08 p_1<0.01	1.43±0.08 p_1>0.05
Total cholesterol, blood plasma, mmol/L			
2.18±0.09	2.21 ±0.17 p_1>0.05	2.46±0.18 p_1>0.05	2.54±0.18 p_1>0.05
Esterified cholesterol, blood plasma, mmol/L			
1.52±0.07	0.91±0.07 p_1<0.05	0.96±0.09 p_1<0.05	1.27±0.08 p_1<0.05
Free cholesterol, blood plasma, mmol/L			
0.66±0.04	1.30±0.11 p_1<0.05	1.50±0.11 p_1<0.05	1.27±0.08 p_1<0.05
HDL-cholesterol, blood plasma, mmol/L			
1.54±0.02	1.34±0.11 p_1>0.05	1.29±0.04 p_1<0.01	1.24±0.04 p_1<0.001
LDL-cholesterol, blood plasma, mmol/L			
0.25±0.07	0.60±0,03 p_1<0.01	0.88±0.13 p_1<0.01	0.97±0.10 p_1<0.01
VLDL-cholesterol, blood plasma, mmol/L			
0.39±0.01	0.27±0.03 p_1<0.02	0.29±0.01 p_1<0.001	0.33±0.04 p_1>0.05

Notes: p_1 — significant difference if compared to the control animals.

cell membrane protectors and also reducing systemic concentrations of toxic components in the body. The use of so-called efferent therapies, including enterosorbtion is one of the approaches to removing foreign substances and metabolic products.

In this study, we used the combination of carnitine and enterosorbent sodium alginate. The changes of total cholesterol in blood plasma of the experimental animals were as follows. During the whole experiment we observed significant and linear decrease of cholesterol contents in the experimental animals compared to the control group. On the third day cholesterol levels were by 75% lower in experimental group, on the fifth — by 62%, and on the seventh — by 56%, which was lower than in the intact animals (Table 1). This decrease was in the free cholesterol, while the concentration of esterified forms significantly increased for the period of the experiment.

In this study, we evidenced the positive effect of combined use of carnitine and sodium on the concentrations of HDL, LDL and VLDL cholesterol. The combined effect of toxicants caused a marked reduction in the concentration of HDL cholesterol at all stages of the experiment compared to the intact animals (Figure 2).

Subsequent treatment with carnitine and Algigel caused a significant increase in these rates. Thus, on the 3rd day it increased by 12%; on the 5th by 22%; and on the 7th by 26% compared to the control animals. These rates, however, were significantly different from the corresponding rates in the intact group.

When treated with the corrective factors, carnitine and Algigel, the animals demonstrated significant decrease of LDLC concentration. In particular, on the 3rd day this rate decreased in 3.6 times in comparison with the control animals. On the 5th day the concentration decreased more and was in 5.8 times lower than in the control animals, and on the 7th day it was in 8 times lower.

In the experimental group, the concentration of VLDL cholesterol on the 3rd day increased by 79% compared to the animals of the control group, and on the 5th and 7th days by 50% and 36% respectively.

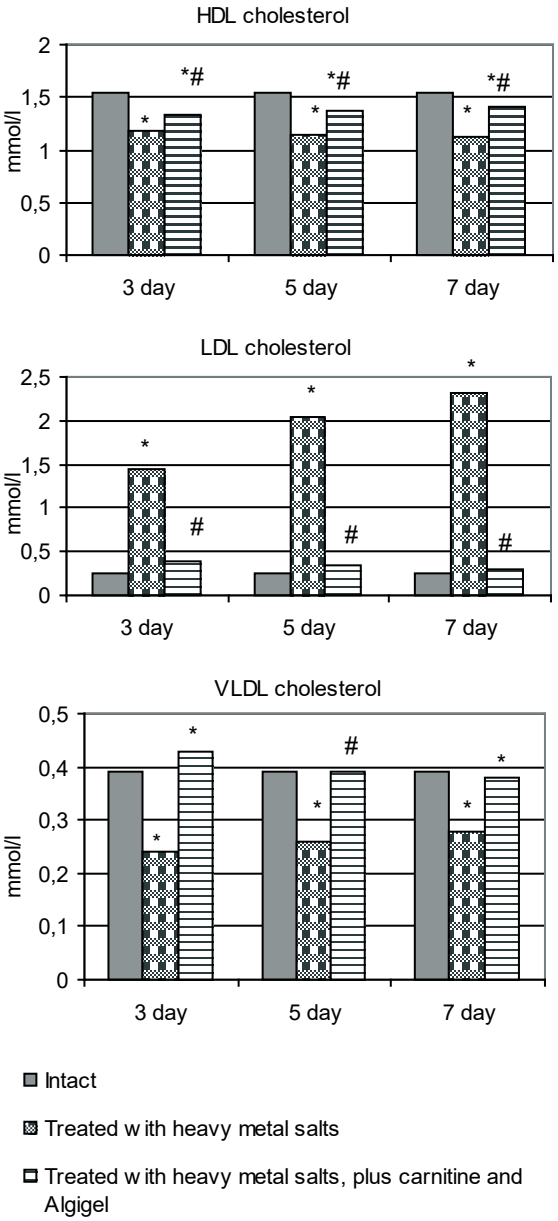

Figure 2. Changes in the concentrations of HDL, LDL and VLDL cholesterol in blood plasma of the animals.
Notes: * — significant difference if compared to the control animals;
— significant difference if compared to the affected animals.

Conclusions

This study proved that carnitine in combination with sodium alginate had a clear positive effect on some parameters of lipid metabolism in blood plasma of rats. The proposed means of correcting biochemical changes caused by poisoning with lead and cadmium salts in subthreshold doses demonstrated the need for further studies to develop possible use in clinical settings for correction of lipid metabolism and reducing toxic effects of xenobiotics.

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AGE RELATED OXIDATIVE PROCESSES AND ENDOGENOUS INTOXICATION DYNAMICS OF RATS ARTER TOBACCO SMOKE AFFECTION

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Background. *In an experiment on rats in the content of reactive oxygen species and lipid peroxidation products in blood and liver of rats of different age groups after 45-day affection with tobacco smoke was studied.*

Objective. *The study was aimed to investigate the rate of reactive oxygen species formation, especially the processes of lipid peroxidation and degree of endogenous intoxication in rats of different age groups in terms of 45 day affection with tobacco smoke.*

Methods. *The content of ROS was determined in blood neutrophils method gradient centrifugation, the activity of free radical processes in rats evaluated the content of TBA - active products (TBA - AP), the degree of endogenous intoxication - the content average molecular weight (AMW) of the two factions – MW1 (dominated by chain amino acids) and MW2 (dominated by aromatic amino acids). Blood, blood serum and liver of the experimental rats were used for the investigation. It was prepared 10% homogenate in saline from liver tissue.*

Results. *We have noticed that the destruction of rats by tobacco smoke for 45 days caused the increase of ROS in blood content, which is the result of toxic effect on the body. The immature rats were the most sensitive to the affection, which ROS contents in neutrophils increased in 2.75 times till the end of the experiment, in the mature animals it was in 1.65 times higher than in the intacts, in the senile it was higher in 2.43 times than normal rate. It was found that the level of oxygen metabolites and TBA-active products increased during the experiment (on the 15th, 30th and 45th day of toxicity).*

Conclusions. *The most pronounced changes were inherent for the immature rats. Accumulation of active toxic metabolites was conducted with endogenous intoxication intensifying that was proved by the high content of catabolism products in the body – middle mass molecules that are likely to grow in serum of rats of all age groups.*

KEY WORDS: **oxidative processes, lipid peroxidation, reactive oxygen species, endogenous intoxication, tobacco smoke, rats.**

Introduction

At present tobacco smoke is one of the most common anthropogenic agents that has a wide range of effects on morphofunctional status of various systems of the body [8, 10, 15, 16]. The share of tobacco smoke in the overall air pollution is quite considerable and each year continues to rise, so it is one of powerful polluters of the environment. It is established that the basis of the pathogenic action of contaminated pollutants or cigarette smoke air is the oxidant aggression on the mucosa of respiratory tract reactive oxygen species, nitrogen dioxide and sulfur and other free radicals, which cause the activation of lipid peroxidation and damage of biological membranes [3, 11]. Smoking generates the reactive oxygen species (ROS: $O_2^{\cdot-}$, O_2^1, OH, H_2O_2, etc.), which are important for many physiological and biochemical processes: the regulation of vascular tone, cell proliferation, prostaglandins synthesis, signals transmission from intercellular signalling molecules of regulatory system that control the expression of phagocytes genes antimicrobial action [6, 9, 14]. Under the influence of extreme factors of various origin (chemical contamination, ionizing radiation, hyper- and hypoxia, toxic substances, inflammatory processes) formation of ROS in organisms is enhanced [12,

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13, 17, 18]. The last causes free radical oxidation activation that leads to increased lipid peroxidation (LPO), oxidative modification of proteins (OMP), degradation of nucleic acids, carbohydrates, increasing of endogenous intoxication in the organism.

However, in literature there is no definitive data on the rate of formation of ROS and processes of lipid peroxidation activation in rats of different ages after prolonged exposure with tobacco smoke.

Accordingly, the study was aimed to investigate the rate of reactive oxygen species formation, especially the processes of lipid peroxidation and degree of endogenous intoxication in rats of different age groups in terms of 45-day affection with tobacco smoke.

Material and Methods

The experiments were conducted on white outbred male rats, which were kept on a vivarium standard diet of Ternopil State Medical University. The rats were divided into three age groups: the first — immature, weight 60–80 g, the second — mature, weight 180–200 g and the third — senile, weight 300–320 g. Each age group consisted of two subgroups: an intact control (C) and an experimental group (E). The rats of the experimental groups were affected with tobacco smoke during 45 days. The model of the chronic smoke was created by means of airtight chamber volume of 30 litres that allowed animals to fumigate free behaviour. Tobacco smoke was formed by smoking of 6 cigarettes Prima sribna (synia) (0.6 mg of nicotine and 8mg of tar), was served into it through openings in the chamber. Six animals were simultaneously in the chamber during 6 minutes. The animals of the control group were also 6 minutes in a sealed chamber, but were not subjected to smoke.

In 15, 30 and 45 days after the beginning of the affecting the animals with tobacco smoke, they were taken out of the experiment by euthanasia, which was performed with thiopental anaesthesia.

Blood, blood serum and liver of the experimental rats were used for investigation. 10% homogenate in saline of liver tissue was prepared.

The content of ROS was determined by blood neutrophils method [6], the activity of free radical processes in rats evaluated the content of TBA-active products (TBA-AP) [5], the degree of endogenous intoxication — the average molecular weight (AMW) content of two factions [2, 7]: MW_1 (dominated by chain amino acids) and MW_2 (dominated by aromatic amino acids).

We followed general principles of animal experiments in the research that were approved at the National Congress on Bioethics (Kyiv, Ukraine, 2001) and consistent with the provisions of the European Convention for the Protection of vertebrate animals used for experimental and other scientific purposes (Strasbourg, France, 1985) [4]. Parametric (according to Student) and non-parametric (according to Wilcoxon) methods for data statistical analysis were used. Changes were considered as significant at $p \leq 0.05$.

Results and Discussion

Any stress reaction normally is accompanied by a brief increase in the number of ROS [6]. This is due to adaptation to extreme conditions in which ROS play a role of a secondary messenger participating in signal transduction and activation of transcription factors and related genes, including those encoding enzymes antioxidants.

We noticed that the affection of the rats by tobacco smoke during 45 days caused the increase of ROS in blood content (Table 1), which is the result of toxic factor on the body. The immature rats were the most sensitive to the affection, the ROS contents in neutrophils increased in 2.75 times at the end of the experiment, in the mature animals it was in 1.65 times higher than in the intacts, in the senile it was in 2.43 times higher than normal rate.

It is established that under the influence of various extreme factors (chemical contami-

Table 1. The contents of ROS (%) in blood neutrophils of the rats affected with tobacco smoke of all ages (M±m; n=72)

Research time, days	Groups of the experimental animals		
	immature rats	mature rats	senile rats
intact rats	15.06±0.71	18.47±0.22	19.87±0.86
15th day of the affection	17.19±0.83	28.58±2.53*	25.38±1.95
30th day of the affection	39.25±1.29*	29.54±0.50*	41.89±0.78*
45th day of the affection	41.52±2.37*	30.63±0.66*	48.32±0.57*

Note: * — differences between the intact rats and the rats affected with tobacco smoke ($p \leq 0.05$).

nation, ionizing radiation, hyper- and hypoxia, toxic substances, inflammatory processes) the formation of ROS in organisms is enhanced. Among the reasons that causes of the increased production of ROS are: violation of electron transport in the respiratory chain of mitochondria and electron transport chain microsomes, the intensification of synthesis and oxidation of catecholamines, the increased degradation adenylate nucleotides and activation of xanthine oxidase, the emergence of a pool of catalytically active metal ions of variable valence (especially Fe^{2+}), the synthesis of prostaglandins from arachidonic acid (reactions catalysed cyclooxygenase and lipoxygenase), the activation of inducible form of nitric oxide synthase, the increased activity of phagocytes [14].

The intensification of free radical oxidation reactions is one of the manifestations of oxygen metabolites toxic effects. Free radical oxidation is a universal mechanism, which controls the most important homeostatic physical and chemical parameters of cells: strength, integrity and selective permeability of cell membranes [3].

A significant increase of ROS, which was evidenced after poisoning the animals with tobacco smoke, caused the intensification of free radical oxidation including lipid peroxidation. As one of the indicators of lipid peroxidation, the contents of TBA-AP significantly increased in serum and liver of the rats after tobacco smoke intoxication (Table 2). In serum of the immature animals on the 15th day of the intoxication, this indicator increased by 30% (changes were not significant). The other age groups in this period were more sensitive: the TBA-AP content in serum increased by 87% in the mature animals and by 58% in the senile ($p \leq 0.05$).

The intoxication with tobacco smoke during 45 days caused the significant activation of lipid peroxidation, which was evidenced by considerable increase of TBA-AP content in serum of the animals of all experimental groups (in the immature rats the indicator increased in 2 times, in the immature — in 2.1 times, in the senile — in 1.8 times).

In liver of the experimental animals a similar increase of investigational product lipid peroxidation was observed during the experiment. By the end of tobacco smoke toxicity (on the 45th day of the research), the increase of TBA-AP content in liver of the mature and senile animals in 1.8 times respectively was evidenced. The immature animals were more sensitive to this indicator and the content of intermediate peroxidation increased in 3.2 times in the test organ. Perhaps this is due to insufficient decontaminating of liver, including oxidation processes and microsomal enzymes that took part in it. In pathological processes the average molecular weight (AMW) is quite important, some its fraction exhibit high biological activity [1]. The spectrum of pathological action of these compounds has a different character: they break the physical and chemical properties of cell membranes, inhibit tissue respiration and oxidative phosphorylation, inhibit ATP activity and DNA synthesis, activate lipid peroxidation and reduce the activity of antioxidant defence cells, stimulate cellular proliferation and inhibiting the process of apoptosis [2]. AMW accumulation is a consequence of the activation of catabolic processes and reduction of liver detoxification function. Metabolic syndrome intoxication is developing, which leads to severe functional impairment and morphological damage to various organs and systems. Activation of ROS-formation processes and lipid peroxidation causes increased proteolysis reactions in the body affected with tobacco smoke, accompanied by accumulation of both factions AMW — AMW_1 (dominated by chain amino acids) and AMW_2 (dominated by aromatic amino acids). The results of the

Table 2. The content of TBA-AP in blood serum (mmol/L) and liver (mmol/kg) of the rats in dynamics of tobacco smoke affection (M±m; n=72)

Research time, days	Groups of the experimental animals		
	immature rats	mature rats	senile rats
	blood serum		
Intact rats	3.28±0.23	1.85±0.14	2.35±0.14
15th day of the affection	4.28±0.31	3.47±0.29*	3.71±0.28*
30th day of the affection	4.85±0.36*	4.28±0.31*	4.42±0.28*
45th day of the affection	6.62±0.08*	3.94±0.27*	4.23±0.17*
	Liver		
Intact rats	15.49±1.28	14.42±0.71	16.55±0.98
15th day of the affection	18.69±1.28	23.50±1.35*	25.12±2.09*
30th day of the affection	30.86±1.55*	28.73±0.78*	29.06±1.46*
45th day of the affection	49.66±3.38*	26.49±1.51*	29.69±2.39*

Table 3. AMW content in blood serum (cu/L) of the rats in dynamics of tobacco smoke affection (M±m; n=72)

Research time, days	Groups of the experimental animals		
	immature rats	mature rats	senile rats
	AMW_1		
Intact rats	14.00±1.15	11.00±0.85	13.66±0.61
15th day of the affection	19.00±1.52	17.66±1.20*	18.00±1.55
30th day of the affection	37.00±1.12*	31.00±1.12*	35.66±0.61*
45th day of the affection	41.66±0.95*	33.00±1.52*	38.00±1.03*
	AMW_2		
Intact rats	16.66±0.84	12.67±0.84	15.33±0.67
15th day of the affection	23.00±2.29	15.33±0.66	20.33±1.82
30th day of the affection	28.67±0.67*	18.66±0.67*	23.33±0.99*
45th day of the affection	36.33±1.20*	21.66±1.20*	29.66±1.20

research of this indicator in blood serum are presented in Table 3.

After 15-day toxicity with tobacco smoke in blood serum of the immature rats the AMW_1 content increased by 35%, AMW_2 – by 38%. By the end of the experiment, these indices increased in 3 and 2.2 times respectively in this age group. At the end of the research in the mature rats the AMW_1-content in blood serum was in 3 times higher than in the intact animals, AMW_2-content was in 1.7 times higher than normal. Similarly, the increased content of MSM in the senile rats in 45 days after the tobacco intoxication in blood serum of this age group the AMW_1-content was in 2.8 times higher than the level of the intact control, the AMW_2-content exceeded the level of the healthy animals in 1,9 times. Analysing the processes of catabolism in the affected organism we can notice that they were activated in all age groups of rats similarly. Obviously, tissue hypoxia is the primary in the development of destructive processes in the organism after tobacco smoke toxicity due to the breach of oxygen transport.

Conclusions

Due to the chronic affection with tobacco smoke on the rats of different ages the massive formation of reactive oxygen species in neutrophils causes activation of lipid peroxidation and permanent changes of endogenous intoxication (the increase of TBA-active products in blood serum and liver of the effected rats, the accumulation of average molecular weight). Immature rats were the most sensitive to tobacco smoke; metabolic disorders were the most pronounced as well.

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LIPID PEROXIDATION IN MULTIPLE ORGAN FAILURE CAUSED BY ASSOCIATED CHEST AND HIP TRAUMA

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Background. *The injured with polytrauma are classified as extremely severe patients, as their emergency care and intensive care is followed by significant difficulties, frequent development of complications, high mortality and survivors disability.*

Objective. *This study is aimed to evaluate the effect of lipid peroxidation in heart, lungs, liver and blood of rats in early and late periods of combined traumatic injury of chest and hips and to establish their influence on the development of multiple organ failure.*

Methods. *The study was conducted on 70 adult male white nonlinear rats. It was determined the content of lipid hydroperoxides and concentration of TBA-active products in erythrocyte mass, heart, lungs and liver.*

Results. *The data prove activation of free radical oxidation at the first day of post-traumatic period. Analysis of the data evidences the increase in of lipid hydroperoxides (HPL) rate in liver homogenate in group E1 in 1.5 times, in all subsequent periods of the research the increase in rate fluctuated within 1.9-2.0 times. In blood and heart the HPL rate increased twice in group E1, reached maximum in group E2 and gradually decreased till the end of the experiment, data exceeded the control group. The highest level of HPL was determined in lung tissues (r≤0.01). In the post traumatic period the highest TBA-AP was detected in lungs and liver.*

Conclusions. *In case of simulated trauma (injury of chest with fractures of both hips) hyperactivation processes of free radical oxidation is observed in 1 day, reaching a peak in 7–14 days of post-traumatic period in blood, tissues, liver, heart and lungs if compared to the control group causing multiple organ failure***.**

KEY WORDS: **trauma, lipid peroxidation, lung, liver, heart.**

Introduction

Nowadays one of the most urgent problems of medicine are multiple and combined injuries [1, 2]. This is due to the constant increase of the number of multisystem and multiorgan injuries on the background of some general manifestations of injuries stabilization, which leads to mortality increase [3, 4]. As a result of constant technological advances the increase in the number of man-made disasters has led to a significant increase in the number of patients with polytrauma. According to WHO trauma is one of the top five causes of death among people aged 15–44; mortality from injuries and accidents takes the first place [5]. The injurees with polytrauma are classified as extremely severe patients, as emergency care and intensive care is accompanied by significant difficulties, frequent development of complications, high mortality and survivors' disability [6]. In case of multiple trauma, wounded are primarily affected by phenomena of traumatic-haemorrhagic shock and tissue hypoxia. In the post-traumatic period unnoticed injuries, infection, SIRS, sepsis and multiple organ failure threaten their life and health [7].

Therefore, our study was **aimed to** evaluate the effect of lipid peroxidation in heart, lungs, liver and blood of rats in early and late periods of combined traumatic injury of chest and hips and establish their influence on the development of multiple organ failure.

Material and Methods

The study was conducted on 70 adult male white nonlinear rats of 200–220 g body weight. Rats were housed under standardized laboratory conditions, with 12 h dark/light cycle and free access to food and tab water ad libitum. All procedures were conducted according to the European Convention for the Protection of

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Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasbourg, 1986) and General Ethical Principles of Experiments using Animals (First National Congress of Bioethics, Kyiv, 2001). The animals were randomly divided into 8 groups: 1 control (C1) and 5 experimental (E1, E2, E3, E4, E5) groups, each comprising 12 animals. Rats of experimental groups. The animals in the experimental group underwent thiopental sodium anaesthesia (40 mg/kg intraperitoneal rat) using a trocar modeled right-closed pneumothorax from fractured ribs and combined them with a broken left and right femur. Skeletal injury was modeled by applying a single shock dosed by a specially designed device on each thigh, which caused a closed fracture [8]. Impact energy was 0.375 J, which corresponded to the injury of moderate severity. Combined injury was modeled by sequential administration of these two injuries. Mortality of animals in each group was E1(12/11), E2(12/9), E3(12/10), E4(12/10), E5(12/9). At the end of the experimental period, the rats were sacrificed by decapitation.

The content of lipid hydroperoxides in erythrocyte mass, heart, lungs and liver was determined by [9]. This method is based on spectrophotometrical optical density measurement of the products of ammonium thiocyanate, hydrochloric acid and Mohr salt reaction. Lipids from the samples were preliminarily extracted with ethanol. Selection of tissue samples and preparation for extraction were performed at 4°C. Ethanol (2.8 ml) and 0.05 ml of 50% trichloroacetic acid (TCA) were added to 0.2 ml of hemolisate (dissolved in buffer solution with pH 7.4), and shaken for 5–6 min. Obtained protein precipitate was separated by centrifugation at 700 g. Ethanol (1.2 ml), 0.02 ml of concentrated HCl, and 0.03 ml of 1% Mohr salt solution in 3% HCl were added to 1.5 ml of supernatant. The mixture was stirred. After 30 s, 0.2 ml of 20% ammonium thiocyanate was added, and then the absorbance of the solution was determined at λ=480 nm. In a control sample, the appropriate amount of bidistilled water was added instead of supernatant. The content of lipid hydroperoxides was calculated by the difference between experimental and control values, and expressed in arbitrary units of optical density for 1 mg of protein.

The concentration of TBA-active products, characterizing the LPO rate, was assessed by Korobeinikova method based on the reaction between malondialdehyde (MDA) together with other products of peroxidation and thiobarbituric acid (TBA), occurring at high temperature and in acidic environment, and forming the colored complex of one MDA and two TBA molecules [10]. Protein concentration was measured by Lowry [11]. All reagents used were obtained from SigmaAldrich and Fluka (USA). The experimental data were processed by variation statistics methods by the OriginPro 8 program. Student t-test was used to determine the likely differences between the means of the samples. In all cases, reliable differences were considered by P value under 5% ($P<0.05$).

Results and Discussion

The data prove activation of free radical oxidation at the first day of post-traumatic period (Table 1 and 2). Activation of peroxide oxidation is one of the factors destabilizing membrane [12]. It is established that the action of hydroxyl radicals in diene conjugates fatty acids, lipid hydroperoxide is formed, which cause conformational irregularities in cell mem-

Table 1. The rates of lipid hydroperoxides in rat tissues (cu/mg protein) after combined trauma of chest and both hips (M±m)

Animal group	Blood	Heart	Lungs	Liver
Control	0.98±0.05	0.83±0.02	0.63±0.02	0.83±0.02
Trauma of chest and hips, 1st day of observation	1.96±0.01* p≤0.01	1.64±0.02* p≤0.01	1.62±0.01* p≤0.01	1.26±0.01* p≤0.05
Trauma of chest and hips, 3rd day of observation	2.36±0.08* p≤0.05	2.20±0.07* p≤0.01	1.79±0.01* p≥0.05	1.58±0.02* p≤0.01
Trauma of chest and hips, 7th day of observation	2.03±0.06* p≤0.01	1.82±0.01* –	2.04±0.05* p≤0.01	1.80±0.01* p≤0.01
Trauma of chest and hips, 14th day of observation	1.88±0.06* –	1.78±0.02* –	1.85±0.01* p≤0,01	1,65±0.01* p≤0,05
Trauma of chest and hips, 28th day of observation	1.71±0.04* –	1.62±0.04* –	1.62±0.01* p≤0.01	1.57±0.02* p≤0,01

Notes. Tables 1 and 2: * — significant differences between the baseline indicators of the studied and control groups; p — significant differences between the experimental groups.

Table 2. The rate of active products of thiobarbituric acid in rat tissues (mmol/mg protein) after combined trauma of chest and both hips (M±m)

Animal group	Blood	Heart	Lungs	Liver
Control	0.22±0.01	0.36±0.01	0.20±0.08	0.40±0.02
Trauma of chest and hips, 1st day of observation	0.40±0.01 p≥0.05	0.70±0.02* p≤0.01	0.42±0.02 p≥0.05	0.87±0.02* p≤0.01
Trauma of chest and hips, 3rd day of observation	0.75±0.02* p≤0.05	0.97±0.03* p≤0.01	0.68±0.02* p≤0.01	1.23±0.02* p≤0.01
Trauma of chest and hips, 7th day of observation	1.06±0.03* p≤0.01	0.80±0.02* p≥0.05	0.67±0.01* p≥0.05	1.76±0.03* p≤0.01
Trauma of chest and hips, 14th day of observation	0.86±0.02* p≤0.01	0.75±0.02* p≥0.05	0.60±0.02* p≤0.01	2.08±0.04* p≤0.05
Trauma of chest and hips, 28th day of observation	0.72±0.03* p≥0.05	0.68±0.03* p≥0.05	0.53±0.02* p≥0.05	1.87±0.02* p≥0.05

branes. Analysis of the data evidences the increase in HPL rate in liver homogenate in group E1 in 1.5 times, in all subsequent periods of observation the increased rate fluctuated within 1.9–2.0 times. In blood and heart the HPL rate increased twice in group E1, reached a maximum in group E2, and gradually decreased until the end of the experiment, data exceeded the control group (Table 1). The highest level of HPL was determined in lung tissues, particularly in E1 it exceeded in 2.6 times the control rate, respectively E2 — in 2.9 times, E3 — in 3.2 times, E4 — in 3.0 times and E5 — in 2.5 times (r≤0.01). Due to the fact that in case of trauma in lung tissues the activation of neutrophils is the main source of reactive oxygen species [13], and taking into account the administered chest injury, we believe that the pathological processes that occur in lungs in case of simulated injuries have a significant contribution to the development of multiple organ failure.

It is established that fatty acids in the ground connection break into fragments of aldehyde groups at the ends to form malonic dialdehyde. Analysing the change of active products thiobarbituric acid in rat tissues, different activity of lipid peroxidation in the studied rat organs was revealed (Table 2). The post traumatic period following combined chest trauma of both thighs on the 1st day surveillance the highest TBA-AP was detected in lungs and liver, respectively, in 3 days — in blood and lungs, in 7 days and in subsequent periods of observation — in blood and liver (Table 2).

For a comparative analysis of the flow of free radical oxidation in various organs combined chest trauma of both thighs was administered, the control group was regarded as 100%. It is established that the phenomenon of lipid peroxidation in heart was the most intense on the 3rd day after polytrauma simulation, respectively, in lungs — from the 3rd to the 7th day, in blood — on the 7th day, in liver — on the 14th day (Fig. 1). Scientists have proved that the maximum intensity of lipid peroxidation coincides with the body's response to inflammation

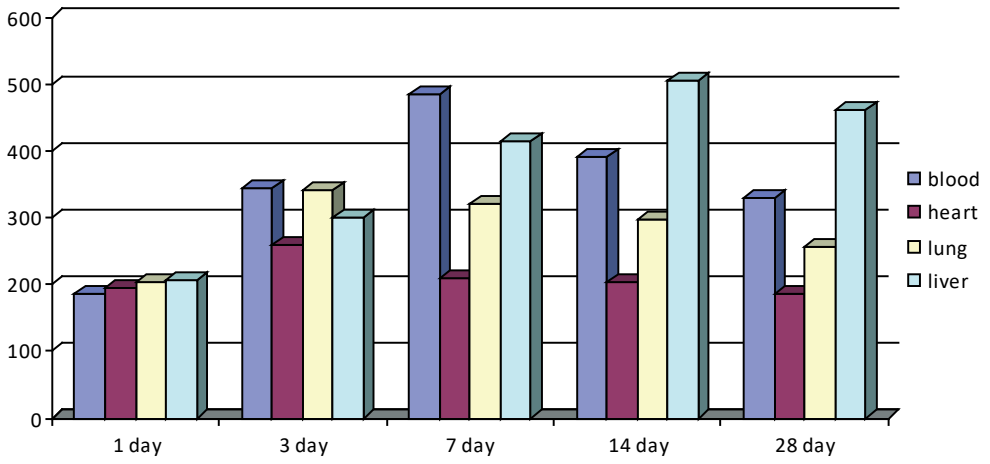

Fig. 1. Severity of the processes of lipid peroxidation (%) in rat tissues (as example, MDA-AP) in post-traumatic period following combined trauma of chest and both hips.

[14]. The development of hypoxemia caused by chest trauma affected lipid peroxidation stimulation causing disruption of cell membranes structure and consequently cell death closing the 'vicious' circle, which in our and other authors opinion cause multiple organ failure with involvement of heart, lungs, liver in the pathological process [15].

The data indicate that the intensity of lipid peroxidation in different tissues of body depends on injury type. Thus, Kozak D.V. in the research [16] notes that the intensity of lipid peroxidation due to blood loss and hip trauma has oscillatory nature of the duration increase up to the third day, the period of temporary welfare in 14 days and repeated increase in 21 days. Our results prove that various tissues experience peroxidation activation and its maximum occurs at different time.

Conclusions

In case of simulated trauma (chest injury with fractures of both hips) hyperactivation processes of free radical oxidation is observed in the 1st day, reaching a peak in 7–14th days of post-traumatic period in blood, tissues, liver, heart and lungs if compared to the control group that causes multiple organ failure.

The future prospects of the research are investigation of oxidant system dynamics after traumatic injuries of chest and hips and establishment of antioxidant-prooxidant disbalance.

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DAMAGING EFFECT OF IMMOBILIZING STRESS ON HYPOXIA HIGH- AND LOW-RESISTANT RATS OF BOTH SEXES

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Background. *Cardiovascular morbidity is a topical issue; stress is an essential contributing factor. Pathogenic links in damaging stress impact on the animal units of different reactivity is promising in disease prevention and development of individual correction methods.*

Objective. *Stress-induced development of cardiovascular pathology is undeniable, the stress impact depending on individual systemic response, age and sex.*

Methods. *96 hypoxia high- and low-resistant (HR and LR, respectively) Wistar rats aged 5.5-6 months were used in experiments. Changes of lipid peroxidation processes, as well as protein oxidative modification, nitrite anion content and the indices of antioxidant defence under immobilizing stress were studied in the research.*

Results. *Immobilizing stress causes the development of oxidative and carbonyl stress in HR and LR rats that is more pronounced in LR group; and the activation of antioxidant defence system. In males, stress development is concomitant with increased catalase activity as well as that of blood peroxidase, ceruloplasmin and reduced glutathione content, whereas increased catalase and ceruloplasmin activity has been found in HR females, and that of superoxide dismutase and ceruloplasmin — in LR group. As compared with the females, more intensive oxidative and nitroxidative stress, protein oxidative modification, and stress-related accumulation of circulating immune complexes have been found in the males.*

Conclusions. *The most intensive oxidative and nitroxidative stress, protein oxidative modification, and stress-related accumulation of circulating immune complexes have been found in the hypoxia low-resistant males.*

KEY WORDS: **immobilizing stress, resistance to hypoxia, heart, damage.**

Introduction

Stresses are common in daily life and work [1], the long-lasting ones being the most dangerous. The same stressor impacts the people of various sex and age differently [2], the aftermath depends on a person's reactivity, nervous and endocrine regulation, immune system, resistance to surroundings, etc. Cardiovascular morbidity is a topical issue; stress is an essential contributing factor [3]. Pathogenic links in damaging stress impact on the animal units of different reactivity is promising in disease prevention and development of individual correction methods.

Therefore, the research was aimed to determine the lipid peroxidation changes, nitrite anion and protein oxidative modification products content as well as of the antioxidant defence in heart of stress-exposed HR and LR rats of both sexes.

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Material and Methods

96 hypoxia high- and low-resistant (HR and LR, respectively) Wistar rats aged 5.5–6 months were used in the research. The animals were divided into groups: control and experimental (exposed to chronic stress), each group comprising 12 males and 12 females. Animal units with different resistance to hypoxia were separated in accordance with V. Ya. Berezovskiy method (1978) [4]. Stress was simulated by immobilization of the rats in supine position (1 hour/4 times, stress episodes at 72-hour interval). 24 hours after the 4th fixation the animals were withdrawn from the procedure [5].

Experiments were performed in a special room within the morning at 18–22 °C, relative humidity 40–60 % and illumination250 lux. The regulations of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Strasbourg, 18.03.1986), Resolution of the I National Congress on Bioethics (Kyiv, 2001) and the order of the Ministry of Health of Ukraine № 690 of 23.09.2009, were strictly followed.

The rats were euthanized by total heart bloodletting and previous thiopental sodium anaesthesia (60 mg.kg^{-1} b.w. intraperitoneally). Blood and heart samples were taken for the research. Concentration of diene conjugates (DC), triene conjugates (TC), Schiff bases [6], TBA-active products [7]; indices of protein oxidative modification (POM 370 and POM 430) [8], nitrite anion [9], circulating immune complexes (CIC) [10], superoxide dismutase (SDA) [11], catalase [12] activity, and ceruloplasmin (CP) content [13] were determined in heart homogenate, whereas peroxidase blood activity (PBA) [14], reduced glutathione (RGI) [15], as well as glutathione peroxidase and glutathione reductase activity[16] were determined in blood serum.

Statistical analyses of digital data was performed by Department of Systemic Statistical Research of the University by means of Excel (Microsoft, USA) and STATISTICA 6.0 (Statsoft, USA). Reliability of the value difference between independent quantities was determined by Student t-test at normal distribution and by nonparametric methods in other cases.

Results and Discussion

lower lipid peroxidation activity was revealed in the control HR males as compared with the LR animals, that was confirmed by 6.78% lower ($p<0.001$) indices of TBA-active products (Table 1).

Under chronic stress, the significant increase of primary and intermediate lipid peroxidation products: DC — in 2.26 times ($p<0.001$), TC — in 3.56 times ($p<0.001$), TBA-active products — in 4.45 times ($p<0.001$), whereas Schiff bases decreased by 32.32% ($p<0.001$) was revealed the HR group. In the LR group the DC value increased in 5.99 times ($p<0.001$), TC — in 6.80 times ($p<0.001$), TBA-active products — in 5.81 times ($p<0.001$), and Schiff bases decreased by 22.84% ($p<0.001$). As compared with the LR group, the HR group presented lower lipid peroxidation values: DC — in 2.73 times ($p<0.001$), TC — in 1.99 times ($p<0.001$), TBA-active products — in 1.39 times ($p<0.001$), and Schiff bases — by 9.35% ($p<0.001$). This data evidenced progressing oxidative and carbonyl stress in the HR and LR male rats that was more pronounced in the last group.

As compared with LR group, higher activity of primary and final lipid peroxidation products (DC — by 14.38%, $p<0.001$, Schiff bases — by 31.12%, $p<0.001$) and lower concentration of TBA-active products (by 8.01%, $p<0.001$) were revealed in the control HR females. The data proved more intensive lipid peroxidation process in the intact HR females, probably due to more potent activity of antioxidant and endocrine system. The former is confirmed by the fact that in HR females TC values were by 14.67% ($p<0.001$) higher and Schiff bases values — by 22.12% ($p<0.001$) lower as compared with the HR males.

Table 1. Stress-related changes of lipid peroxidation indices in HR and LR animals of both sexes M±m (n=12)

Group	Index			
	Diene conjugates, standard unit. g.$^{-1}$	Triene conjugates standard unit. g.$^{-1}$	TBA-a active products mcmol/kg	Schiff bases standard unit
Males				
HR				
Control	0.975±0.021	0.994±0.013	0.906±0.012	1.413±0.100
Stress	2.209±0.057*	3.537±0.103*	3.537±0.103*	0.956±0.009*
LR				
Control	1.005±0.010	0.994±0.013	0.967±0.006**	1.354±0.055
Stress	6.025±0.794*,**	7.039±0.701*,**	5.625±0.163*,**	1.044±0.019*,**
Females				
HR				
Control	0.952±0.024	1.140±0.032#	0.899±0.002	1.100±0.016#
Stress	1.023±0.001*,#	0.972±0.005*,#	1.319±0.008*,#	1.043±0.006*,#
LR				
Control	0.861±0.006**,#	0.966±0.017#	0.971±0.005**	0.932±0.016**,#
Stress	1.027±0.013*,#	1.074±0.006*,**,#	1.865±0.014*,**,#	1.038±0.003*

Notes: * — reliable indices as compared with the controls;
** — reliable indices as compared with the HR;
# — reliable indices as compared with the males of corresponding group.

As compared with the HR females, TC indices lower by 14.67% (p<0.001) and Schiff bases values higher by 22.12% (p<0.001) were revealed in the control HR males. In comparison with the LR females, DC indices higher by 14.38% (p<0.001) were revealed in the LR males, whereas TC and Schiff bases values were by 6.63% (p<0.002) and 31.12% (p<0.001) higher, respectively. These findings indicated more intensive lipid peroxidation process in females alongside with potent activity of antioxidant system that provided neutralization of lipid peroxidation products through decreased Schiff bases' production. More intensive lipid peroxidation course under stress was revealed in the males. As compared with HR females, the increase in DC in 2.16 times (p<0.001) was revealed in the HR males, whereas TC increased in 3.64 times (p<0.001), TBA-active products — in 3.06 times (p<0.001), and Schiff bases — by 9.06% (p<0.001). In comparison with the LR females the increase in DC in 5.86 times (p<0.001) was evidenced in the LR males, whereas TC increased in 6.56 times (p<0.001), and TBA-active products — in 3.01 times (p<0.001). Thus, stress impact is responsible for more intensive lipid peroxidation course in males that can be attributed to activated sympathetic division of autonomic nervous system, as well as to the less potent or more intensive antioxidant discharge, and to the decrease of sex hormones protective effect.

The study of protein oxidative modification revealed reduced POM 370 and POM 430 values in the control HR males as compared with the LR group (by 7.23%, p<0.001 and by 10.11%, p<0.001, respectively, table 2). Under stress impact, POM 370 decreased by 11.64%, p<0.001 in the HR group, while POM 430 increased by 65.69%, p<0.001. The POM 370 index decreased in the LR group by 24.32%, p<0.001; POM 370 and POM 430 values were higher in the HR group (by 8.17%, p<0.001 and by 26.63%, p<0.001, respectively).

As against the LR group, higher POM 370 (by 13.57%, p<0.001) was revealed in the control HR females. Under stress impact, the decreased POM 370 (by 13.07%, p<0.001) and increased POM 430 (by 69.19%, p<0.001) were revealed in the HR group, whereas in the LR group POM 370 decreased by 23.59%, p<0.001, but POM 430 decreased by 46.39%, p<0.001. POM 370 and POM 430 were higher in the HR group (by 24.02%, p<0.001 and 16.14%, p<0.001, respectively).

As compared with the females, higher POM 370 and POM 430 indices (in the HR – by 11.57%, p<0.001 and by 15.41%, p<0.001; in the LR – by 28.72%, p<0.001 and by 25.59%, p<0.001, respectively) were revealed in the control males. Under stress impact, higher POM 370 and POM 430 indices were revealed in the HR males in comparison with the females (by

Table 2. Stress-related changes in the indices of protein oxidative modification nitric oxide anion-radical, and circulating immune complexes (CIC) in HR and LR rats of both sexes, M±m (n=12)

Group	Index			
	POM 370, mmol/g of protein	POM 430, mmol/g of protein	Nitrite anion, $x10^{-3}$, mkmol'/l	CIC, standard unit
Males				
HR				
Control	1175.08±3.71	712.69±11.09	1.044±0.075	57.66±1.67
Stress	1038.32±7.84*	1180.86±4.74*	9.564±0.301*	118.00±3.85*
LR				
Control	1260.00±3.51**	784.74±3.80**	1.189±0.083	56.25±0.84
Stress	953.53±38.05*,**	866.38±53.34**	5.410±0.124*,**	103.25±1.63*,**
Females				
HR				
Control	1039.11±17.20##	602.83±13.05##	0.988±0.059	54.00±1.41
Stress	903.26±8.45*,##	1019.42±20.91*,##	1.792±0.048*,##	86.00±1.33*,##
LR				
Control	898.14±35.92**,##	583.93±11.77##	1.156±0.039**	50.00±0.77**,##
Stress	686.31±13.02*,**,##	854.83±14.20*,**	1.876±0.038*,##	83.50±1.66*,##

Notes: * –reliable indices as compared with the controls;
** – reliable indices as compared with the HR;
– reliable indices as compared with the males of corresponding group.

13.01%, p<0.001 and by 13.67%, p<0.001, respectively), whereas in the LR males POM 370 index was higher by 28.025%, p<0.001.

Thus, decreasing POM 370 index was revealed in all groups due to the stress effect, whereas POM 430 increased in all HR rats and LR females, indicating the development of oxidative stress, and is likely to be related to free-radical oxidation of lipids which act on the proteins as potent oxidizers.

No reliable difference in nitrite anion values between the control HR and LR males was found. Under stress impact, the index increased in the HR group in 9.2 times, p<0.001, and in the LR group — in 4.5 times, p<0.001 that was higher by 43.43%, p<0.001 in the HR group. The values of nitrite anion of the control HR females were by 16.95%, p<0.005 lower as compared with the LR group. Under stress, the value increased in 43.84 times, p<0.001 in the HR group and in 32.66 times, p<0.001 — in the LR group. No significant difference in nitrite anion values as against the females was revealed in the control males. Under stress, the values of the females increased as compared to the males (in the HR group — by 81.26%, p<0.001, in the LR group — by 65.32%, p<0.001).

Thus, in comparison with the males, more intensive nitrite anion accumulation has been found in the females that may evidence the development of active oxygen forms in the females.

As against the LR group, CIC values in the control HR males were similar. Under stress, they increased in the HR group in 2.0 times (p<0.001) and in the LR group — in 1.8 times (p<0.001) that was by 12.50% (p<0.001) lower in the LR group. In the control HR females the CIC value was 7.41% (p<0.001) higher as compared with the LR group. Under stress, the value increased in 2.90 times (p<0.001) in the HR group and in 3.29 times (p<0.001) in the LR group that was higher in the latter by 5.16% (p<0.001).

As compared with the females, the CIC value was by 11.11% (p<0.001) higher in the control LR males. Under stress, the CIC values were by 27.12% (p<0.001) higher in the HR males as compared with the females, and in the LR group — by 19.13% (p<0.001) higher.

The research findings proved more significant CIC increase in the males as compared with the females, and in the HR males against the LR males. The CIC increase is fraught with the development of autoimmune diseases.

The study of antioxidant defence enzymatic link (Table 3) revealed that SOD activity in the control HR males was by 37.33% (p<0.001) higher as compared with the LR group, whereas ceruloplasmin content — by 15.90% (p<0.001) higher, and blood peroxidase activity (BPA) — by18.13% (p<0.001). These findings evidence the minor lipid peroxidation products content in the LR males as compared with the HR group.

Table 3. Stress-related changes in antioxidant state in heart of HR and LR rats of both sexes, M±m, n=12

Group	Index			
	SOD standard unit, mg^{-1}	Catalase, mcat/kg	Ceruloplasmin, mg/l	BPA, mg/l
Males				
HR				
Control	0.98±0.02	1.61±0.01	2.35±0.09	342.90±1.21
Stress	0.19±0.01*	2.21±0.04*	14.85±0.44*	788.28±7.99*
LR				
Control	0.71±0.01**	1.26±0.06	2.03±0.05**	322.48±3.38**
Stress	0.08±0.02*,**	2.58±0.04*,**	13.89±0.14*,**	599.71±6.99*,**
Females				
HR				
Control	0.81±0.01#	0.35±0.03#	3.63±0.17#	283.71±1.97#
Stress	0.79±0.02#	0.88±0.01*,#	6.50±0.07*,#	97.43±2.79*,#
LR				
Control	0.65±0.01**,#	1.09±0.01**,#	2.43±0.06**,#	270.38±3.76**,#
Stress	0.81±0.02*,#	0.24±0.01*,**,#	5.95±0.05*,**,#	122.57±5.42*,**,#

Notes: * — reliable indices as compared with the controls;
** — reliable indices as compared with the HR;
# — reliable indices as compared with the males of corresponding group.

In the males, stress impact caused the decrease in SOD activity (in the HR group — by 80.99%, p<0.001; in the LR group — by 89.22%, p<0.001), increase in catalase activity (in the HR group — in 1.38 times, p<0.001, in the LR group — in 2.04 times, p<0.001), ceruloplasmin content (in the HR group — in 6.32 times, p<0.001, in the LR group — in 6.85 times, p<0.001), and BPA (in the HR group — in 2.30 times, p<0.001, in the LR group — in 1.86 times, p<0.001). In the HR group, SOD activity was by 58.72% (p<0.001) higher, as well as ceruloplasmin content (by 6.48%, p<0.05) and BPA (by 23.92%, p<0.001). However, catalase activity was by 16.62% (p<0.001) lower. The data prove that minor lipid peroxidation products content was provided by higher activity of antioxidant systemic defence in the HR rats.

As against the LR group, the increase in ceruloplasmin content by 25.65% (p<0.001), the increase in SOD activity by 33.13% (p<0.001), increase in BPA by 4.70% (p<0.01) in the control HR females were revealed, whereas catalase activity decreased in 3.07 times (p<0.001). These findings evidenced the intensive lipid peroxidation process and adequate antioxidant defence in the HR rats. As compared with the females, higher SOD activity (by 16.79%, p<0001 in the HR group and 9.32%, p<0.001 — in the LR group), catalase activity (by 77.95%, p<0.001 in the HR group, by 13.72%, p<0.01 — in the LR group), BPA (by 17.26%, p<0.001 in the HR group and by 16.15%, p<0.001 in the LR group) were found in the intact males. Meanwhile, higher ceruloplasmin content (by 54.56%, p<0.001 in the HR group and by 19.78%, p<0.001 — in the LR group) was evidenced in the females.

In the HR females under stress, catalase activity increased in 2.48 times (p<0.001), ceruloplasmin content — in 1.79 times (p<0.001), whereas BPA decreased by 65.66% (p<0.001). In the LR females, catalase activity decreased by 78.13% (p<0.001) and BPA decreased by 54.67% (p<0.001), while SOD activity increased by 25.38% (p<0.001) and ceruloplasmin content increased in 2.45 times (p<0.001). As compared with the LR females, higher catalase activity by 72.92% (p<0.001) and higher ceruloplasmin content by 8.42% (p<0.001) were revealed in the HR group, while BPA was by 25.81% (p<0.001) lower. This data indicated that minor lipid peroxidation products content in the HR group was provided by more potent activity of antioxidant system.

In comparison with the males, SOD activity was higher in the females (in 4.27 times, p<0,001 and in 10.55 times, p<0,001 in the HR and LR groups, respectively). However, higher catalase indices were in the males (by 60.22%, p<0.001 in the HR group and by 90.76%, p<0.001 in the LR group), ceruloplasmin (by 56.26%, p<0.001 in the HR rats and 57.16%, p<0.001 in the LR animals), and BPA (87.64 %, p<0.001 in the HR group and 79.56%, p<0.001 in the LR group. This

Table 4. Stress-related changes in glutathione system in heart of the HR and LR rats of both sexes, M±m, n=12

Group	Index		
	Reduced glutathione, mcmol/g	Glutathione peroxidase, mcmol/min.kg	Glutathione reductase mcmol/min.kg
Males			
HR			
Control	776.32±22.04	0.441±0.002	0.621±0.004
Stress	1393.27±40.89*	0.199±0.004*	0.319±0.001*
LR			
Control	625.73±25.31**	0.226±0.004**	0.616±0.004
Stress	1057.02±12.80*,**	0.290±0.001*,**	0.254±0.001*,**
Females			
HR			
Control	820.17±13.68	0.512±0.001##	0.303±0.012##
Stress	486.84±24.63*,##	0.132±0.002*,##	0.155±0.011*,##
LR			
Control	644.74±13.68**	0.228±0.003**	0.281±0.004##
Stress	451.75±19.92*,##	0.094±0.002*,**,##	0.094±0.002*,**,##

Notes: * — reliable indices as compared with the controls;
** — reliable indices as compared with the HR;
— reliable indices as compared with the males of corresponding group.

data evidenced minor lipid peroxidation activation due to SOD effect that provided minor accumulation of secondary and final lipid peroxidation products in the females.

During the study of glutathione system indices (Table 4), the reduced glutathione (RG) was revealed by 24.06% ($p<0.001$) higher in the HR males as compared with the LR group, glutathione peroxidase values higher by 95.57% ($p<0.001$). In the HR females the reduced glutathione values were by 21.39% ($p<0.001$) higher and glutathione peroxidase values were by 55.46% ($p<0.001$) higher. Enzyme activity was lower in the HR females than in the males (glutathione peroxidase by 16.17%, $p<0.001$, glutathione reductase by 51.27%, $p<0.001$), and glutathione reductase in the LR females (by 54.41%, $p<0.001$).

In the males, stress impact caused the increase in reduced glutathione values (the HR group — in 1.79 times, $p<0.001$, the LR group — in 1.69 times, $p<0.001$) and decrease in glutathione reductase activity (the HR group — by 48.62%, $p<0.001$, the LR group — by 58.80%, $p<0.001$), while glutathione peroxidase values decreased in the HR group by 54.89%, $p<0.001$ and increased in the LR group by 28.67%, $p<0.001$.

Stress caused the decrease of reduced glutathione indices (in the HR group — by 40.64%, $p<0.001$, in the LR group — by 29.93%, $p<0.001$), while glutathione reductase activity decreased in the HR group (by 80.26%, $p<0.001$) and increased in the LR group (by 88.39%, $p<0.001$). In the HR group glutathione peroxidase activity remained higher (by 28.79%, $p<0.001$), as compared with the LR group, but glutathione reductase activity was higher (by 39.26%, $p<0.001$). All the studied indices in the females under stress impact were lower in comparison with the females (reduced glutathione index in HR group — by 65.06% ($p<0.001$), glutathione peroxidase — by 66.33% ($p<0.001$), and glutathione reductase — by 51.53% ($p<0.001$). In the LR group, the indices were 57.26% ($p<0.001$), 67.61% ($p<0.001$) and 62.96 % ($p<0.001$), respectively.

Reduced glutathione increase has been noted in the males only; it evidenced of increasing hydroxyl radical which is crucial for the modification of amino acid residues. Besides, in the males the decrease and in the females — increase of SOD-enzyme were revealed that inactivates superoxide-anion radical. Apparently, the processes of free radical oxidation progress differed in the HR and LR rats of both sexes.

Conclusions

As compared with the LR animals of the same age, minor activity of lipid peroxidation processes owing to TBA-active products were revealed in the intact adult HR male rats, whereas increased activity due to diene conjugates and Schiff bases were evidenced in the female rats. Immobilizing stress causes the development of oxidative and carbonyl stress in the HR and LR rats, more pronounced in the LR group, and the activation of antioxidant defence system. In the males, stress development was concomitant with the increased catalase activity as well as blood peroxidase, ceruloplasmin and reduced glutathione content, whereas the increased catalase and ceruloplasmin activity was evidenced in the HR females, and that of superoxide dismutase and ceruloplasmin — in the LR group, thus failing to provide neutralization of lipid peroxidation products in the latter group. As compared with the females, more intensive oxidative and nitroxidative stress, protein oxidative modification, and stress-related accumulation of circulating immune complexes has been found in the males.

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ENDOGENOUS INTOXICATION IN ANIMALS OF DIFFERENT AGE GROUPS IN CASE OF POLYTRAUMA

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Background. *Associated injury is a worldwide social and economic problem. Age related aspects of endogenous intoxication are not studied comprehensively. Annually, from 44 000 to 65 000 citizens die because of traumatic injuries. As a result, this number increased by 32.6% for the last 10 years. The detoxification system, as a component of the functional systems of the organism, experiences significant changes in case of polytrauma.*

Objective. *The study was aimed to discover pathogenetic peculiarities of the multiple trauma in age aspect in different disease periods and to explore the level of endogenous intoxication in this condition.*

Methods. *The experiments were performed on 72 white male rats aged 3, 6 and 12 months, which underwent simulation of severe skeletal trauma and examination of the contents of middle mass molecules and endogenous intoxication index (markers of endogenous intoxication) in 1, 4 and 24 hours after the associated injury.*

Results. *The most significant increase of the middle mass molecules was fixed in 24 hours after modeling of severe skeletal injuries in all groups of animals, especially it was the most pronounced in 12-month-old animals. The erythrocyte intoxication index reached the highest level in 4 hours after the injury, its increase was most significant in sexually mature adult animals.*

Conclusions. *A significant increasing of endogenous intoxication markers in 12-month-old rats, if compared to 3- and 6-month-old animals, can be caused by the decrease in compensatory protection mechanisms.*

KEY WORDS: **multiple trauma, endogenous intoxication, age.**

Introduction

These days, the increased number of associated is one of the most important social and economic problems [1–7].

In Ukraine the mortality rate, as a consequence of injury, is 91.8% of cases per 100 000 citizens. Annually, from 44 000 to 65 000 citizens die because of traumatic injuries. As a result, this number increased by 32.6% for the last 10 years.

The detoxification system, as a component of the functional systems of the organism, experiences significant changes in case of multiple trauma. The disturbance of functioning of this system leads to the development of endogenous intoxication syndrome (EIS), which causes the primary diseases and the complications due to increased tissue decay, escalation of catabolic processes, insufficient functioning of internal organs [11, 12]. Among the causes of death during terminal stage are multiple organ failure and consistent progressive failure of organ systems as a result of injury.

Nowadays the age-related mechanisms of EIS development are not studied comprehensively. There are no complete pathogenetic approaches to the explanation and prognosis of early and late complications of multiple trauma.

The study was **aimed to** discover pathogenetic peculiarities of the multiple trauma in age aspect in different disease periods and to explore the level of endogenous intoxication in this condition.

Material and Methods

In this experimental study we used 72 white rats of the age of 3, 6, and 12 months old. The experimental model of multiple trauma was implemented during the study [9]. The control group was comprised of intact animals, which

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were kept under the standard vivarium conditions. Decapitation was accomplished under thiopental anaesthesia on 1st, 4th, and 24th hour of the experiment in accordance to The *European Convention* for the *Protection* of Vertebrate *Animals* Used for Experimental and other Scientific Purposes (Strasbourg, 1986). We used blood serum for the research. The degree of toxic syndrome was evaluated by the level of middle mass molecules (MMM) [8] and erythrocyte intoxication index (EII) [10]. The received data was processed by method of statistical variation and Student t-test. The results were considered as significant at the value $p<0.05$.

Results and Discussion

In our experiment we detected the significant changes of MMM in all 3 animal groups and compared the results with the intact ones.

In the 3-month-old rats the increase of MMM_{254} was observed during the 1st hour after multiple trauma. The level of these molecules increased by 39%, and after 4 hours it was 48% if compared to the intact animals. On the 1st day after the multiple trauma MMM_{254} was on 43% higher than normal rates.

The similar changes were observed in the 6-month-old rats. During the 1st hour the MMM_{254} was increasing even by 73%, in 4 hours — by 83% and in 1 day of the experiment it was by 79% higher than in the intact group.

The most significant increase in MMM_{254} was evidenced in the 12-month-old animals. After the 1st hour it increased by 93%, in 4 hours — by 96% if compared to the intact animals, in 1 day — more than in 2 times.

The increase of MMM level in animals after multiple trauma proved the enforcement of catabolic processes. The rise of MMM levels, which can include oligopeptides, fragments of nucleic acids, fatty acids, and triglycerides, can prove the injury of the hepatocytes membranes and MMM_{254} that include purine bases, uric acid and aromatic amino acids — the suppression of the detoxifying function of liver [13].

The differences in the dynamics of changes of MMM_{280} between the 1st, 2nd and 3rd groups were manifested (Fig. 2).

After the multiple trauma the level of MMM_{280} in the 3-month-old animal group was higher by 82% if compared to intact animals. In 4 hours it was exceeding the level of control group by 108%, in 1 day — by 98%.

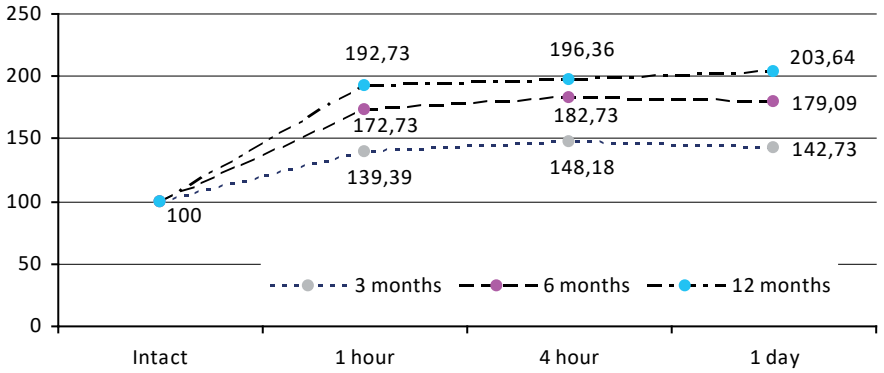

Fig. 1. The level of MMM_{254} in rats of different age after multiple trauma (in percentage if compared to the level of the intact animals) in dynamics.

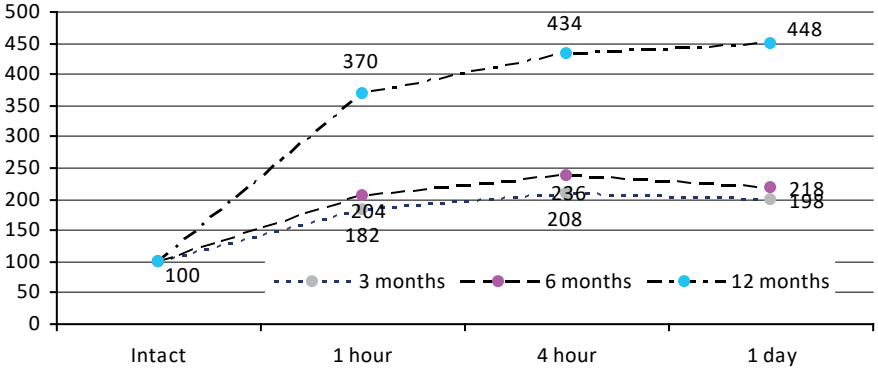

Fig. 2. The level of MMM_{280} in rats of different age after multiple trauma (in percentage if compared to the level in the intact animals) in dynamics.

In the 6-month-old group MMM_{280} was higher by 104% if compared to the control group, in 4 and 24 hours — by 136% and 98% respectively.

The highest level of MMM_{280} was observed in the 12-month-old animals in all periods of the experiment. In 1 hour after multiple trauma, it was exceeding the level of the intact animals by 270%, in 4 hours — by 344%, in 1 day — by 448%.

We argue that the increase in MMM levels is a manifestation of catabolic processes enforcement in cells of vital tissues. The possible cause for these changes can be the activation of intracellular, particularly lysosomal proteases under the influence of these toxins [14].

The multiple trauma also influenced on the level of erythrocyte membranes impairment (Fig. 3).

EII significantly increased during the 1st hour after multiple trauma in the 3-month-old rats and exceeded the level of the intact animals by 85%. In 4 and 24 hours of the experiment it increased by 97% and 92% respectively.

In the 6-month-old rats, in 1 hour the EII was higher than in the intact animals by 107%, in 4 hours — by 124%, in 1 day – by 213%.

In the 12-month-old rats, in 1 hour the EII was higher by 127% if compared to the intact rats, in 4 hours — by 245%, and in 1 day — by 149%. In case of MMM the EII reached the highest level.

The data obtained prove the action potentiation of endogenous toxins that are excreted to blood in case of multiple trauma; it can be accompanied by either high level of catabolic processes or the suppression of the detoxification system functional activity [15].

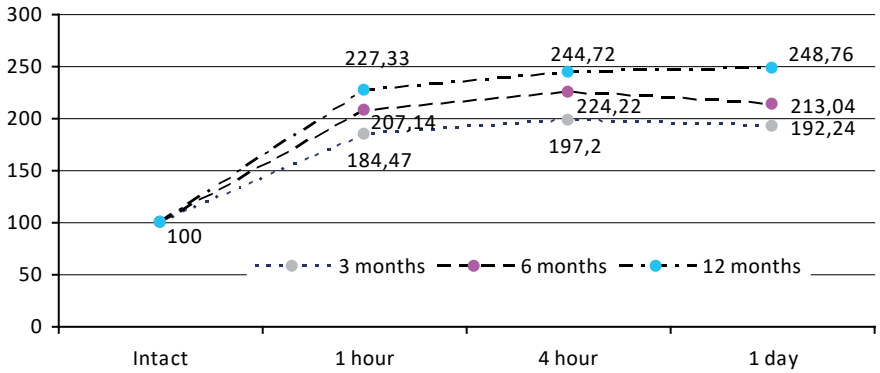

Fig. 3. The level of EII in rats of different age after multiple trauma (in percentage if compared to the level of the intact animals) in dynamics.

Conclusions

MMM and EII proved serious age-related differences between the animals with multiple trauma. In all animal groups the highest value was reached during the 1st hour after the injury and continued with concomitant slow decline, especially in the 3- and 6-month-old animals in 4 hours after the injury. However, in the 12-month-old rats the increase of MMM and EII continued; it proved prolongation of endogenous intoxication.

The further research on endogenous intoxication will broaden the view on the pathogenesis of traumatic diseases in people of different age and in various periods of the diseases that will provide the opportunity to make prognoses on further course of the disease.

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