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SUBCLINICAL CARDIAC DAMAGE IN CARDIOPULMONARY POLYMORBIDITY (REVIEW, PART 2)

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Abstract

Hypertension and chronic obstructive pulmonary disease are frequent comorbid conditions in the internal medicine and are subject to meaningful cooperation among physicians, cardiologists, and pulmonologists.

A combination of chronic obstructive pulmonary disease and hypertension presents certain diagnostic and therapeutic challenges. These conditions share common risk factors, similar clinical presentations and some common parts of pathogenesis. A problem of association between chronic obstructive pulmonary disease and hypertension may be currently discusses both as a simple combination of various clinical entities, and as chronic obstructive pulmonary disease resulting in development of factors contributing to hypertension. One way or another, either a simple combination, or a mutually aggravating syndrome, but we state there is a cardiorespiratory continuum where chronic obstructive pulmonary disease acts as a valid component of hypertension development, and vice versa. Thus, it seems to be relevant to study peculiarities of the structural and functional status of the cardiovascular system and microcirculation, systemic remodeling mechanisms, endothelial dysfunction and inflammation in presence of chronic obstructive pulmonary disease-associated hypertension. Problems of additional cardiovascular risk marker development, treatment efficiency assessment remain topical.

The use of electrocardiography and echocardiography with dopplerometry has been an important diagnostic principle of subclinical cardiovascular damage in presence of hypertension and chronic obstructive pulmonary disease comorbidity. Non-invasive imaging methods play a central part in diagnosis of subclinical target organ damage. Wide implementation thereof is based on high diagnostic accuracy, common availability, safety and relatively low price.

Key words: *hypertension, chronic obstructive pulmonary disease, comorbidity, electrocardiography, echocardiography with dopplerometry.*

Echocardiography

There are currently several options for echocardiographic studies available: Two-Dimensional Echocardiography, M-Mode, Doppler Echocardiography (Pulsed Wave – PW), High Pulse Repetition Frequency Doppler (HPRF), Continuous Wave (CW), Color Doppler, Color M-mode Doppler, Power Doppler, Color Tissue Velocity Imaging (Color TVI), Tissue Nonlinear Velocity Imaging, or C-Mode, Pulsed Wave Tissue Velocity Imaging, Tissue Tracking, Strain and Strain Rate, Vector Velocity Imaging or Vector Analysis of Endocardium Movement Velocity, Transesophageal Echocardiography, Stress Echocardiography, Three- and Four-Dimensional Cardiac Modelling, Intravascular Ultrasound, Contrast Echocardiography [1].

Echocardiographic signs of Right Atrial Hypertrophy

Up to day, only few studies have focused on the right atrial (RA) role in pathological conditions [2]. Right atrial dimensions are most often assessed through apical access in a four-chamber

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view. In this view, the RA area is assessed using planimetry [3]. The maximum distance along the RA long axis (longitudinal dimension) from the center of the tricuspid ring to the center of the upper wall of the AR "roof" is drawn parallel to the interatrial septum. A lesser transverse dimension through the middle of the RA is determined from the middle of the RA free lateral wall to the middle of the interatrial septum at right angle to the long axis. The RA area is outlined at the end of the ventricular systole from the lateral edge of the tricuspid ring to its septal edge, excluding an area between the valve leaflets and the fibrous ring along the AR endocardial edge [4].

Echocardiographic signs of Right Ventricle Hypertrophy

Unlike the RA, measuring the right ventricular (RV) dimensions requires using numerous echocardiographic views, including the parasternal view along the long and short axis view, RV inflow tract view, apical four-chamber and subcostal views. Change in the RV dimensions and function suggests increased pulmonary vascular resistance and load from the left heart chambers. Rapid increase in the RV afterload is known to present as the RV dilation, and a chronic one presents as concentric hypertrophy. In normal condition, the RV free wall thickness is less than 0.5 cm when measured both with M-mode, and with two-dimensional echocardiography. It is preferable to measure the RV wall from the subcostal view at a tricuspid valve chord level [5]. End-diastolic median and basal RV diameter measurement in the apical four-chamber view is a simple RV quantification method. Moreover, the RV longitudinal dimension may be measured in this view (*Table 2*).

Echocardiographic signs of Left Atrium Hypertrophy

Up to day, increase in the left atrial (LA) dimensions is known to be a marker both for severity and duration of diastolic dysfunction and pressure increase degree in the LA and is associated with development of adverse cardiovascular events [6]. Standard of the LA assessment is measurement of the anterior-posterior dimension with parasternal access to the LA long axis in M- or B-modes. In M-mode, it was measured from the anterior edge of the posterior aortic wall to the anterior edge of the LA posterior wall which, if normal, is 2.7–3.8 cm for women and 3.0–4.0 cm for men [6].

Echocardiographic signs of Left Ventricle Hypertrophy

The major diagnostic method for subclinical cardiovascular damages is assessment of the left ventricular (LV) dimensions, both linear dimensions, and LV myocardial mass (LVMM).

When echocardiographic findings are described, severity of damage is indicated as "minor", "moderate" or "major", allowing making conclusions about the damage intensity.

For linear and volumetric measurements of the LV (interventricular septum (IVS), posterior wall (PW), LV internal dimensions (end-systolic dimension [ESD] and end-diastolic dimension [EDD]), it is preferable to assess images in the parasternal view along the LV long axis.

It is recommended to measure the LV internal dimensions (ESD and EDD) and wall thickness at the LV minor axis level, at about mitral valve leaflet tips level. These linear dimensions may be measured both directly in the B-mode, and M-mode under B-mode control. An image made

Table 1

Right Atrial Cavity Dimensions

Dimension	Lower Normal Limit (95% CI)	Upper Normal Limit (95% CI)
RA Longitudinal Dimension (mm)	34 (32–36)	53 (51–55)
RA Transverse Dimension (mm)	26 (24–29)	44 (41–46)
RA End-Systolic Area (cm^2)	10 (8–12)	18 (17–20)

Table 2

Normal RV dimensions measured in the apical four-chamber view [6]

Dimension	Lower Normal Limit (95% CI)	Upper Normal Limit (95% CI)
Basal RV transverse dimension	24 (21–27)	42 (39–45)
Mean RV Transverse Dimension (mm)	20 (15–25)	35 (30–41)
RV Longitudinal Dimension (mm)	56 (50–61)	86 (80–91)

Table 3

The most frequently used parameters for LV dimensions description are provided in the table [1]:

Parameter	Men				Women			
	Normal	Minor Damage	Moderate Damage	Major Damage	Normal	Minor Damage	Moderate Damage	Major Damage
M-Mode LVMM, g	67–162	163–186	187–210	≥211	88–224	225–258	259–292	≥293
LVMM/body surface area (BSA), g/m^2	43–95	96–108	109–121	≥122	49–115	116–131	132–148	≥149
Interventricular septal thickness, cm	0.6–0.9	1.0–1.2	1.3–1.5	≥1.6	0.6–1.0	1.1–1.3	1.4–1.6	≥1.7
LV posterior wall thickness, cm	0.6–0.9	1.0–1.2	1.3–1.5	≥1.6	0.6–1.0	1.1–1.3	1.4–1.6	≥1.7
B-mode LVMM, g	66–150	151–171	172–192	≥193	96–200	201–227	228–254	≥255
LVMM/BSA, g/m^2	44–88	89–100	101–112	≥113	50–102	103–116	117–130	≥131
End-diastolic volume (EDV), ml	56–104	105–117	118–130	≥131	67–155	156–178	179–201	≥201
End-systolic volume (ESV), ml	19–49	50–59	60–69	≥70	22–58	59–70	71–82	≥83

in the parasternal view along the short axis in B-mode or in M-mode under B-mode control may also be used when the view is positioned at the right angle to the IVS and LV PW. Measurements of the small axis in B-mode turn out to be smaller than measurements made in M-mode with the upper normal limit for EDD 5.2 cm vs 5.5 cm for M-mode. Normal LV EDD and ESD measurements make up 4.7±0.4 cm and 3.3±0.5 cm, respectively [7, 8]. LV internal dimension and IVS and PW thickness are measured at end-diastole and end-systole in a two-dimensional and M-modes [7].

Prolonged stable blood pressure increase and absence of adequate antihypertensive therapy result in the LV shape change. LV remodeling type, its geometric model are determined based on the LV myocardial mass (MM) and LV relative wall thickness (RWT). There are two popular methods of LVMM calculation: Penn and method, proposed by the American Society of Echocardiography (ASE).

According to the updated ASE recommendations to linear cardiac chamber measurement, the upper normal limit for LVMM is > 95 g/m^2 for women and > 115 g/m^2 for men [7].

There are two formulae for LV RWT calculation:

1) PW thickness x 2/diastolic LV diameter;

2) (IVS thickness + PW thickness)/diastolic LV diameter.

Patients with the concentric-type hypertrophy typically display high overall peripheral vascular resistance, subnormal systolic output and increased pulse pressure (PP) [9].

Patients with the eccentric-type hypertrophy typically display increased left ventricular cavity, high systolic output, relatively low overall peripheral vascular resistance with simultaneous relatively low PP. The latter is conditional upon arterial blood stream compliance in absence of significant vasospastic reactions. Increased venous tone or circulating blood volume are considered to be hemodynamic factor of LV eccentric hypertrophy development (e.g., in hypertensive disease patients) [9].

In concentric remodeling, LV wall thickness and myocardial mass are not increased, and decreased EDD and left ventricular cavity volume are the major remodeling signs.

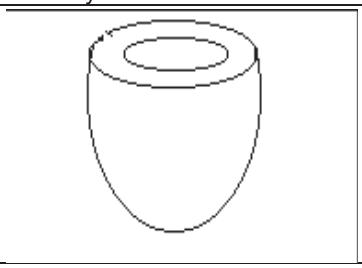
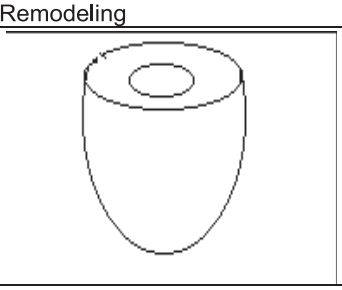
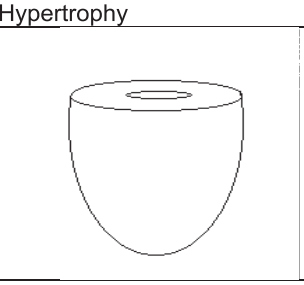
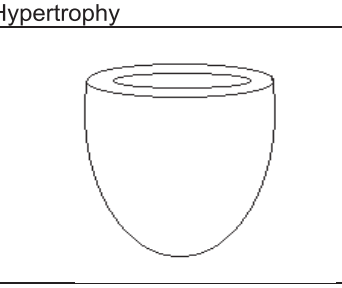
There is an alternative LV geometry classification based on LVMM identification (along the vertical axis), LV volume (along the horizontal axis) and RWT (or LV mass to LV volume ratio) and includes eight types of ventricular remodeling [7, 8] (Table).

As the proposed classification suggests, a non-dilated ventricle has the following features: normal morphology, concentric remodeling/hypertrophy, confirmed LV hypertrophy and RWT > 0.42. A dilated, non-hypertrophied LV typically displays presence of eccentric remodeling with RWT < 0.32. A dilated LV with increased muscular thickness typically displays eccentric hypertrophy (RWT < 0.32), mixed hypertrophy (RWT > 0.42) or physiological hypertrophy (RWT 0.32-0.42).

EchoCG in COPD patients reveals pulmonary heart disease characterized by dilation of the right cardiac chambers, right ventricular wall hypertrophy, presence of pathological tricuspid regurgitation. All these signs suggest presence

Table 4

Description of Classic Remodeling Types

Type 1 – Normal Geometry	
LVMM ≤ 115 g/m^2 (men) or ≤ 95 g/m^2 (women) LV RWT < 0.42	
Type 2 – Concentric Remodeling	
LVMM ≤ 115 g/m^2 (men) or ≤ 95 g/m^2 (women) LV RWT > 0.42	
Type 3 – Concentric Hypertrophy	
LVMM ≤ 115 g/m^2 (men) or ≤ 95 g/m^2 (women) LV RWT > 0.42	
Type 4 – Eccentric Hypertrophy	
LVMM ≤ 115 g/m^2 (men) or ≤ 95 g/m^2 (women) LV RWT < 0.42	

of pulmonary hypertension. Paradoxical diastolic movement of the interventricular septum toward the left ventricle associated with pulmonary hypertension may be observed. However, a number of patients with chronic non-specific pulmonary conditions display only right ventricular wall hypertrophy, right atrial dilation, and pathological tricuspid regurgitation. The systolic function of the left and right ventricles may be normal or decreased. The diastolic function of the left ventricle typically has the first-type impairment. The diastolic function of the right ventricle may also have the first-type impairment, however, no clear correlation with the right ventricular systolic function impairment and pulmonary hypertension severity has not been determined so far. Studies are still conducted in this area [1].

Modern preventive and treatment strategy for the cardiopulmonary polymorbidity considers pathophysiological mechanisms of effects caused by risk factors early identification and elimination of which will contribute to significant improvement of prognosis. Therefore, it is very important to identify early markers of cardiopulmonary impairments which may help to determine a risk group at a preclinical level. Cardiovascular and pulmonary disease prevention is one of the highest priorities for the modern medicine [10].

Functional markers of biventricular dysfunction

Functional markers of both left and right ventricular dysfunction are widely used in addition to structural abnormalities to further stratify cardiovascular risk in patients with cardiopulmonary pathology.

Left ventricular systolic function

Estimation of functional state of the left ventricle should always include thorough evaluation of its systolic and diastolic function [11]. Markers that reflect left ventricular contractile capacity may be determined using a variety of imaging modalities, with sonographic techniques and MRI being the most widely used. Among these, transthoracic echocardiography is remaining the most cost-effective technique to be used with a purpose of screening of the patients, at the same time presenting an extensive set of parameters that may be acquired during a routine study.

Ejection fraction (EF) of the LV is the most widely used index of its global systolic function. EF prognostic role has been validated in a large number of prospective trials for patients with different types of myocardial impairment, with no data showing its limited accuracy or prognostic value in concomitant bronchopulmonary pathology [12, 13].

There is a strong data supporting the negative prognostic value of LVEF < 40% that has its reflection in the current guidelines on management of heart failure, with the values < 35% being even less favorable and therefore granting a more aggressive therapeutic approach to decrease the risk of sudden cardiac death [14]. The values of LVEF > 50% are considered normal, with a "grey zone" of 40 to 50% being defined as the mid-range ejection fraction (mrEF); for these patients with heart failure, up to date there is no sufficient data to recommend any kind of outcome-modifying treatment but presence of mrEF (even in a patient with no symptoms) should be perceived as a sign of subclinical myocardial impairment and therefore warrant further diagnostic search.

The pitfalls of using LVEF as a marker of systolic function include significant inter-observer variability [11] and technical limitations in case of suboptimal acoustic window; partially, these problems are being slowly overcome by introduction of the software for 2D and especially 3D automatic volumetry. LVEF measured by an MRI is considered the gold standard for left ventricular volumetry but remains too expensive to be used for screening purposes and lacks proper validation as a treatment-guiding modality (as all the studies justifying the cut-off values of the LVEF were performed using EchoCG) [15, 16].

The next widely used marker of LV global function is its global longitudinal strain (GLS), calculated directly using speckle tracking techniques or indirectly via tissue Doppler measurements [17, 18]. Being a more sensitive tool than EF, GLS tends to deteriorate earlier and therefore could be used for screening of subclinical myocardial impairment in a way similar to the one validated for patients undergoing chemotherapy [19], with an additional benefit of more precise estimation of local contractile abnormalities compared to traditional visual assessment. The pitfalls of GLS include lower availability in older scanners and significant inter-vendor variability [20] that limits the possibilities of comparing the results obtained by different scanners. The current guidelines estimate the lower range of normal values of the GLS as 17%, with additional remark regarding the need of using the same scanner in dynamic observation of a single patient; no data is available regarding the need of use of different cut-off value in bronchopulmonary pathology [21].

Other existing markers of LV systolic function, in general, do not have added diagnostic or prognostic value compared to EF and GLS, but there are two of them that may serve as a surrogate indices of LV global longitudinal contractility when assessment of GLS is technically impossible. Those are mitral annular peak systolic velocity (S', derived in pulse-wave tissue Doppler mode) and excursion (MAPSE, M-mode derived and hence ready for acquisition on all available scanners). Lower normal limits for them are 7.0/10.0 cm/sec (for medial/lateral portions) and 16 mm (measured at lateral portion), appropriately. There is some data on negative prognostic value of both low MAPSE and S' [14], both markers are easily obtainable and reproducible, and hence ready for use in dynamic observation of patients with a purpose of early detection of subclinical LV damage.

Left ventricular diastolic function

According to the current standards, evaluation of the LV diastolic filling should be routinely performed in every EchoCG study [21]. In patients with bronchopulmonary pathology, it is especially important in the context of differential diagnosis of post-capillary pulmonary hypertension due to impaired LV filling and pre-capillary PH being a result of pulmonary parenchymal and microvascular remodeling.

The current guidelines on the evaluating of LV diastolic filling [21] include a comprehensive algorithm for detecting and grading of diastolic dysfunction that is far from perfect due to a lot of patients being classified as having indeterminate diastolic filling, is being widely

debated and the description of which is not within this paper's purpose. Still, there is solid data on a set of functional markers that may be reliably used (with the exception of some specific settings) in most of the patients to detect and further observe the changes of LV diastolic function.

Thus, tissue Doppler-derived mitral annular peak early diastolic velocity (E') is an index of early diastolic relaxation of the myocardium of LV, with the cut-off values of 7,0 and 10,0 cm/sec for the medial and the lateral portions, appropriately. Late diastolic filling pressures is best correlated with the E/E' ratio (with E being a peak velocity of early diastolic transmitral flow). The current guidelines recommend to use the mean (medial/lateral) E' value in the calculation and to take 13 [14] to 14 [21] as the upper limit of normal for the E/E' ratio. Additional diastolic indices include late diastolic transmitral flow peak (A) and its annular equivalent (A'), E peak deceleration time (DTE), E/A ratio, isovolumetric relaxation time (IVRT) derived from transmitral flow or mitral annular motion, pulmonary veins flow pattern and some easy functional maneuvers that can be additionally used when estimating the LV filling state.

It cannot be stressed enough that the current approach is only able to estimate LV diastolic function in the setting of the left chambers morphology, with left ventricular hypertrophy supporting the presence of diastolic dysfunction in general, and left atrial enlargement > 34 ml/m^2 being one of the markers of elevated LV filling pressure [14, 21]. It is important to draw attention to the fact that the third index of elevated LVEDP, the peak tricuspid regurgitation velocity, should not be used in patients with bronchopulmonary disease, being a non-specific marker of pulmonary hypertension.

The diastolic indices presented above also have a wide set of limitations, the most frequently met of which are atrial fibrillation, calcification of the mitral annulus and significant mitral disease [21]. Still, even in these settings E' and E/E' can be used in a longitudinal monitoring of LV early and late diastolic function (given the stable state of mitral leaflets opening, stable severity of mitral regurgitation, and stable HR for atrial fibrillation).

Right ventricular systolic function

Proper evaluating of right ventricular performance should be and essential part of echocardiographic study in a patient with bronchopulmonary pathology, with right chambers being a target for adverse cardiac remodeling in pulmonary hypertension, and with a set of RV functional indices having been shown to have great clinical utility and prognostic value [22].

Tricuspid annular plane systolic excursion (TAPSE) is an easily obtainable with any scanner, highly reproducible parameter that mainly reflects the global longitudinal function of the RV but has shown good prognostic value and correlations with volumetric and planimetric estimates of RV contractility [23, 24]. According to the current consensus, TAPSE values < 16 [22] to 17 [11] mm should be interpreted as a sign of RV systolic dysfunction.

Peak systolic velocity of the tricuspid plane derived in a pulsed wave tissue Doppler mode at the lateral portion of tricuspid annulus (S') is another marker of the right ventricular global longitudinal contractility. The cut-off value for differentiating normal and abnormal function is 9.5 [25] to 10 cm/sec [22].

Both S' and TAPSE share the same innate limitations, with somewhat lower correlation to MRI- and scintigraphy-derived RV EF in states with regional wall motion abnormalities and inhomogeneous morphologic remodeling of the RV, including severe tricuspid regurgitation, acute pulmonary embolism, RV myocardial infarction, etc. [22].

Fractional area change (FAC) of the RV during systole, defined as (end-diastolic area – end-systolic area) / end-diastolic area × 100 (with the cut-off value of 35%), is up to date the most reliable and accurate index of RV global systolic performance that accounts for the possible regional motion abnormalities and correlates well with MRI-derived RV EF [26, 27]. The main major limitation of the FAC is that the technique of its obtaining is highly demanding to the quality of the RV endocardial tracing and hence the accuracy of measurements tends to dramatically drop in technically challenging cases, which is frequently essential in patients with pulmonary emphysema.

3D RV volumetric EF and RV free wall strain measurements are promising techniques that will probably help to overcome the limitations listed above in evaluating the RV systolic function [28, 29]. Still, up to date these techniques are not available in the majority of routinely used scanners, lack of validated cut-off values (which is of essential importance with regard to acknowledged inter-vendor variability [20], and therefore should be reserved mainly for research use.

Right ventricular diastolic function

Similar to LV function, changes in diastolic filling of the RV precede the decline of its contractility, and hence parameters reflecting RV

diastole could be used for longitudinal monitoring to detect early, subclinical changes in the RV function.

Unlike systolic function, where different shape and structure of the two ventricles lead to impossibility of use of some of the validated LV indices (e.g., 2D-derived EF) to adequately reflect the contractility of the RV, for thorough evaluating of diastolic function it is recommended to use mostly the same parameters as for LV.

As such, parameters like tricuspid E/A ratio (with a normal range of 0,8–2,1), E' (lower reference value 8 cm/sec), E/E' ratio (normal values < 6,0) can be monitored routinely, being the best validated and highly reproducible indices,

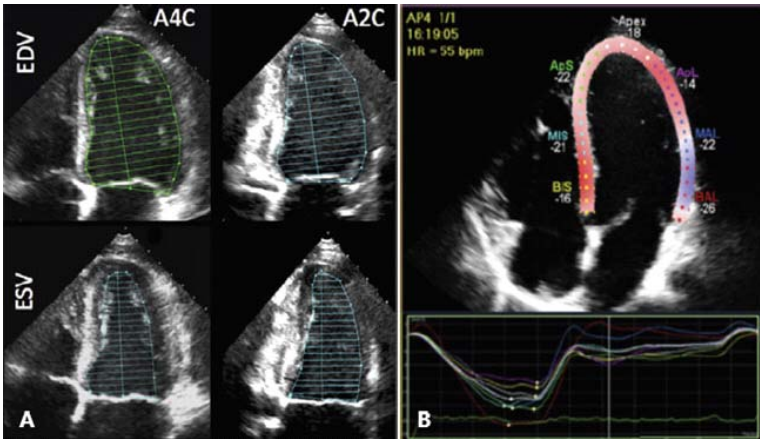


Fig. 1. Main parameters of the left ventricular global systolic function. A – LV ejection fraction using biplane simpson method; B – LV longitudinal strain in apical 4-chamber view. Adapted from [11]

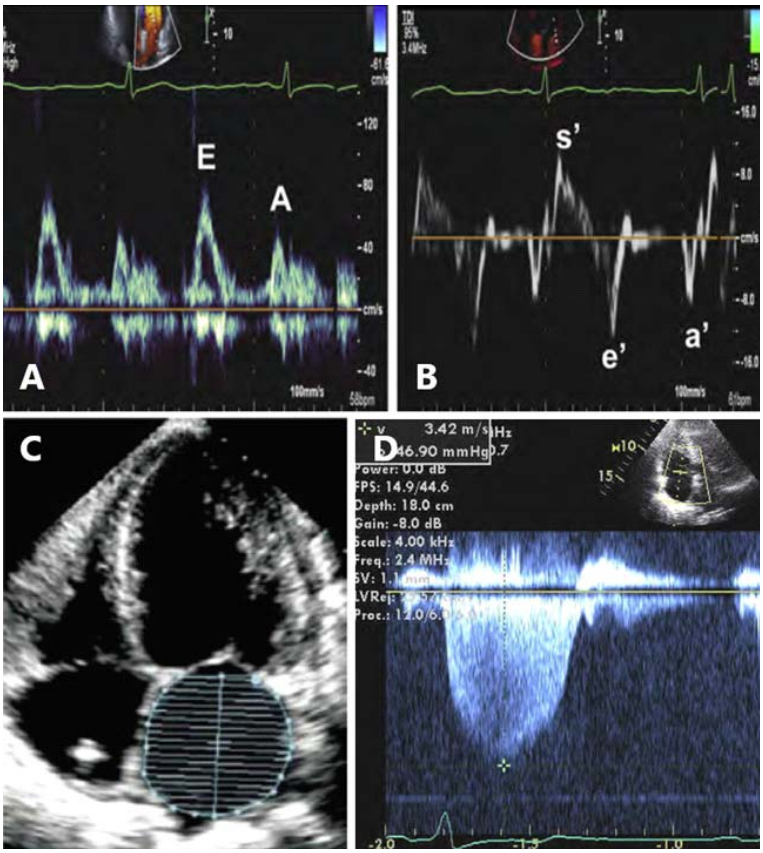


Fig. 2. Main parameters of the left ventricular diastolic function. A – PW Doppler-derived transmitral flow velocities; B – PW tissue Doppler-derived mitral annular motion velocities;
C – left atrial maximal volume using Simpson method;
D – CW Doppler-derived peak tricuspid regurgitation velocity. Adapted from [11, 14]

with a possible estimation of E peak deceleration time and IVRT in challenging cases [22].

Pulmonary artery pressures

Being highly dependent not only on cardiac function but also on the state of pulmonary microcirculation that is being altered at advanced stages of most bronchopulmonary diseases, pulmonary artery pressure should not be taken as a reliable index of the global LV function.

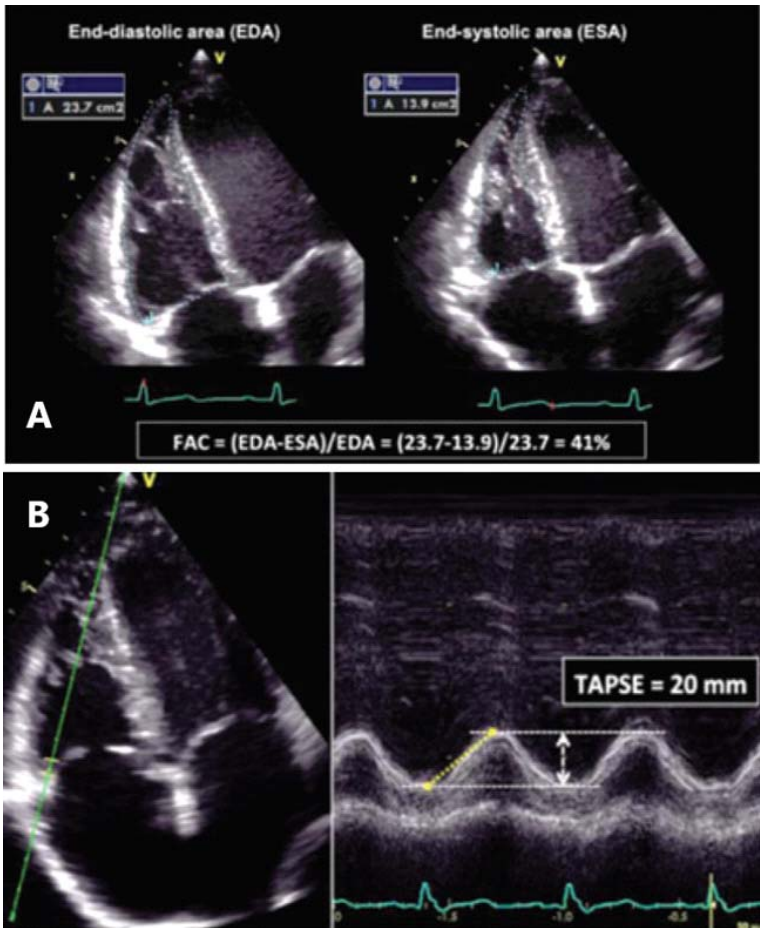


Fig. 3. Main parameters of the left ventricular global systolic function. A – RV fractional area change; B – tricuspid annular plane systolic excursion. Adapted from [11]

At the same time, the information on RV afterload might be essential in understanding the mechanisms of RV structural remodeling and functional failure and hence should be routinely incorporated in every EchoCG report [22].

Of the indices used, systolic pulmonary artery pressure (SPAP) calculated as a sum of estimated right atrial pressure and RV systolic pressure by the peak tricuspid regurgitation speed (using modified Bernoulli equation) is the most reproducible and best validated index, with upper reference value of 35 mmHg [30, 31]. Indirect estimation of mean PA pressure (mPAP) using right ventricular outflow tract acceleration time might serve to outrule the pulmonary hypertension in technically challenging cases where tricuspid regurgitation jet is difficult to detect; the upper reference value for mPAP is 20 mmHg, with pressures over 25 mmHg being defined as pulmonary hypertension and 20–25 mmHg range falling into the "grey zone". These techniques [32] should not be taken as a substitute to SPAP measurement as their accuracy tends to dramatically decrease in high PA pressures.

Thus, detailed evaluation of the systolic function of both ventricles as well as LV diastolic filling should be an essential part of every EchoCG study, including those performed in patients with bronchopulmonary disease. With a purpose of early detection of subclinical functional alterations, additional monitoring of the RV diastolic indices might be advised in this category of patients.

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INFLUENCE OF HORMONAL DISORDERS ON ENDOTHELIAL DYSFUNCTION IN PATIENTS WITH ARTERIAL HYPERTENSION AND COMORBIDE ENDOCRINOPATHIES

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Abstract

Purpose: to investigate the effect of hormonal changes on endothelial dysfunction (ED) in patients with a comorbid course of hypertension (H), type 2 diabetes mellitus (T2DM) and subclinical hypothyroidism (SHT). **Methods:** 183 patients with H stage II were divided into 3 groups: Group 1 (n=50) – with isolated H (comparison group); Group 2 (n=63) – with a combined course of H and T2DM; Group 3 (n=70) – with comorbidity of H, T2DM and SHT. Blood pressure levels, carbohydrate, lipid and thyroid metabolism, plasma insulin concentration, insulin resistance (IR) the HOMA-IR index, vascular endothelial growth factor (VEGF-A) plasma levels were investigated. **Results:** Dyslipidemia was more pronounced in group 2 than in group 1. The addition of SHT was accompanied by a tendency to increase all the atherogenic lipids. IR was observed in all patients groups and was significantly higher than in control group ($p<0.05$). Significant increase of VEGF-A levels in all patients groups in comparison with the control ($p<0.05$) was found. In group 2 VEGF-A was lower than in group 1, which is probably due to the protective effect of metformin. Analysis of the influence of thyroid dysfunction degree on ED revealed significant increase of VEGF-A levels in TSH>6.0 ?MU/ml subgroup (352.55±17.64 pg/ml vs 461.74±20.13 pg/ml ($p<0.05$)). **Conclusion:** Hormonal disorders contribute to aggravation of endothelial dysfunction in patients with hypertension and comorbid endocrinopathies – type 2 diabetes mellitus and subclinical hypothyroidism. Even minor decrease in thyroid function lead to the progression of endothelial dysfunction.

Key words: *hypertension, type 2 diabetes mellitus, subclinical hypothyroidism, endothelial dysfunction.*

Endothelial dysfunction (ED) is a complex of multi-stage balance disorders of opposing factors: relaxation and vasoconstriction, procoagulation and anticoagulation, factors of hyperproliferation and inhibition of endothelial growth, etc. Various triggers can lead to ED development, e.g. changes of blood flow velocity, increased pressure, hypoxia, hyperhomocysteinemia, increased processes of hyperperoxidation, hormonal imbalance, etc. [1]. The occurrence and progression of ED is the basis of many diseases development and formation of various vascular complications. The leading role of ED in development of arterial hypertension (H), atherosclerosis, diabetes mellitus (DM), chronic heart failure, and others has been proved [2]. It is important that ED develops already in the early stages of pathological processes and precede the clinical manifestations of the disease. Therefore, detection of ED in the early stages has a great diagnostic and prognostic significance.

Achievements of modern medicine have significantly expanded the capabilities of diagnosis, treatment and prevention of various diseases, however, the issues of effective treatment of the most common and socially significant diseases, the ability to influence the course of the disease, prognosis and the quality of life are still relevant. Treatment of patients with

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comorbid diseases such as H and the most common endocrinopathies – type 2 diabetes mellitus (T2DM) and hypothyroidism, including subclinical one (SHT), which often affect able-bodied people, is of particular importance and determines their great social significance.

The combined course of these diseases greatly increases the risk of cardiovascular complications (CVC) development. And if, in the combined course of H and T2DM, similar pathogenetic disorders develop, the adverse effect of which on CVC formation is realized, including through ED, then in cases of H and thyroid imbalance, many questions still remain unclear. It is known that the thyroid system, actively interacting with other neurohormonal factors, also influences the processes of vascular regulation.

It is believed that T2DM with hypothyroidism, including subclinical one, make a significant contribution to formation of cardiometabolic risk factors, which determine the course and prognosis of various cardiovascular diseases (CVD) and CVC development.

Until now the data on the impact of thyroid dysfunction, developing against a background of SHT, on the state of endothelium and ED formation are very diverse and remain unclear [3]. It is suggested that a decrease in endothelium-dependent vasodilation in patients with hypothyroidism may be a manifestation of free triiodothyronine deficiency. There is also evidence of vascular endothelial damage due to immune complexes in the presence of chronic autoimmune thyroiditis (AIT), which can enhance ED manifestations [4].

Numerous factors and conditions which effect ED development, such as age, hormonal changes, in particular postmenopause, dyslipidemia (DL), especially hypercholesterolemia and hypertriglyceridemia, T2DM, smoking, H have been established . The risk of ED development progressively increases depending on the presence of different risk factors combination.

One of the important and widely investigated during the recent years marker of ED is Vascular Endothelial Growth Factor-A (VEGF-A), which is considered to be one of the earliest blood serum marker of ED, the changes of which appear even ahead of active intravascular inflammation[5–7].

The multifactorial mechanisms of ED development in comorbid pathology are complex and not sufficiently investigated. The study of comorbid conditions, each of them is characterized by the presence of ED in the pathogenesis and factors, exacerbating this dysfunction, which can lead to changes in VEGF-A expression and, probably, will allow better understanding the common pathogenetic processes and development of more effective preventive and therapeutic measures, is of particular interest.

2. Purposes, subjects and methods:

2.1. Purpose was to investigate the effect of hormonal changes on endothelial dysfunction in patients with a comorbid course of hypertension, type 2 diabetes mellitus and subclinical hypothyroidism.

2.2. Subjects & Methods

The study involved 183 patients (of them 114 women) aged 40–75 (mean age 57.4±5.2 years) with stage II H. Depending on the comorbid pathology all patient were divided into 3 groups: Group 1 (n=50) – with isolated H (comparison group); Group 2 (n=63) – with a combined course of H and T2DM; Group 3 (n=70) – with comorbidity of H, T2DM and SHT, which developed as a result of autoimmune thyroiditis (AIT).

Non-inclusion criteria were symptomatic H, diabetes type 1 and other endocrine disorders, clinical signs of coronary heart disease or severe concomitant chronic diseases, pregnancy. Patients with diagnosed manifested hypothyroidism or with treated SHT, after surgical treatment of thyroid gland were not included. Administration of iodine preparations, glucocorticoids, amiodarone, lithium medicines, estrogens was also exclusion criteria. The control group consisted of 20 age- and sex-matched volunteer patients, and without cardiovascular diseases and endocrinopathies.

Against a background of dietary recommendations, all patients received basic antihypertensive therapy in individually selected doses in accordance with international and national Guidelines [8–10]. Antidiabetic therapy included Metformin in individually selected doses from 1000 to 2500 mg/day.

The survey program included a single list of laboratory and instrumental studies. Blood pressure levels were assessed by means of blood pressure obtained from three measurements at 2-minute intervals in a sitting position.

Assessment of carbohydrate metabolism included fasting glucose (analyzer "Humolizer", Germany); glycosylated hemoglobin level (HbA1c) with "Hummer" kits (USA); lipid metabolism assessment included: total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-cholesterol) in blood serum (by enzymatic colorimetric method with kits "Human", Germany); very low density lipoproteins

cholesterol (VLDL-cholesterol), low density lipoprotein cholesterol (LDL-cholesterol) by standard methods; plasma insulin concentration – by enzyme immunoassay (Insulin ELISA, DRG, Germany). To determine insulin resistance (IR) the HOMA-IR index was used according to the formula: (fasting glucose) x (fasting insulin)) mmol/ml/22.5). For verification of the diagnosis of SHT and AIT the concentration of thyroid stimulating hormone (TSH), free thyroxine (fT4) and antibodies to thyroid peroxidase (AT-TPO) in serum with enzyme immunoassay were measured using diagnostic kits reagents, "Granum" Ltd (Ukraine). Ultrasound examination of the thyroid gland was performed according to the standard procedure on the device "LOGIQ5".

The level of VEGF-A in blood plasma was studied by an immunoassay (ELISA) kit IBL International Gmbh (Germany) on a semi-automatic immunoassay microplate analyzer "ImmunoChem-2100", HighTechnology, Inc. (USA).

Statistical data were processed using SPSS 21.0 software. Qualitative data were presented as percentages; quantitative in the form of the mean and standard error (M±m). The Student criterion was used to estimate the differences between groups in the distribution close to normal. The differences were considered statistically significant at $p<0.05$.

The study was performed in compliance with the basic provisions of the World Medical Association (WMA), Helsinki Declaration on ethical principles for medical research involving human subjects (1964–2000) and MOH of Ukraine Order No. 690 dated September 23, 2009. The study was approved by the Bioethics Commission of Kharkiv National Medical University in accordance with the principles set forth in Helsinki Declaration. All the patients signed the inform consent.

Conflict of interests

The authors declare that they have no competing interests.

3. Results and discussion

To objectify the study all selected patients had controlled H, which made it possible to minimize the effect of blood pressure (BP) on the studied parameters. Thus, in all groups of examined patients BP levels differed non significantly ($p>0.05$, see Table).

DL was observed in all groups of examined patients: in patients of group 2, DL was more expressed than in patients of group 1. Addition of SHT in patients of group 3 was accompanied by a tendency to increase all the atherogenic lipids.The presence of more expressed DL in patients of group 3, obtained in our study, coincides with the results of HUNT study, which showed a direct relationship between TSH levels and blood lipids [11] and again confirm the existing data on the presence of atherogenic dyslipidemia on the background of incomplete compensation of thyroid metabolism, including with comorbid pathology, as a risk factor for the progression of atherosclerosis and, accordingly, the risk of its complications [12].

IR developed in all patients groups and was significantly higher than control group (HOMA-IR in groups 1, 2 and 3, respectively: 4.12±0.48; 8.24±1.29; 6.27±1.38; control – 2.23±0.36 ($p<0.05$)), significant differences between the examined patients with the control group and at the same time minimal differences between the plasma insulin levels in different groups (respectively in groups 1, 2 and 3 (μIU/ml): 15.28± 2.11 ; 18.54±2.17; 18.12±2.14; control -9.8±1.16 ($p<0.05$)) was found.

The obtained data on the presence of IR require clarification. The occurrence of IR with T2DM is an expected sign, but lower level of IR in patients with a comorbid course of H, T2DM and SHT is not completely clear. Xu C. et al. [13] have re-analyzed the data of a mature database (NHANES, 1999 ~ 2002) and found that both fasting plasma glucose levels and the proportion of hyperglycemic subjects among SHT patients were higher than that found in euthyroid controls. These authors confirmed increased HOMA-IR index in the SHT state in their study.

Contrariwise, the presence of significantly lower values of the HOMA-IR index in group 3 may be explained by the favorable effect of metformin, which was accepted by all participants in the study with diabetes. In recent years, there has been a number of studies indicating the positive effect of Metformin not only on carbohydrate metabolism but also on the thyroid gland [14], which may have a greater positive effect on the degree of IR than in the case of impaired carbohydrate metabolism alone at T2DM, but these data are quite contradictory and need further studying [15].

At the same time, the decreased dynamics of HOMA-IR in patients of group 3 can be explained by bi-directional effect of thyroid hormones: on the one hand, they have a counter-insular effect, and on the other hand, they can improve the transport and utilization of glucose in the periphery, i.e. have a synergistic effect with insulin, which probably explains such changes in IR.

ED was assessed by changing the VEGF-A levels and a significant increase in all groups of patients was found in comparison with the controls (respectively, control, groups 1, 2 and 3 (pg/ml): 270.11±18.34 pg/ml; 379.24±19.17 pg/ml; 327.10±26.25 pg/ml; 480.19±29.12 pg/ml; ($p < 0.05$)). In group 2, VEGF-A was lower than in group 1, which is probably due to the protective effect of Metformin which was an inclusion criteria for T2DM patients, on the functional state of endothelium [16, 17]. The maximum level of VEGF-A was observed in patients of group 3. The explanation for this may be a combination of adverse effects on the endothelium of several factors: the effect of thyroid hormones directly on ED, as well as the damaging effect of the immune complexes formed against a background of AIT.

To detail the effect of thyroid disorders on ED, the patients of the 3 groups were additionally divided into 2 subgroups depending on the level of TSH: 3^a subgroup (n=36) with a slight increase in TSH (≤ 6.0 μMU/ml) and 3b subgroup (n=34) with a significant increase in TSH (from 6.1 to 10 μMU/ml).

An additional analysis of the effect of the thyroid status on ED revealed significant differences in the level of VEGF-A in 3^a and 3b subgroups (respectively 3^a and 3b subgroups, pg/ml: 352.55±17.64 pg/ml vs 461.74±20.13 pg/ml ($p < 0.05$)), which confirms the negative effect of thyroid disorders on ED even at the stage of SHT despite the presence of factors contributing to its improvement (in our study, taking metformin). It is important to note that aggravation of ED occurred even with a slight increase in TSH.

Moreover, similar data were already obtained in another study conducted earlier, where a complex of carotid intima-media was studied as a marker of ED, in particular, its thickness in patients with the same pathology [18], and, the pathogenetic mechanisms of carbohydrate metabolism disorders, their relationship with ED were clarified [19].

Conclusions:

1. Hormonal disorders contribute to aggravation of endothelial dysfunction in patients with hypertension and comorbid endocrinopathies – type 2 diabetes mellitus and subclinical hypothyroidism.

2. The degree of negative impact of type 2 diabetes mellitus and subclinical hypothyroidism on endothelial dysfunction in patients with hypertension has certain differences. Endothelial dysfunction is more expressed with a combination of hypertension, type 2 diabetes and subclinical hypothyroidism.

3. Even minor changes in thyroid status contribute to the progression of endothelial dysfunction in patients with comorbid pathology.

4. Metformin may have a protective effect on endothelial dysfunction in patients with comorbid pathology.

5. Early detection of endothelial dysfunction and timely treatment of patients with a combination of hypertension and endocrinopathies – type 2 diabetes mellitus and subclinical hypothyroidism will not only reduce the risk of cardiovascular complications, but also change the course of the disease and improve the quality of the patient's life.

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CYTOKERATIN-18 FRAGMENTS FOR PREDICTING STEATOHEPATITIS IN PATIENTS WITH NONALCOHOLIC FATTY LIVER DISEASE

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Abstract

The aim of the study was to investigate metabolic parameters, cytokeratin 18 (CK-18) levels in blood plasma and the state of the liver parenchyma of patients with non-alcoholic fatty liver disease (NAFLD) in non-alcoholic steatohepatitis (NASH) stage. **Materials and methods:** Fasting serum CK-18 was measured in 30 NASH patients (mean age (49.3±3.6). The control group consisted of 20 practically healthy volunteers. The diagnosis of NADH was established on the basis of clinical, biochemical and instrumental data. In addition to basic laboratory tests and ultrasonographic examination, the Fibromax test (Biopredective, France) was performed. **Results:** In patients with NAFLD, the lipid profile showed a tendency to hyper- and dyslipidemia. The obtained data showed a significant increase in the parameters of enzymatic activity of the liver, the average level of aspartate aminotransferase (AST) exceeded the control group 2.5 times, the alanine aminotransferase (ALT) – 2.9 times, respectively (< 0.05). In NAFLD in NASH stage CK-18 levels in blood plasma were significantly increased, exceeded the results of the control group 3 and more times, and allowed to establish NASH in patients of the main group. Conclusions: Increased levels of CK-18 in NASH patients as well as its association with atherogenic dyslipidemia and increasing of liver enzymatic activity were revealed. The obtained data showed the possibility of using biomarker for non invasive diagnostics of NASH and represent the prospect of further research.

Key words: *nonalcoholic fatty liver disease, nonalcoholic steatohepatitis, noninvasive diagnosis, cytokeratin-18.*

Introduction

The prevalence of non-alcoholic fatty liver disease (NAFLD) around the world is approximately 30 %. However, the true prevalence of NAFLDs and associated diseases is unknown, mainly due to the lack of reliable and widely used diagnostic tests. NAFLD is traditionally considered as a consequence of metabolic syndrome (MetS). However, the link between the components of MetS and NAFLD, in particular type 2 diabetes mellitus (T2DM), hypertension (HTN) and cardiovascular disease (CVD), is more complicated than previously thought. NAFLD-associated diseases affect various systems and have different pathogenetic pathways with a large number of clinical evidence, indicate that NAFLD can precede and promote the development of T2DM, HTN, atherosclerosis and CVD. The risk of developing these cardiometabolic diseases is directly related to the severity of NAFLD. Existing data suggest that the presence and severity of NAFLD are associated with an increased risk of T2DM and HTN. Moreover, long-term prospective studies indicate that the presence and severity of NAFLD are independently associated with episodes of lethal and non-fatal cardiovascular events [4, 10, 11].

Fatty liver can be diagnosed using ultrasound, while liver biopsy remains the gold standard for the evaluation of fibrosis and NASH. Although other non-invasive tests have been proposed, they still need to be tested in large multicenter studies. Since steatosis, NAFLD and NASH and associated metabolic diseases are an economic burden, the search for inexpensive NAFLD diagnostic methods and NASH directly is a priority [2, 7, 9].

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The invasiveness, the complexity of the procedure and the mistakes in the interpretation of the biopsy require development of new safe and accurate methods of NAFLD diagnosis. Therefore, there is a growing need for non-invasive diagnosis using biomarkers. Previous studies have shown that some serum biomarkers have the potential for NAFLD and NASH diagnosis.

Povsic, M. et al. (2018), in their meta-analysis of all the biomarkers for NAFLD diagnosis, concluded that cytokeratin 18 (CK18) is a marker of hepatocyte apoptosis and is the most widely studied biomarker of steatohepatitis [12].

CK-18 has been widely studied since 2009 and has showen its high specificity and sensitivity both for NAFLD and NASH in many studies. In recent years, studies of CK-18 in NAFLD patients have been combined with other markers and scales, and aimed at increasing of diagnostic value of NASH and liver fibrosis progression. Thus, Juan Pablo Arab (2018) et al. in their research analyzed CK-18 levels in 41 patients of the Chilean population with a biopsy-proven NAFLD and showed that CK-18 levels were significantly higher in patients with NASH than those in patients without NASH (183.6 U/L [97.4-734.4] vs. 117.2 U/L [83.8–954.8], p = 0.016). The level of CK-18 was a reliable predictor of NASH with 0.732 AUC (95% CI, 0.572–0.897). The CK-18 cut-off of 130.5 U/L had a sensitivity of 92.9%, a specificity of 63%, a positive predictive value of 56.5%, and a negative predictive value of 94.4%. Scientists concluded that CK-18 is a reliable non-invasive marker for NASH and can be used to predict both NASH and liver fibrosis in patients with NAFLD, CK-18 and NAFLD score [1].

Darweesh, Samar K. et al. (2019) studied CK-18 diagnostic value in combination with FibroScan and showed improved accuracy of diagnosis and strong direct link of the marker with NAFLD progression [6].

Toshifumi Tada et al. (2018) in their research with 130 patients histologically verified NASH, created a new scoring system, FIC-22, to predict NASH using CK18 levels and the FIB-4 index. The areas under the ROC (AUROC) FIC-22 curve and the NAFIC score estimation were 0.82 (95% CI, 0.75–0.89) and 0.71 (95% CI, 0.62–0.78) (p = 0.044). In addition, AUROC FIC-22 score for predicting the presence of fibrosis (F ≥ 1) was 0.78 (95% CI, 0.70–0.85). It indicates a high predictive accuracy of the FIC-22 scale for patients with NAFLD not only for steatohepatitis, but also for the presence of liver fibrosis [14].

Therefore, the study of the possibility of using non-invasive biomarkers for the diagnosis of liver fibrosis and the progression of NASH in conjunction with HTN is a promising area of research.

2. **Purposes, subjects and methods:**

2.1. Purpose of the study was to investigate metabolic parameters, cytokeratin 18 (CK-18) levels in blood plasma and the state of the liver parenchyma of patients with NAFLD in the stage of NASH.

2.2. Subjects & Methods

30 patients with NAFLD were examined, aged 30 to 60 years (mean age (49.3±3.6) years). The control group consisted of 20 practically healthy volunteers. The gender distribution of patients admitted to the study was presented by 56.6 % (17/30) of males and 43.3 % (13/30) of females. Statistically significant differences in the gender distribution of patient groups were not obtained. Clinical laboratory parameters of blood, urine and biochemical parameters of blood were determined in all patients. The diagnosis of NAFLD was established in accordance with the criteria outlined in the clinical protocol of the European Association of Gastroenterologists, in common with the Association for the Study of Diabetes and the Association for the Study of Obesity [8]. The blood plasma levels of CK-18 fragments were carried out using the enzyme immunoassay method with the ELISA kit. The condition of liver parenchyma was studied using biochemical test Fibrotest (Biopredictive). An echosonographic examination of the abdominal cavity was performed after fasting using an ultrasound scanner of the expert class "Vivid-3" ("General Electric", USA).

Exclusion criteria were diffuse connective tissue diseases, oncological diseases, acute inflammatory diseases, history of viral hepatitis, toxic (alcohol), drug-induced, congenital metabolic diseases of the liver.

Statistical processing of the results was carried out using "Excel 2010" (Microsoft) and SPSS 19 software packages. Continuous variables are represented as medians and values of 25^{th} and 75^{th} percentiles – Me (Q1-Q3). Student's criterion (t-criterion) was used in order to determine the statistically significant difference in continuous variables in two independent groups for dependent and independent samples, and non-parametric Mann–Whitney and Wilcoxon criteria were used for the distribution of the investigated characteristic, different from the normal one. The difference was considered to be reliable at the

bilateral level of significance $p < 0.05$ for all conducted analyzes.

Conflict of interests

The authors declare that they have no competing interests.

3. Results and discussion

Given the typical metabolic profile of patients with NASH, determination of anthropometric parameters, lipid and carbohydrate profiles is of particular interest. The average BMI of the patients in the main group corresponded to the classes of people with increased weight, in particular 30% of patients (n = 6) were overweight and 30 % (n = 6) had obesity of the 1st degree (*Table 1*).

Table 1

Anthropometric indices of patients in the main group and control group

Index	NAFLD group (n=30)	Control group (n=20)	p
BMI, kg/m2	26.9 (24.8; 28.9)	25.35 (24.3; 27.6)	
WHR	1.02 (0.98; 1.06)	0.89 (0.87; 0.91)	< 0.001

Note: *p < 0.05 – the difference between the values of group's indicators is statistically significant.

The waist to hip ratio (WHR) in NAFLD patients demonstrated the presence of abdominal obesity in comparison with the control group, in which this index exceeded the normal value in 10 % (n = 2) cases of overweight.

Comparison of indicators of functional state of the liver and markers of its inflammation in patients with NASH and control group is presented in *Table. 2.*

The obtained data showed the increased enzymatic activity of the liver in patients with NAFLD. Alanine transaminase (ALT) was 2.9 times, aspartate transaminase (AST) – 2.5 times and gamma-glutamyltransferase (GGT) – 2.8 times higher compared to those in practically healthy individuals. The inflammatory liver markers and total bilirubin did not exceed the generally accepted normal values, but significantly differed between the groups. Thus, the median of alkaline phosphatase in the main group was 1910 units, the thymol test was 2.1 U versus 1205 units and 1.4 U in the control group, respectively ($p < 0.001$).

It is well known that the presence of NAFLD is associated with hyperlipidemia, metabolic syndrome and obesity. In recent years, evidence

Table 2

Indicators of the functional state of the liver, lipid and carbohydrate profiles in patients of the main group and control group

Index	NAFLD group (n=30)	Control group (n=20)	p
ALT, U/l	72.0 (60.7; 84.5)	24.5 (20.2; 29.2)	0.03
AST, U/l	57.5 (48.5; 66.7)	22.5 (17.0; 27.7)	0.007
GGT, U/l	63.0 (59.7; 70.5)	22.5 (18.0; 26.7)	0.002
Alkaline phosphatase	1910 (1745;2127)	1205 (990;1432)	< 0.05
Thymol test, U	2.1 (1.7; 3.5)	1.4 (0.74; 2.0)	< 0.05
Total bilirubin, mkmol/l	14.2 (12.4; 17.2)	9.1 (8.4; 9.3)	< 0.05
Total cholestirol, mmol/l	5.4 (5.0; 6.2)	4.3 (3.7; 4.7)	0.001
TG, mmol/l	1.55 (1.3;1.75)	0.8 (0.6;1.0)	0.001
HDL, mmol/l	3.3 (3.0; 3.9)	2.3 (1.8; 2.7)	0.001
Aterogenic ratio	0.7 (0.60;0.80)	0.4 (0.3;0.52)	0.001
LDL, mmol/l	0.59 (0.38;0.8)	1.38 (1.2;1.5)	0.001
TC, mmol/l	3.1 (2.2; 4.0)	2.04 (1.5; 2.5)	0.001
Glucose, mmol/l	5.4 (5.1; 5.6)	4.47 (4.1; 4.97)	0.001
Insulin, U/l	22.7 (21.2; 25.4)	13.4 (11.6; 17.1)	0.002
HOMA-IR	5.47 (5.0; 6.03)	2.7 (2.1; 3.4)	0.001

based data claimed that lipid metabolism disorders could be present in NAFLD patients without obesity [17]. Comparison of the lipid profile of patients in the main group and control group showed a significant increase in the parameters of total cholesterol, triglycerides (TG), LDL-cholesterol and lowering of antiatherogenic HDL-cholesterol ($p < 0.001$). The atherogenic ratio of the main group 1.5 times exceeded that of healthy volunteers ($p < 0.001$). Changes in all parameters of lipid profile can be explained by the presence of NASH and overweight in examined patients.

The state of carbohydrate metabolism in NASH patients characterized by hyperinsulinemia compared with the control group, and as a consequence, insulin resistance. All indications were significantly different in comparison between the groups, but an increase in fasting glucose level was not higher than normal values, and the levels of insulin and the HOMA-index in 1.5 and 2 times exceeded the results obtained in the control group, respectively. In patients involved in the study, there were stages of liver fibrosis, which on the international scale Metavir correspond to the values F0, F1, F2–3. Stage F4 of fibrosis was not detected. The distribution of the stages of liver fibrosis among patients in the comparison group found that the overwhelming number of patients had the stages F1 and F2–3, namely 80 %. According to the results of the analysis, the total sample of patients was distributed as follows: 0 stage of fibrosis was detected in 20% of patients with NAFLD, F1 in 30 % and F2–3 in 50% of patients (*Fig. 1*).

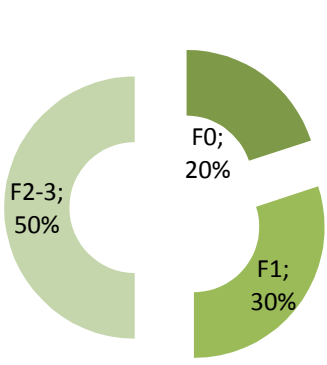

Fig. 1. Distribution of stages of liver fibrosis in NASH patients

Analysis of the enzymatic activity of the liver indicated its growth with an increase in the stage of fibrosis. Thus, the values of AST and ALT were significantly higher in patients with F2–3 compared to those at the F0 stage of fibrosis, that was also confirmed in comparison of the groups F1 and F2–3 ($p2 < 0.05$, $p3 < 0.05$), whereas comparing the results of patients with the stage F0 of fibrosis with the F1 group revealed only a tendency to increase the activity of the inflammatory process in the liver. The obtained results and their association with the stage of fibrosis by such indicators as alkaline phosphatase, thymol test and total bilirubin were non-specific and no significant differences were found.

Similar results were obtained in evaluation of the lipid profile, where the HDL cholesterol index increased with liver fibrosis stage in comparing groups, except comparison of the groups with stages F0 and F2–3. Also, the increase in the rates in comparison of the F1 and F2–3 groups was determined by the level of platelets, which were significantly higher with fibrosis growth ($p < 0.05$). According to the carbohydrate profile, there was no significant difference between the groups.

Determination of the level of cytokine CK-18 in the blood plasma of patients with NAFLD showed its increase. It is noteworthy that the levels of CK-18 in 3 and more times exceeded those of practically healthy individuals.

Discussion. Related data was obtained and published by Li Xue et al. (2018) in the histological study of liver tissue in rats, levels of CK-18 increased with progression of liver disease [15]. Feldstein et al. in 2008 at first found the properties of circulating fragments of CK-18 to reflect NASH in patients with NASH. Since then, intensive biomarker studies have been ongoing, indicating a high diagnostic value of CK-18 and its link with NASH [16].

Cusi K. et al. (2014) in their study found that levels of CK-18 in the blood plasma of patients with NAFLD increased with steatosis, inflammation and liver fibrosis stages growth reflecting the severity of the disease. CK-18 AUROC for predicting NAFLD, NASH, or fibrosis was 0.77 (95% CI = 0.71-0.84), 0.65 (95% CI = 0.59-0.71), and 0.68 (95% CI = 0.61–0.75) respectively. Overall sensitivity/specificity for NAFLD, NASH and fibrosis was 63% (57–70%) / 83% (69–92%), 58% (51–65%)/68% (59–76%) and 54% (44–63%) / 85% (75–92%) respectively [5].

Speliotes E. et al. in the study of paired liver biopsies, found a correlation between the level of CK-18 fragments in blood plasma and NASH in patients with NAFLD [13]. Chalasani N. et al. studied the cohort of NAFLD patients: levels of CK-18 fragments in plasma ≥225 U / L, ≥250 U/l or ≥300 U/l had sensitivity of 70%, 60%, or 53%, respectively; Specificity 82%, 93%, or 100%;

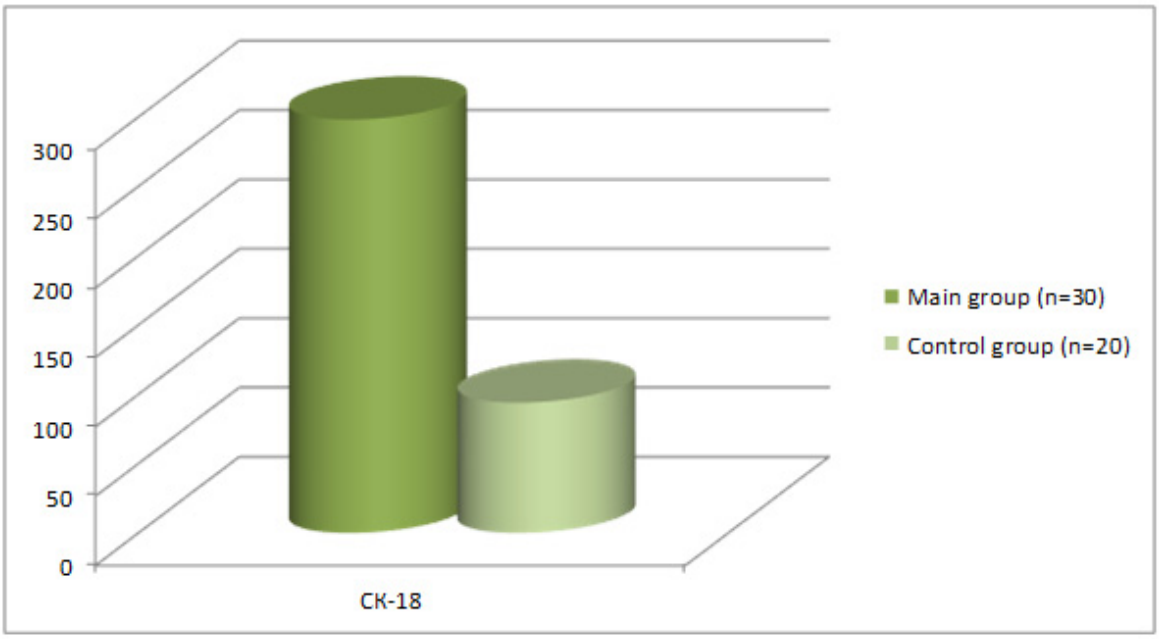

Fig. 2. Levels of CK-18 in patients of the main group and control group
Note: $p < 0.02$ – statistically significant differences in comparison with control group indicators.

positive predictive value 84%, 95%, and 100%; and a negative predictive value of 73%, 69%, and 67% for the diagnosis of NASH, respectively [3]. It makes possible using of CK-18 fragments for NAFLD activity determination and treatment response.

Conclusions

The metabolic profile of NAFLD patients with NASH is characterized by an increase in enzymatic activity of the liver, elevated cholesterol and its fractions and hyperinsulinemia. The levels of CK-18 in the blood plasma of the examined patients were significantly higher than those in the control group, and were associated with metabolic state disorders.

The CK-18 is a promising predictive marker and can be used in NASH diagnosis. Further evidence is needed to improve our understanding of NASH diagnostic, especially as fibrosis stages advance.

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CURRENT CLINICAL AND PATHOMORPHOLOGICAL FEATURES OF THE COURSE OF EXPERIMENTAL EXTRAPULMONARY TUBERCULOSIS

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Abstract

The **purpose** of this study was to describe extra-pulmonary tuberculosis modeling and its features in guinea pigs for further use of the results of the experiment in medical and scientific practice. The study involved 40 sexually mature guinea pigs. Modeling of tuberculous spondylitis was carried out on the basis of the method developed by us (Patent No. 112423 (UA) Ukraine). All animals underwent a dynamic follow-up with clinical, X-ray, pathomorphological and laboratory tests. The withdrawal of experimental animals from the experiment was carried out according to the previously developed schedule, in identifying signs of the studied stages of the tuberculous process.

The study gave a possibility to trace the stage of development of tuberculous spondylitis in guinea pig and to trace the current features of the clinical, radiological and pathomorphological course. The identity of the model of the main clinical forms of tuberculous spondylitis in guinea pig and humans was shown.

This study showed that modern intensive specific antibiotic therapy in the conditions of experiment resulted in a delimitation of the destructive process in relatively early stages of development of the disease (4–5 weeks).

The new knowledge about pathomorphological features of the course of tuberculous spondylitis makes it possible to carry out radical surgical interventions on the locomotor apparatus without the risk of generalizing the tuberculous process at an earlier term.

Key words: *experimental modeling, extrapulmonary tuberculosis, tuberculous spondylitis, treatment.*

Introduction

At present, Ukraine belongs to the group of countries with high levels of tuberculosis cases with a significantly higher incidence than in the vast majority of countries in Central and Eastern Europe [13, 14].

The current epidemiological situation is characterized by spread of tuberculosis infection in all its manifestations, which is also accompanied by a gradual increase in the incidence of extrapulmonary tuberculosis (EPT). The difficulties of early detection lead to a relatively late diagnosis of the disease as late as in the period of development of the destructive process, abscesses and fistulas. Diagnostic errors in the early stages of the examination of patients with EPT play a leading role in development of advanced tuberculosis [3, 12, 16, 17].

It is important to note that at the present time the system of provision of specialized medical care to patients with EPT does not work in our country. The problems of treatment and diagnosis of pulmonary tuberculosis are addressed to the Ministry of Health of Ukraine and specialized medical institutions, while the problems of EPT treatment remain beyond the attention of these institutions [6, 7, 13].

In the context of the current epidemiological situation in Ukraine, osteoarticular tuberculosis (OAT) is ranked first in the structure of morbidity of extrapulmonary tuberculosis, and in the structure of the total incidence of tuberculosis, the share of extrapulmonary disease accounts for 10.6% [7, 13].

The basic information about the pathogenesis of tuberculosis as a chronic specific infection from the time of Robert Koch to the present day has been obtained through experimental studies, and

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the spine according to the literature, the form and size of vertebrae in guinea pigs differ from human ones, but the general plan of the anatomical structure of the spine is similar [4]. The selection of animals is also due to high susceptibility to MTB.

Modeling of tuberculous spondylitis was conducted on the basis of the original method (Patent No. 112423 (UA) Ukraine) [11]. The utility model was based on the task of creating a model of tuberculous spondylitis maximally close to the natural process according to its clinical and radiological signs.

According to the utility model, the animal is immobilized and in the position on the right or left side, the skin in the region of the lumbar spine projection above the ilium is removed from fur and treated with iodine solution, the surgical field is separated by sterile napkins, and layered external abdominal access to the anterior-lateral surface of the bodies of L2–L4 segments is performed by stratification of the muscles of the anterior abdominal wall and pressing of the parietal layer of the peritoneum along with the contents of the abdominal cavity to the medial side. After removal of the anterior-lateral surface of the vertebral bodies, segmental vessels are visualized, the latter are taken on the holders on both sides, banded and cut, then 0.5 ml of mycobacterium tuberculosis culture of M. bovis suspension of Valle strain is administered under visual control with a syringe into the body of the vertebra. The dressing of segmental vessels significantly degrades circulation in the vertebrae, thereby creating favorable conditions for development of a specific inflammatory process. The postoperative wound is sutured, this is followed by dynamic clinical and radiological control until the appearance of clinical and radiological signs of tuberculous spondylitis.

The study involved 40 guinea pigs. The animals were divided into 4 equal groups.

Groups 1, 2, 3 (main) received an injection of 0.5 ml of M. tuberculosis suspension (0.1 mg of dry weight in 1 ml) into the vertebral body according to the procedure.

Group 4 was the control one. Animals were injected with a sterile physiological solution (0.9% – 0.5 ml) into the vertebral body.

Group 1 included 10 guinea pigs receiving specific first-line antibacterial agents (ABA) (isoniazid, streptomycin, rifampicinum).

Group 2 comprised 10 guinea pigs receiving specific second-line antibacterial agents (ABA) (amikacinum, rifabutin, ofloxacin).

Group 3 involved 10 guinea pigs receiving no treatment.

The dose of specific ABAs was calculated based on the animal weight (parenterally) daily.

The choice of the site of infection was determined by a high frequency> 50% of localization of primary osteitis in the subchondral zone of the vertebral body in patients with TS.

Experimental modeling of EPT was carried out by introducing M.bovis suspension of Valle strain (0.1 mg dry weight in 1 ml) intra-rectally according to the procedure.

In the first stage of the experiment, after the detection of the signs of spondylitis stage of tuberculous spondylitis (in 1 month), 16 animals were removed from the experiment (4 in each group). In the second stage, after the detection of clinical radiological spondylitic stage, 16 animals were removed likewise. The withdrawal from the experiment was carried out by ether overdosing.

Later, we were waiting for the appearance of the post-spondylitic stage or remission of the disease, but the overwhelming number of animals in which EPT was modeled died within 3 to 4 months. The cause of the death of these animals, as confirmed by macroscopic examination on autopsy, was generalization of the infectious process with subsequent damage to the vital organs and systems.

Post-spondylitic stage of the disease was obtained only in 2 guinea pigs of the second group, receiving specific second-line ABAs. Two guinea pigs in the control group were not found to have any signs of the infectious process at the end of the experiment.

All animals underwent follow-up examination with a detailed clinical assessment, weighing, evaluation of the function of the spine and lower extremities. Clinical examination of the animals involved assessment of the behavior, posture and nature of the movements.

All the animals that were taken out of the experiment underwent a pathomorphological study.

Conflict of interests

The authors declare that they have no competing interests.

3. Results and discussion

One month after infection, in all the animals of the main observation group, an increase in general temperature was observed on average by 0.5°, accompanied by an increasing restriction of movements in the large joints of the lower extremities. Loss of body weight of animals ranged from 20.0 to 40.0 grams. Six guinea pigs

were found to have abscesses on the anterior and medial surfaces of the thighs. Development of abscesses could result from the destruction of the cortical layer near the destruction site and subsequent involvement in the inflammatory process of the perivertebral tissues. X-ray of the spine showed local osteoporosis with focus of damage with a destruction of the subchondral zone.

The next examination of experimental animals showed formation of tuberculous osteitis which later increased in size (only within the vertebra).

Over the next 4–6 weeks, symptoms of intoxication continued to increase (lethargy, appetite loss), weight loss was an average of 60 grams. Some animals were found to have thickening and infiltration of soft tissues in the area of postoperative access, and paravertebrally there was a flexural contracture in the joints of the lower extremities. A marked lameness was detected; the limb of the animal was not loaded, pulling it behind the body (*Fig. 1*). In some cases, abscesses spread to the thigh area, fistulas were formed. Radiograms registered an increase in destructive centers, occupying the entire vertebra with the transition to an adjacent segment. The foci of destruction contained sequesters of various forms and sizes (*Fig. 2*).

The withdrawal of experimental animals from the experiment was carried out according to a previously developed schedule. Before euthanasia, the animals were thoroughly examined, and after that, blocks of segments of the lumbar spine affected by the destructive process were produced, the macroscopic specimens were subjected to X-ray and pathomorphologic examination, and pathomorphological studies of the internal organs were performed.

Pathomorphological study

Anatomical preparation and macroscopic study of spinal specimens were carried out immediately after withdrawal from the experiment. The study involved macroscopic evaluation of the condition of the spine, musculo-ligamentous apparatus, detection of congested abscesses, preparation of macroscopic specimens of vertebrae (*Fig. 3*).

Fig. 1. Appearance of guinea pigs in the spondylitic stage of the disease

For histological examination, the vertebrae of guinea pigs and adjoining muscles were isolated and fixed in a solution with a mass fraction of neutral formalin 10%. To study the inflammatory process in the vertebrae, decalcification of the bones was performed in a solution with a mass fraction of 4% nitric acid at the temperature ranging from 18 to 22°C. The bones after decalcification and muscles adjacent to vertebrae were dehydrated in alcohols of increasing concentration (50°, 70° alcohols and twice at 96°) and in alcohol with ether (1:1 solution), and enclosed in celloidine. Histologic slices were made using Reichthert sledge microtome and stained with hematoxylin and eosin and by Van Gieson. Histological analysis was performed using Axio Star Plus (Carl Zeiss) light microscope, taking photos with Canon Power Shot A610 digital camera and AxioVision computer software.

Histological examination in 1 month after the inflammatory process modeling clearly showed the signs of inflammation (infectious process) in all animals.

Histological specimens made from the lumbar spine of the guinea pigs infected with mycobacterium tuberculosis and treated with specific first-line ABAs in the spongy tissue of the vertebral body, showed foci of specific inflammatory process, manifested by formation of epithelioid (center – epithelioid cells) and necrotic (center – caseous necrosis) tubercles. Around the centers of the tubercles there was a shaft of epithelioid cells followed by lymphocytes, macrophages, plasma cells, and polynuclear giant Pirogov–Langhans cells (*Fig. 4*).

After the treatment of animals with specific second-line ABA, microscopic analysis revealed the changes typical for cessation of the inflammatory process. There was sclerosis of trabeculae and cortex, forming on the border with the area of impairment of newly formed spongy bone tissue with a significant density of brightly colored osteocytes on the surface. The cells did not form lacunae and contained large hyperchromic nuclei and basophilic cytoplasm. Intertrabecular spaces contained reticulofibrous tissue.

However, restoration of the integrity of the vertebral body was not observed. Connective

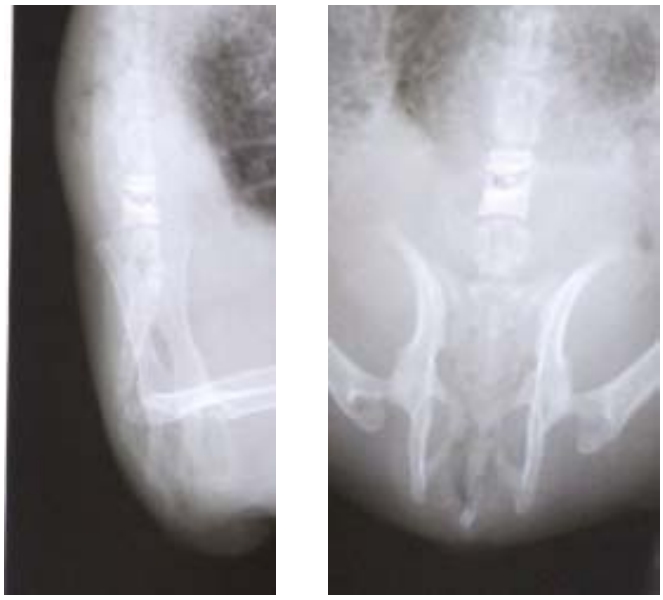


Fig. 2. X-ray of the spine. Contact destruction of the vertebrae

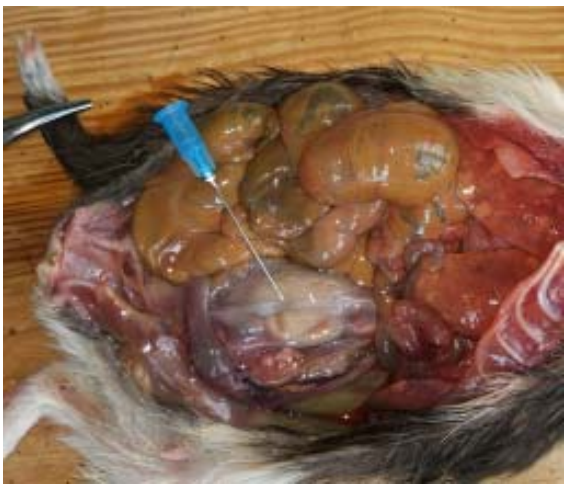


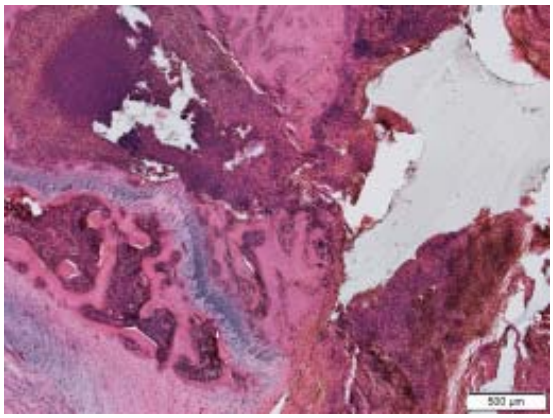
Fig. 3. Visualization of bilaterally congested abscesses of the spine of the guinea pig of the first group

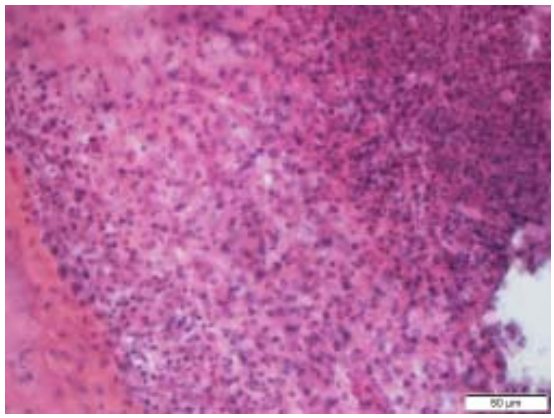
tissue of varying degrees of maturity with a significant number of vessels of different diameters, densely packed fibroblasts, the presence of lymphoid and plasma cells, formed in the area of impairment by its tuberculous inflammation after the action of specific second-line ABA.

The pathological changes found 1 month after EPT modeling in the affected vertebrae of guinea pigs that did not receive specific ABA, were characterized by the following pattern: evaluation of the specimen at the level of destruction showed that infiltrate was located subchondrally. Among infiltrates there was a small necrotic focus with the presence of elements of decay.

Among inflammatory infiltrates there were necrotic foci with granular disintegration of cellular elements of granulation tissue. Along with

a





b

Fig. 4. A fragment of the vertebral body of the lumbar spine of a guinea pig after modeling of tuberculosis and treatment with specific first-line ABA: a) focus of a specific inflammatory process, destruction of the cortical layer; b) peripheral part of necrosis tubercle, macrophages, lymphocytes, plasmocytes. H&E stain

infiltrates there was an inflammatory edema of loose connective tissue. In the subchondral bone at the border with the subchondral tuberculosis described above there were necrotic changes in the bone trabeculae.

In the tubercle there was a high density of epithelioid cells with a characteristic structure, namely a small weakly basophilic core, surrounded by abundant cytoplasm.

In the intertrabucular space, destruction of the bone marrow and formation of productive inflammation foci and epithelioid-cell tubercles, which differ from each other, was observed. Some of them did not contain all the elements of the tubercle. They were represented by epithelioid cells with light oval nuclei. In the marginal parts of the tubercles there was a high density of lymphocytes, macrophages and fibroblasts. Plasma cells were singular.

Thus, morphological study showed the presence of an active tuberculous process in the bodies of vertebrae and paravertebral tissues in animals with modeled tuberculosis and treatment with specific first-line ABAs, as well as in animals without specific treatment.

It is important to note that the degree of severity of destructive changes in the affected vertebrae in untreated animals and those receiving first-line ADAs was practically the same.

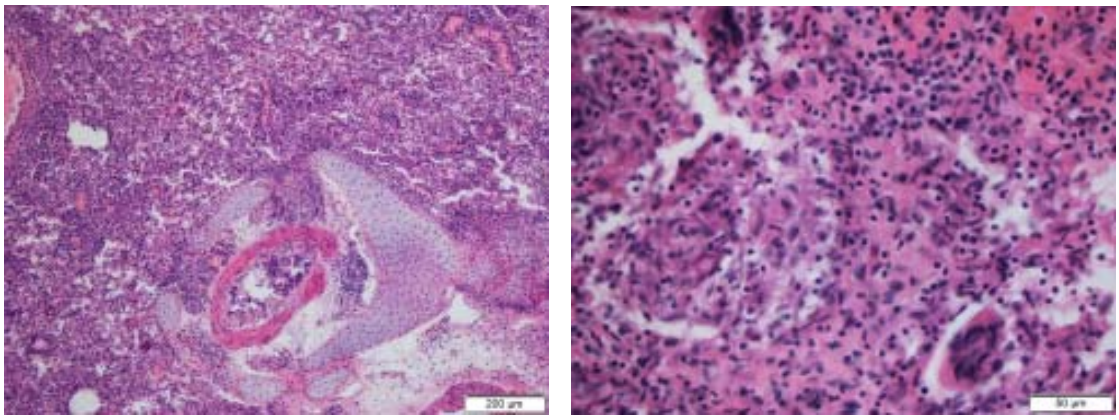
Animals with modeled tuberculosis treated with specific second-line ABAs were found to have inhibition of the pathological process with the formation of young bone and connective tissue of varying degrees of maturity, and the presence of an area separating the inflammatory focus from healthy tissue in the early stages of the disease (one month).

Microscopic examination of the internal organs of animals with modeled EPT and treatment with specific first-line ABAs and untreated animals showed pathological changes characteristic of generalization of the tuberculous pathological process.

Pathomorphological study showed structural impairment of the lungs: alveolar structure was not traced;, desquamation of the epithelium in bronchioles and bronchi, their lumen was filled with fluid, peri-bronchial foci of lymphocytes and plasmocytes. There was productive specific inflammation. In all fields of view, there were multiple tubercles of epithelioid-cellular structure (*Fig. 5 a*). There were several layers of epithelial cells, macrophages, lymphocytes and plasma cells. There were polynuclear Pirogov–Langhans cells among epithelioid cells (*Fig. 5, b*).

In contrast to the animals with modeled tuberculosis treated with first-line agents and the untreated ones, the alveoli in the lungs of the control group and of animals receiving second-line ABAs, were in a state of dystelectasis, partially expanded, filled with insignificant amounts of fluid. Interalveolar partititions were thin. Alveolocytes were located in one row on the basement membrane, had an eosinophilic cytoplasm and a round small hyperchromic nucleus. Bronchioles had a folded inner membrane with a cylindrical single-row epithelium, the nuclei were located on the basement membrane.

Microscopic examination of the liver in animals of experimental groups showed structural impairment: capillary sinuses were not defined, diffused inflammatory infiltrates around isolated preserved liver triads, hepatocytes were located in separate islets without the formation of a trabecular structure. Thus, multiple productive



a *b*

Fig. 5. A fragment of a guinea pig lung after modeling of tuberculosis and treatment with first-line ABAs: a) absence of an alveolar structure, a productive specific inflammation; b) productive epithelial tubercle with a giant Pirogov–Langhans cell. H&E stain

necrotic tubercles found around the central veins were found to have necrotized, or epithelioid-cellular center. Around it there was a shaft of epithelial cells, macrophages, lymphocytes, and plasma cells (*Fig. 6*). The revealed morphological picture is characteristic of tuberculous inflammation.

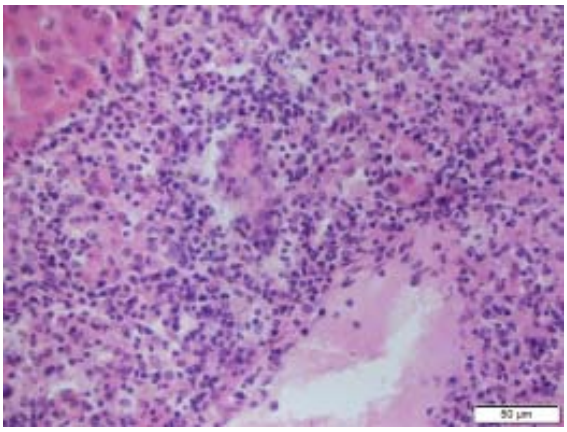


Fig. 6. Fragment of a guinea pig liver with modeled tuberculosis following administration of first-line ABAs (Group 1): epithelioid, lymphoid cells, macrophages, dystrophically altered hepatocytes. H&E stain

On histological specimens of the liver of control animals and animals receiving specific second-line ABAs, the connective tissue capsule and hepatic lobes were clearly differentiated. Central veins were located in the center of the lobes, hepatocytes radially departed from it, forming a trabecular structure. Cytoplasmic membrane of cells was clearly visualized, small eosinophilic inclusions were noted in the cytoplasm. The described structure is characteristic of the norm, which indicates the high efficiency of specific therapy with second-line ABAs, and the absence of generalization of the tuberculous process secondary to treatment with these agents.

Summing up the observations of guinea pigs, we conclude that in all cases (30 guinea pigs), under this method of infection, tuberculous spondylitis was confirmed clinically and pathohistologically.

In our experiment it was possible to trace the stage of development of tuberculous spondylitis in guinea pig and to correlate the phases of its evolution with the stages of development of TS, set forth in the generally accepted classification of E. M. Belendir [2] in accordance with the tasks set.

The identity of the tuberculosis model of the spinal cord in guinea pig with intravertebral infection was revealed in the human with the main clinical forms of TC: tuberculous osteitis developed up to 4 weeks (1 stage according to the classification of E.M. Belendir), up to 8–9 weeks – there was progression of osteitis with the onset of spondylitis (2 stage in this classification) with subsequent distribution of destruction on adjacent segments of the spine [1, 3].

It should be noted that fundamental research regarding the features of the current course of extrapulmonary tuberculosis and its pathomorphologic features is not performed in our country. Recent Russian-language publications on experimental modeling of this disease date back to the 1960s. There are practically no publications on EPT modeling in the last 30 years in foreign literature.

Crucially important, in our view, is the lack of experimental studies in the world scientific literature on the study of the impact of not only modern antibacterial anti-tuberculosis agents (amicacinum, rifabutinum, ofloxacin), but also agents which have been introduced as part of standard treatment over the last decade, on the destructive specific tuberculous inflammatory process of the spine. The only exception is the study of the effect of streptomycin on the course of tuberculous inflammation of the musculoskeletal system, conducted by O.P. Skoblin in 1953 [14].

Scientific works of the leading experts of Leningrad Institute of Tuberculosis Surgery state that pathogenesis of tuberculosis is the most important violation of microcirculation around the lesion, which plays a major role in the spread of infection, its localization in organs and tissues, in the very development and course of tuberculosis inflammation, the role of tuberculosis inflammation, and the role of tuberculosis inflammation, primary focal lesions of organs [2, 5, 8]. The procedure of ligature of segmental vessels in guinea pigs, which allowed us to create an experimental model of pulmonary tuberculosis in all experimental animals, was aimed at the violation of microcirculation in the zone of infection during the experiment.

Conclusions

Based on the histological examination of vertebrate bodies, the animals treated with specific first-line ABAs were found to have evident morphological features of tuberculous inflammation. The animals treated with specific second-line ABAs were shown to have inhibition of the pathological process with the formation of young bone and connective tissue of varying

degrees of maturity but without restoring the integrity of the vertebral body.

Microscopic study of the internal organs of the guinea pigs showed a specific productive inflammation in animals with modeled tuberculosis in which generalization of tuberculosis occurred in the lungs and the liver, morphological features of which were the same in both groups.

The foregoing indicates the low effectiveness of the antibacterial action of specific first-line ABAs, and the high efficiency of the second-line ABAs, the absence of generalization of the tuberculous process secondary to treatment with these agents.

Thus, this study showed that modern intensive specific antibiotic therapy in the conditions of experiment allows to achieve a delimitation of destructive process in relatively early stages of development of the disease (4–5 weeks).

The new knowledge about the pathomorphological features of the EPT course secondary to specific antibiotic therapy allows for radical surgical intervention on the locomotor apparatus without the risk of generalization of the tuberculosis process at an earlier term.

In our opinion, the prospects for further research in experimental EPT are as follows: testing of diagnostic tests, testing of medicinal products, development of surgical methods of treatment, testing of the use of various pathogenic methods of treatment.

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METHOD OF MORPHOMETRIC ANALYSIS OF THE CORPUS CALLOSUM FORM ON THE BASIS OF MR-IMAGES AND APPLICABLE TO ITS NATURAL PREPARATIONS

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Abstract

Further development of neurosurgery requires increased knowledge of the anatomy of the corpus callosum. That is why the current direction of modern morphological research is the study of the sexual dimorphism of the corpus callosum in the age aspect, taking into account individual variability. The purpose of the study was to develop an integrated approach to digital quantization of the corpus callosum, which will allow to solve the problem of defining the characteristics of the individual variation of the human corpus callosum sexual dimorphism in the age aspect in more detailed and comprehensive way. The material used were 40 MRI images of male and female heads of the II period of mature age without a pathology of the central nervous system and 44 brain preparations of men and women of the II period of mature age, who died for reasons not connected with the pathology of the central nervous system either. As a result of the study, the longitudinal-altitudinal index of the corpus callosum was calculated and its three main forms were distinguished: low convex, medium convex and high convex. By isolating the two thighs in the trunk of the corpus callosum (anterior and posterior), we obtained additional data specifying the true length of the corpus callosum. We also resorted to the topological transformation of a complicated configuration of the corpus callosum shape into a simple planimetric figure, which is a circle, by determining its radius according to the formula $R=L/2\pi$, where L is the total length of the corpus callosum perimeter. This provides an opportunity to express the nuances of its individual, sexual and age variability in more visual form in diagrams. Conclusions. To obtain an optimal morphometric characteristic of the planar projection of the sagittal profile of the corpus callosum, we offer a simple geometric analysis that is applicable not only for MRI images, but also for anatomical preparations on the basis of photographs of the medial surface of the cerebral hemispheres. Thus, we get the opportunity to find out how the corpus collosum differs in vivo from the postmortem state.

Key words: *corpus callosum, individual variability, sexual dimorphism, morphometry.*

Introduction

In order to study sexual dimorphism and age-related differences of the corpus callosum, it is necessary to have reliable quantitative criteria about its shape [1–6]. It can be achieved by conducting a well-known morphometric analysis based on conventional reference points, which are usually unequally distant points and straight-line distances between them [7-13]. An example of this approach is the content of the patent for an invention owned by A.N. Biryukov [14] named "Method of the corpus callosum size determination in vivo". All measurements were carried out by the author on the midline images of the brain obtained using magnetic resonance imaging, where the corpus callosum quantization is made on the basis of the measurements of the thickness of its individual parts (genu, trunk and splenium), as well as its length and height. It is quite obvious that this principle can also be applied to natural preparations of the cerebral hemispheres, where the medial surface demonstrates the desired formations.

It should be noted that this method is an example of a simplified approach to the analysis of such a complex geometric formation as the sagittal profile

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of the corpus callosum. Therefore, in the literature there are some other attempts to get closer to a more comprehensive coverage of its form. For example, to quantify it, F. Tomaiuolo, S. Campana and D. Collins [15] used a rectangular contour covering the corpus callosum on midline MRI scans of the cerebral hemispheres, making it possible to determine the angle of its bend by calculating the angle at the top of an isosceles triangle that has the same base and height as the rectangle outlined around the corpus callosum. Obviously, this method can be applied as an additional metric parameter to the previous method.

In the pursuit of a more comprehensive quantitative analysis of the shape and size of the corpus callosum, some authors [16] used multifactorial mathematical apparatus. For this purpose, its shape is modeled using a configuration of conditional marks, which include the center of the genu, the center of the splenium and 50 half-marks, evenly spaced on the circles of the dual contour of the corpus callosum. These configurations are registered by procrust overlay, shifting the coordinate system to a single centroid, by scaling them in accordance with a single centroid size, and also by their rotation in order to minimize the remaining parts of the smallest squares between the corresponding marks. In this case, the centroid size is calculated as the square root of the sum of the squares of all marks from their centroid. Due to the fact that the results obtained by the authors objectively do not differ a lot from those obtained by other authors using simpler methods of morphometric analysis, we consider this method to be excessively complicated.

Thus, the above methods of the morphometric analysis of the corpus callosum do not satisfy the intention to achieve the most optimal result of its quantitative assessment using relatively simple mathematical operations.

Purposes, subjects and methods:

2.1. The purpose of the work was to develop an integrated approach to the digital quantization of the corpus callosum, which will allow to solve the problem of defining the characteristics of the individual variation of the human corpus callosum sexual dimorphism in the age aspect in more detailed and comprehensive way.

2.2. Subjects and methods

The material used were 40 MRI images of the male and female heads of the II period of mature age without the central nervous system pathology, obtained from LLC "Hemo Medica Kharkov". The material obtained from Kharkiv Regional Office of the Chief Medical Examiner was presented by 44 brain preparations of men and women of the II period of mature age, who died for reasons not connected with the pathology of the central nervous system either.

In the the course of study of corpus callosum anatomical preparations, the brain was dissected in a longitudinal split of the cerebrum into two halves after washing and two-week fixation in 10% formalin solution. The medial surfaces of the halves were photographed using a digital camera at the same focal length using a vertically mounted tripod. The image of the corpus callosum sagittal profile obtained in this way corresponded to those ones of MP-tomograms. Morphometric analysis of the sagittal profile of the corpus callosum was performed using the RadiAnt Dicom Viewer and Adobe Photoshop CS6 Extended software.

Conflict of interests. There is no conflict of interests.

3. Results and discussion

Our approach is based on a preliminary geometric analysis of the longitudinal (sagittal) profile of the corpus callosum, which has complex outlines, bordered by two closed (in the region of the rostrum and splenium) arcuate circles, which are smooth curved conjugation of curved lines with different radii of the circles. First of all, its analysis requires the use of relatively conditional, compatible with the shape, coordinates. The most acceptable for this purpose are the rectangular coordinates, where the initial points (marks) used for measurements are determined by the distance between them along straight lines. To do this, we fit the contour profile of the corpus callosum into the limits of an unequal rectangle, the long sides of which are equal to its longitudinal size, and the short sides are equal to the maximum height of its upper trunk bulge (*Fig. 1*). In this case, we will consider the lower long side under the name of the tightening chord of its two arcuate circles (upper and lower), because it is shorter than the length of each of these arcs. We also restrict ourselves to only a few basic dimensional quantities, which include: 1 – the longitudinal dimension along the chord (the length of the corpus callosum as it is usually mentioned in the literature); 2 – the maximum height of its trunk bulge (the vertical distance between the most protruding point of the trunk and the chord); 3 – the thickness of the corpus callosum in the area of the genu, the trunk (in the area of maximum bulge) and the splenium [17, 18].

Using the metric values of length (L) on the constricting chord and the maximum height (H) of

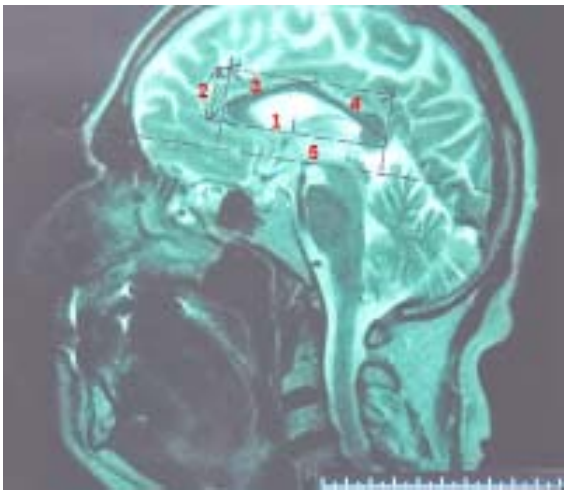


Fig. 1. MRI-image of the male head of the II period of the mature age in the lateral projection; the principle of geometric analysis of the corpus callosum form: 1 – the longitudinal size of the corpus callosum on the constricting chord, 2 – the maximum height of the trunk convex of the corpus callosum, 3 – the length of the anterior trunk thigh, 4 – the length of the posterior trunk thigh, 5 – the largest anteroposterior diameter of the brain skull. A scale metric bar is added below on the right

convexity of the corpus callosum, it is possible to calculate its longitudinal-altitudinal index in accordance with the common formula I=H/L·100. By its value one can determine the individual forms which supposedly will be distributed within the three main ones, which we mention under the name of low convex, medium convex, and high convex forms of the corpus callosum. This is the first supplement we introduce to the method of the morphometric analysis of the corpus callosum previously known in the literature. With these data, it is possible to relate its longitudinal-altitudinal index to the shape of the brain skull of a given individual.

But by determining the length of the lateral profile of the corpus callosum, as it is described in the literature, one ought not speak of its true length, since in its trunk section, approximately in the middle, it is "humped", forming an obtuse angle open downwards. Consequently, the vertex of its angular bend is in accordance with the well-known maximally elevated point of its convexity, which makes it possible to distinguish two thighs in the trunk of the corpus callosum - the anterior and the posterior. Their length can be separately determined by two straight lines connecting a given angular point with the anteriorly projecting point of the genu and posteriorly projecting point of the splenium (*Fig. 2*). It is quite obvious that their total length will be greater than that of the constricting chord, which in this case can be regarded as a hypotenuse with respect to the sides. Thus, we will receive additional data specifying the true length of the corpus callosum, which enables us to obtain

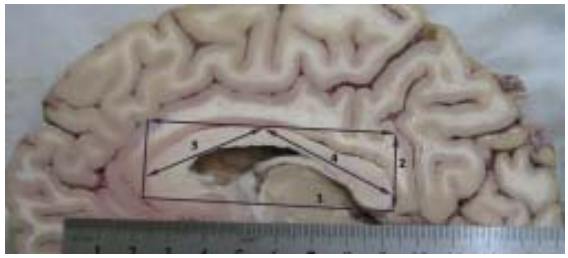


Fig. 2. The medial surface of the cerebral hemisphere of the men of the second period of the mature age. 1 – the longitudinal size of the corpus callosum on the pulling chord; 2 – maximum height of the stem convexity of the corpus callosum; 3 – length of the anterior stem hips; 4 – length of the posterior stem hips

more accurate information when studying its individual variability, sexual dimorphism, and age transformation [19]. By this we introduce the second supplement to the methodology of the corpus callosum morphometry.

But this data do not exhaust the opportunities to expand the metric base of the corpus callosum in its versatile study. For some reason researchers overlooked the opportunity to resort to a planimetric estimation of the area occupied by the corpus callosum in its sagittal section which is currently practically feasible with the help of "Inobitec DICOM Viewer" software [20]. Concurrently, it is possible to measure the length of the outer contour of the corpus callosum (*Fig. 3*).

Having these metric data, we can resort to a topological transformation of a complicated configuration of the corpus callosum shape into a simple planimetric figure, which is a circle, by determining its conditional (isometric) radius (CRCC) according to the formula R=L/2π, where

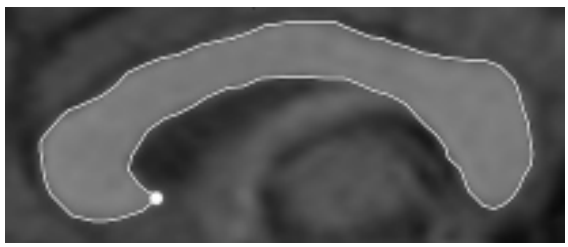
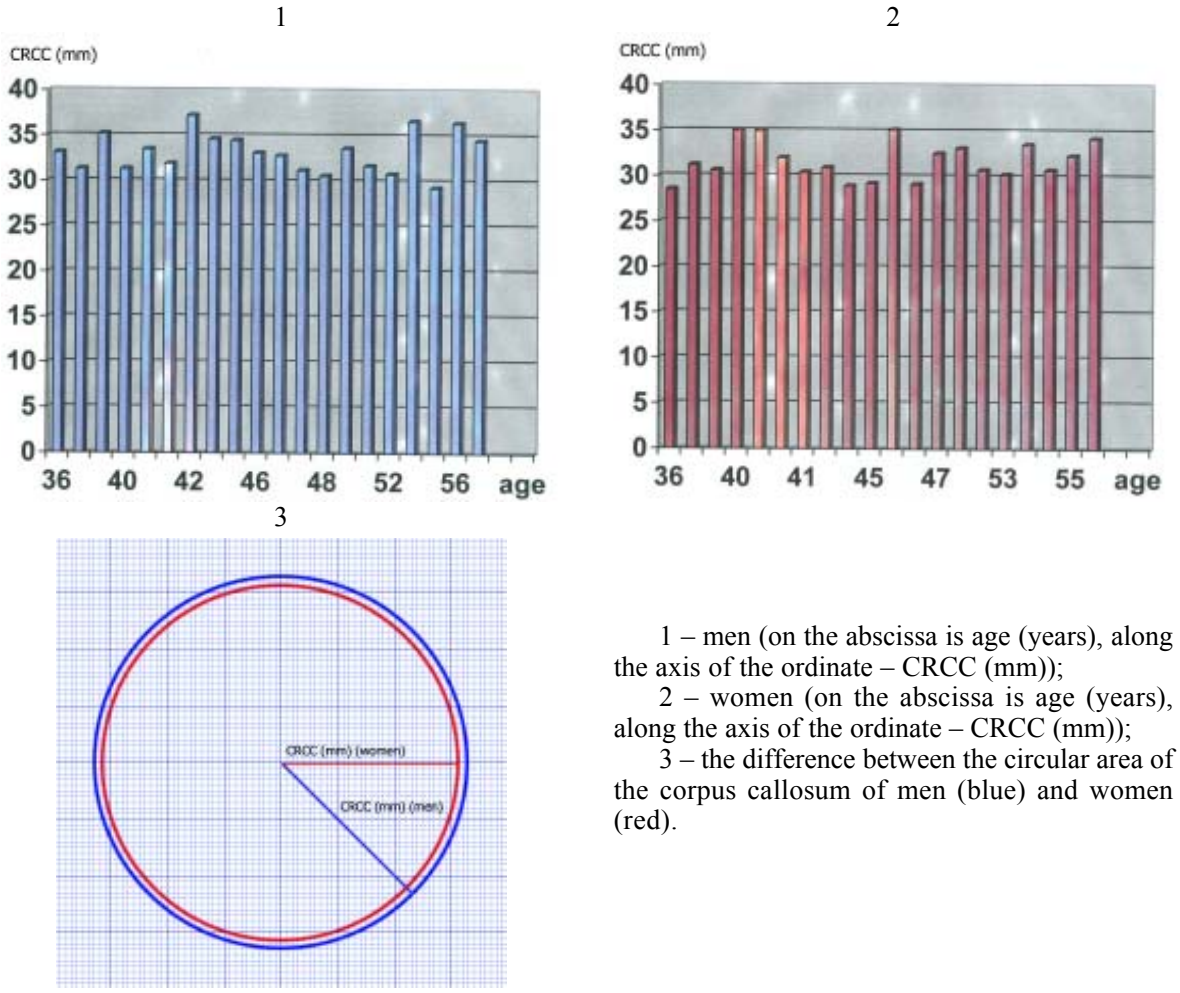


Fig. 3. MR-images of the human corpus callosum, outlined along the perimeter with a white line, interrupted in the terminal part of the beak by a reference point when measuring the length of its contour circumference

L is the total length of the corpus callosum perimeter; this provides an opportunity to express the nuances of its individual, sexual and age variability in more visual form in diagrams (*Fig. 4*). Such a planimetric method, which involves the topological transformation of a complicated configuration of the corpus callosum outline into a circle that makes it more understandable for the visual assessment. As our experience shows, this method is highly productive and has no analogues in the literature, as evidenced by the results of the patent-information retrieval.



1 – men (on the abscissa is age (years), along the axis of the ordinate – CRCC (mm));
2 – women (on the abscissa is age (years), along the axis of the ordinate – CRCC (mm));
3 – the difference between the circular area of the corpus callosum of men (blue) and women (red).

Fig. 4. Individual variability and sexual dimorphism of the medial profile of the corpus callosum, formally expressed by the calculation of its conditional (isometric) radius

Conclusions. Thus, to obtain an optimal morphometric characteristic of the planar projection of the lateral (sagittal) profile of the corpus callosum, we offer a simple geometric analysis that is applicable not only for MRI images, but also for anatomical specimens on the basis of photographs of the medial surface of the cerebral hemispheres. Thus, we get the opportunity to find out how the corpus collosum differs in vivo from the postmortem state according to the following metric parameters: 1 – the length of the corpus callosum along the constricting chord; 2 – the maximum height of its trunk bulge; 3 – the length of the anterior and posterior trunk thighs of the corpus callosum and their total value; 4 – the maximum thickness of the corpus callosum in the area of its genu, trunk and splenium; 5 – the total area of the sagittal profile of the corpus callosum; 6 – the total length of its outer circumference, whose value is used for the topological transformation of the actual form of the corpus callosum into an isometric circle. It should be noted that for this purpose other two-dimensional geometric shapes can be used, such as a square or an equilateral triangle. In these cases, it is necessary to divide the total length of the outer circumference into four or three parts, respectively. In our studies, we preferred the circle, since it is figuratively more similar to the shape of the corpus callosum.

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MENSTRUAL CYCLE-RELATED CHANGES IN BLOOD SERUM TESTOSTERONE AND ESTRADIOL LEVELS AND THEIR RATIO STABILITY IN YOUNG HEALTHY FEMALES

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Abstract
The role of testosterone in females has not been fully elucidated. Studies usually involved postmenopausal women. Literature data on age-related changes of testosterone levels are contradictory. The application of sex hormones and their combination in medical practice increases the importance of study of the menstrual cycle fluctuations in testosterone, populational variability of testosterone and estradiol levels and their ratio in healthy females to prevent the excessive doses of sex steroids and provide the using of optimal their doses in different phases of menstrual cycle during treatment. The objective of our research was to evaluate testosterone and estradiol levels, their interrelation and their ratio in different stages of menstrual cycle in young healthy women. Twenty-two young Ukrainian females aged 18 to 22 years were enrolled in this study. Testosterone and estradiol levels in blood serum were determined by Estradiol ELISA and Testosterone ELISA kits (Italy). Both estradiol and testosterone levels depended on menstrual cycle phases. The highest testosterone level was revealed in ovulation. No correlation between blood serum testosterone and estradiol levels was found in all menstrual cycle phases. Differences in testosterone and estradiol levels between Ukrainian women and some other populations of women were noted, indicating that such differences must be taken into account when treating women of different populations. Testosterone/estradiol ratio was not changed during menstrual cycle. Because of the constancy of the ratio of testosterone to estradiol during menstrual cycle and the age-related change in that ratio, this must be taken into account in the treatment of elderly women in order to create a testosterone-estradiol ratio that is characteristic of young women.
Key words: *Testosterone, estradiol, menstrual cycle phases, women.*

Introduction

Testosterone is an essential hormone for women [1, 2]. Its physiological effects are mediated directly or via aromatization to estradiol in peripheral tissues [1]. However, little attention has been paid to functions of endogenous androgens in females [3]. Conclusions about roles of endogenous androgens are usually based on clinical investigations [1, 4–6] or on data obtained on postmenopausal women [3, 7–9]. Information on healthy young women is scarce [10–12]. Moreover, age-related changes in blood testosterone levels have been demonstrated. However, data on the dynamics of such changes are contradictory [3]. Testosterone and estradiol and their combination are used not only in female sexual dysfunction [13, 14], but also may become a new therapeutic strategy in depression [15]. However the menstrual cycle-related fluctuations of testosterone, populational variability of testosterone and estradiol levels and their ratio in healthy females remain insufficiently investigated. These studies are necessary to prevent the excessive doses of sex steroids and

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to provide the physiological menstrual cycle-related fluctuations during the treatment.

2. **Purposes, subjects and methods:**

2.1. Purpose

Given the facts mentioned above, the aim of our study was to assess testosterone and estradiol levels, their interrelation and their ratio in different stages of menstrual cycle in young healthy females.

2.2. Subjects & Methods

The study comprised 22 young healthy Ukrainian women aged 18 to 22 years with body mass index of 19–24. All females had the regulatory 28–32-day-long menstrual cycle. They had no surgery and traumas in anamnesis. They have not been taking contraceptives and medicines that may affect their hormonal profile by any way for last 3 months. Blood samples were collected 3 times: in follicular, ovulation and luteal phases of menstrual cycle at the same time of day (8.00 – 9.00 a.m.). Blood serum was prepared and used for testosterone and estradiol level determination.

Testosterone and estradiol levels in blood serum were determined by Estradiol ELISA and Testosterone ELISA kits purchased from *DiaMetra* (Italy). Both kits were based on the quantitative sandwich enzyme immunoassay technique. All procedures were carried out in accordance with the instructions provided by the manufacturer. As soon as the color development was stopped, the optical density of the solutions was determined with the help of the Awareness Technology Stat Fax 303 Plus Microstrip Reader (USA). Testosterone concentrations in blood serum were expressed in nmol/l, while the level of estradiol was expressed in pmol/l.

All procedures and manipulations were carried out in accordance with the ethical standards of the Committee of Ethics and Bioethics of Kharkiv National Medical University and the revised Declaration of Helsinki (2000). All subjects signed a written informed consent.

Statistical analysis was performed using nonparametric statistical methods for dependent variables with the help of the *Statistica* 6.0 software (StatSoft, USA). A nonparametric analogue of the dispersion analysis - the Friedman ANOVA test – was used to reveal the dependence of parameters on the menstrual cycle phase. Wilcoxon test was used to compare parameters in various phases of the cycle. Correlation analysis according to Spearman was used to compare parameters of the same group.

Conflict of interests

The authors declare that they have no competing interests.

3. **Results and discussion**

In order to detect the dependence of the content of sex hormones on the phases of the menstrual cycle, we used a Friedman ANOVA test. Friedman ANOVA test is a nonparametric alternative to one-way repeated measures analysis of variance. According to our results, both estradiol and testosterone levels depended on the menstrual cycle phases: Chi Sqr. = 8,000000, $p<0.01832$ for testosterone; Chi Sqr. = 6,888889, $p<0.03192$ for estradiol.

No correlation between blood serum testosterone and estradiol levels was found in all menstrual cycle phases.

The highest β-estradiol level was observed in ovulation (*Fig. 1*). The differences between estradiol levels in ovulation and the follicular phase, in ovulation and the luteal phase were statistically significant ($p=0.003511$ and $p=0.012793$, respectively). The difference between estradiol levels in the follicular and luteal phases was statistically insignificant ($p=0.656642$).

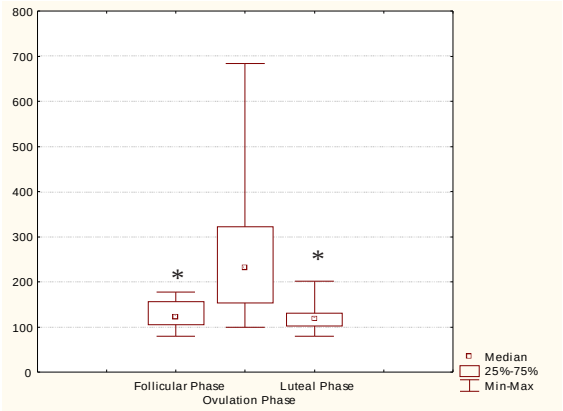

Fig. 1. β-Estradiol levels (pmol/l) in blood serum of women in different phases of the menstrual cycle (Me [25%; 75%], min and max; * – $p<0.05$ versus the ovulation phase)

Follicular phase: testosterone Mean+St.Dev. 0.3286+0.1491 nmol/l; estradiol Mean+St.Dev. 121.7629+33.0308 pmol/l; testosterone/estradiol ratio 2.69.

Ovulation phase: testosterone Mean+St.Dev. 0.5476+0.192 nmol/l; estradiol Mean+St.Dev. 244.1746+138.949 pmol/l; testosterone/estradiol ratio 2.91.

Luteal phase: testosterone Mean+St.Dev. 0.4251+0.2384 nmol/l; estradiol Mean+St.Dev. 122.1754+30.2833 pmol/l; testosterone/estradiol ratio 3.58.

The highest testosterone level was revealed in ovulation (*Fig. 2*). The difference between testosterone levels in the follicular and ovulation

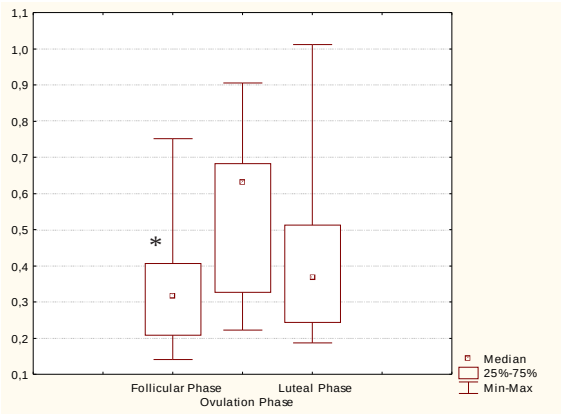

Fig. 2. Testosterone levels (nmol/l) in blood serum of women in different phases of the menstrual cycle (Me [25%; 75%], min and max; * – p<0.05 versus the ovulation phase)

phases was significant (p=0.002162). The differences between testosterone levels in the follicular and luteal phases, in ovulation and luteal phases were almost significant (p=0.062).

The differences between the testosterone/estradiol ratios in various phases of the menstrual cycle were insignificant.

Discussion. The β-estrogen level was higher in Ukrainian women compared with the Norwegian ones [10] and lower than in Chinese [16] or Greek [5] population. The testosterone level was lower in women of Ukrainian origin in comparison with the Norwegian [10], Spanish [16] or Greek [5] women. Our findings of population differences in the testosterone and estradiol levels suggests that such differences must be taken into account when interpreting data during medical tests and discussing the findings of scientific researches.

Our findings concerning the dynàmics of estradiol during the menstrual cycle correspond to the data of other resåàrchers. In pàrticular, the låvels of estradiol in the follicular and luteal phases did not differ [16]. Estradiol peak was observed in ovulàtion.

According to î ur data, not only estradiol but also testosterone levels depend on the menstrual cycle phases. Similarly to estradiol, the peak of testosterone was observed during ovulation. As in the case of estradiol, there was no difference in the content of testosterone in the follicular and luteal phases. Unlike estradiol, differences in testosterone levels during ovulation and in the luteal phase were insignificant. Statistically significant elevation of mid-cycle testosterone concentrations was found by other authors, but they bålieve that that elevation is not clinically relevant since day-to-day variation is higher and independent of the menstrual cycle [17]. The påàk of testosterone in ovulation may be due to a control of androgen production by luteinizing hormone in gî nadotropin-dependent stages of folliculogenesis [18].

In our study, the testosterone/estradiol ratio was not changed during the menstrual cycle. According to our data, testosterone levels in women are on average 3 times higher than the estradiol level. It has been reported that the testosterone level exceeds the estradiol content in blood serum dozens-fold, but this difference is observed in the elderly women [5]. At the same time, a decrease in the content of both hormones is observed, but the estradiol level is reduced more noticeably.

Higher levels of testosterone compared with estradiol in women, dependence of testosterone levels on the menstrual cycle, the pråsence of androgen receptors in womån, and the localization of androgen receptor gene on the X chrî mosome [5] suggest that tåstosterone performs certain functions in females.

Only several researches have focused on the role of testosterone in healthy women. Thus, information on this subject is limited. In particular, it has been demonstrated that endogenous testosterone levels corrålate positively with amygdala reactivity in female adolescents and middle-àged women. After receiving a single nasal dose of testosterone, the amygdala reactivity in the middle-aged women rapidly increases to a level comparable to the yî ung women [19]. Exogenî us testosterone attenuates the integrated central stress response in healthy young females [12]. Authî rs believe that androgens promote dynamic regulation of the stress system via central nåuropeptidergic pathways that control corticotropin-releasing hormone and arginine vasopressin expression. A research performed on the androgen receptor knockout female mice revealed that the androgen receptors have a functional rî le in neuroendocrine regulation and timing of the ovulatory luteinizing hormone surge as well as the antràl/preovulatory follicle development [20].

Most of the data on the role of testosterone in the body of women was obtained in clinical studies. Such findings are frequently contradictory.

Different authors have opposite opinions about the testosterone influence on depression development. According to some of them, testosterone prevents the development of dåpression, since the low testosterone levels are

observed in depressed women compared with women in the cî ntrol group and its elevated levels are found after pharmacotherapy [4]. On the other hànd, it has been show that testosterone does not influence the depression development [21] or even worsens symptoms of depression [22].

According to some clinical studies, exogenous testosterone enhances cî gnitive performance and improves musculoskeletal health in postmenopausal women [1]. However, the other resåarches do not support the hypothesis that increasing testosterone concentrations prevent cognitive decline in men and women over 65 years of age [23]. Moråover, the results of other studies support the hypothesis that the free testosterone has a sex-specific impact on cognitive performance. Positive correlation between free testosterone levels and visual-spatial abilities, semantic memory, and episodic måmory, with gråater positive influence with increasing age wàs demonstrated in males. In women, free testosterone negatively correlated with verbal fluency, semantic memory, and episodic memory [24]. It has been reported in another study that in older women the endogenous testosterone has a negative association with verbal memory, which is usually one of the first functions to dåcline in dementia [6]. Some functions of testosterone in women are provided by its conversion to estradiol. This reaction is catàlyzed by aromatase. In the hippocampus, it is upregulated in postmenopausal women and downregulated in Alzheimer's disease [25].

Although not all the effects of testosterone in females are provided by its transformation into estradiol. This is indirectly evidenced by our data on the lack of correlation between the testosterone and estradiol levels in the blood serum.

Obviously, the optimal ratio between testosterone and estradiol is of huge importance, which is indirectly confirmed by the constancy of this ratio throughout the menstrual cycle. It is also confirmed by literature data. It is believed that relative but no absolute levels of sex hormones play more important role in etiology of depression [26]. According to Huang Y et al. (2019), in men the testosterone/estradiol ratio correlated significantly with age, human leukocyte telomerase reverse transcriptase mRNA levels, telomerase activity of peripheral blood mononuclear cells and telomere length [27].

Conclusions

The level of testosterone in blood serum of young women depends on the phase of the menstrual cycle. Population differences in the testosterone and estradiol levels suggests that such differences must be taken into account during treatment of women from different populations.

No correlation between serum levels of testosterone and estradiol in all phases of the menstrual cycle is found indirectly indicating that testosterone in females has specific functions not associated with its conversion to estradiol.

The testosterone/estradiol ratio does not depend on the menstrual cycle phase. The age-related changes in the testosterone/estradiol ratio should be taken into account in the older women treatment in order to create the optimal the testosterone/estradiol ratio characteristic for young women from the same population.

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EXPERIMENTAL INVESTIGATION OF THE EFFECT OF PHARMACEUTICAL COMPOSITION ON THE CENTRAL NERVOUS SYSTEM

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Abstract

It is known that combination of non-steroidal anti-inflammatory drugs with other drugs can cause complementary effects, or potentiate the effects of each other. **Object and methods.** The objective of this experiment is to analyze the use of a new pharmaceutical composition consisting of nonsteroidal anti-inflammatory drug of the coxib group (rofecoxib) and an immunomodulator of lycopid (glucosaminylmuramyl dipeptide). An experiment on laboratory animals was conducted in order to view the effect of rofecoxib, licopid and their pharmacological composition on the central nervous system and on emotional-behavioral reactions in an "open field" test under formalin edema conditions. **Results** Previously, we studied the pharmaceutical compositions of nonsteroidal anti-inflammatory drugs of different chemical structure with the psychostimulant caffeine. The results indicated that caffeine potentiated the pharmacological effects of nonsteroidal anti-inflammatory drugs. In this study, we set a goal to create a pharmaceutical composition of rofecoxib with licopid and investigated its effect on the emotional-behavioral reactions of rats under formalin edema conditions. Ànalysis of the experimental results show that the addition of licopid to rofecoxib contributed to an increase in rat's locomotor and orienting-research activities and also an increase in the indicators of their emotional reactions (urination and defecation) compared to the mono-administration of rofecoxib. **Conclusions.** The pharmaceutical composition of rofecoxib and licopid is expedient and promising for the study of anti-inflammatory and analgesic effects.

Keywords: *"open field", licopid, rofecoxib, pharmaceutical composition, formalin edema.*

Introduction

The inflammatory process in the human body leads to a disruption of many systems in the body, including immune, endocrine and nervous systems. It is often accompanied by severe pain, edema, spasms, etc. [1, 2]. Therefore, an urgent problem of the pharmaceutical development is the search for new effective compositions containing known non-steroidal, anti-inflammatory drugs (NSAIDs), whose pharmacological effects are achieved through rational choice of chemicals. The benefit of combining drugs in comparison to monotherapy is that they can effectively eliminate pain and inflammation, rather than administering each component individually [3]. Such combinations enable the addition of active substances in drugs using lower doses, which reduces toxicity and, thus, the negative side effects [3, 4].

It is known that NSAIDs are often combined with other drugs that may cause complementary effects, or potentiate the effects of each other [5–7]. The fact that there are no combined rofecoxib based medicines is the reason we have selected coxib as our study object (namely, rofecoxib).

Rofecoxib-([4-[4-(Methylsulfonyl)phenyl]-3-phenyl-2(5H)-furanone]) NSAIDs- is a synthetic preparation of the coxib group and it contains a sulfone side chain in its structure:

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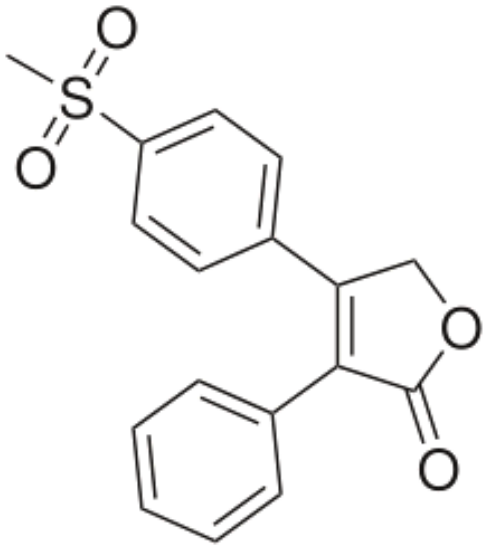

Fig. 1. Rofecoxib (rofecoxibum, 4-(4-methylsulfonylphenyl)-3-phenyl-5H-furan-2-one) C17H14O4S

Rofecoxib is a highly selective inhibitor of cyclooxygenase-2 isoform (COX-2), which is formed exclusively at inflammatory sites and provides the conversion of arachidonic acid to prostaglandin (PG). COX-2 participates in the formation of inflammatory mediators: PG, prostacyclin and thromboxane. The activity of rofecoxib, as well as other preparations of the group of coxibs, isoform COX-2, is due to the peculiarity of their active center structures of COX-2 and the molecule of coxibs. In the molecules of coxibs, there is a rigid side chain, which allows these drugs to penetrate into the cavity of the COX-2 molecule, and interact precisely with this isoform COG [8]. Rofecoxib has a pronounced analgesic and anti-inflammatory effect, compared with other NSAIDs, it has a chondroprotective effect, which reduces the concentration of PG in the cerebrospinal fluid, leading to the suppression of secondary hyperalgesia development, therefore, allowing the drug to be used for trauma and postoperative pain syndrome [9].

The second component of the potential analgesic drug is the immunomodulator, licopid (Glucoseminylmuramildipeptidum (GMDP), [4-O-(2-Acetylamino-2-deoxy-beta-D-glucopyranosyl)-N-acetylmuramyl]-L-alanyl-D-α-glutamylamide, $C_{25}H_{43}N_5O_{15}$), which belongs to the group of cytokines and immunostimulants [10]. The mechanism of its action resembles the process of natural immunoregulation [11, 12]. Licopid exhibits antibacterial and antiviral activity and exerts leukopoietic effect. Licopid is a synthetic GMDP free from bacterial impurities. Licopid stimulates all parts of the immune system, but primarily activates phagocytosis, that is, the ability of cells to absorb foreign cells, bacteria and mushrooms. In addition, it activates macrophages to begin synthesizing immune mediators, cytokines, that enhances the ability of lymphocytes to kill foreign cells, to produce protective antibodies and to increase the formation of new leukocytes involved in phagocytosis. Licopid is a highly effective and safe immunotropic drug; it is convenient to use, and it can be tolerated [8, 12].

2. Purposes, subjects and methods:

2. Purposes, subjects and methods:

2.1. Purpose – to search, create and study a new pharmaceutical composition containing NSAIDs from the group of coxib (rofecoxib) and licopid by influencing the central nervous system (CNS) and emotional-behavioral reactions (EBR) in an experiment on laboratory animals in an "open field" test.

2.2. Subjects & Methods

Experimental justification for the use of a new pharmaceutical composition consisting NSAIDs of the coxib group (rofecoxib) and licopid.

Experimental studies of pharmacological activity were carried out on laboratory animals (36 white WAG rats weighing 180–280 g of both sexes) at the Department of Medical and Bioorganic Chemistry of Kharkiv National Medical University. The research was conducted in accordance with the methodological recommendations of the State Pharmacological Center of the Ministry of Health of Ukraine [13]. Recount from human doses to rats was carried out using a coefficient of species sensitivity according to Yu.R. Rybolovlev [14]. When choosing the number of animals and their distribution in groups, the economical approach, bioethical rules and statistical requirements were taken into account.

The study was carried out on laboratory animals from the experimental biological clinic of KhNMU taking into account the norms of storage, care and feeding (air temperature – 23–25°Ñ, lighting - in the room 100 lx, in the cell – 20–40 lx) [15] Length of stay of laboratory animals - 1.5 months; period of acclimatization – 2 weeks; main ration – vegetables, fodder beets; water source – tap water. The rats were kept in vivarium in accordance with the rules of humane treatment of laboratory animals. The studies were conducted in compliance with the principles of the "European Convention for the Protection of Vertebrate Animals used for Experimental and

Scientific Purposes" (Strasbourg, 1986) [16], Directive 2010/63 / EU of the European Parliament and of the Council of the European Union "On the Protection of Animals Used for Scientific Purposes" "(Brussels, 2010) [17] and" General Ethical Principles of Experiments on Animals "(Kyiv, 2001), the Decree of the First National Congress on Bioethics (Kyiv, 2009) [18]. Experiments were conducted in the first half of the day, which according to the literature agrees with the dependence of the main pharmacological parameters and pharmacological activity of the drugs taken from the circadian rhythms [19, 20].

Statistical processing of the data was carried out using generally accepted methods of statistical analysis (mean, average error, probability criterion of Fisher-Stuent) using MS Excel and Stat Graphics Plus 2.1 programs [21].

Studies of the effect of rofecoxib, licopid and their pharmaceutical composition on rat's EBR were also conducted under formalin edema conditions. The rats were divided into 6 groups of 6 animals each. Animals of the 1st intact group intragastrically received single dose of 3% starch mucus (2 ml per 200 g of rat's weight). Animals of the 2nd group received 3% starch mucus and the formalin induced edema was modeled by sub-planar administration 0.1 ml of 2% formalin solution into hind paw of rat [13]. Animals from experimental groups 3–6 were intragastrically administered studied drugs in the form of a suspension of 3% starch mucus: animals of the 3rd group – rofecoxib in the dose of 1.3 mg per 1 kg of rat's weight), 4th – licopid (0.6 mg/kg), 5th group received composition of rofecoxib (1.3 mg/kg) with licopid (0.6 mg/kg), 6th group – reference drug sodium diclofenac (8 mg/kg). Maximum development of formalin induced edema is observed 4 hours after its modeling [13]. 3% starch mucus, drugs and their pharmaceutical composition were administered 1 hour before, taking into account their pharmacokinetic and pharmacodynamic characteristics.

Interaction of animal and the environment is based on mechanisms of nervous, humoral and immune regulation. Depending on the genetically determined characteristics of these processes, animals react differently to environmental changes. This allows them to distinguish different individual-typological properties. In addition, individual peculiarities of animals are determined by the general condition of the animal at the time of exposure to extreme conditions, a preliminary study of the decision of a stressful situation and other features acquired during life.

While studying the mechanisms of pharmaceutical action of drugs, it is important to study their effect on characteristics of animal behavior. Absence of verbal contact limits number of possible tests. The most popular and informative is an "open field" test [22, 23].

Evaluations of the influence of the drugs and their composition on the characteristics of animal's behavior was carried out by comparing groups 3–5 with the control group (group 1), and with formalin edema (group 2) and with reference drug diclofenac sodium (group 6). Observation of the parameters of the approximate research activity of the rats in the "open field" test [22, 23] and the multi-parameter method for evaluating anxiety-phobic states according to the generally accepted method was carried out within 3 minutes [24].

The parameter of the orienting-research activity of the rats in "open field" test is characterized by a number of variables: the number of crossed squares (locomotor activity), vertical racks and surveyed openings (routine-research activity), washings (grooming), ovaries and boluses (emotional reactions) according to the generally accepted the method [23, 24].

Conflict of interests

The authors declare that they have no competing interests.

3. Results and discussion

The influence of the drugs under study and their composition on rat's locomotor activity (number of crossed squares). Formalin induced edema (group 2) reduced the rat's locomotor activity in 1.9 times relative to the control group (*Table*).

The mono-administration of rofecoxib (group 3) and licopid (group 4) was not significantly different statistically from the rats' locomotor activity of that of group 2, the findings are significantly different statistically from the control values (group 1). The composition of rofecoxib with licopid increased the rat's locomotor activity relative to groups 2–4, which did not significantly differ statistically from the reference drug (group 6), that is, there was a normal locomotor activity (see *Table*).

An analysis of the rat's orienting-research activities in "open field" test, in terms of the number of sets and explored holes, reveals the motivational component of the animal's characteristics. In this case, they try to enter into indirect contact with objects located at a distance: the rats sniffed to objects located outside the "open field".

The number of crossed squares. Formalin induced edema (group 2) contributed to

Indices of the rats' behavioral activity and emotional reactions under formalin edema according to "open field" method

№	Groups of rats	Locomotor activity	Orientation-research activity		Emotional reactions		
		number of crossed squares	number of sets	number of explored holes	number of washing (grooming)	number of urinations	number of defecations
1.	Control (n = 6)	18.50±0.62 **/***/****	4.83±0.65**	7.17±1.01 **/***/****	8.50±2.99	1.17±0.31	4.17±0.48
2.	Formalin-induced edema (n = 6)	10.00±0.52 */******/*****	2.17±0.79 */*****/*******	2.17±0.65 */******	1.67±1.05	1.00±0.00	2.67±0.42
3.	Rofecoxib (n = 6)	10.25±0.63 */*****/******	3,50±0,87 ******	1.00±0.41 */*****/******	0.67±0.67	1.00±0.48	2.75±0.48
4.	Licopid (n = 6)	11,67±2,28 */******	2.67±0.62 *****	2.17±0.48 */******	1.50±0.96	1.17±0.17	2.83±0.95
5.	Rofecoxib + licopid (n = 6)	18.50±1.20 **/***/****	6.33±1.12 **/***/****/******	3.83±0.87 ***	0.83±0.40	1.50±0.22	2.83±0.87
6.	Sodium diclofenac (n = 6)	18.00±0.82 **/***/****	6.25±0.25 **/***/****/*****	6.50±0.65 **/***/****	5.75±2.10	1.50±0.29	2.50±0.87

Notes (mean ± error in mean).

n – number of rats in each group;

* – the difference is significant as compared to the control group, $p < 0.05$;

** – the difference is significant as compared to formalin-induced edema, $p < 0.05$;

*** – the difference is significant as compared to the mono-administration of rofecoxib, $p < 0.05$;

**** – the difference is significant as compared to the mono-administration of licopid, $p < 0.05$;

***** – the difference is significant as compared to the administration of the rofecoxib and licopid composition, $p < 0.05$;

****** – the difference is significant as compared to the mono-administration of sodium diclofenac, $p < 0.05$.

statistically significant reduction in the number of sets to group 2 (see *Table*). The mono-administration of rofecoxib (group 3) there was an increase in the number of racks by 1.6 times relative to group 2, which did not significantly differ statistically from the values of both control and reference drug. The mono-administration of licopid (group 4) increased the number of sets by 1.2 times compared with group 2, and the findings were also significantly statistically different from the control values (group 1) and the reference drug (group 6). Composition of rofecoxib and licopid showed a statistically significant increase in the number of explored holes relative to the experimental groups 2–6 and remained at the level of animals in group 6 (reference drug) (see *Table*).

The number of explored holes. A variety of rat's orienting-research activities is the number of examined openings – an indicator of the renal reflex, which indicates the ability of the animal to explore the "open field", in particular, to look into the openings. The number of examined openings characterizes cognitive activity of rats.

Formalin induced edema (group 2) contributed to statistically significant reduction in the number of examined openings by 3.3 times in comparison with the control group. The mono-administration of rofecoxib (group 3) has contributed to a decrease in the number of examined holes in rats relative to groups 1 and 2. The mono-administration of licopid did not affect the number of explored holes in the conditions of formalin edema. Introduction of the pharmaceutical composition of rofecoxib with lycopid (group 5) increased the cognitive activity of rats in 1.8 times relative to group 2, in 3.8 times relative to group 3 (mono-administration of rofecoxib). These indications did not significantly differ statistically from the control group and the index of the reference drug, that is, the introduction of this composition contributed to the normalization of vertical activity (see *Table*).

Emotional reactions in rats are an important characteristic of animal behavior in the "open field". The level of emotional state of rats is estimated by the number of washings (grooming), ovaries and boluses (defecations).

Cosmetic behavior of rats (grooming). Traditionally, rats devote most of their time to scrubbing their body, as compared with moving in space. Grooming closely correlates with motor activity. Therefore, in the study of medicinal products, this behavioral characteristic is particularly interesting.

Formalin induced edema shows that there was a decrease in the amount of washings by 5.1 times relative to control (group 1). The mono-administration of rofecoxib, licopid and their

pharmaceutical composition did not positively affect grooming in the conditions of formalin edema (see *Table*).

Diuresis and defecation. The level of the rat's emotional state is measured by observing the urination and defecation number. The rate of the rat's emotional stress is evaluated by these numbers.

Formalin induced edema (group 2) contributed to the reduction of urinations and defecation, in 1.2 times and 1.6 times respectively, relative to the control. The mono-administration of rofecoxib, licopid at the formalin-induced edema did not affect the number of urinations and defecations. Introduction of the composition of rofecoxib and licopid contributed to the increase in diuresis and the number of defecations movements relative to group 2 (correspondingly), which was not significantly, statistically different from the reference drug (see Table).

Previously, at the Department of Medical and Bioorganic Chemistry of Kharkiv National Medical University (KhNMU) we studied the pharmaceutical compositions of NSAIDs of different chemical structure (N-(4-hydroxyphenyl) acetamide, 4-hydroxy-2-methyl-N-2-pyridinyl-2H-1,2-benzothiazine-3-carboxamide-1,1-dioxide, [o-(2,6-dichloro-aniline)-phenyl] acetic acid sodium salt, 4-hydroxy-2-methyl-N-(5-methyl-2-thiazolyl)-2H-1,2-benzothiazin-3-carboxamide-1,1-dioxide) with the psychostimulant caffeine (1,3,7-trimethylxanthine) [2–6]. The results indicated that caffeine potentiated the pharmacological effects of NSAIDs of different chemical structure. In this study, we set a goal to create a pharmaceutical composition of the NSAIDs group of coxib (rofecoxib) with a new adjuvant licopid and investigated its effect on the EBR of rats under the conditions of formalin edema, which has not been experimentally tested previously.

Earlier, employees of D.O.Alpern Department of Pathological Physiology of KhNMU studied l³copid in an animal experiment: the possibility of its influence on the leukocyte reaction of peripheral blood in case of carrageenan secondary chronic inflammation was evaluated [25, 26]; studied the effect of l³copid on the central link of the blood system, i.e. bone marrow hematopoiesis [27]. The results of these studies show that the use of glucosaminylmuramyl dipeptide leads to a decrease in the chronization of the process, which shows the advisability of using it to prevent chronic inflammation. The authors consider promising future studies related to the improvement of pathogenetic therapy, as well as the prevention of chronic inflammation.

Conclusions

A study was conducted to evaluate the effect of rofecoxib and licopid and their pharmaceutical composition on EBR indices in rats by the "open field" method under the conditions of formalin edema, which showed that the introduction of the pharmaceutical composition of rofecoxib and licopid contributed to an increase in rat's locomotor and orientation-research activity, in its indicators of emotional reactions (diuresis and defecations) compared with the mono-administration of rofecoxib.

The pharmaceutical composition of rofecoxib and lycopodis is expedient and promising for the study of anti-inflammatory and analgesic effects.

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EFFICACY OF FIBRIN MATRIX WITH NEUROINDUCED MESENCHYMAL STEM CELLS TRANSPLANTATION FOR RESTORATION SCIATIC NERVE FUNCTION AFTER ITS COMPLETE RUPTURE IN RATS

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Abstract

Peripheral nerves damage is a frequent pathology with significant socio-economic significance. The aim is to study the possibility of using fibrin matrices filled with neuroinduced mesenchymal stem cells (nMSC) to restore integrity of peripheral nerves. Methods. The study was carried out on 40 mongrel female rats. Sciatic nerves (SN) of all rats were intersected and then reconstituted using various methods. nMSC were obtained from rats` bone marrow and cultivated by special method. Results. Total anatomical rupture of SN without treatment led to persistent neurologic deficit (SFI = -98) in E1 group. Partial restoration of SN function increased to SFI = -37 on the 30th day in E2 (operative reconstruction) group. Partial restoration of SN function occurred after 20 days (SFI = -64) in E3 group (transplanted acellular fibrin matrix). Partial restoration of SN function started at the 3rd day, stably increased to SFI=-27 on 30th day in E4 group (transplanted fibrin matrix with nMSC). Histological evaluation showed: there were alternating portions of connective tissue with portions of nerve fibers in E2 group; in E3 group large scar was formed at the place of transplanted fibrin matrix; in E4 were found spindle-shaped and stellate cells with long processes running from one side of SN to another, cells of connective tissue and thin nerve fibers. Conclusions. It has been shown that transplantation of the fibrin matrix with nMSC was more effective for treatment of SN trauma than transplantation of cell-free fibrin matrix and close to the results of surgical reconstruction.

Keywords: *mesenchymal stem cells, sciatic nerve, fibrin matrix.*

Introduction

Peripheral nerves damage is a frequent pathology with accounting for 13 to 23 cases per 100 people a year [1].

Even insignificant injury of the musculoskeletal system can be accompanied by the damage to peripheral nerves and cause partial or complete loss of limb function. The number of such victims is increasing annually due to increasing frequency of technogenic injuries and complex combined injuries of the musculoskeletal system. Rapid urbanization leads to increasing neurotraumatism on average by 2% per year [2]. In Ukraine, injuries occur every year in 2500–3000 people affected, 60–75% of them are disabled.

Functional recovery has paramount importance for the rescue of limbs in the treatment of peripheral nerves injuries. [1] They can range from compression of the nerve to complete nerve transection. Trauma can affect different nerves causing respective regional paralysis. It is characterized by muscle weakness, reflexes changes, numbness, severe pain, motor dysfunction and prolonged disability.

In recent years, fundamentally new opportunities have been opened up in the reconstructive surgery of injuries and treatment of compression-ischemic lesions of nerve trunks mainly through the improvement of surgical techniques [10]. In most cases, trauma of nerves due to the specific nature of anatomical and topographic relationships is rarely isolated. Very

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often is it accompanied by damage to the blood vessels, bones and soft tissues.

Pathomorphological changes that develop after the damage of arteries and peripheral nerves are characterized by replacement of these structures with fibrous tissue and the development of tendogenic and arthrogenic contractures, delayed consolidation of bone structures as a consequence.

Despite the achievements of microsurgical techniques in the imposition of primary epineural suture or autotransplantation, the functional results are often unsatisfactory, especially if there is a significant size of the nerve defect. The effectiveness of surgical interventions on peripheral nerves depends on the type of a damage and the nature of the operational technique [5].

In connection with the foregoing, the issue of saving and adequate treatment after various injuries with the damage of peripheral nerve trunks requires development of fundamentally new methodological approaches to treatment [19].

The known surgical methods for replacing a nerve defect are mainly aimed at transplanting the nervous or any other tissue into a gap between the central and peripheral segments, as well as various artificial materials. Nerves, muscles, vessels, tubes of polymers of lactic and glycolic acids, polyphosphoether polymers are used for this. However, these techniques also have a number of drawbacks, such as development of necrosis and graft rejection. Germination of regenerating axons under these conditions is difficult and often gives complications.

Development of cellular technologies and tissue engineering methods gave new prospects for effective methods of regenerative medicine development for the restoration of peripheral nerves, such as, autotransplantation using new biopolymer materials, stem cells and tissue engineering structures [3, 14, 17].

2. Purposes, subjects and methods:

2.1. Purpose of the work was to study the possibility of using biodegradable fibrin matrices filled with neuroinduced mesenchymal stem cells (MSC) of the bone marrow to restore the anatomical and functional integrity of the peripheral nerves in rats [17, 18].

2.2. Subjects & Methods

The study was carried out on 40 mongrel female rats aged 4–5 months weighing 250±50 g., which were contained in the standard conditions of the KhNMU vivarium, in compliance with the current bioethical standards (Council Directive 86/609/EEC of 24 November 1986 on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes; European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes Strasbourg, 18.III.1986; Law of Ukraine No. 3447-IV "On the Protection of Animals from cruelty", 2006).

Sciatic nerve injury model in rats. Surgery was performed under general anesthesia (intraperitoneal administration of a mixture of solutions of xylazine – Sedazin, Biowet, Poland, 15 mg kg and ketamine – Calipsol, Gedeon Richter, Hungary, 70 mg/kg body weight). The animal was fixed on the operating table in the mid-physiological position. Observing the rules of asepsis and antiseptics, after the treatment of the operating field, a linear cut of the skin was made along the lateral surface of the left thigh in the projection of the sciatic nerve.

With the tools ("mosquito" type, surgical clamp, surgical forceps) left sciatic nerve (SN) was picked up and mobilized. At a distance (20±1.5) mm from the exit point of the SN from the small pelvis cavity a fragment (7±2) mm in length was cut by blade. After thorough hemostasis in animals of all groups, the surgical wound was closed with one row of nodular sutures using an atraumatic needle with a monofilament polyamide thread No. 3/0 (Olimp, Ukraine). Cephthriaxone ("Darnitsa", Ukraine, 20 mg/kg body weight) was used to prevent infectious complications. Solution of dexamethasone (KRKA, Slovenia, 6 mg/kg body weight) was administered intraperitoneally for anti-inflammatory and anti-edematous effect. The animals were kept in a room with an increased air temperature (30 °C). Nerve transection and repair was done on the left limb, and the contralateral limb served as a non-transected nerve control.

MSC preparation and administration

To obtain MSC culture of rats, bone marrow was flushed from the tibia, washed twice in Hanks' solution, centrifuged at 430 g for 10 minutes. The cells were sowed in ratio of 50 million per culture flask (75 cm^2, Nunc) and cultured in DMEM/F12 medium (1/1) with 10% FBS (fetal bovine serum) in a CO2 incubator. After 24 hours the culture medium with non-adherent cells to the substrate was removed, fresh culture medium was added and the remaining fibroblasts-like MSCs had been cultivated for the next 14 days, changing the medium every three days.

After formation of the monolayer, MSC were removed from the bottom of the vials and resuspended in Hanks solution to the required concentration. All reagents, serums and media came from SIGMA-ALDRICH (USA).

Ñulture medium with 2% fetal bovine serum and a solution of retinoic acid (10-6M) were used for neuroinduction of bone marrow MSC of rats [6].

Neuroinduced MSCs were resuspended in Hanks solution (500,000 cells/ml each) and mixed with blood plasma in a ratio of 1:2. Calcium chloride was added to the mixture to the concentration of 0.3% in solution and mixed. Then mixture was taken into a sterile silicone tube of 3 mm in diameter and incubated at 37 °C for 20 minutes to form a fibrin gel. After polymerization, the resulting matrix was extruded from the tube using a syringe and cut into transplants of the required size. The cell-free matrices were prepared similarly by mixing Hanks' solution with blood plasma and calcium chloride in the same proportions.

The animals were divided into 4 groups of 12 individuals each.

The sciatic nerve was cut through in the animals of the first group (E1) crossed without subsequent restoration (*Fig. 1*) The animals of group 2 (E2) recovered the excised fragment of the nerve trunk by epineural neuroraphy end-to-end using atraumatic needle with a monofilament polyamide thread No. 9/0 (DEVTS Olimp, Ukraine) under the magnification of an operating microscope (magnification × 12) (*Fig. 2*). Animals of group 3 (E3) restored the integrity of the nerve trunk with fibrin cell-free matrix (*Fig. 3*) In animals of group 4 (E4), the integrity of the nerve trunk was restored with fibrin matrices filled with neuroinduced mesenchymal bone marrow stem cells of rats (*Fig. 3*). In Experiments 3 and 4, the ends of the biomatrix were connected to the ends of the nerve trunk with the help of fibrin glue, which was prepared 15 minutes before application.

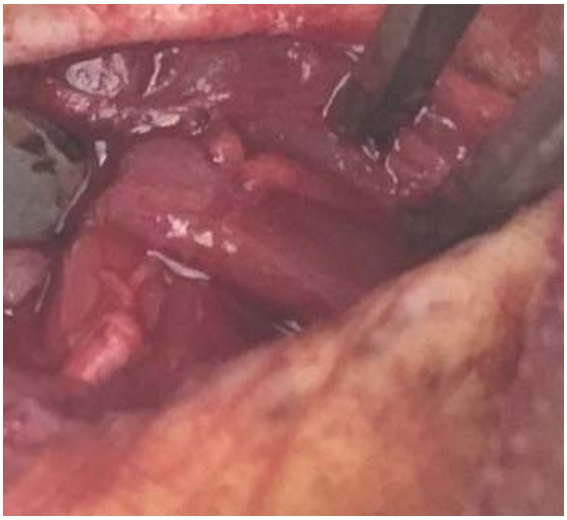

Fig. 1. Rupture without treatment

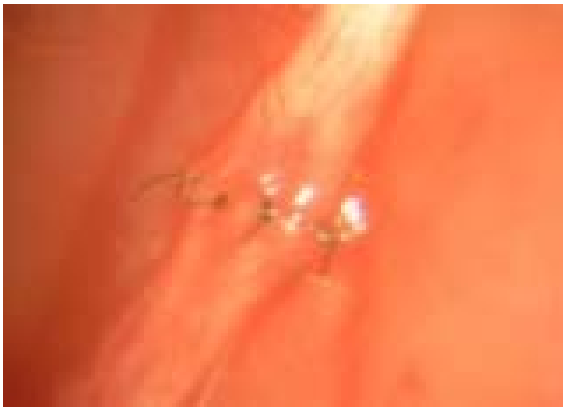

Fig. 2. Surgical Reconstruction (mag. ×10)

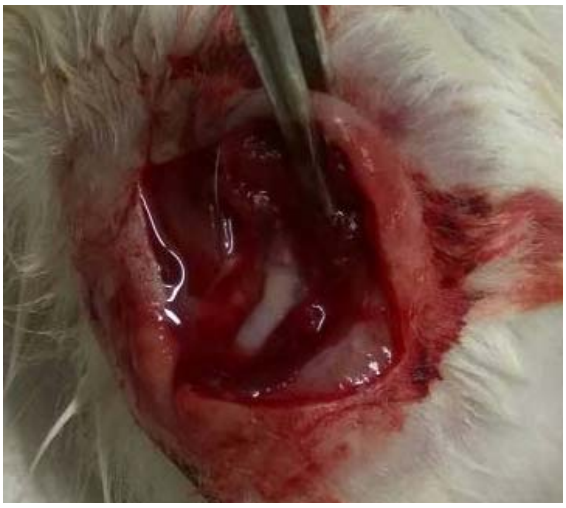

Fig. 3. Reconstruction with acellular fibrin matrix and fibrin matrix with nMSC

Assessment of neurological deficits. The degree of the sciatic nerve recovery was assessed by the results of neurological tests, which were performed 10, 20 and 30 days after the operation. To control the functional recovery we used "Walking track analysis" according to the method of Johnston et al. [15, 16] (*Fig. 4*) As a dye, an emerald green alcohol solution was used.

Plantar surfaces of the left (experimental, letter E before the indicator) and right (intact, letter N before the index designation) of the limbs in the same animal were measured after obtaining footprints:

PL (print length) – the distance in mm from the heel to the III finger

TS (toe spread) – the distance in mm from I to V finger ITS (intermediate to toe spread) – distance in mm from II to IV finger.

Fig. 4. "Walking track analysis" test

The data were calculated using Bain–Mackinnon–Hunter formula and then functional index of the sciatic nerve (SFI-sciatic functional index) was evaluated.

SFI= -38.3 ((EPL - NPL)/NPL)) + 109,5 ((ETS - NTS)/NTS)) + 13,3 ((EIT - NIT/NIT)) - 8.8 [2].

The obtained data were systematized, both the average value and the standard deviation of the index were calculated for all groups of animals.

After SFI count, the results were evaluated. Result "0" was the normal function of the sciatic nerve, "-100" – was a total function failure. The reliability of the differences in the SFI values obtained among animal groups was measured using the Mann–Whitney U test.

Histological methods of evaluation. The animals were removed from the experiment 30 days after the operation as a result of an thiopental anesthesia overdose. The tissues from the sciatic nerve rupture zone (E1), its operative recovery (E2), or biomatrix transplantation (E3, E4) were fixed in a 4% solution of paraformaldehyde and enclosed in paraffin blocks. The sections were stained with hematoxylin-eosin for a general cytomorphological evaluation of the tissues and with Mallory staining for the nerve fibers detection.

Conflict of interests

The authors declare that they have no competing interests.

3. Results and discussion

As a result of evaluation of the neurological condition of animals, it was found that a total anatomical rupture (8–10 mm) of SN without treatment in animals of the E1 group leads to a persistent neurologic deficit that has been persisting for 30 days of observation (SFI = -98) in all animals. In experimental group of animals E2 partial restoration of SN function began on the 10th day of evaluation and increased to SFI = -37 on 30th day after operative reconstruction of nerve trunk. In E3 group of animals with a transplanted acellular fibrin matrix, the partial restoration of SN function (SFI = -64) occurred in animals after 20 days and remained stable on 30th day after the operation. In E4 experiment, partial restoration of CH function was started in 80% of the animals on the 3rd day of observation, stably increased on the 10th, 20th and 30th days after the operation and reached values (SFI=-27), comparable with the results in the E2 group. The results of all groups are graphically displayed for comparison (*Fig. 5*).

Analysis of histological specimens of tissue from the trauma zone showed that in group E3 with complete anatomical rupture of SN

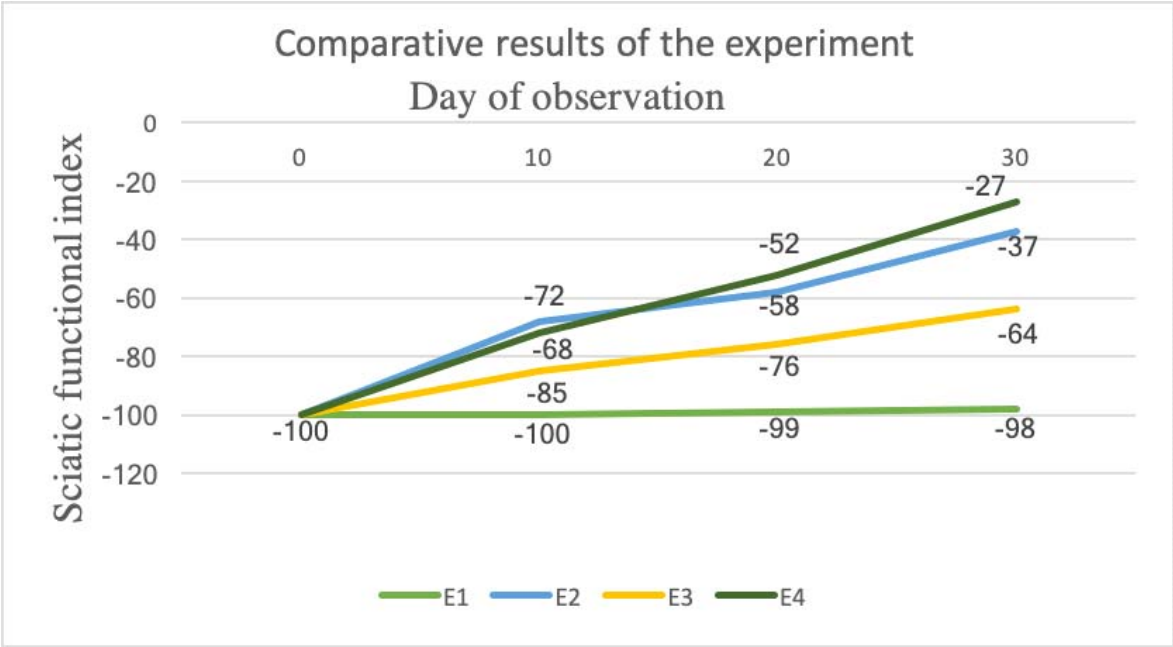

Fig. 5. The results of all groups in comparison

spontaneous recovery of nerve fibers does not occur and connective tissue is formed at the site of SN disruption. On the tissue specimens of SN (E2) there were alternating portions of connective tissue scar with portions of nerve fibers. Apparently, it can explained the partial restoration of SN function in many animals from this group. In most animals of E3 group the formation of a large scar at the site of the transplanted fibrin matrix was observed. Only in 3 animals from this group among the fibroblasts clusters, thin nerve fibers were found. Probably, the fibrin cell-free matrix may be a substrate for the growth of nerve fibers from one end of SN to the other in some cases. Spindle-shaped and stellate cells with long processes running from one end of SN to the other, cells of connective tissue and thin nerve fibers except of MSC (*Fig. 6*) were found on

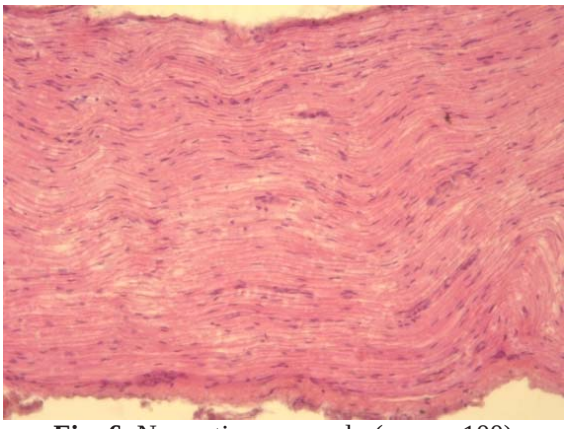

Fig. 6. Nerve tissue sample (mag. ×100)

the specimens of grafts with induced MSC from the trauma zone on the 30rd day after the operation. This can explain the high efficiency of restoring the function of SN after transplanting the matrix with neuroinduced MSC into the zone of rupture.

Currently, the effectiveness of SN regeneration after transplantation of matrices based on collagen and neural stem cells polyglycolic acid and Schwann cells was shown [7]. More than 10 years ago, it was shown that bone marrow MSC can be differentiated into Schwann cells and thus support the regeneration of the peripheral nerve [8]. Therefore, the efficiency of restoring the neurological functions of the nerve with transplanted fibrin matrices with MSC in our work can be related not only with neuronal cells differentiating in the matrix and also to their transformation into Schwann cells. As methods for restoring the ends of the ruptured SN thin microsurgical methods of nerve fiber stitching, epinevrium and even the method of tissue welding were suggested [9]. In our work, we not only confirmed the possibility of effective treatment of SN trauma with fibrin matrix and MSC of the bone marrow, but also offered a simple method of transplanting this matrix with fibrin glue.

Conclusions:

1. The proposed model of damage to the sciatic nerve in rats by operative removal of its part (8–10 mm) leads to a persistent neurologic deficit, which persists for 30 days.

2. A method for obtaining tissue-engineering structure based on blood plasma and neuroinduced MSC of rat bone marrow for reparation of the sciatic nerve was developed.

3. This method of recovery in peripheral nerve damage utilizing MSCs is attractive, feasible, and promising. MSC therapy improved limb functional recovery in peripheral nerve damage. Research of novel approaches such as mesenchymal stem cell transplantation is expected to make a significant impact in the clinical outcome of nerve injuries.

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RISK FACTORS OF DEVELOPMENT OF HALLUCINATORY-PARANOIDAL DISORDERS IN PATIENTS WITH MIXED DEMENTIA

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Abstract

Seventy-two patients with Alzheimer's disease (AD) with late onset and vascular brain disease, dementia with hallucinatory-paranoid symptoms (the main group) were examined. The control group included 61 patients with AD with late onset and vascular disease of the brain without hallucinatory-paranoid symptoms. The risk factors for the development of hallucinatory-paranoid disorders in patients with mixed dementia were determined. These factors included: female gender; age of 78.6±7.3 years; female history of personality disorders; genetic predisposition to cognitive disorders; neuropathic constitution in childhood; retardation of psychophysical development in childhood; low levels of academic performance; absence of family; secondary and secondary specialized education; a physical type of a job; acute psychogenic factors associated with the worsening of financial conditions, conflicts in the family, and divorce.

Keywords: *mixed dementia, hallucinatory-paranoid disorders, risk factors, diagnosis.*

Introduction

Modern socio-demographic processes are characterized by an increase in the proportion of elderly persons in populations of different countries [1, 2]. The results of Ukrainian and foreign studies indicate a high prevalence of cognitive disorders among the elderly persons [3]. The number of patients with mixed dementias (MD), according to various epidemiological data, ranges from 4% at the age of 70 years and more to 32% at the age of more than 85 years [4]. Recently, dementia has been defined as one of the main causes of disability and mortality of patients [1, 5].

Along with this, in the current literature there is not enough reliable information about the risk factors of the development of psychotic disorders, and in particular hallucinatory-paranoid disorders, in patients with dementia of different origin [3–5]. In contemporary gerontopsychiatry, this issue remains poorly understood, and the available data describe the risk factors for developing psychosis irrespective of the genesis of dementia. Therefore, the study and determination of the main risk factors of formation of hallucinatory-paranoid disorders in patients with MD is relevant at the modern stage of development of psychiatry.

2. Purposes, subjects and methods:

2.1. Purpose – to determine the risk factors for the development of hallucinatory-paranoid disorders in patients with MD.

2.2. Subjects & Methods

The study of risk factors for the development of hallucinatory-paranoid disorders (HPD) in patients with MD was carried out on a sample of 72 patients with F00.1 (1-2); F01.3 (1–2), which formed the main group. Control group included 61 patients with MD without HPD (F00.1; F01.3).

This study involved a set of research methods, which included anamnestic, socio-demographic, clinical-psychopathological, psychometric and mathematical-statistical methods.

The anamnestic method was used to identify the anamnestic factors in the development of HPD in patients with MD. The socio-demographic method involved the study of characteristics such as distribution of patients by age, level of

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education, marital status, educational level, types of job. Clinico-psychopathological method was based on generally accepted approaches to psychiatric examination by interviewing and observing. The survey was conducted using diagnostic and research criteria of ICD-10. Selection of patients with MD implied the employment of both the ICD-10 criteria and the criteria developed by the research group of the National Association of Neurological Diseases and Stroke – the International Association for Neuroscience Research and Education (NINDS-AIREN) [6]. Psychometric study included the study of the severity of cognitive impairments (normal, physiological aging, mild memory impairment, mild, moderate, moderately severe and severe dementia) using the GDS scale [7]. Statistical data were processed using mathematical t-test method [8]. The method was to conduct a comparative study on the t-criterion according to the traditional method for parametric statistics. Also, the data were processed using t-test to determine the probability of disagreement between the groups [8].

Conflict of interests

There is no conflict of interests.

3. Results and discussion

The investigation of the main characteristics of the object of study (constitutional-biological, socio-demographic and psychosocial) allowed us to identify the main risk factors for the development of HPD in patients with MD.

The results of the analysis of gender distribution among patients with MD of the main and control group are presented in *Figure 1*.

Among patients with MD and psychotic disorders, women prevailed (76.40%), while among patients with MD without psychotic disorders the number of women did not exceed the level of 49.20% ($p<0.05$). The number of men in the main group of patients with MD with HPD did not exceed 23.60%, and in the control group the number of men reached 50.80% ($p<0.01$).

Analysis of the average age ($M \pm \sigma$ years) of patients with MD complicated by HPD in comparison with patients with MD without psychotic disorders allowed to determine that patients of age of 78.7±6.5 years predominated (at $p>0.5$) in the main group, whereas in the control group they were patients of age 76.3± 6.2 years (at $p>0.5$).

The results of the analysis of family history of mental disorders, alcohol and drug dependence among patients with MD with psychotic disorders showed that patients of the main group had family history of hereditary burden of personality disorders (mainly emotionally unstable, paranoid and schizoid) (29.17%), while in the control group, family history of personality disorders did not exceed the level of 11.48% of cases ($p<0.01$) (*Table 1*).

Family history (genetic predisposition) of cognitive disorders in the patients of the main and control groups was registered in the presence of cognitive disorders ranging from mild cognitive disorders to severe dementia in histories of the closest relatives of these patients.

Assessment of cognitive impairments in the first-line relatives of patients with MD with HPD and patients with MD without HPD, which was

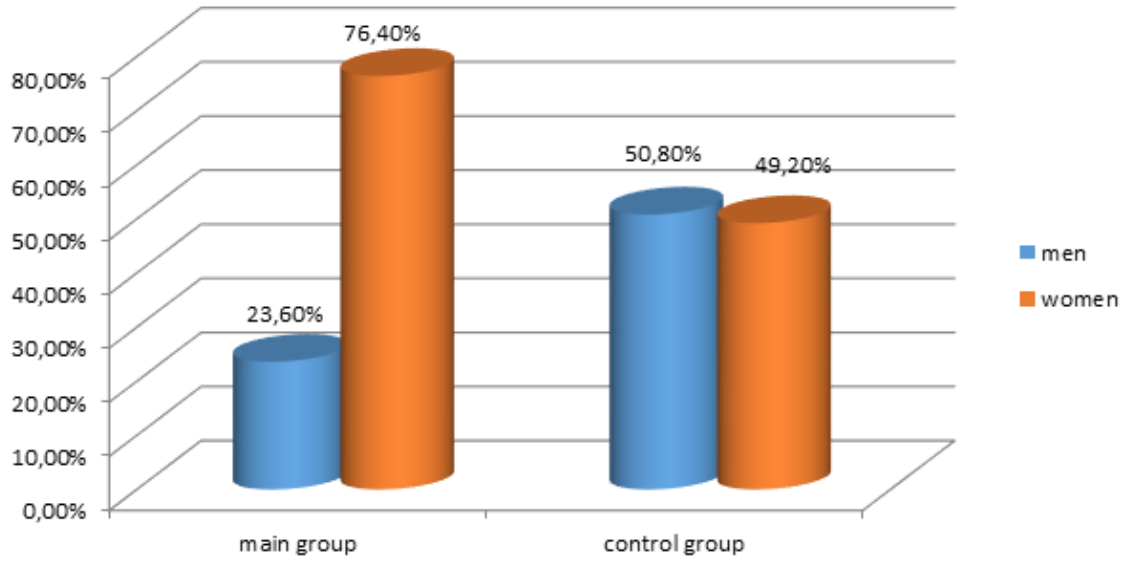

Fig. 1. Distribution of patients with mixed dementia by gender

Table 1

Number of patients with dementia with family history of mental disorders, alcohol and drug dependency

Name of parameters	Main group n=72		Control group n=61	
	Abs.	%	Abs.	%
Schizophrenia	1	1.39	1	1.64
Epilepsy	–	–	1	1.64
Somatoform disorders	7	9.72	6	9.84
Neurotic disorders	3	4.17	4	6.56
Personality disorders	21	29.17*	7	11.48*
Affective disorders	12	16.67	7	11.48
Alcohol dependence	3	4.17	3	4.92
Drug dependence	–	–	1	1.64
Symbols: * – differences are statistically significant at p <0.05				

performed using clinical and anamnestic methods, showed that in patients of the main group, a genetic predisposition to cognitive disorders was observed in 38.9% of cases, whereas in patients with MD without psychotic disorders it was observed in 21.3% of cases (p<0.05).

The results of the investigation of perinatal development (the presence of perinatal pathology in history) among patients with MD with psychotic disorders (the main group) and patients with MD without psychotic disorders (the control group) demonstrated, that 9.7% of patients in the main group had perinatal pathology, whereas in the control group, perinatal pathology was observed in 21.3% of patients (p<0.01).

33.3% of patients, while in patients with MD without psychotic disorders there was retardation of childhood psychophysical development in 11.5 % of cases only (p<0.01).

The analysis of the level of academic performance (at school, secondary specialized and higher educational institutions) of patients with MD revealed that 40.3% of patients in the main group and 18.0% of patients in the control group had low levels of academic performance (p <0.01).

The analysis of the results of investigations of the marital status of patients with MD is presented in *Table 2*. The table demonstrates, that among patients with MD complicated by HPD, 58.33%

Table 2

Marital status of patients with mixed dementia (% ± m)

Marital status	Main group n=72		Control group n=61	
	Abs.	%	Abs.	%
Married	42	58.33	44	72.13
Not married	30	41.67	17	27.87
Symbols: * – differences are statistically significant at p <0.05				

The results of the analysis of the prevalence of neuropathic constitution in childhood among patients with MD showed, that in patients with MD complicated by psychotic disorders, neuropathic constitution in childhood was observed in 30.6% of cases, and in 9.8% of patients in the control group (p<0.01). It was also determined that retardation of psychophysical development in childhood was observed in 33.3% of patients with MD complicated by HPD, and in 11.5% of cases in the group of patients with MD without psychotic disorders (p<0.01).

An investigation of the level of psychophysical development in childhood in patients with MD with psychotic disorders showed retardation of psychophysical development in childhood in

of patients were married and 41.67% were not married (single, divorced, widowers / widows), while among patients with MD without psychotic disorders, 72.13% of patients were married (p<0.05) and 27.87% were not married (p<0.05).

The results of the analysis of an educational level of patients with MD are presented in *Fig. 2*, which shows that patients with secondary (55.6%) and secondary specialized (29.2%) educational level prevailed in the main group, while in the control group the number of patients with secondary and secondary specialized education was significantly less (29.5%, p<0.01 and 19.7%, p<0.05, respectively), and with higher (incomplete higher) education was significantly greater (50.80%, p<0.01).

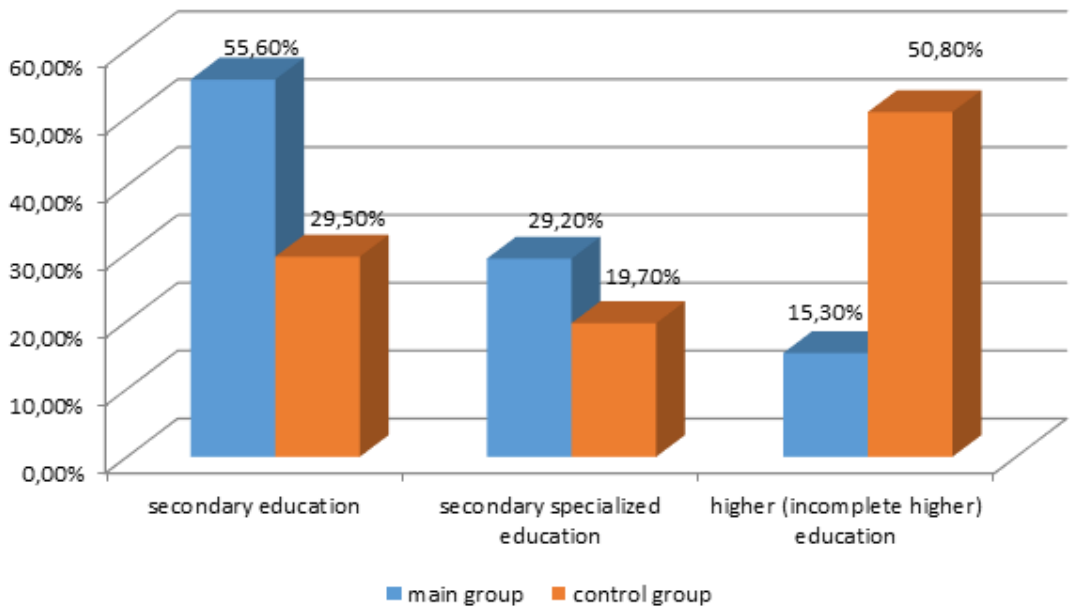

Fig. 2. Educational levels in patients with mixed dementia

The results of investigations of types of job in patients with dementia with psychotic disorders showed that patients with a physical type of job (69.4%) predominated among patients with MD complicated by HPD, while in the group of patients with MD without psychotic disorders, there were 52.6% of patients who worked physically ($p<0.05$). Accordingly, the number of patients with an intellectual type of job was 30.6% in the main group and 47.5% in the control group ($p<0.05$).

The analysis of the main psychogenic factors in patients with MD complicated by HPD showed, that predominant psychogenies in the main group were as follows: a worsening of financial conditions (65.3%) and conflicts in the family, divorce (63.9%), whereas in the group of patients with MD without psychotic disorders the psychogenic factor of worsening of financial conditions was registered only in 44.3% of patients ($p<0.05$), and psychogenies related to conflicts in family and divorce were observed in 24.6% of patients only in the control group ($p<0.01$). It should be noted, that among patients with MD complicated by HPD, 48.6% had a chronic, and 51.4% had an acute character of psychotraumas, whereas in the group of patients with MD without psychotic disorders, a chronic character of psychotrauma in 75.4% ($p<0.05$) and an acute character in 24.6% of cases ($p<0.01$) were defined.

The data are consistent with the results of studies conducted by the commission *Lancet* which published the nine factors that increase the risk of dementia: social isolation; low level of education; physical inactivity; hypertension; type 2 diabetes; obesity; smoking; ignoring the first signs of depression and middle-aged hearing loss [9]. Authors stated that these factors, which are generally modifiable lifestyle issues, account for about 35% of the overall risk of dementia [10].

Conclusions

The study identified risk factors (constitutional-biological, socio-demographic and psychosocial) of the development of HPD in patients with MD.

These risk factors included:

– female gender;

– age of 78.6 ± 7.3 years;

– family history of personality disorders (mostly emotional-unstable, paranoid and schizoid spectra);

– genetic predisposition to cognitive disorders;

– neuropathic constitution in childhood;

– retardation of psychophysical development in childhood;

– low levels of academic performance;

– absence of family (single, divorced, widower/widow patients);

– secondary and secondary specialized levels of education;

– a physical type of the job;

– acute psychogenic factors associated with a worsening of financial conditions, conflicts in the family, and divorce.

Thus, these identified risk factors for the development of hallucinatory-paranoid disorders in patients with MD should be used to establish algorithms for the diagnosis of HPD in patients with MD and to develop a personalized program for their psychosocial rehabilitation.

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ORAL DISORDERS RISK PREDICTION IN MACROSOMIC NEWBORNS AND INFANTS USING THEIR HEIGHT-WEIGHT INDEX AT BIRTH

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Abstract

The purpose of the study was to identify the risk factors and to assess the differences in the risk of hard tooth tissue and periodontal tissue diseases formation in macrosomic newborns or infants, depending on their weight-height index at birth, with the help of questionnaire survey.

Objects and Methods. The study involved 151 newborns or infants (aged from one day up to 6 months) during the period of 2014–2019. Sixty-eight children born macrosomic comprised the Main Group (MG), and the children of the corresponding age with normal weight-height parameters at birth (83 children) were included into the Comparison Group (CG). The groups were equally represented by male and female participants. MG children were additionally subdivided into 4 subgroups based on the weight-height parametres of a newborn child, using the classification proposed by Kharkiv scholars. The survey was carried out using previously developed questionary, which included 70 questions, divided into 7 scales.

Results and Conclusions. The hygienic state of the oral cavity of parents, the presence of concomitant pathology of parents, bad habits and the degree of locomotor activity before pregnancy, and during pregnancy, peculiarities of nutrition and medical therapy of parents influence practically identically on the formation of oral disorders in children born normomic or in children born macrosomic, regardless of their weight-height index at birth. Reliable differences between groups and subgroups were not detected.

The effect of the total paternal factor on the formation of oral disorders in individuals with macrosomia at birth, can be different from the effect of this factor in normosomic-at-birth persons, but this difference is related to the same phenomenon that we are studying - the fetal macrosomia, but the paternal one. The highest risk of the oral pathology formation have children whose parents were born macrosomic.

A person born macrosomic, on the average, has twice as many complaints about oral health compared to a person born normosomic.

Keywords: *oral cavity pathology, newborn, fetal macrosomia, questionnaire.*

Introduction

Intrauterine processes leads to fetal macrosomia formation (the body weight of a newborn child is more than or equal to 4,000 g [1]) and creates prerequisites for the onset of numerous systemic diseases and pathological conditions [2]. Children with macrosomia at birth have inherent propensity to metabolic syndrome, obesity, diabetes and many other diseases [3–5].

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Dental abnormalities in such children are manifested by high intensity of deciduous and permanent teeth caries and a high prevalence of mallocclusions [6–8]. Due to the variety of reasons associated with macrosomia formation, and significant spread of the weight-height index values in macrosomic newborns, children whose antropometric indices at birth were higher than normal, are not a homogeneous group. Our previous studies has proved that the weight-height index of a child at birth is a reliable "indicator" of severity of oral disorders forming later [9]. In our opinion, the oral health in macrosomic-at-birth children also depends on their intrauterine development (well-balanced growth and body

weight gain, or intrauterine obesity, or relative insufficiency of body weight).

There can be another view to this situation: parents' pathology of hard tooth and periodontal tissues, malloclusions, state of oral hygiene, comorbidity, their social habits, locomotor activity before pregnancy and during it, or the nature of nutrition and medical therapy influences the poor state of oral health in children. The dependence of the carious process intensity in children on the condition of their parents' oral cavity can not be denied [10]. Mallocclusion, in most cases, is also hereditary [11]. Limited locomotor activity (hypodynamia) of parents or parents' smoking may affect the timing of teeth eruption in children [12].

We have developed the questionnaire [13] that allows predicting the degree of risk of oral pathology formation in newborns, due to their higher parameters at birth associated with all the above-mentioned factors. It also helps highlight the most significant factors in their parents.

2. Purposes, subjects and methods:

2.1. Purpose of the study was to identify risk factors and to assess the differences in the risk of hard tooth tissue and periodontal tissue diseases formation in newborns or infants born macrosomic, depending on their weight-height index at birth, with the help of questionnaire survey method.

2.2. Subjects & Methods

The parents of 151 newborns or infants (aged from one day up to 6 months) were interviewed during the period of 2014–2019. Sixty-eight children born macrosomic comprised the Main Group (MG), and the children of the corresponding age with normal weight-height parameters at birth (83 children) were included into the Comparison Group (CG). The diagnoses were verified by the neonatology physicians of the Kharkiv Municipal Perinatal Centre.The groups were equally represented by male and female participants. MG children were additionally subdivided into 4 subgroups based on the weight-height parametres of a newborn child, using the classification proposed by Kharkiv scholars [14].

Thirty seven children were assigned to the subgroup I (SG-I). At birth they were tall and harmoniously developed (weight-height index of such children at birth was comparable to that in the CG). Subgroup II (SG-II) included 14 children who were tall with relatively lower body weight (weight-height index was significantly lower than in children with CG) at birth. Subgroup III (SG-III) consisted of 10 children who at birth were tall and had obesity (the weight-height index at birth was significantly higher than that of the CG). Subgroup IV (SG-IV) included 7 children, whose body length at birth matched the body length in the CG, and the weight-height index indicated an intrauterine obesity. The principle of research participants distribution into the subgroup was described in details earlier [15].

The survey was carried out using the original questionary [13], which included 70 questions, divided into 7 scales. **Scale 1** refers to the identification of risk factors of hard tooth tissue and periodontal tissue pathology development according to their past history data and complaints of the child's parents. It is evaluated by the answers to questions 1–10. **Scale 2** is intended for the detection of anatomical and functional factors of malocclusion in the child's parents. It is evaluated by the answers to questions 11–20. **Scale 3** refers to the identification of the hygienic state of the oral cavity of the child's parents. It is evaluated by the answers to questions 21–30. **Scale 4** can identify factors of the family hereditary predisposition for a child's being macrosomic. It is evaluated by the answers to questions from 31 to 40. **Scale 5** is intended to identify the factors of concomitant parent's pathology. It is evaluated by the responses to questions from 41 to 50. **Scale 6** refers to the detection of the effects of the social habits and physical activity before and during pregnancy. It is evaluated by the answers to questions 51–60. **Scale 7** is designed to identify the nutritional factors and drug therapy of the child's parents. Itis evaluated by the answers to questions 61–70. The text of the questionnaire is given in abbreviated form (*Fig. 1*).

The degree of risk of hard tooth tissue and periodontal tissue pathology formation is evaluated as follows: risk is absent – 0 points; minimal risk – from 1 to 10 points; moderate risk – from 11 to 20 points; significant risk – from 21 to 30 points; high risk – more than 30 points.

Test results of the MG and CG participants, after checking on the Pearson criterion for the correspondence of their distribution to the Gauss law, were compared with each other by means of parametric statistics (Student t-criterion). Due to the small number of participants in subgroups, the study of differences in scales between MG subgroups and CG was conducted using nonparametric statistics (Mann–Whitney criterion). The differences were considered reliable if the probability of error p did not exceed 0.05. Confidence intervals (CI) for the percentage of positive answers (points) to questions in groups and subgroups were calculated on the assumption of binomial distribution of a random variable with probability of error $p<0.05$ [16].

Information about a newborn or an infant:

Date of birth _____ gender_____ place of birth_____
blood group, Rh factor _____ body weight _____ body length _____ head circumference _____ chest circumference _____ gestational age _____ timely delivery _____ premature birth _____ delayed birth _____
vaginal delivery_____ physiological _____ pathological _____
delivery by a c-section: _____ planned operation _____ urgent surgery _____
based on the fetus condition _____ based on the mother condition _____
a *1* min *Apgar score* _____ a *5* min *Apgar score*_____
Whether the child was put to the breast in the delivery room _____
Whether the child was taken to the intensive care unit or a newborn resuscitation unit (indicate the number of days) after delivery _____ Whether the child is breastfed or not _____
Diagnosis (from the Neonatal Case Record, Child's Record or Hospital Sheet)_____

Information about newborn's parents:

marital status _____ mother's age _____ father's age_____
height / weight of mother _____ height / weight of father _____
height / weight of mother at birth _____ height / weight of father at birth _____
blood group, Rh factor of the mother _____ blood group, Rh factor of the father _____
Information on previous pregnancies of the newborn's mother: the delivery number _____ the pregnancy number _____ time interval after the previous delivery_____
Describe whether the course of previous pregnancy was complicated by any factors, namely, whether there was: toxicosis, anemia, gestational diabetes, small vein or polyhydramnios of amniotic fluid, pre-eclampsia, manifested by increased blood pressure, edema, the presence of protein in the urine; whether the mother had infectious diseases during pregnancy; whether she was in hospital for pregnancy maintenance; whether there was a threat of miscarriage; whether there were complications during delivery? _____
If mother, father, siblings or other close relatives of the newborn had the weight of 4 kg, or more at their birth, indicate who exactly _____

Questions
1. Did the child's mother have delayed eruption of primary or permanent teeth?
2. Did the child's father have delayed eruption of primary or permanent teeth?
3. Did the child's mother have premature eruption of primary or permanent teeth?
4. Did the child's father have premature eruption of primary or permanent teeth?
5. Does mother's DMF index (decayed, missing, and filled permanent teeth) exceed 13 points?
6. Does father's DMF index (decayed, missing, and filled permanent teeth) exceed 13 points?
7. Was the oral cavity of the child's mother not restored to health during pregravidar preparation and pregnancy?
8. Was the oral cavity of the child's father not restored to health during mother's pregravidar preparation and pregnancy?
9. Did the child's mother need to visit a dentist for teeth treatment or teeth removal during the last year?
10. Did the child's father need to visit a dentist for teeth treatment or teeth removal during the last year?
11. Does the child's mother have malocclusion, tremas or diastema, anomalies of the shape or the amount of teeth, crowding of teeth? (underline your indications)
12. Does the child's father have malocclusion, tremas or diastema, anomalies of the shape or the amount of teeth, crowding of teeth? (underline your indications)
13. Does the child's mother have bruxism accompanied with muscular and articular pain in the area of the mandible or temporomandibular joint?
14. Does the child's father have bruxism accompanied with muscular and articular pain in the area of

the mandible or temporomandibular joint?

15. Does the child's mother have periodontal diseases manifested as gingival bleeding while toothbrushing accompanied with mobile teeth and early tooth loss?

16. Does the child's father have periodontal diseases manifested as gingival bleeding while toothbrushing accompanied with mobile teeth and early tooth loss?

17. Does the child's mother mark crunch in the temporomandibular joint, change in the motion of the mandible when opening or closing the mouth?

18. Does the child's father mark crunch in the temporomandibular joint, change in the motion of the mandible when opening or closing the mouth?

19. Does the child's mother or her close relatives have abnormal tooth abrasion?

20. Does the child's father or his close relatives have abnormal tooth abrasion?

21. Does the child's mother visit a dentist for preventive examinations, less often than once or twice a year?

22. Does the child's father visit a dentist for preventive examinations, less often than once or twice a year?

23. Does the child's mother brush her teeth less often than twice a day?

24. Does the child's father brush his teeth less often than twice a day?

25. Does the child's mother avoid changing her toothbrush once every 3-4 months?

26. Does the child's father avoid changing his toothbrush once every 3-4 months?

27. Does the child's mother neglect special medications for prevention of periodontal and hard tooth tissue diseases such as remineralizing elixirs, balms, and rinses and additional hygiene tools such as floss, intradental brushes, massagers and others?

28. Does the child's father neglect special medications for prevention of periodontal and hard tooth tissue diseases such as remineralizing elixirs, balms, and rinses and additional hygiene tools such as floss, intradental brushes, massagers and others?

29. Does the child's mother have dental braces or other removable and non-removable orthodontic or orthopedic appliances

30. Does the child's father have dental braces or other removable and non-removable orthodontic or orthopedic appliances?

31. Does the child's mother or her close relatives suffer an endocrine pathology, namely thyroid disease?

32. Does the child's father or his close relatives suffer an endocrine pathology, namely thyroid disease?

33. Does the child's mother or her close relatives have diabetes?

34. Does the child's father or his close relatives have diabetes?

35. Does the child's mother have obesity of 1st stage or more at the moment, precisely whether the mother's weight exceeds 20% of the physiological norm?

36. Does the child's father have obesity of 1st stage or more at the moment, precisely whether the father's weight exceeds 20% of the physiological norm?

37. Does the child's mother have following anthropometric characteristics: height is above 170 cm, weight is more than 80 kg?

38. Does the child's father have following anthropometric characteristics: height is above 180 cm, weight is more than 90 kg?

39. Is the child's mother older than 30?

40. Is the child's father 40 or older?

41. Does the child's mother have atopic dermatitis, asthma, allergic rhinitis and other allergic manifestations?

42. Does the child's father have atopic dermatitis, asthma, allergic rhinitis and other allergic manifestations?

43. Does the child's mother have hip dysplasia or dysplasia of other joints, common dislocation of the

joints, stretch marks on the skin, flatulence, and abnormal chord of the left ventricle, mitral valve prolapse, or myopia? (underline your indications)
44. Does the child's father have hip dysplasia or dysplasia of other joints, common dislocation of the joints, stretch marks on the skin, flatulence, and abnormal chord of the left ventricle, mitral valve prolapse, or myopia? (underline your indications)
45. Does the child's mother have brittle nails or dry skin, violation of the hair structure (hypotrichosis), poor growth and hair loss?
46. Does the child's father have brittle nails or dry skin, violation of the hair structure (hypotrichosis), poor growth and hair loss?
47. Does the child's mother belong to a category of frequently ill people, namely whether she comes to hospital complaining for one and the same disease more o than 4 times a year, or more than 6 times a year complaining for different diseases?
48. Does the child's father belong to a category of frequently ill people, namely whether she comes to hospital complaining for one and the same disease more o than 4 times a year, or more than 6 times a year complaining for different diseases?
49. Does the child's mother suffer from hypotension (the blood pressure is equal to 90/60 and lower)?
50. Does the child's father suffer from hypotension (the blood pressure is equal to 90/60 and lower)?
51. Did the child's mother use to smoke (including passive smoking) during pregnancy or 5 years before it?
52. Did the child's father use to smoke (including passive smoking) during mother's pregnancy or 5 years before it?
53. Does the child's mother suffer hypertonic disease (the blood pressure is equal to 140/90 and higher)?
54. Does the child's father suffer hypertonic disease (the blood pressure is equal to 140/90 and higher)?
55. Can the child's mother lifestyle be characterized as hypodynamic?
56. Can the child's father lifestyle be characterized as hypodynamic?
57. Was the child's mother often in stressful conditions at work or at home during pregnancy and 5 years before it?
58. Was the child's father often in stressful conditions at work or at home during mother's pregnancy and 5 years before it?
59. Did the child's mother drink alcohol during pregnancy or 5 years before it?
60. Did the child's father drink alcohol during pregnancy or 5 years before it?
61. Does the child's mother prefer fatty food?
62. Does the child's father prefer fatty food?
63. Does the child's mother prefer fried food?
64. Does the child's father prefer fried food?
65. Does the chid's mother consume sugary non carbonated drinks or carbonated drinks, sugary juices, syrups?
66. Does the chid's father consume sugary non carbonated drinks or carbonated drinks, sugary juices, syrups?
67. Does the child's mother eat snacks in between food intakes?
68. Does the child's father eat snacks in between food intakes?
69. Does the child's mother use corticosteroids, immunosuppressants, anti-depressants, salicylates, aerosol medications to control asthma, hormonal contraceptives or other medications?
70. Does the child's mother use corticosteroids, immunosuppressants, anti-depressants, salicylates, aerosol medications to control asthma, hormonal contraceptives or other medications?

Fig. 1. Prognosis of the Risk Degree of Oral Pathology Formation in Macrosomic Newborns or Infants (Appendix to the Neonatal Case Record, Child's Record or Hospital Sheet). The text of the questionnaire is given in an abbreviated form

Declarations. The Ethical and bioethical committee of the Kharkiv National Medical University (Record No. 5 dated 10 May 2016) confirms that the techniques used in this study have beenapplied with the respect to human rights in accordancewith the current legislation in Ukraine, meet international ethicalrequirements and do not violate ethical norms inscience and standards for conducting biomedical research. The parents of each child gave written consent to participate in the study.

List of abbreviations. Confidence intervals (CI); Main Group (MG); Comparison Group (CG); Subgroup (SG); total parental factor (PF); percentage number of cases (PNC).

Conflict of interests

The authors declare that they have no competing interests.

3. Results and discussion

The results of comparing the total score of the dental anomalies risk factors in the MG and CG children (see *Table 1*) obtained from a big number of participants, confirmed the risk predominance in MG children and agreed with the results obtained earlier [17].

However, it should be noted that in 32 (47.1% CI: 36.2–58.1%) MG children and in 8 (9.6% CI: 5.1–16.6%) CG children at least one of the parents had a weight-height parameters at birth that corresponded to macrosomic. Therefore, these parents had significant dental problems which were proved by our previous studies [18]. Children of SG-I had macrosomic-at-birth parents in 18 (48.6% of CI: 34.4–63.1%) cases, SG-II hadsuch parents in 6 (42.9% CI: 23,0–64,9%) of cases, SG-III included 6 (60,0% CI: 34.8– 81.3%), and SG-IV comprised 2 (28.6% CI: 9.9– 57.9%) cases. This fact may be one of the explanations for the risk prevalence among the participants in SG-I, SG-II and SG-III over the CG. It should be noted that higher risks are also observed in children aged from 4 to 17 who were born with signs of intrauterine obesity in the background of acceleration (subgroup III) [19].

The test results analysis (*Table 2* and *Table 3*) revealed that the points scored by the participants

Table 1

Tabulated Scores of the Test Results of the CG, MG and Subgroups Partisipants, Depending on the Risk Degree

Groups and Subgroups	Minimal Risk Degree	Moderate Risk Degree	Significant Risk Degree	High Risk Degree
Comparison Group	7 (8.4% CI: 4.3–15.1%)	42 (50.6% CI: 40.6–60.6%)	32 (38.6% CI: 29.2–48.7%)	2 (2.4% CI: 0.8–6.5%)
Main Group	2 (2.9% CI: 0.9–7.9%)	20 (29.4% CI: 20.2–40.1%)*	41 (60.3% CI: 49.2–70.6%)*	5 (7.4% CI: 3.3–14.4%)
Subgroup I	1 (2.7% CI: 0.7–9.5%)	9 (24.3% CI: 13.8–38.2%)*	24 (64.9% CI: 50.2–77.5%)*	3 (8.1% CI: 3.0–18.2%)
Subgroup II	0 (0.0% CI: 0.2–23.2%)	6 (42.9% CI: 23.0–64.9%)	7 (50.0% CI: 28.9–71.1%)	1 (7.1% CI: 1.8–23.2%)
Subgroup III	0 (0.0% CI: 0.3–30.8%)	2 (20.0% CI: 6.7–44.5%)	6 (60.0% CI: 34.8–81.3%)	2 (20.0% CI: 6.7–44.5%)*
Subgroup IV	1 (14.3% CI: 3.7–41.0%)	3 (42.9% CI: 18.4–71.0%)	3 (42.9% CI: 18.4–71.0%)	0 (0.0% CI: 0.4–41.0%)

* – The difference from the CG is significant (within the 0.95 confidence interval).

Table 2

Tabulated Scores of the Test Results of CG, MG and Subgroups Participants Depending on the Scale of the Questionnaire

Groups and Subgroups	Scale 1	Scale 2	Scale 3	Scale 4	Scale 5	Scale 6	Scale 7
Comparison Group	2.65±0.35	1.76±0.28	3.80±0.49	0.93±0.22	1.35±0.23	3.94±0.41	4.59±0.48
Main Group	3.50±0.36*	2.38±0.37	4.43±0.53	1.51±0.32*	1.71±0.34	3.93±0.43	5.37±0.47
Subgroup I	3.54 (p=0.0026)*	2.59 (p=0.0161)*	4.57	1.49 (p=0.0054)*	1.51	4.16	5.46
Subgroup II	3.50 (p=0.0413)*	2.50 (p=0.0227) † (p=0.0381)*	4.71	1.43	1.79	3.93	5.07
Subgroup III	3.70	2.10	4.50	2.00 (p=0.0389)*	2.30 (p=0.0461)*	3.60	5.90
Subgroup IV	3.00	1.43	3.00	1.14	1.71	3.29	4.71

* – The difference from the CG is significant (within the 0.95 confidence interval).

† – The difference from the SG -IV is significant (within the 0.95 confidence interval).

Table 3

Tabulated Scores of the Test Results of CG, MG and Subgroups Participants Depending On the Question

Question №	Comparison Group	Main Group	Subgroup I	Subgroup II	Subgroup III	Subgroup IV
1	6 (7.2)	8 (11.8)	3 (8.1)	1 (7.1)	2 (20.0)	2 (28.6)
2	2 (2.4)	5 (7.4)	2 (5.4)	0 (0.0)	2 (20.0) *	1 (14.3)
3	8 (9.6)	7 (10.3)	5 (13.5)	0 (0.0)	1 (10.0)	1 (14.3)
4	6 (7.2)	4 (5.9)	0 (0.0)	3 (21.4)	1 (10.0)	0 (0.0)
5	19 (22.9)	25 (36.8)	15 (40.5)	5 (35.7)	3 (30.0)	2 (28.6)
6	15 (18.1)	27 (39.7) *	14 (37.8)	7 (50.0) *	4 (40.0)	2 (28.6)
7	26 (31.3)	30 (44.1)	16 (43.2)	6 (42.9)	5 (50.0)	3 (42.9)
8	37 (44.6)	33 (48.5)	19 (51.4)	6 (42.9)	5 (50.0)	3 (42.9)
9	55 (66.3)	50 (73.5)	28 (75.7)	12 (85.7)	7 (70.0)	3 (42.9)
10	46 (55.4)	49 (72.1)	29 (78.4)	9 (64.3)	7 (70.0)	4 (57.1)
11	31 (37.3)	31 (45.6)	18 (48.6)	6 (42.9)	5 (50.0)	2 (28.6)
12	19 (22.9)	17 (25.0)	12 (32.4)	3 (21.4)	2 (20.0)	1 (14.3)
13	7 (8.4)	9 (13.2)	5 (13.5)	2 (14.3)	0 (0.0)	1 (14.3)
14	10 (12.0)	9 (13.2)	5 (13.5)	1 (7.1)	2 (20.0)	1 (14.3)
15	35 (42.2)	41 (60.3)	24 (64.9)	9 (64.3)	5 (50.0)	3 (42.9)
16	21 (25.3)	19 (27.9)	9 (24.3)	6 (42.9)	4 (40.0)	0 (0.0)
17	16 (19.3)	19 (27.9)	13 (35.1)	3 (21.4)	2 (20.0)	1 (14.3)
18	1 (1.2)	8 (11.8) *	7 (18.9) *	1 (7.1)	0 (0.0)	0 (0.0)
19	3 (3.6)	5 (7.4)	2 (5.4)	2 (14.3)	1 (10.0)	0 (0.0)
20	3 (3.6)	4 (5.9)	1 (2.7)	2 (14.3)	0 (0.0)	1 (14.3)
21	36 (43.4)	43 (63.2)	25 (67.6)	9 (64.3)	6 (0.60)	3 (42.9)
22	46 (55.4)	44 (64.7)	25 (67.6)	11 (78.6)	6 (0.60)	2 (28.6)
23	27 (32.5)	26 (38.2)	16 (43.2)	5 (35.7)	4 (40.0)	1 (14.3)
24	28 (33.7)	30 (44.1)	18 (48.6)	7 (50.0)	4 (40.0)	1 (14.3)
25	29 (34.9)	26 (38.2)	17 (45.9)	4 (28.6)	2 (20.0)	3 (42.9)
26	34 (41.0)	28 (41.2)	17 (45.9)	6 (42.9)	2 (20.0)	3 (42.9)
27	39 (47.0)	34 (50.0)	13 (35.1)	71.42857	8 (0.80)	3 (42.9)
28	39 (47.0)	38 (55.9)	15 (40.5)	11 (78.6)	8 (0.80)	4 (57.1)
29	24 (29.0)	18 (26.5)	15 (40.5)	1 (7.1)	2 (20.0)	0 (0.0)
30	13 (15.7)	14 (20.6)	8 (21.6)	2 (14.3)	3 (30.0)	1 (14.3)
31	8 (9.6)	12 (17.6)	5 (13.5)	4 (28.6)	3 (30.0)	0 (0.0)
32	3 (3.6)	3 (4.4)	2 (5.4)	0 (0.0)	1 (10.0)	0 (0.0)
33	2 (2.4)	4 (5.9)	2 (5.4)	0 (0.0)	2 (20.0) *	0 (0.0)
34	0 (0.0)	1 (1.5)	0 (0.0)	0 (0.0)	1 (10.0)	0 (0.0)
35	2 (2.4)	7 (10.3)	3 (8.1)	2 (14.3)	1 (10.0)	1 (14.3)
36	4 (4.8)	3 (4.4)	1 (2.7)	1 (7.1)	1 (10.0)	0 (0.0)
37	4 (4.8)	11 (16.2) *	7 (18.9)	1 (7.1)	2 (20.0)	1 (14.3)
38	15 (18.1)	22 (32.4)	10 (27.0)	6 (42.9)	4 (40.0)	2 (28.6)
39	30 (36.1)	28 (41.2)	18 (48.6)	4 (28.6)	4 (40.0)	2 (28.6)
40	9 (10.8)	12 (17.6)	7 (18.9)	2 (14.3)	1 (10.0)	2 (28.6)
41	14 (16.9)	14 (20.6)	7 (18.9)	3 (21.4)	2 (20.0)	2 (28.6)
42	9 (10.8)	13 (19.1)	7 (18.9)	1 (7.1)	4 (40.0) *	1 (14.3)
43	18 (21.7)	15 (22.1)	5 (13.5)	4 (28.6)	3 (30.0)	3 (42.9)
44	6 (7.2)	11 (16.2)	4 (10.8)	4 (28.6)	2 (20.0)	1 (14.3)
45	7 (8.4)	11 (16.2)	7 (18.9)	2 (14.3)	2 (20.0)	0 (0.0)
46	5 (6.0)	8 (11.8)	5 (13.5)	1 (7.1)	2 (20.0)	0 (0.0)
47	5 (6.0)	12 (17.6)	6 (16.2)	3 (21.4)	2 (20.0)	1 (14.3)
48	6 (7.2)	5 (7.4)	2 (5.4)	2 (14.3)	1 (10.0)	0 (0.0)
49	39 (47.0)	25 (36.8)	11 (29.7)	5 (35.7)	5 (0.50)	4 (57.1)
50	3 (3.6)	2 (2.9)	2 (5.4)	0 (0.0)	0 (0.0)	0 (0.0)
51	36 (43.4)	34 (50.0)	22 (59.5)	5 (35.7)	4 (40.0)	3 (42.9)
52	54 (65,1)	61,76471	25 (67,6)	7 (50,0)	7 (0,70)	3 (42,9)
53	11 (13,3)	12 (17,6)	6 (16,2)	2 (14,3)	2 (20,0)	2 (28,6)
54	10 (12,0)	12 (17,6)	6 (16,2)	2 (14,3)	2 (20,0)	2 (28,6)
55	15 (18,1)	9 (13,2)	5 (13,5)	3 (21,4)	1 (10,0)	0 (0,0)
56	14 (16,9)	11 (16,2)	6 (16,2)	3 (21,4)	2 (20)	0 (0,0)
57	37 (44,6)	37 (54,4)	18 (48,6)	10 (71,4)	5 (50,0)	4 (57,1)
58	39 (47,0)	36 (52,9)	17 (45,9)	7 (50,0)	7 (70,0)	5 (71,4)
59	52 (62,7)	34 (50,0)	23 (62,2)	8 (57,1)	1 (10,0) *	2 (28,6)
60	59 (71,1)	41 (60,3)	26 (70,3)	8 (57,1)	5 (50,0)	2 (28,6) *
61	29 (34,9)	28 (41,2)	14 (37,8)	5 (35,7)	7 (70,0)	2 (28,6)
62	49 (59,0)	41 (60,3)	22 (59,5)	7 (50,0)	8 (0,80)	4 (57,1)
63	51 (61,4)	47 (69,1)	27 (73,0)	10 (71,4)	7 (70,0)	3 (42,9)
64	57 (68,7)	53 (77,9)	29 (78,4)	10 (71,4)	9 (0,90)	5 (71,4)
65	59 (71,1)	55 (80,9)	32 (86,5)	11 (78,6)	7 (70,0)	5 (71,4)
66	46 (55,4)	46 (67,6)	27 (73,0)	8 (57,1)	7 (70,0)	4 (57,1)
67	42 (50,6)	42 (61,8)	22 (59,5)	10 (71,4)	7 (70,0)	3 (42,9)
68	40 (48,2)	43 (63,2)	22 (59,5)	9 (64,3)	7 (70,0)	5 (71,4)
69	6 (7,2)	5 (7,4)	3 (8,1)	1 (7,1)	0 (0,0)	1 (14,3)
70	2 (2,4)	5 (7,4)	4 (10,8)	0 (0,0)	0 (0,0)	1 (14,3)

***** – The difference from the CG is significant (within the 0.95 confidence interval).

on scale 1 were significantly different from the points scored by the CG participants (see *Table 2*). While analyzing the differences in subgroups, we found out that the "predominance" of complaints was achieved precisely because of the answers of those parents who were born macrosomic. In particular, the following responses were significantly different: they indicated that the newborn's father had a delay in the timing of the deciduous or permanent teeth eruption in 2 persons in the SG-III (20.0% CI: 6.7–44.5%) versus 2 people in the CG (2.4 % CI: 0.8–6.5%). The fact that the total number of carious, sealed and extracted teeth (DMF index) in the newborn's father exceeded 13, reported 27 MG people (39.7% CI: 29,4–50.8%) and 7 people in SG-II (50.0% CI: 28.9–71.1%) against 15 people in the CG (18.1% CI: 11.4–26.7%).

There was no significant difference between the points scored by the MG and the CG participants in the questionnaire scale 2 (*Table 2*). Subgroup scores analysis showed that there were more complaints in the SG-I, SG-II and SG-III parents than in the CG parents. These differences were true for SG-1 and SG-II. In particular, there were significantly more cases where a newborn's father noted pain or crunch in the temporomandibular joint, changes in the movement of the mandible during opening or closing the mouth, namely 8 MG participants (11.8% CI: 6.2–20, 1%) and 7 SG-I participants (18.9% CI: 9.8–32.0%) against CG (1.2% CI: 0.3–4.3%).

The results questionnaires analysis in the part of the total parental factor (PF) influence on the risk of oral disorders formation in newborns (summarized results of scale 1 and scale 2), children (scale 1) and adults (scale 1) [18], are presented in *Table 4*.

According to our data for macrosomic newborns, the average score of PF is significantly higher than for normosomic newborns. But does PF really affect the risk of oral disorders formation in macrosomic newborns more than in normosomic? Note that one of the parents, regardless whether their newborn is macrosomic or normosomic, can also be normosomic-at birth, and macrosomic-at-birth. Data on each age category of a person participating in the survey on the total number of respondents, the number of macrosomic-at-birth parents and their relative number are presented in *Table 5*.

Since the state of the oral health (hence the number of complaints) in macrosomic- and normosomic-at-birth is different, and the relative number of macrocomic-at-birth parents in different categories of participants is also not the same, differences in the average score of the PF (*Table 4*) may occur due to this factor. Let us consider this question in details. We introduce the following notation: bn and bm - the average scores on the scale of the PF for cases where the father

Table 4

The Mean Score of the Total PF Influence on the Risk of Emergence and Formation of Oral Diseases in Macrosomic-At-Birth- Or Normosomic-At-Birth Participants of Different Age Categories

Age Categories	Mean Score of the Total PF in Macrosomic-At-Birth Participants (MG), B_1	Mean Score of the Total PF in Normosomic-At-Birth Participants (CG), B_2
Newborns	2.94 ±0.27 (mean scale 1 score: 3.50 ±0.36 mean scale 2 score: 2.38 ±0.37)	2.20±0.23 (mean scale 1 score: 2.65 ±0.35 mean scale 2 score: 1.76 ±0.28)
Children	3.49 ±0.44	3.15 ±0.65
Adults	3.12 ±0.38	2.80 ±0.36

Table 5

The Data on the Absolute and Relative Number of Macrosomic-At-Birth Parents Among the Survey Participants Depending on the Age Category and the Study Group

Age Categories	Macrosomic-at-birth person (MG)			Normosomic-at-birth person (CG)		
	Total number of respondents, Σ_1	Number of macrosomic-at-birth pearents, m_1	Relative number of macrosomic at-birth pearents, m_1/Σ_1	Total number of respondents Σ_2	Number of macrosomic-at-birth pearents, m_2	Relative number of macrosomic-at-birth pearents m_2/Σ_2
Newborns	68	32	0.471	83	8	0.096
Children	82	33	0.402	41	10	0.244
Adults	114	33	0.290	127	10	0.079

and mother of the participant are the normosomic and when one of them is macrosomic-at-birth respectively; n and m is the number of cases where the father and mother of the participant are the normosomic and when one of them was born with macrosomia, respectively. Then for the average score of the PF you can write:

$$B = \frac{b_n n + b_m m}{n + m} = \frac{b_n n + b_m m}{\Sigma},$$

where Σ = n + m is the total number of respondents. Let the indexes 1 and 2 refer to the cases of the participant who was macrosomic-at-birth and the normosomic-at-birth person, respectively. Then the ratio of the average score of the PF for these cases has the form:

$$\frac{B_1}{B_2} = \frac{1 + (k-1)\frac{m_1}{\Sigma_1}}{1 + (k-1)\frac{m_2}{\Sigma_2}}.$$

Here m / Σ is the relative number of cases where any of the parents was at birth macrosomic; $k = b_m / (b_n)$ is a coefficient that shows how much more average dental complaints are in the macrosomic than in the normosomic-at-birth partisipants. To begin with, we define this coefficient, for example, for the category "children"[19]:

$$k = 1 + \frac{1 - \frac{B_1}{B_2}}{\frac{m_2}{\Sigma_2}\frac{B_1}{B_2} - \frac{m_1}{\Sigma_1}} \approx 1 + \frac{1 - \frac{3.49}{3.15}}{0.244\frac{3.49}{3.15} - 0.402} \approx 1.82.$$

Thus, the macrosomic-at-birth parents complain almost 2 times more than the normosomic-at-birth parents. Now we calculate by the formula

$$B_1 = B_2 \frac{1 + (k-1)\frac{m_1}{\Sigma_1}}{1 + (k-1)\frac{m_2}{\Sigma_2}}$$

The average score for the macrosomic-at-birth person in all age categories is compared with the data given in *Table 4*. The results of calculations are given in *Table 6*. We can see that the estimated values are quite close to those obtained from the questionnaires [18, 19], which indicates the adequacy of the hypothesis about the reason for the variability of the average score of the PF, at least within the same age category.

Consequently, the score for scales 1 and 2 should be adjusted taking into account the weight-height parameters of the parents at birth.

The survey showed that according to scale 3, the MG participants, together and in the subgroups, had no significant difference in the number of points compared to the CG (*Table 2*).

Scale 4 includes a set of questions aimed at identifying the factors of the child's family-genetic predisposition to the fetal macrosomia. A significant difference was found between the CG and MG (*Table 2*).

Comparative analysis in the subgroups revealed a significantly higher number of points in individuals of SG-I and SG-III. In these subgroups we found a significantly higher percentage of parents born macrosomic.

Parents of SG-III children have the highest percentage number of cases (PNC) of big anthropometric parametres among all participants in the study (*Table 3*), which has also been confirmed in other age groups [19], as well as PNC of diabetes. In particular, the PNC of diabetes mellitus was significantly higher in newborn's mothers (Table 3) of SG-III and comprised 2 persons (20.0% CI: 6.7–44.5%) versus 2 persons in the CG 2 (2.4% CI: 0.8–6.5%). This subgroup is also the "leader" among participants in other age categories (children, adults) [19].

The overall assessment of the true difference between the MG and CG on the scale 5 was not detected which can be seen in *Table 2*. But the PNC of the parents concomitant pathology was significantly higher in SG-III than the same factor in CG. In particular, PNC of atopic dermatitis, bronchial asthma, allergic rhinitis or other allergic manifestations in the newborn's father was significantly higher in SG-III - 4 persons (40.0% CI: 18.7–65.2%) versus 9 persons in the CG (10.8% CI: 5.9–18.1%). It is appropriate to say that every father, who indicated the presence of

Table 6

Comparison of Actual and Estimated Average Parental Factor Scores in Different Age Categories and Groups of Participants Under Study

Age Categories	Average score of the PF for the macrosomic-person (actual), B_1	Average score of the PF for the macrosomic-person (calculated), B_1
Newborns	2.94 ±0,27	2.83
Children	3.49 ±0.44	3.49
Adults	3.12 ±0.38	3.26

allergic diseases, was found to be born macrosomic. The facts we have discovered are confirmed by other scholars. It is known that allergic reactions occur much more often if a person before the age of 2 was obese [20]. The interrelation of obesity and asthma is also confirmed by the authors of the study [21].

Significant difference between the MG and CG in scale 6 also was not detected (*Table 2*). However, the PNC of alcoholic beverages consumption by mothers during pregnancy or during 5 years before pregnancy in SG-III was significantly lower than in the CG: 1 person (10.0% CI: 2.5–30.8%) against 52 persons (62.7% CI: 52.6–71.9%), respectively. PNC of alcoholic beverages consumption by fathers during 5 years before pregnancyof a newborn's mother in SG-IV was significantly lower than in the CG: 2 persons (28.6% CI: 9.9–57.9%) versus 60 persons (72.3 % CI: 62.7–80.5%), respectively. There was no significant difference, or tendencies in one or another direction, between subgroups and CG in all other questions related to this scale.

There was no significant difference between the MG and subgroups and CG (*Table 2, Table 3*) in the scale 7.

Discussion. Until recently, research papers concerned with the study of the features of dental disorders formation in individuals born with macrosomia have been extremely few. Now the situation is starting to change and many research papers that confirm the high intensity and prevalence of caries, as well as other dental disorders, in macrosomic at birth persons have been published. [22, 23].

As mentioned above, there is genetic predisposition for certain dental disorders [24, 25]. But this tendency is manifested in both the group of macrosomic newborns and the group of normosomic newborns.

Taking into account the drastic variety among macrosomic newborns, in particular, due to their different height-weight index at birth [26], the dental state specific to each subgroup is subsequently formed [8, 9, 15, 19]. We hoped to reveal some differences in the parents of macrosomic children in the different subgroups, namely: eating habits, bad habits, features of dental status, etc. However, taking into account that some parents were also born macrosomic, it has turned out that the survey of parents of macrosomic children in the different subgroups shows no certain difference in the results. That is, poor oral health that occur in ontogeny in macrosomic at birth persons is most likely more closely related to the same metabolic patterns that summed into the fetal macrosomia [27, 28] but not to a set of factors grouped in our questionnaire (parents' pathology of hard tooth and periodontal tissues, malloclusions, state of oral hygiene, comorbidity, their social habits, locomotor activity before pregnancy and during it, or the nature of nutrition and medical therapy).

After a detailed analysis of the subgroups and comparison group of these data with the data of the questionnaire of older age groups, it was concluded that the effect of the total paternal factor on the formation of oral disorders in individuals with macrosomia at birth, can be different from the effect of this factor in normosomic-at birth persons, but this difference is related to the same phenomenon that we are studying – the fetal macrosomia, but the paternal one.

Perhaps, one of the factors, which influence the onset of dental disorders in ontogeny, is the reduced bone mineral density in macrosomic newborns, which is associated with the features of macrosomic fetus formation and intrauterine metabolic and immune shifts in the macrosomic fetus [29–31].

Conclusions

1. The hygienic state of the oral cavity of parents, the presence of concomitant pathologyof parents, bad habits and the degree of locomotor activity before pregnancy, and during pregnancy, peculiarities of nutrition and medical therapy of parents influence practically identically on the formation of oral disorders in the first year of life in children born macrosomic, regardless of the weight-height index at birth, or in children born normomic. Reliable differences between groups and subgroups were not detected.

2. According to the past history data and complaints of parents, the highest risk of the oral pathology formation have children whose parents were born macrosomic.

3. Factors influencing the formation of fetal macrosomia in the intrauterine period, in addition to heredity, defects in oral hygiene or concomitant pathology, ground the patterns of "disturbed" oral health. A person born macrosomic, on the average, has twice as many complaints about oral health compared to a person born normosomic.

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