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# **ВІСНИК МОРФОЛОГІЇ REPORTS OF MORPHOLOGY**

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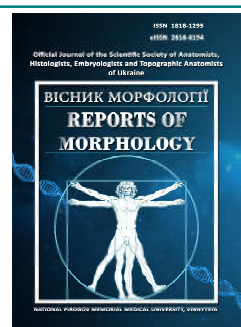




## REPORTS OF MORPHOLOGY

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# Features of hemodynamics in the comorbid course of essential hypertension and type 2 diabetes in men, residents of Podillia, carriers of polymorphic variants of the brain natriuretic peptide gene

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Essential hypertension (EH) and type 2 diabetes (T2D) are important risk factors for the development of chronic heart failure (CHF). The early detection of CHF, especially under comorbidity, remains a challenge. To solve it in today's conditions are used not only instrumental diagnostic methods (Echo-CG), but also the assessment of plasma levels of biomarker - brain natriuretic peptide (BNP), the expression of which is determined by the corresponding gene (locus T-381C) and may depend on its structural organization. It is proved that deregulation of the natriuretic peptide system (NP) is an important factor in the initiation and progression of myocardial dysfunction and energy imbalance, but the role of genetic preconditions for these disorders, including the peculiarities of polymorphic variants of the most physiologically significant gene is still not enough clear. The aim of the work was to investigate the presence of associations between indicators of systemic and intracardiac hemodynamic and the carrier of polymorphic variants of the BNP gene (T-381C) in men, residents of Podillia with the comorbid course of EH 2 and type 2 diabetes mellitus. We examined 132 middle-age men: 62 patients with EH 2 and chronic heart failure (CHF) 0-I functional classes (FC) according to NYHA Classification and 70 - with EH 2 combined with T2D and CHF FC I-II. Patients with EH 2 included in the first group and patients with EH 2 and T2D included in the second group of comparison. Parameters of intracardiac hemodynamics were determined on the basis of pulsed Doppler echocardiography. The genomic DNA of the BNP gene (T-381C) for the determination of its alleles was isolated by PCR. The mathematical processing was performed using the standard statistical package Statistica 10. We calculated the primary statistical indicators, identified differences between groups on statistical signs, performed correlation and discriminant analysis. The calculation of the relative risk with a 95% confidence interval was performed using an online calculator (<https://medstatistic.ru/calculators/calcrisk.html>). Among men living in Podillya with EH, both in the presence and absence of diabetes mellitus 2, the T381C genotype of the BNP gene ( $p>0.05$ ) dominates. In the group of comorbid patients diastolic dysfunction of the left ventricle (DD LV) was diagnosed in 90% of people ( $n=63$ ), while in the isolated course of EH it was found only in 43.55% ( $n=28$ ). Although among homozygotes T381T BNP gene its symptoms were 100% ( $n=24$ ), and among carriers of the C allele - in 84.78% ( $n=39$ ) of patients. Carriers of the T381T genotype of the BNP gene dominated among persons with DD grade II: 41.66% against 10.87% of carriers of the C allele ( $p<0.05$ ), while among persons with DD LV grade I there were more carriers of the C allele. Homozygous T381T genotype with EH 2 and T2D had a higher level of pulse blood pressure ( $p<0.01$ ), a higher probability of developing eccentric left ventricular hypertrophy ( $p?0.05$ ) and more pronounced diastolic changes in the myocardium, as compared with carriers of the C allele and can be allocated to the priority group of observation for the organization of targeted measures aimed at preventing the development and progression of CHF.

**Keywords:** essential hypertension, type 2 diabetes, the brain natriuretic peptide gene polymorphism, diastolic dysfunction.

## Introduction

The combination of essential hypertension (EG) and type 2 diabetes (T2D) as an additional factor of specific adverse effects on the myocardium several times increases the risk of cardiovascular complications due to the mutually aggravating course of the disease and damage to common target organs, including blood vessels and heart [13, 22, 24]. The inevitable end and the leading cause of death, as in almost all cardiovascular diseases and T2D is the development of chronic heart failure (CHF), the course of which in comorbid patients is more severe and characterized by a worse prognosis [12, 13, 16, 23].

In order to improve the individual strategy for the prevention of CHF, especially in the comorbid course of EG and T2D, there is an urgent need to use effective diagnostic biomarkers of myocardial dysfunction at the stage of reverse changes [33, 36]. Natriuretic peptides (NP) are considered to be important markers, as well as biological properties of direct antagonists of RAAS activity and regulators of heart structure and function [21]. Currently, the assessment of the informativeness of brain natriuretic peptide (BNP) remains relevant, the plasma level of which may depend on hereditary, sexual, age characteristics of patients and metabolic changes in T2D [5, 10, 15, 32]. It is known that the genotype is an important determinant of BNP levels in the general community and explains some variability in its plasma concentration [8, 11, 17]. That is why there is a need to clarify the diagnostic significance of allelic polymorphism of the BNP gene (locus T-381C-SNPs 198389) in left ventricular hypertrophy (LVH) and the detection of early signs of CHF. This clarification seems especially important in patients with T2D, because it is proved that the violation of the regulation of the NP system is a significant factor not only in the initiation and progression of myocardial dysfunction, but also energy imbalance [6, 33, 39].

The growing number of genetic and epidemiological studies in recent years suggests that BNP involved in cardiac stress is inversely associated with risk factors for diabetes - with metabolic syndrome and insulin resistance, but the expected association remains unclear [3, 7, 19]. Because metabolic processes, in turn, are closely linked to the development of cardiovascular disease, interest in the study of humoral agents that combine them has not abated. That is why the role of BNP in the development of remodeling and myocardial dysfunction, as well as the genetic polymorphism of the gene, which may cause its regulation, requires further evaluation under the conditions of comorbidity of EG and T2D.

Studies conducted in this area in Ukraine are few. In previous works, employees of the Department of Internal Medicine of the Medical Faculty №2 National Pirogov Memorial Medical University, Vinnytsia evaluated the features of the structural and functional state of the myocardium in carrying different variants of the BNP gene

in men, residents of Podillya in the isolated course of EG II with different stages of CHF, but patients with T2D were not included in the study [2, 25, 26, 27].

Continuing research to improve understanding of pathogenetic mechanisms as a possible individual basis for the development of CHF, taking into account genetic, sex and population differences in the combined course of EG and T2D may complement existing data and provide new perspectives for early detection, timely and rational treatment of these comorbid patients.

*Purpose of study:* to investigate the presence of associations between indicators of systemic and intracardiac hemodynamics and the carrier of polymorphic variants of the BNP gene (T-381C) in men, residents of Podillya with the comorbid course of EG II and T2D.

## Materials and methods

The study was approved by the local ethics commission, as well as the informed consent of all patients. Surveyed 132 middle-aged men living in the Podillya region of Ukraine in the third generation, at a distance of more than 5 km from each other and which are not relatives. A comprehensive clinical-anamnestic, anthropometric and laboratory-instrumental examination was performed, on the basis of which the diagnosis of EG II and CHF was established in accordance with the recommendations of the European and Ukrainian Association of Cardiologists for the diagnosis and treatment of hypertension and CHF [29, 31, 37].

Verification of the diagnosis of T2D was performed according to the WHO criteria and according to the Order of the Ministry of Health of Ukraine dated 21.12.2012 №1118 [9, 34]. The parameters of intracardiac hemodynamics were determined on the basis of echocardiography. The criterion of LVH for men was considered to be  $LV\ weight/height^{2.7} > 50\ g/m^{2.7}$  according to the recommendations of the European Association of Cardiologists for the diagnosis and treatment of hypertension (2018) [37]. Diastolic LV function was assessed according to current guidelines using pulsed Doppler echocardiography [18]. Exclusion criteria were: symptomatic hypertension, severe CHF (III-IV FC according to NYHA) with reduced left ventricle ejection fraction (<40%), T1D, T2D decompensation, insulin therapy, diabetic nephropathy 4-5 degree, chronic kidney disease of non-diabetic origin, liver failure, chronic obstructive pulmonary disease and bronchial asthma, acquired heart disease, tumors, diseases of the blood system, concomitant inflammatory and other endocrine diseases, except T2D.

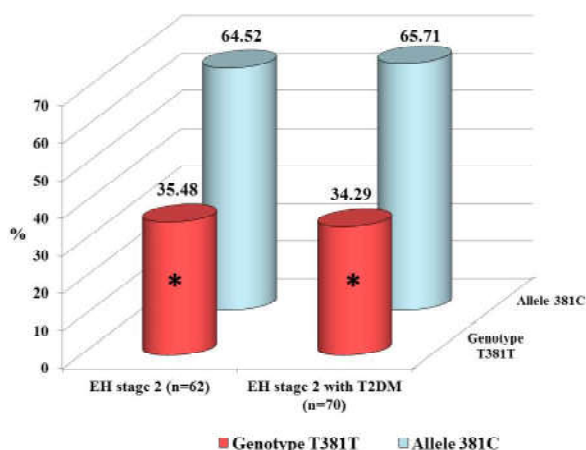
Patients were divided into 2 groups. 62 individuals with EG II and CHF 0-I FC according to NYHA were included in the first, and 70 men with EG II in combination with T2D and CHF I-II FC - in the second comparison group. The genomic DNA of the BNP gene for the determination of alleles of the polymorphic region (T-381C) was isolated by

PCR in collaboration with the Research Institute of Genetic and Immunological Basis of Pathology and Pharmacogenetics of the Ukrainian Medical Dental Academy, Poltava (Head of Laboratory - Doctor of Medicine, Professor I.P.Kaidashev).

Mathematical processing of the results was performed using the standard statistical package Statistica 10. Primary statistical indicators were calculated, differences between groups on statistical features were revealed, correlation and discriminant analysis was performed. Relative risk (RR) with a 95% confidence interval is calculated using an online calculator (<https://medstatistic.ru/calculators/calcrisk.html>).

### Results

The frequency distribution of polymorphic genes in the population was checked in accordance with Hardy-Weinberg equilibrium law. Analysis of the frequency distribution of genotypes of the BNP gene revealed the dominance of the T381C variant ( $p \leq 0.05$ ) in both comparison groups in the absence of a significant difference between the number of carriers of individual polymorphic variants in each group. For greater accuracy of comparative analysis due to the small number of individuals - homozygotes C381C they are combined with carriers of the genotype T381C to one common group - carriers of the C allele of the BNP gene (Fig. 1). In order to identify hereditary preconditions for myocardial remodeling and dysfunction



**Fig. 1.** Frequency of distribution of alleles of the BNP gene in comparison groups among men living in Podillya (%). The difference is significant ( $p \leq 0.05$ ) when compared with \* - carriers of the C allele within the group.

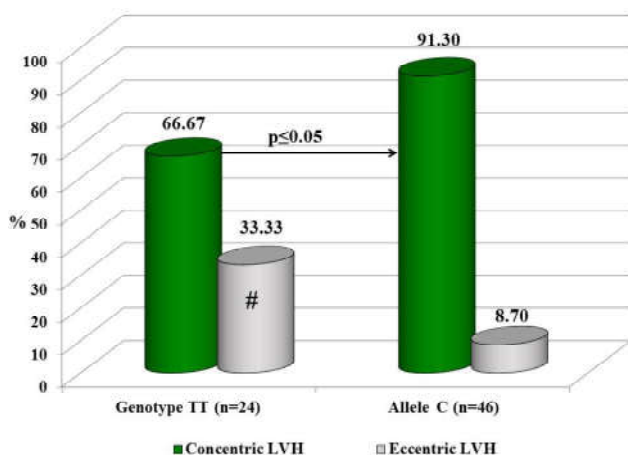
in men with EG II in the presence of comorbid T2D, the parameters of systemic hemodynamics and structural and functional parameters of the myocardium in comparison groups depending on the structural organization of the BNP gene were determined (Table 1).

The Kendall rank correlation method was used to investigate the possible relationship between the carrier of polymorphic variants of the BNP gene and the values of

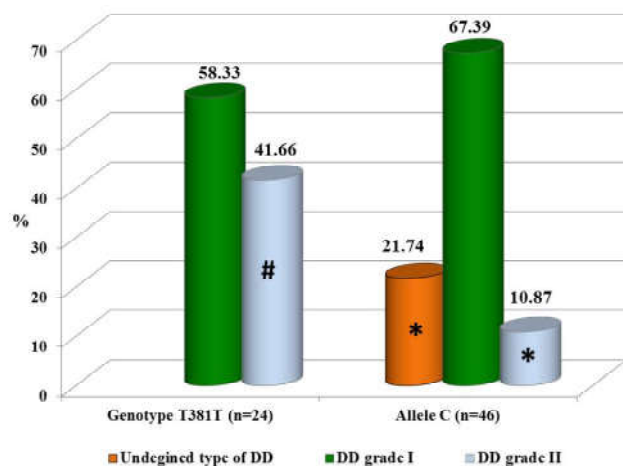
**Table 1.** Systemic hemodynamic parameters and structural and functional parameters of the myocardium in men with EG II, and EG II in combination with T2D, carriers of different genotypes of the BNP gene, (M±m).

Indicator	1. Patients with EG II, homozygotes T381T (n=22)	2. Patients with EG II, carriers of the C allele (n=40)	3. Patients with EG II and T2D, homozygotes T381T (n=24)	4. Patients with EG II and T2D, carriers of the C allele (n=46)	p
SBP, mm Hg	159.80±1.11	141.60±2.05	173.96±2.32	166.48±1.80	$p_{2-1}^*$ ; $p_{3-1}^\#$ ; $p_{4-2}^\#$ ; $p_{4-3}^*$
DBP, mm Hg	91.25±1.39	84.60±1.06	103.25±1.37	100.46±0.79	$p_{2-1}^*$ ; $p_{3-1}^*$ ; $p_{4-2}^\#$ ; $p_{4-3}^*$
Pulse pressure, mm Hg	68.55±1.23	57.00±1.99	70.71±1.15	66.02±1.29	$p_{2-1}^*$ ; $p_{3-1}^*$ ; $p_{4-2}^\#$ ; $p_{4-3}^*$
Heart rate, for 1 min.	78.16±0.12	74.37±1.43	83.00±2.74	78.43±1.82	$p_{3-1}^*$ ; $p_{4-3}^*$
Left ventricular posterior wall thickness, cm	1.32±0.02	1.18±0.02	1.36±0.04	1.17±0.03	$p_{2-1}^*$ ; $p_{4-3}^*$
Interventricular septal wall thickness, cm	1.36±0.03	1.22±0.02	1.33±0.03	1.26±0.04	$p_{2-1}^*$ ; $p_{4-2}^\#$
Relative wall thickness, c.u.	0.54±0.03	0.51±0.01	0.51±0.03	0.52±0.02	$p_{3-1}^*$ ; $p_{4-2}^*$
LVMI, g/m <sup>2.7</sup>	62.35±2.81	60.89±2.32	71.41±3.06	66.22±1.92	$p_{3-1}^*$ ; $p_{4-2}^\#$ ; $p_{4-3}^*$
End diastolic volume index, mL/m <sup>2</sup>	61.07±1.67	53.20±0.92	56.29±2.65	55.52±1.97	$p_{2-1}^*$ ; $p_{3-1}^*$ ; $p_{4-2}^\#$
End systolic volume index, mL/m <sup>2</sup>	26.28±1.26	21.14±0.80	21.71±1.61	21.36±1.31	$p_{2-1}^*$ ; $p_{4-2}^*$
Emission fraction, %	60.29±1.24	62.06±1.83	58.34±1.38	62.39±1.43	$p_{4-3}^*$
Left atrium, cm	3.82±0.10	3.58±0.08	4.00±0.05	3.80±0.08	$p_{2-1}^*$ ; $p_{4-2}^\#$ ; $p_{4-3}^*$
E/A, c.u.	0.89±0.07	0.83±0.06	1.09±0.13	0.73±0.05	$p_{3-1}^*$ ; $p_{4-2}^*$ ; $p_{4-3}^*$
E/E', c.u.	7.77±0.38	6.25±0.24	10.13±0.50	7.69±0.23	$p_{2-1}^\#$ ; $p_{3-1}^\#$ ; $p_{4-2}^\#$ ; $p_{4-3}^\#$
DT, ms	249.02±3.43	254.07±4.27	194.79±6.37	255.07±5.12	$p_{3-1}^\#$ ; $p_{4-3}^\#$
IVRT, ms	95.35±2.09	91.46±2.14	109.15±2.82	119.11±2.23	$p_{2-1}^*$ ; $p_{3-1}^*$ ; $p_{4-2}^\#$ ; $p_{4-3}^\#$

**Notes:** \* - difference of indicators is statistically significant at ( $p < 0.05$ ); # - difference of indicators is statistically significant at ( $p < 0.01$ ).



**Fig. 2.** Types of LV remodeling in men with EG II in combination with T2D. Carriers of different genotypes of the BNP gene (%). The difference was significant ( $p < 0.05$ ) when compared with # - individuals with eccentric LVH in different study groups.



**Fig. 3.** Distribution of variants of transmitral blood flow in men with EG II in combination with T2D. Carriers of different variants of the BNP gene (%). The difference is significant ( $p < 0.05$ ) when comparing: \* - with persons with DD I degree within one genotype/allele; # - with persons with DD II degree in carriers of other genotypes/alleles of the BNP gene.

individual indicators of systemic and intracardiac hemodynamics. In men with a comorbid course of EG II and T2D, the presence of a correlation between the carrier of polymorphic genotypes of the BNP gene and the level of DBP ( $\tau = -0.53$ ,  $p < 0.05$ ), interventricular septal wall thickness indicators ( $\tau = 0.17$ ,  $p < 0.05$ ), size of left atrium ( $\tau = 0.19$ ,  $p < 0.05$ ), LVMI ( $\tau = 0.18$ ,  $p < 0.05$ ), E/A ( $\tau = -0.42$ ,  $p < 0.05$ ), DT ( $\tau = 0.62$ ,  $p < 0.01$ ), IVRT ( $\tau = 0.41$ ,  $p < 0.01$ ), E/E' ( $\tau = -0.60$ ,  $p < 0.01$ ) was found. The obtained data indicate that the carrier of different variants of the BNP gene is to some extent associated with indicators that characterize systemic hemodynamics, the degree of myocardial remodeling and the severity of LV DD.

Analysis of systemic hemodynamic parameters (Table 1) showed that the level of pulse blood pressure was higher

in T381T homozygotes than in carriers of the 381C allele of the BNP gene:  $70.71 \pm 1.15$  mm Hg against  $66.02 \pm 1.29$  mm Hg ( $p < 0.05$ ). In addition, the mean values of systolic blood pressure (SBP), diastolic blood pressure (DBP) and pulse pressure among carriers of the T381T genotype were higher in the group of comorbid patients ( $p < 0.05$ ,  $p < 0.01$ ).

Among men with EG II and T2D, carriers of the T381T genotype had higher values of left ventricular posterior wall thickness, left atrium, LVMI, and E/A and E/E' indices ( $p < 0.01$ ), while the IVRT was lower than that of C allele carriers. ( $p < 0.01$ ), which may be a sign of more pronounced disorders of the structural and functional state of the myocardium. The emission fraction index in carriers of the T381T genotype was lower than in carriers of the 381C allele of the BNP gene ( $p < 0.05$ ).

When analyzing the distribution of individuals by types of LV remodeling, it was found (Fig. 2) that in the comorbid course of EG II and T2D, regardless of the inheritance of a particular variant of the BNP gene, individuals with concentric LVH ( $p < 0.05$ ) predominated, but among T381T homozygotes found more people with eccentric LVH, which is characterized by a less hemodynamically effective type of remodeling - 33.33% vs. 8.7% in the group of carriers of the C allele ( $p < 0.05$ ).

In the group of comorbid patients DD LV was diagnosed in 90% of people ( $n = 63$ ), while in men with isolated EG II only in 43.55% of people ( $n = 28$ ), although among homozygotes T381T its signs had 100% ( $n = 24$ ), and among carriers of the C allele - 84.78% of patients ( $n = 39$ ).

The next step was to study the presence of associations between the polymorphism of the BNP gene and variants of transmitral blood flow (TBF) among men with a combined course of EG and T2D (Fig. 3).

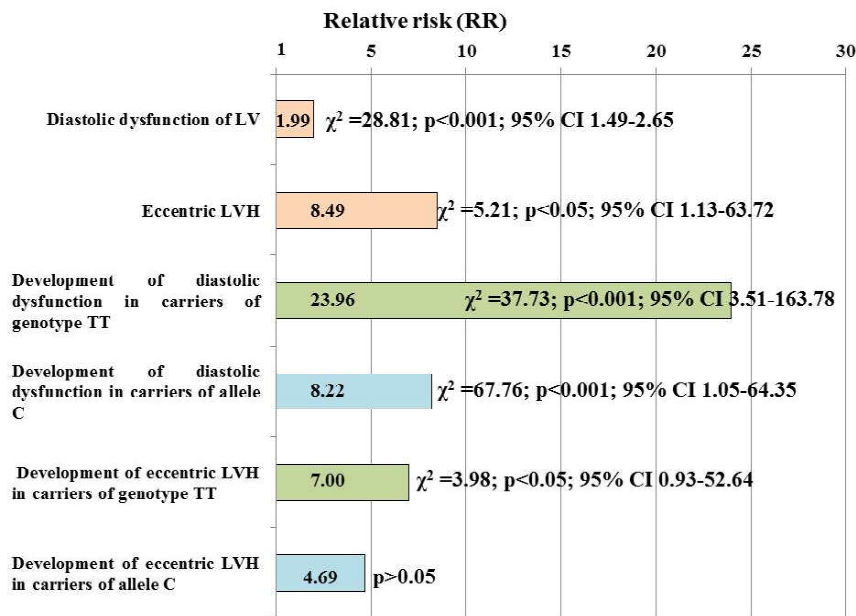
It should be noted that among persons with DD of the II degree carriers of the T381T genotype of the BNP gene prevailed: 41.66% against 10.87% of carriers of the C allele ( $p < 0.05$ ), while among persons with less pronounced disorders of LV diastolic function carriers predominated allele C.

In order to assess the significance of the impact of the studied indicators on the risk of structural and functional changes of the heart in men, residents of Podillya with EG II in combination with T2D relative risk (RR), which also indicates that the carrier of the T381T genotype higher risk of formation of eccentric LVH and LV DD than the carrier of the C allele (Fig. 4).

The obtained results can confirm the presence of associations between the corresponding genetic component - the carrier of the T381T genotype of the BNP gene and the tendency to more pronounced negative structural and functional changes of the myocardium under the conditions of comorbidity of EG and T2D.

Thus, among men living in Podillya, carriers of the T381T genotype of the BNP gene constitute a special cohort with a high risk of myocardial remodeling and an unfavorable course of CHF. Such patients with EG and T2D can be





**Fig. 4.** Relative risk of development of structural and functional changes of the heart on the background of EG II and T2D among men, residents of Podillya (RR=1 - no association. RR>1 - increased risk of pathology. RR<1 - negative association).

assigned to the priority group of observation with the organization of targeted measures aimed at preventing the development of CHF and premature mortality.

The above data suggest that taking into account genotype, in addition to age and sex, when using biomarkers may improve their test characteristics to detect LV dysfunction in men with comorbid pathology and increase the effectiveness of screening among asymptomatic individuals and in expert cases.

### Discussion

For many years, the focus of numerous scientific studies remains the system of natriuretic peptides, one of which is the brain natriuretic peptide. In addition to the classic hemodynamic effects, they have been shown to regulate many physiological functions that control energy metabolism: they can activate lipolysis, lipid oxidation and mitochondrial respiration, darkening of white adipose tissue, protect against obesity, caused by diet, and insulin resistance. These metabolic processes, in turn, are closely linked to the development of cardiovascular disease. Today it is believed that due to the secretion of NP the heart can play a central role in regulating the energy balance [20, 35]. Given the above, the scientific interest in terms of studying the role of BNP in the regulation of cardiohemodynamic and energetic processes is a cohort of patients with mutually aggravating comorbid pathology, namely with EG in combination with T2D. A number of authors note that the reasons for the increase in the level of circulating BNP in patients with T2D, even without concomitant heart failure and clinically significant decrease in renal function, are not

fully understood [30]. Of great interest are the population and sexual characteristics of the inheritance and expression of BNP, which led to the involvement in the study of persons with comorbid pathology only in males and residents of one region of Ukraine.

We did not find a statistically significant difference in the distribution of genotyping and frequency of alleles between groups of patients, which coincides with the results of researchers from Iran in 70 cardiac patients (acute coronary syndrome, etc.) and in the control group of healthy [1]. The authors also indicate that the T-381C polymorphism in the BNP gene affects the plasma level of the biomarker, where the CC genotype and the C allele are associated with its higher levels.

The results of our study of comorbid patients with EG II and T2D with signs of LV DD and early stages

of CHF indicate the presence of associations between carriers of polymorphic variants of the BNP gene and certain parameters of intracardiac and systemic hemodynamics. Based on the obtained data, we can predict that the presence of the T-allele of the T-381C polymorphism of the BNP gene may adversely affect the course of disease and progression of HF.

Similar data were obtained by E.N.Berezikova in the Russian population of patients with coronary heart disease. It is noted that the T allele and the TT genotype of the T-381C polymorphic locus of the BNP gene were associated with a high risk of development, severity and adverse course of CHF, and the C allele and the CC genotype proved to be protective factors [4]. In the South Chinese population, it was also found that the rs198389 polymorphism of the BNP locus may be an additional additive genetic factor influencing the progression of LV dysfunction in patients with coronary heart disease and dyslipidemia [38]. The association of genetic variations in the NP system with cardiovascular effects and T2D in the New Zealand population was studied. Variants of rs198388 and rs198389 of the BNP gene were found to be associated with decreased blood pressure, decreased remodeling, improved LV function, and lower incidence of T2D [14].

Interesting but ambiguous correlations have been obtained in a number of studies on the plasma level of BNP and associations with the carrier of certain genotypes of the corresponding gene in individuals living in different areas. A mixed cohort of North Americans with EG found that inheritance of BNP genotypes with the presence of the 381C allele was associated with high plasma peptide

concentrations [11]. However, a large, prospective EPIC-Norfolk study (USA) in a mixed cohort of individuals found no significant association between rs198389 genotypes and the risk of CHF. The mean follow-up was 12.6 years. The results did not differ significantly in the presence of hypertension, obesity and coronary heart disease. According to the authors, a possible explanation may be that the physiological activation of BNP in conditions of enhanced mechanical deformation of the heart may block small genetically determined differences in biomarker levels. In addition, it is possible that a slight genetic influence on certain subtypes of heart failure syndrome, which is associated with polymorphism of the gene, may not affect the risk of its development as a whole [28].

As the literature data differ markedly, a further study of the determining role of the BNP gene polymorphism involved in the coding of the corresponding biomarker and determining the need to take into account genetic features in the diagnostic process in EG, including comorbid T2D is a promising approach.

Continuation of population studies to establish patterns between the carrier of polymorphic variants of the BNP gene, the plasma level of the biomarker and the structural and functional state of the myocardium in different variants of

comorbid diseases will further provide additional information that may be particularly useful for improving individual diagnosis and diagnosis. appropriate secondary prevention measures.

### Conclusions

1. Carrying of the T381T genotype of the BNP gene, in middle-aged men, residents of Podillya with comorbid EG II and T2D, is associated with a significantly higher level of pulse blood pressure ( $p < 0.01$ ), a higher probability of developing eccentric LVH ( $p \leq 0.05$ ) and with more pronounced diastolic changes in the myocardium, compared with the carrier of the allele C.

2. Among comorbid male patients with EG and T2D, residents of Podillya carriers of the T381T genotype of the BNP gene can be allocated to the priority observation group with the organization of targeted measures aimed at preventing the development of CHF and premature mortality.

3. Taking into account the genotype, in addition to age and sex, the use of biomarkers can improve their test characteristics to detect LV dysfunction in men with comorbid pathology and increase the effectiveness of screening among asymptomatic individuals and in expert cases.

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### ОСОБЛИВОСТІ ГЕМОДИНАМІКИ ЗА КОМОРБІДНОГО ПЕРЕБІГУ ЕСЕНЦІАЛЬНОЇ ГІПЕРТЕНЗІЇ ТА ЦУКРОВОГО ДІАБЕТУ 2 ТИПУ У ЧОЛОВІКІВ, МЕШКАНЦІВ ПОДІЛЛЯ, ПРИ УСПАДКУВАННІ ПОЛІМОРФНИХ ВАРІАНТІВ ГЕНА МОЗКОВОГО НАТРІЙУРЕТИЧНОГО ПЕПТИДУ

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Есенціальна гіпертензія (ЕГ) та цукровий діабет 2 типу (ЦД 2) є визнаними вагомими факторами ризику хронічної серцевої недостатності (ХСН) раннє виявлення якої, особливо за умов коморбідності, залишається складним завданням. Сьогодні для його вирішення поряд з інструментальними методами діагностики (Ехо-КГ), застосовують оцінку плазматичного рівня біомаркера - мозкового натрійуретичного пептиду (МНУП), експресія якого детермінується відповідним геном (локус Т-381С) і може залежати від його структурної організації. Доведено, що порушення регуляції системи натрійуретичних пептидів є вагомим фактором в ініціації, прогресуванні міокардіальної дисфункції та енергетичного дисбалансу, однак роль генетичних передумов цих порушень, в тому числі особливостей носійства поліморфних варіантів найбільш фізіологічно значимого гена МНУП, до цього часу залишається не до кінця зрозумілою. Метою роботи було: дослідити наявність асоціацій між показниками системної і внутрішньосерцевої гемодинаміки та носійством поліморфних варіантів гена МНУП (Т-381С) у чоловіків, мешканців Поділля, за умов коморбідного перебігу ЕГ II та ЦД 2. Обстежили 132 чоловіки середнього віку: 62 особи з ЕГ II і ХСН 0-ІФК за НУНА склали першу, а 70 - з ЕГ II у комбінації з ЦД 2 і ХСН I-ІІФК другу групу порівняння. Параметри внутрішньосерцевої гемодинаміки визначали на основі імпульсної доплер-ЕхоКГ. Геномну ДНК гена МНУП для визначення алелей поліморфної ділянки (Т-381С) виділяли методом ПЛР. Математичне опрацювання виконали, використовуючи стандартний статистичний пакет Statistica 10. Розраховано первинні статистичні показники, виявлено відмінності між групами за статистичними ознаками, здійснено кореляційний та дискримінантний аналіз. Відносний ризик з 95% довірчим інтервалом розраховано за допомогою онлайн-калькулятора (<https://medstatistic.ru/calculators/calcrisk.html>). Встановлено, що серед чоловіків, мешканців Поділля з ЕГ як за наявності, так і за відсутності ЦД 2 домінує генотип Т381С гена МНУП ( $p > 0,05$ ). У групі коморбідних хворих діастолічну дисфункцію лівого шлуночка (ДД ЛШ) діагностовано у 90% осіб ( $n=63$ ), тоді як за ізольованого перебігу ЕГ лише у 43,55% ( $n=28$ ) при тому, що серед гомозигот Т381Т гена МНУП її ознаки мали 100% ( $n=24$ ), а серед носіїв алелю С - 84,78% ( $n=39$ ) пацієнтів. Серед осіб із ДД II ступеня переважали носії генотипу Т381Т гена МНУП: 41,66% проти 10,87% носіїв алелю С ( $p < 0,05$ ), тоді як серед осіб з ДД ЛШ I ступеня було більше носіїв алелю С. Чоловіки, гомозиготи Т381Т гена МНУП з ЕГ II в сполученні з ЦД 2, вирізняються вищим рівнем пульсового АТ ( $p < 0,01$ ), більшою ймовірністю розвитку ексцентричної гіпертрофії ЛШ ( $p \leq 0,05$ ) та більш вираженими діастолічними змінами в міокарді, порівняно з носіями алелю С і можуть бути виділені у пріоритетну групу спостереження для організації цільових заходів, спрямованих на профілактику розвитку і прогресування ХСН.

**Ключові слова:** есенціальна гіпертензія, цукровий діабет 2 типу, поліморфізм гена мозкового натрійуретичного пептиду, діастолічна дисфункція.

### ОСОБЕННОСТИ ГЕМОДИНАМИКИ ПРИ КОМОРБИДНОМ ТЕЧЕНИИ ЭСЕНЦИАЛЬНОЙ ГИПЕРТЕНЗИИ И САХАРНОГО ДИАБЕТА 2 ТИПА У МУЖЧИН, ЖИТЕЛЕЙ ПОДОЛЬЯ, ПРИ НАСЛЕДОВАНИИ ПОЛИМОРФНЫХ ВАРИАНТОВ ГЕНА МОЗГОВОГО НАТРИЙУРЕТИЧЕСКОГО ПЕПТИДА

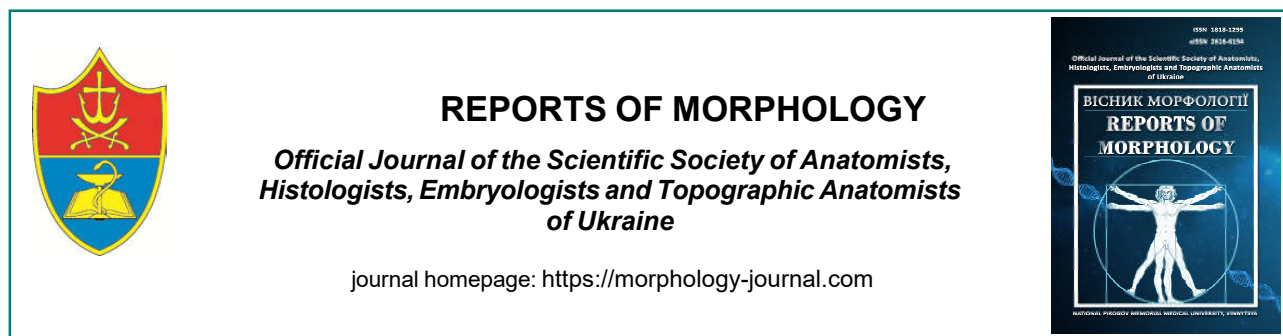
**Антонюк Я.А., Пашкова Ю.П., Гуменюк А.Ф., Сакович Е.А., Жебель В.Н.**

Эссенциальная гипертензия (ЭГ) и сахарный диабет 2 типа (СД 2) являются признанными весомыми факторами риска хронической сердечной недостаточности (ХСН), раннее выявление которой, особенно в условиях коморбидности, остается сложной задачей. Для ее решения в современных условиях, наряду с инструментальными методами диагностики (ЭхоКГ), применяется оценка плазменного уровня биомаркера - мозгового натрийуретического пептида (МНУП), экспрессия которого детерминирована соответствующим геном (локус Т-381С) и может зависеть от его структурной организации. Доказано, что нарушение регуляции системы натрийуретических пептидов является важным фактором в инициации и прогрессировании миокардиальной дисфункции и энергетического дисбаланса, однако роль генетических предпосылок этих нарушений, в том числе особенностей носительства полиморфных вариантов наиболее физиологически значимого гена МНУП до сих пор остается не до конца понятной. Целью работы было: исследовать наличие ассоциаций между показателями системной и внутрисердечной гемодинамики и носительством полиморфных вариантов гена МНУП (Т-381С) у мужчин, жителей Подолья, в условиях коморбидного течения ЭГ II и СД 2. Обследовали 132 мужчин среднего возраста: 62 человека с ЭГ II и ХСН 0-ІФК по НУНА составили первую, а 70 - с ЭГ II в сочетании с СД 2 и ХСН I-ІІФК вторую группу сравнения. Параметры внутрисердечной гемодинамики определяли на основе импульсной доплер-ЭхоКГ. Геномную ДНК гена МНУП для определения аллелей полиморфного участка (Т-381С) выделяли методом ПЦР. Математическую

обработку выполнили, используя стандартный статистический пакет Statistica 10. Рассчитаны первичные статистические показатели, выявлены различия между группами по статистическим признакам, осуществлен корреляционный и дискриминантный анализ. Расчет относительного риска с 95% доверительным интервалом осуществлен с помощью он-лайн калькулятора (<https://medstatistic.ru/calculators/calcrisk.html>). Установлено, что среди мужчин, жителей Подолья с ЭГ как при наличии, так и при отсутствии СД 2 доминирует генотип Т381С гена МНУП ( $p > 0,05$ ). В группе коморбидных больных диастолическая дисфункция левого желудочка (ДД ЛЖ) диагностирована у 90% лиц ( $n=63$ ), тогда как при изолированном течении ЭГ только у 43,55% ( $n=28$ ) при том, что среди гомозигот Т-гена МНУП ее признаки имели 100% ( $n=24$ ), а среди носителей аллели С - 84,78% ( $n=39$ ) пациентов. Среди лиц с ДД II степени преобладали носители генотипа Т381Т гена МНУП: 41,66% против 10,87% носителей аллели С ( $p < 0,05$ ), тогда как среди лиц с ДД ЛЖ I степени было больше носителей аллели С. Мужчины, гомозиготы Т381Т гена МНУП с ЭГ II в сочетании с СД 2, отличаются высоким уровнем пульсового АД ( $p < 0,01$ ), большей вероятностью развития эксцентрической гипертрофии ЛЖ ( $p \leq 0,05$ ) и более выраженными диастолическими изменениями в миокарде по сравнению с носителями аллели С и могут быть выделены в приоритетную группу наблюдения для организации целевых мероприятий, направленных на профилактику развития и прогрессирования ХСН.

**Ключевые слова:** эссенциальная гипертензия, сахарный диабет 2 типа, полиморфизм гена мозгового натрийуретического пептида, диастолическая дисфункция.

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# Features of the frequency of angio-, retino- and neuropathy, general clinical and anthropometric parameters in patients with type 1 diabetes with different levels of albumin in the urine depending on the level of cystatin C

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*Cystatin C improves the risk stratification among people with diabetic nephropathy, the risk of mortality, cardiovascular disease, disorders of the visual organ and nervous system in the preclinical and early stages. The aim of the study was to study the frequency of angio-, retino- and neuropathy, as well as differences in general clinical and anthropometric parameters in patients with type 1 diabetes (T1D) with different levels of albumin in the urine depending on cystatin C. The sample was 78 men and 62 women aged 22-26 years, patients with T1D, who were hospitalized in the therapeutic department №1 and №2 of the Vinnytsia Regional Highly Specialized Endocrinology Center. The control group consisted of 8 healthy men and 13 healthy women of the same age. The level of microalbuminuria and cystatin C was determined for all patients by enzyme-linked immunosorbent assay. The frequency of angio-, retino- and neuropathy, general clinical (systolic, diastolic, pulse) and anthropometric (height, weight, body surface area, waist circumference, body mass index) was assessed indicators. Statistical processing of the obtained results was performed in the license package "Statistica 5.5", using non-parametric evaluation methods. It was found that in the group of men with cystatin C<0.9, and in women with cystatin C>0.9 with increasing albuminuria, the percentage of patients with more severe microvascular complications of T1D. In patients with diabetes mellitus 1 compared with the control group systolic blood pressure is significantly higher and increases with increasing albumin levels in the urine (with cystatin C<0.9 - in men with normo-, microalbuminuria and proteinuria by 7.14%, 8.1% and 10.8%; in women with normo-, microalbuminuria by 7.5% and 10.0%, with cystatin C>0.9 - in men with normo-, microalbuminuria by 4.9% and 7.2%, in women with proteinuria by 19.5%). Similar changes were found for diastolic blood pressure (with cystatin C<0.9 - in men with proteinuria by 13.0%; in women with normo-, microalbuminuria by 11.4% and 13.4%; with cystatin C>0.9 - in men with microalbuminuria by 9.0%; in women with normo- and proteinuria by 9.5% and 21.5%) and heart rate (with cystatin C<0.9 - in men with microalbuminuria and proteinuria by 18.4% and 12.6%, in women with microalbuminuria by 9.13%; with cystatin C>0.9 - in men with microalbuminuria by 12.0%, in women with normo-, microalbuminuria and proteinuria by 10.1%, 16.3% and 25.3%). In patients with T1D compared to the control group, the length of the body is significantly smaller and decreases with increasing levels of albumin in the urine (with cystatin C<0.9 - in men with normo-, microalbuminuria and proteinuria by 3.6%, 6.7% and 9.0%; women with microalbuminuria by 1.9%, with cystatin C>0.9 - in men with normo-, microalbuminuria by 5.2% and 7.3%, in women with normoalbuminuria and proteinuria by 2.6% and 4.3%). Similar changes were found in men for body weight (with cystatin C<0.9 - with microalbuminuria and proteinuria by 13.6% and 30.1%; with cystatin C>0.9 - with normo- and microalbuminuria by 10.2% and 25.4%) and body surface area (with cystatin C<0.9 - with normo-, microalbuminuria and proteinuria by 5.8%, 10.8% and 18.9%; with cystatin C>0.9 - with normo- and*

*microalbuminuria by 8.2% and 16.2%). The size of the waist circumference in patients with T1D with normoalbuminuria is significantly higher, and in patients of other groups significantly less than in healthy subjects (with cystatin C<0.9 - with normo-, microalbuminuria and proteinuria by 3.8%, 1.2% and 5.2%; cystatin C>0.9 - with microalbuminuria by 5.1%). Compared to healthy women, the waist circumference was significantly higher in sick women (with cystatin C<0.9 - with normo- and microalbuminuria by 11.2% and 10.7%; with cystatin C>0.9 - with normo- and proteinuria by 9.7% and 6.0%). In patients with T1D men with proteinuria compared with the control group, the value of the body mass index was significantly lower by 9.7% (cystatin C<0.9). The value of the body mass index was significantly higher in patients with normoalbuminuria and microalbuminuria - by 11.8% and 17.7% (cystatin C<0.9), respectively, and in patients with proteinuria by 7.2% (cystatin C>0.9) compared with healthy women. Thus, between healthy and patients with T1D with varying degrees of albuminuria, differences in general clinical and anthropometric parameters were found, and they are greater the higher the level of cystatin C.*

**Keywords:** *type 1 diabetes, cystatin C level, urinary albumin level, angio-, retino- and neuropathy, general clinical indicators, anthropometric indicators.*

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## Introduction

Today, type 1 diabetes (T1D) occupies one of the first places in terms of severity of its complications and mortality, and the scale of the prevalence of this pathology and its complications is becoming threatening [7].

The study of the relationship between anthropo-somatotypological features of man and the course of disease is undoubtedly an important step in the development of modern medicine. There are numerous data in the scientific and literature on the course of diseases of the respiratory system, cardiovascular and nervous system, gastrointestinal tract, etc., depending on the characteristics of anthropo-somatotypological indicators [21]. However, there is virtually no such work on the course of nephropathy, angiopathy, retinopathy and neuropathy in T1D. Identification of these patterns is a valuable contribution to the early diagnosis of these complications of T1D [13].

Currently, preclinical markers of structural and functional changes in the kidneys, retina, blood vessels, nervous tissue, myocardium, and others are being actively searched for. As such markers, along with traditional methods of early biochemical diagnosis, the definition of cystatin C is proposed. Cystatin C is a low molecular weight protein with a molecular weight of 13.4 kDa, belongs to the family of cysteine proteinase inhibitors. An increase in serum cystatin C is observed with a decrease in renal filtration function, and an increase in its urinary excretion indicates dysfunction of proximal tubular cells [11].

Serum cystatin C, an alternative marker of renal function, both alone and in combination with serum creatinine and/or albuminuria, has recently been shown to be a better predictor of adverse effects compared to serum creatinine [15]. This protein is found in almost all human tissues and body fluids, which, unlike creatinine, is less susceptible to age, race or muscle mass. Studies have shown that elevated levels of cystatin C are associated with the severity of retino-, angio- and neuropathy and are an independent risk factor for these complications along with the duration

of diabetes and glycosylated hemoglobin levels [4].

The patterns described above were obtained mainly in the study of foreign samples. If confirmed in the domestic population, such an approach may change clinical practice and serve as a basis for the inclusion of cystatin C as a marker to improve the stratification of the risk of neuropathy, angiopathy and retinopathy in people with diabetic nephropathy. This group of high-risk individuals can benefit from more careful monitoring and more timely intervention before irreversible and threatening complications occur.

*The aim* of the study was to study the frequency of angio-, retino- and neuropathy, as well as the differences in general clinical and anthropometric parameters in patients with T1D with different levels of albumin in the urine depending on the level of cystatin C.

## Materials and methods

The sample consisted of 78 men and 62 women aged 22-26 years, patients with T1D, who underwent inpatient treatment in the therapeutic departments №1 and №2 of the Vinnytsia Regional Highly Specialized Endocrinology Center. The control group consisted of 8 practically healthy men and 13 practically healthy women of the same age.

All patients underwent the procedure of determining the level of microalbuminuria and cystatin C by enzyme-linked immunosorbent assay using spectrophotometry (reagents from ORGenTec, Germany). Regulatory values of microalbumin in the set of reagents used - 0-25 µg/ml.

Diagnosis of vascular lesions of the lower extremities was performed by determining the pulsation of the vessels of the lower extremities and the patient's subjective complaints of intermittent claudication, pain in the legs while walking, feeling cold in feet.

The condition of the vessels of the fundus was assessed by an ophthalmologist after medical dilation of the pupils using direct ophthalmoscopy. In order to diagnose diabetic retinopathy, the classification of E. Kohner, M. Porte (1992) was used: Stage I - non-



proliferative, Stage II - preproliferative, Stage III - proliferative.

Diabetic neuropathy was diagnosed by a neurologist in the presence of characteristic subjective sensations in patients, as well as based on the signs of impaired sensitivity or reduction/loss of tendon reflexes detected as a result of objective neurological examination.

Blood pressure was measured on the arteria brachialis according to the method of Korotkov (1905), according to the recommendations of EHS experts (2007). ECG recording was performed according to the generally accepted method in 12 standard leads using a diagnostic automated system.

Anthropometric research included the determination of length, weight, body surface area, waist circumference and calculation of body mass index (the ratio of body weight in kilograms to height in meters squared). Body length was determined using a height meter (accuracy - up to 0.01 m) when the shoes were removed. Body weight was measured using medical scales (accuracy - up to 0.5 kg) without outer clothing in the morning. Waist circumference was measured with a centimeter tape.

Statistical data processing was performed in the license package "Statistica 5.5" using non-parametric methods of evaluation of the obtained results.

## Results

Among patients with normoalbuminuria and cystatin C level  $>0.9$ , a higher ( $p=0.059$ ) percentage of men without angiopathy and a lower ( $p=0.075$ ) percentage with grade 3 angiopathy were found compared to women (37.5% and 7.7% and 12.5% and 38.5% respectively).

When comparing the frequency of absence or presence of angiopathy between men with different albumin levels, in whom the level of cystatin C was  $<0.9$ , a lower ( $p<0.05$ ) percentage with grade 3 angiopathy in the group with normoalbuminuria compared with proteinuria (12.5% and 50.0%, respectively) was found.

When comparing the frequency of absence or presence of angiopathy between men or women with different albumin levels, in which the level of cystatin C was  $>0.9$ , found: in men - a higher ( $p=0.056$ ) percentage with no angiopathy in the group with normoalbuminuria compared with microalbuminuria (respectively 37.5% and 0%); lower ( $p=0.054$ ) percentage with grade 3 angiopathy in the group with normoalbuminuria compared with microalbuminuria (12.5% and 38.9%, respectively); in women - a higher ( $p=0.065$ ) percentage with grade 2 angiopathy in the group with microalbuminuria compared with proteinuria (75.0% and 28.6%, respectively); lower ( $p<0.05$ ) percentage with grade 3 angiopathy in the group with microalbuminuria compared with proteinuria (16.7% and 71.4%, respectively).

Among patients with microalbuminuria and cystatin C  $<0.9$ , a higher ( $p=0.085$ ) percentage of men with grade 3 retinopathy was found compared to women of the same group (71.4% and 27.3%, respectively). Among patients with normoalbuminuria and cystatin C  $>0.9$ , a lower ( $p=0.086$ )

percentage of men with grade 3 retinopathy was found compared to women (4.2% and 23.1%, respectively).

Among men with microalbuminuria and retinopathy of the 3rd degree, a higher ( $p=0.058$ ) percentage of patients with cystatin C level  $<0.9$  was found in comparison with patients with cystatin C level  $>0.9$  (71.4% and 27.8%, respectively). Among women with normoalbuminuria and grade 3 retinopathy, a lower ( $p<0.05$ ) percentage of patients with cystatin C  $<0.9$  was found compared to patients with cystatin C  $>0.9$  (0% and 23.1%, respectively). Among women with microalbuminuria and without retinopathy, a lower ( $p=0.062$ ) percentage of patients with cystatin C level  $<0.9$  was found compared to patients with cystatin C level  $>0.9$  (18.2% and 58.3%, respectively).

When comparing the frequency of absence or presence of retinopathy between men or women with different albumin levels, in which the level of cystatin C was  $<0.9$ , it was found: in men - a higher ( $p<0.01-0.001$ ) percentage without retinopathy in the group with normoalbuminuria compared with microalbuminuria and protein (respectively 75.0%, 0% and 0%); a higher ( $p=0.092$ ) percentage with grade 1 retinopathy in the group with microalbuminuria compared with proteinuria (28.6% and 0%, respectively); higher ( $p<0.001$  in both cases) percentage with grade 2 retinopathy in the group with proteinuria compared with normoalbuminuria and microalbuminuria (100%, 0% and 0%, respectively); higher ( $p<0.01$  in both cases) percentage with grade 3 retinopathy in the group with microalbuminuria compared with normoalbuminuria and proteinuria (71.4%, 12.5% and 0%, respectively); in women - a higher ( $p<0.05$ ) percentage without retinopathy in the group with normoalbuminuria compared with microalbuminuria (61.1% and 18.2%, respectively); a higher ( $p<0.05$ ) percentage with grade 3 retinopathy in the group with microalbuminuria compared with normoalbuminuria (27.3% and 0%, respectively).

When comparing the frequency of absence or presence of retinopathy between men or women with different albumin levels, in which the level of cystatin C was  $>0.9$ , it was found: in men - a higher ( $p<0.05$ ) percentage without retinopathy in the group with normoalbuminuria compared with microalbuminuria (66.7% and 27.8%); higher ( $p<0.05$ ) percentage with grade 3 retinopathy in the group with microalbuminuria compared with normoalbuminuria (27.8% and 4.2%, respectively); in women - a higher ( $p=0.078$ ) percentage without retinopathy in the group with microalbuminuria compared with proteinuria (58.3% and 14.3%, respectively); higher ( $p<0.001$ ) percentage with grade 2 retinopathy in the group with proteinuria compared with normoalbuminuria and microalbuminuria (85.7%, 0% and 0%, respectively).

When comparing the frequency of absence or presence of neuropathy between men or women with different albumin levels, in which the level of cystatin C was  $<0.9$ , men found a higher ( $p<0.001$  in both cases) percentage with grade 2 neuropathy in the group with normoalbuminuria and



microalbuminuria compared with proteinuria (75.0%, 100%, and 0%, respectively) and a lower ( $p<0.001$  in both cases) percentage with grade 3 neuropathy in the group with normoalbuminuria and microalbuminuria compared with proteinuria (0%, 0%, and 90.0%, respectively).

When comparing the frequency of absence or presence of neuropathy between men or women with different albumin levels in which the level of cystatin C was  $>0.9$ , it was found: in men - a lower ( $p<0.05$ ) percentage with grade 2 neuropathy in the group with normoalbuminuria compared with microalbuminuria (respectively 58.3% and 88.9%); in women - a higher ( $p<0.01$  and  $p<0.001$ ) percentage with grade 2 neuropathy in the group with normoalbuminuria and microalbuminuria compared with proteinuria (76.9%, 91.7% and 14.3%, respectively); lower ( $p<0.001$ ) percentage with grade 3 neuropathy in the group with normoalbuminuria and microalbuminuria compared with proteinuria (0%, 0% and 71.4%, respectively).

Patients with microalbuminuria and cystatin C level  $>0.9$  had a higher ( $p<0.05$ ) value of this indicator compared with women of the same comparison group ( $119.2\pm 13.0$  and  $109.6\pm 13.2$ , respectively).

The average value of systolic blood pressure in healthy men ( $110.6\pm 5.6$ ) was lower ( $p<0.05$ ;  $p<0.05$  and  $p=0.062$ ) compared with sick men with normo- ( $119.1\pm 9.9$ ), microalbuminuria ( $120.3\pm 12.7$ ) and proteinuria ( $124.0\pm 16.5$ ), in which the level of cystatin C was  $<0.9$ . Healthy women had lower ( $p<0.05$  in both cases) mean systolic blood pressure ( $110.4\pm 9.7$ ) compared with sick women with normoalbuminuria ( $119.4\pm 11.0$ ) and microalbuminuria ( $122.7\pm 12.9$ ), in whom the level of cystatin C was  $<0.9$ .

The average value of systolic blood pressure in healthy men ( $110.6\pm 5.6$ ) was lower ( $p<0.05$  and  $p<0.01$ ) in comparison with sick men with normo- ( $116.3\pm 7.8$ ) and microalbuminuria ( $119.2\pm 13.0$ ), in whom the level of cystatin C was  $>0.9$ . Healthy women had a lower ( $p<0.001$ ) mean systolic blood pressure ( $110.4\pm 9.7$ ) compared to sick women with proteinuria ( $137.1\pm 7.0$ ), in whom the level of cystatin C was  $>0.9$ .

In patients with proteinuria in whom the level of cystatin C was  $>0.9$ , found a higher ( $p<0.01$  and  $p<0.001$ ) average systolic blood pressure ( $137.1\pm 7.0$ ) compared with patients with normoalbuminuria ( $113.5\pm 11.1$ ) and microalbuminuria ( $109.6\pm 13.2$ ).

Women with microalbuminuria with cystatin C  $<0.9$  had a lower ( $p<0.05$ ) mean systolic blood pressure compared to women with microalbuminuria with cystatin C level  $>0.9$  ( $122.7\pm 12.9$  and  $137.1\pm 7.0$ , respectively).

Patients with microalbuminuria and cystatin C level  $>0.9$  had a higher ( $p<0.05$ ) value of this indicator compared to women of the same comparison group ( $75.56\pm 8.20$  and  $68.33\pm 8.35$ , respectively).

There was a lower ( $p<0.01$ ) mean value of diastolic blood pressure in healthy men ( $68.75\pm 5.60$ ) compared with sick men with proteinuria ( $79.00\pm 6.99$ ), in whom the level of cystatin C was  $<0.9$ . Patients with proteinuria with cystatin

C  $<0.9$  had a higher ( $p<0.05$ ) mean diastolic blood pressure ( $79.00\pm 6.99$ ) compared with patients with normoalbuminuria ( $70.63\pm 8.14$ ).

Healthy women had lower ( $p<0.01$  in both cases) mean diastolic blood pressure ( $66.15\pm 5.83$ ) compared to sick women with normoalbuminuria ( $74.44\pm 8.38$ ) and microalbuminuria ( $76.36\pm 7.10$ ), in whom the level of cystatin C was  $<0.9$ .

The mean value of diastolic blood pressure in healthy men ( $68.75\pm 3.54$ ) was lower ( $p<0.01$ ) compared with sick men with normo- ( $71.67\pm 6.20$ ) and microalbuminuria ( $75.56\pm 8.20$ ), in whom the level of cystatin C was  $>0.9$ . Patients with microalbuminuria with cystatin C  $>0.9$  had a higher ( $p<0.05$ ) mean diastolic blood pressure ( $75.56\pm 8.20$ ) compared with patients with normoalbuminuria ( $71.67\pm 6.20$ ). In healthy women, the average value of diastolic blood pressure ( $66.15\pm 5.83$ ) was lower ( $p<0.05$  and  $p<0.001$ ) compared with sick women with normoalbuminuria ( $73.08\pm 6.93$ ) and proteinuria ( $84.29\pm 5.35$ ), in whom the level of cystatin C was  $>0.9$ . Patients with proteinuria with cystatin C  $>0.9$  had a higher ( $p<0.01$  in both cases) mean diastolic blood pressure value ( $84.29\pm 5.35$ ) compared with patients with normoalbuminuria ( $73.08\pm 6.93$ ) and microalbuminuria ( $68.33\pm 8.35$ ).

Women with microalbuminuria with cystatin C  $<0.9$  had a lower ( $p<0.05$ ) mean diastolic blood pressure value compared to women with microalbuminuria with cystatin C  $>0.9$  ( $76.36\pm 7.10$  and  $84.29\pm 5.35$ , respectively).

Patients with microalbuminuria and cystatin C  $<0.9$  had a higher ( $p=0.070$ ) heart rate compared to women in the same comparison group ( $88.86\pm 8.20$  and  $78.73\pm 11.00$ , respectively).

There was a lower ( $p<0.01$ ) mean heart rate in healthy men ( $72.50\pm 5.73$ ) compared with sick men with microalbuminuria ( $88.86\pm 9.92$ ) and proteinuria ( $83.0\pm 8.81$ ), in whom the level of cystatin C was  $<0.9$ . Patients with microalbuminuria with cystatin C  $>0.9$  had a higher ( $p<0.05$ ) mean heart rate ( $88.86\pm 9.92$ ) compared with patients with normoalbuminuria ( $76.31\pm 11.32$ ).

Healthy women had a lower ( $p=0.052$ ) mean heart rate ( $71.54\pm 5.50$ ) compared to sick women with microalbuminuria ( $78.73\pm 11.00$ ), in whom the level of cystatin C was  $<0.9$ .

There was a lower ( $p<0.05$ ) mean heart rate in healthy men ( $72.50\pm 5.73$ ) compared with sick men with microalbuminuria ( $82.39\pm 12.10$ ), in whom the level of cystatin C was  $>0.9$ . Patients with microalbuminuria with cystatin C  $>0.9$  had a higher ( $p<0.05$ ) mean heart rate ( $82.39\pm 12.10$ ) compared with patients with normoalbuminuria ( $75.00\pm 9.29$ ). Healthy women had lower ( $p=0.065$ ,  $p<0.01$  and  $p<0.001$ ) mean heart rate ( $71.54\pm 5.50$ ) compared to sick women with normoalbuminuria ( $79.54\pm 10.90$ ), microalbuminuria ( $85.42\pm 13.57$ ) and proteinuria ( $95.71\pm 4.96$ ), in which the level of cystatin C was  $>0.9$ . In patients with proteinuria, in whom the level of cystatin C  $>0.9$ , found a higher ( $p<0.01$  and  $p=0.091$ ) mean heart rate ( $95.71\pm 4.96$ ) compared with

patients with normoalbuminuria ( $79.54 \pm 10.90$ ) and microalbuminuria ( $85.42 \pm 13.57$ ).

Men with microalbuminuria with cystatin C level  $<0.9$  had a higher ( $p < 0.05$ ) mean heart rate value compared with men with microalbuminuria with cystatin C  $>0.9$  ( $88.86 \pm 9.92$  and  $82.39 \pm 12.10$ , respectively).

Healthy men had higher ( $p < 0.001$ ) body length values compared to healthy women ( $183.5 \pm 3.0$  and  $165.7 \pm 3.5$ , respectively). A higher body length was found in patients with normoalbuminuria ( $177.2 \pm 7.1$ ;  $p < 0.001$ ) and microalbuminuria ( $172.0 \pm 11.4$ ;  $p = 0.085$ ) with cystatin C  $<0.9$  compared to women in the same comparison group ( $162.7 \pm 5.5$  and  $162.5 \pm 6.1$ , respectively). A greater value of body length was observed in patients with normoalbuminuria ( $174.4 \pm 6.0$ ;  $p < 0.001$ ) and microalbuminuria ( $171.1 \pm 6.1$ ;  $p < 0.001$ ) with cystatin C level  $>0.9$  compared to women of the same comparison group ( $161.5 \pm 6.1$  and  $160.8 \pm 7.9$ , respectively).

Healthy men had a higher ( $p < 0.05$ - $0.01$ ) mean body length ( $183.5 \pm 3.0$ ) compared with sick men with normoalbuminuria ( $177.2 \pm 7.1$ ), microalbuminuria ( $172.0 \pm 11.4$ ) and proteinuria ( $168.3 \pm 9.6$ ), in whom the level of cystatin C was  $<0.9$ . Patients men with proteinuria in whom the level of cystatin C was  $<0.9$  had a lower ( $p < 0.05$ ) mean body length ( $168.3 \pm 9.6$ ) compared with patients with normoalbuminuria ( $177.2 \pm 7.1$ ). Healthy women had a higher ( $p = 0.056$ ) mean body length ( $165.7 \pm 3.5$ ) compared with sick women with microalbuminuria ( $162.5 \pm 6.1$ ), in whom the level of cystatin C was  $<0.9$ .

Healthy men had a higher ( $p < 0.001$  in both cases) mean body length ( $183.5 \pm 3.0$ ) compared with sick men with normoalbuminuria ( $174.4 \pm 6.0$ ) and microalbuminuria ( $171.0 \pm 6.1$ ), in whom the level of cystatin C was  $>0.9$ . Patients with microalbuminuria with cystatin C  $>0.9$  had a lower ( $p < 0.05$ ) mean body length ( $171.0 \pm 6.1$ ) compared with patients with normoalbuminuria ( $174.4 \pm 6.0$ ). Healthy women had higher ( $p = 0.055$  and  $p < 0.05$ ) mean body length ( $165.7 \pm 3.5$ ) compared with sick women with normoalbuminuria ( $160.8 \pm 7.9$ ) and proteinuria ( $158.9 \pm 7.6$ ), in whom the level of cystatin C was  $<0.9$ .

Healthy men had higher ( $p < 0.001$ ) body weight values compared to healthy women ( $83.13 \pm 4.05$  and  $60.46 \pm 3.18$ , respectively). A higher body weight was found in patients with normoalbuminuria ( $77.41 \pm 9.99$ ;  $p < 0.05$ ) with cystatin C level  $<0.9$  compared with women in the same comparison group ( $66.75 \pm 12.85$ , respectively). A higher value of this indicator was observed in patients with normoalbuminuria ( $75.46 \pm 10.46$ ;  $p < 0.001$ ) and microalbuminuria ( $66.31 \pm 12.59$ ;  $p = 0.069$ ) with cystatin C  $>0.9$  compared to women of the same comparison group ( $60.23 \pm 9.75$  and  $57.75 \pm 10.23$ , respectively).

Healthy men had higher ( $p < 0.05$  and  $p < 0.001$ ) mean body weight ( $83.13 \pm 4.05$ ) compared with sick men with microalbuminuria ( $73.21 \pm 15.67$ ) and proteinuria ( $63.90 \pm 7.37$ ), in whom the level of cystatin C was  $<0.9$ . In patients with proteinuria, in whom the level of cystatin C

was  $<0.9$ , found a lower ( $p < 0.01$ ) average value of this indicator ( $63.90 \pm 7.37$ ) compared with patients with normoalbuminuria ( $77.41 \pm 9.99$ ).

Healthy men had a higher ( $p < 0.05$  and  $p < 0.01$ ) mean body weight ( $83.13 \pm 4.05$ ) compared with sick men with normoalbuminuria ( $75.46 \pm 10.46$ ) and microalbuminuria ( $66.31 \pm 12.59$ ), in whom the level of cystatin C was  $>0.9$ . Patients with microalbuminuria with cystatin C level  $>0.9$  had a lower ( $p < 0.05$ ) mean value ( $66.31 \pm 12.59$ ) compared with patients with normoalbuminuria ( $75.46 \pm 10.46$ ).

Women with microalbuminuria with cystatin C level  $<0.9$  had a higher ( $p < 0.05$ ) mean body weight compared with women with microalbuminuria with cystatin C level  $>0.9$  ( $71.18 \pm 16.49$  and  $57.75 \pm 10.23$ , respectively).

Healthy men had higher ( $p < 0.001$ ) body surface area compared to healthy women ( $2.056 \pm 0.048$  and  $1.671 \pm 0.057$ , respectively). A larger value of body surface area was found in patients with normoalbuminuria ( $1.944 \pm 0.137$ ;  $p < 0.001$ ) with the level of cystatin C  $<0.9$  compared with women of the same comparison group (respectively  $1.712 \pm 0.162$ ). A higher value of this indicator was observed in patients with normoalbuminuria ( $1.900 \pm 0.148$ ;  $p < 0.001$ ) and microalbuminuria ( $1.769 \pm 0.164$ ;  $p < 0.01$ ) with cystatin C  $>0.9$  compared to women in the same comparison group (respectively  $1.632 \pm 0.120$  and  $1.598 \pm 0.159$ ).

In healthy men, higher ( $p = 0.054$ ,  $p < 0.05$  and  $p < 0.001$ ) average body surface area ( $2.056 \pm 0.048$ ) was found compared with sick men with normoalbuminuria ( $1.944 \pm 0.137$ ), microalbuminuria ( $1.856 \pm 0.238$ ) and proteinuria ( $1.729 \pm 0.147$ ), in which the level of cystatin C was  $<0.9$ . Patients with proteinuria in whom the level of cystatin C was  $<0.9$ , had a lower ( $p < 0.01$ ) average value of this indicator ( $1.729 \pm 0.147$ ) compared with patients with normoalbuminuria ( $1.944 \pm 0.137$ ).

In healthy men, a higher ( $p < 0.01$  and  $p < 0.001$ ) mean body surface area ( $2.056 \pm 0.048$ ) was found compared with sick men with normoalbuminuria ( $1.900 \pm 0.148$ ) and microalbuminuria ( $1.769 \pm 0.164$ ), in whom the level of cystatin C was  $>0.9$ . Patients with microalbuminuria with cystatin C  $>0.9$  had a lower ( $p < 0.05$ ) mean value ( $1.769 \pm 0.164$ ) compared with patients with normoalbuminuria ( $1.900 \pm 0.148$ ). In healthy women, a higher ( $p = 0.096$ ) mean value of body surface area ( $1.671 \pm 0.057$ ) was found in comparison with sick women with normoalbuminuria ( $1.632 \pm 0.120$ ), in whom the level of cystatin C was  $>0.9$ .

In women with microalbuminuria, in whom the level of cystatin C was  $<0.9$ , the average value was higher ( $p = 0.062$ ) compared with women with microalbuminuria, in whom the level of cystatin C was  $>0.9$  (respectively  $1.758 \pm 0.209$  and  $1.598 \pm 0.159$ ).

Healthy men had higher ( $p < 0.001$ ) waist circumference values compared to healthy women ( $78.25 \pm 1.39$  and  $67.69 \pm 3.04$ , respectively). A higher waist circumference was found in patients with normoalbuminuria ( $81.31 \pm 5.06$ ;  $p < 0.05$ ) with cystatin C  $<0.9$  compared to women in the

same comparison group (76.22±8.48, respectively). A higher value of this indicator was observed in patients with men with microalbuminuria (74.44±6.23;  $p<0.05$ ) with a level of cystatin C>0.9 compared with women in the same comparison group (respectively 70.42±4.96).

In healthy men, the mean value of waist circumference (78.25±1.39) was lower ( $p=0.062$ ) compared with sick men with normoalbuminuria (81.31±5.06) and higher ( $p=0.064$  and  $p=0.051$ ) compared with microalbuminuria (77.29±10.45) and proteinuria (74.40±4.35), in which the level of cystatin C was <0.9. In patients with proteinuria, in whom the level of cystatin C was <0.9, found a lower ( $p<0.05$  and  $p<0.01$ ) average value of this indicator (74.40±4.35) compared with patients with normoalbuminuria (81.31±5.06) and microalbuminuria (77.29±10.45). Healthy women had a lower ( $p<0.01$  in both cases) average waist circumference (67.69±3.04) compared to sick women with normoalbuminuria (76.22±8.48) and microalbuminuria (75.82±8.05).

In healthy men, a higher ( $p<0.01$ ) average value of waist circumference (78.25±1.39) was found in comparison with sick men with microalbuminuria (74.44±6.23), in whom the level of cystatin C was >0.9. Patients with microalbuminuria with cystatin C>0.9 had a lower ( $p<0.05$ ) mean value (74.44±6.23) compared with patients with normoalbuminuria (79.29±8.24). Healthy women had a lower ( $p<0.05$  in both cases) average waist circumference (67.69±3.04) compared to sick women with normoalbuminuria (74.92±8.34) and proteinuria (72.00±4.40), in whom the level of cystatin C was >0.9.

Women with microalbuminuria with cystatin C<0.9 had a higher ( $p<0.05$ ) average waist circumference compared with women with microalbuminuria with cystatin C>0.9 (75.82±8.05 and 70.42±4.96, respectively).

Healthy men had higher ( $p<0.001$ ) body mass index values compared to healthy women (24.69±1.40 and 22.02±0.96, respectively). A higher value of this indicator was observed in patients with normoalbuminuria (24.60±2.51;  $p=0.095$ ) with a level of cystatin C>0.9 compared with women in the same comparison group (respectively 23.52±3.99).

Healthy men had higher ( $p<0.001$ ) body mass index values compared to healthy women (24.69±1.40 and 22.02±0.96, respectively). A higher value of this indicator was observed in patients with normoalbuminuria (24.60±2.51;  $p=0.095$ ) with a level of cystatin C>0.9 compared with women in the same comparison group (respectively 23.52±3.99).

In healthy men, a higher ( $p<0.05$ ) mean value of the body mass index was found (24.69±1.40) compared with sick men with proteinuria (22.51±1.62), in whom the level of cystatin C<0.9. In patients with proteinuria, in whom the level of cystatin C<0.9, the average value of this indicator was lower ( $p=0.087$ ) (22.51±1.62) compared with patients with normoalbuminuria (24.36±2.76). Healthy women had a lower ( $p<0.05$  in both cases) mean body mass index

(22.02±0.94) compared with sick women with normoalbuminuria (24.96±4.02) and microalbuminuria (26.75±4.93).

Patients men with microalbuminuria with cystatin C>0.9 had a lower ( $p=0.055$ ) mean value (22.56±4.16) compared with patients with normoalbuminuria (24.60±2.51). In healthy women, a lower ( $p<0.05$ ) mean value of the body mass index (22.02±0.94) was found in comparison with sick women with proteinuria (23.73±2.38), in whom the level of cystatin C was >0.9.

In women with microalbuminuria, in whom the level of cystatin C was <0.9, the average value of the body mass index was higher ( $p<0.05$ ) compared to women with microalbuminuria, in whom the level of cystatin C was >0.9 (26.75±4.93 and 22.41±3.07, respectively).

## Discussion

Because macro- and microvascular complications of T1D are better treated in the early stages, the problem of using markers that are more effective than determining urinary albumin excretion is very relevant. Such a marker is thought to be cystatin C. Measurement of serum cystatin C levels may be useful for screening for mild to moderate stages of diabetic angio-, retino-, nephro-, and neuropathy. An increase in its level indicates that the lesions of the target organs are progressing [2].

In a pairwise comparison of groups of men or women who took into account the level of cystatin C, with the corresponding sex groups of subjects, in which this biochemical indicator was not taken into account, found similar patterns and trends [12]. Thus, in the group of men with cystatin C levels <0.9 and in women with cystatin C levels >0.9, the percentage of patients with severe microvascular complications of diabetes increased with increasing albuminuria.

According to scientific studies, an increase in serum cystatin C in the absence of patients with T1D microalbuminuria should be regarded as a predictor of the formation of its complications [19]. Therefore, the focus of our study was on comparing the percentage of patients in groups with different degrees of diabetic complications at a level of less than and greater than 0.9.

There was a significantly lower percentage of patients with cystatin C level <0.9 compared with patients with cystatin C level >0.9 among groups of men with microalbuminuria and retinopathy of the 3rd degree and women with normoalbuminuria and retinopathy of the 3rd degree, with microalbuminuria and without retinopathy. In addition to sex differences, it is noteworthy that only in the case of retinopathy, the percentage of patients "responds" to the gradation of cystatin C. According to medical and epidemiological studies, retinopathy develops (or rather clinically manifested) earlier than complications such as neuro- and angiopathy, its initial manifestations are correlated with the appearance of microalbuminuria [8]. Unfortunately, it was not possible to compare a similar

picture in the groups with proteinuria, as in women with cystatin C<0.9 and men with cystatin C>0.9, the number of observations was only 1 and 3 people, respectively.

Hypertension is an important risk factor for the development of many serious complications of T1D and its deterioration, including diabetic retinopathy, nephropathy, micro- and macroangiopathy [1]. Diabetes mellitus itself is also a factor in the development of arterial hypertension, because in this endocrine pathology there is damage to arteries and, especially, small arteries - arterioles, which contributes to the deposition of atherosclerotic plaques in them [3]. When glycemia decompensates, the body increases the level of constrictor hormones - adrenaline, norepinephrine, thyroxine, cortisol. Elevated adrenergic background is manifested by tachycardia and hypertension [16]. Another explanation is that the parasympathetic nervous system in diabetes is affected earlier than the sympathetic, so the initial sign of autonomic neuropathy may be resting tachycardia [6].

Examination of the general population revealed that cystatin C levels were positively associated with hypertension and pulse. Scientists believe that there are significant differences between filtration in healthy and T1D and believe that serum cystatin C reliably reflects the glomerular filtration rate in these categories of individuals [10].

In our study, in patients with T1D compared with the control group, the value of systolic blood pressure was significantly higher (respectively, in men with normo-, microalbuminuria and proteinuria by 7.14%, 8.1% and 10.8%; in women with normo-, microalbuminuria - 7.5 and 10.0% (cystatin C<0.9); in men with normo-, microalbuminuria - by 4.9% and 7.2%; in women with proteinuria - by 19.5% (cystatin C>0.9) and increases with increasing albumin levels in the urine.

In patients with T1D compared with the control group, the value of diastolic blood pressure is also significantly higher (respectively in men with proteinuria by 13.0%; in women with normo-, microalbuminuria - by 11.4% and 13.4% (cystatin C<0.9); in men with microalbuminuria - by 9.0%; in women with normo- and proteinuria - by 9.5% and 21.5% (cystatin C>0.9) and increases with increasing albumin levels in the urine.

Regarding the pulse, a similar pattern was observed - its value in patients with T1D is significantly higher than in healthy subjects and increases synchronously with an increase in urinary albumin levels (respectively in men with microalbuminuria and proteinuria by 18.4% and 12.6%; in women with microalbuminuria - by 9.13% (cystatin C<0.9); in men with microalbuminuria - by 12.0%; in women with normo-, microalbuminuria and proteinuria by 10.1%, 16.3% and 25.3% (cystatin C>0.9)).

Diastolic blood pressure in men with microalbuminuria was 5.2% higher than in men with normoalbuminuria (cystatin C>0.9). The magnitude of the pulse is also higher in the first group of sick men compared with other (respectively by 14.1% (cystatin C<0.9) and 8.9% (cystatin

C>0.9)).

In women, no comparison of similar differences in blood pressure and heart rate between similar groups was found. At the same time, between groups of women with normo-, microalbuminuria (cystatin C>0.9) such differences are significant. Thus, in women with proteinuria compared with normo-, microalbuminuria systolic pressure values are higher by 17.2% and 20.1%, respectively; diastolic pressure - by 13.3% and 18.9%; heart rate - by 16.9% and 10.6%.

Shulkina S.G. and Smirnova O.M. [19] proved that the value of blood pressure has direct correlations with the level of cystatin C. Thus, with increasing systolic and diastolic pressure, there should be an increase in this biochemical indicator. Thus, in women with microalbuminuria and cystatin C level >0.9 systolic pressure is lower by 12.0%, and diastolic - by 11.8% compared with patients with cystatin C level <0.9. In men with microalbuminuria and cystatin C levels >0.9, the pulse was lower by 7.9% compared with men with cystatin C levels <0.9.

A sufficient amount of information has been accumulated on the relationship between the clinical course and the peculiarities of susceptibility to different types of diseases of persons of different somatotypes. Thus, among asthenics, the most common diseases are varicose veins, gastritis, hypotension, chronic adrenal insufficiency and T1D in particular [14].

A number of scientists in a study of people with T1D, found that they are characterized by lower body length, increased waist circumference and increased accumulation of abdominal fat. In women, low bone and muscle mass were found along with high fat mass. Men in all components had a moderately low weight [5, 17].

According to the results of our study in patients with T1D compared to the control group, the length of the body is significantly less (respectively in men with normo-, microalbuminuria and proteinuria by 3.6%, 6.7% and 9.0%; in women with microalbuminuria - by 1.9% (cystatin C<0.9); in men with normo-, microalbuminuria - by 5.2% and 7.3%; in women with normoalbuminuria and proteinuria - by 2.6% and 4.3% (cystatin C>0.9)) and decreases with increasing levels of albumin in the urine.

Compared with the control group, patients with T1D had significantly lower body weight (13.6% and 30.1%, respectively, in men with microalbuminuria and proteinuria) and 10.2% and 25.4% in men with normo- and microalbuminuria (cystatin C>0.9)) and decreases with increasing albumin levels in the urine.

In patients with T1D compared to the control group, the size of the body surface area is also significantly smaller (respectively in men with normo-, microalbuminuria and proteinuria by 5.8%, 10.8% and 18.9% (cystatin C<0.9); in men with normo- and microalbuminuria - by 8.2% and 16.2% (cystatin C>0.9)) and decreases with increasing albumin levels in the urine.

Regarding waist circumference, the following pattern was observed - its value in patients with T1D and normoalbuminuria was significantly higher, and in patients of other groups significantly lower compared to healthy subjects (respectively, in men with normo-, microalbuminuria and proteinuria by 3.8%, 1.2% and 5.2% (cystatin C<0.9); in men with microalbuminuria - by 5.1% (cystatin C>0.9)). Compared to healthy women, the waist circumference was higher in sick women (11.2% and 10.7%, respectively in women with normo- and microalbuminuria (cystatin C<0.9); 9.7% and 6.0% in women with normo- and proteinuria (cystatin C>0.9)).

In men with T1D and proteinuria compared with the control group, the value of the body mass index was significantly lower by 9.7% (cystatin C<0.9). The value of the body mass index was significantly higher in patients with normoalbuminuria and microalbuminuria - by 11.8% and 17.7% (cystatin C<0.9), respectively, and in patients with proteinuria by 7.2% (cystatin C>0.9) compared with healthy women. In men with microalbuminuria, the value of the body mass index was 9.0% significantly lower than in men with normoalbuminuria (cystatin C>0.9).

Summarizing the above-described regularities concerning the features of anthropometric parameters at different levels of cystatin C, it should be noted that in the case when cystatin C<0.9, significant differences were found only for waist circumference in men with normoalbuminuria and microalbuminuria. When the level of cystatin C is >0.9, in this group of men there are significant differences in all anthropometric parameters.

In early studies, it was concluded that serum cystatin C does not depend on anthropometric parameters [18]. However, then in a number of other studies found a small but significant positive correlation between the level of this biochemical indicator and body mass index, length and surface area of the body [9]. A similar trend was found for the general population [10]. When observing the Japanese sample (596 people aged 30-75 years), it was shown that the level of cystatin C is positively correlated with the amount of adipose tissue. More recently, a positive association between obesity and serum cystatin C levels has also been established [19]. Moreover, there have been reports that a

significantly elevated body mass index may affect serum cystatin C levels [20].

In women with microalbuminuria and cystatin C level <0.9, significantly higher values of the following anthropometric indicators were found in comparison with women in whom cystatin C>0.9: body weight - by 23.2%; body surface area - by 4.9%; waist circumference - by 7.7%; body mass index - by 19.4%.

In our study, unfortunately, it was not possible to conduct a complete analysis of changes in general clinical and anthropometric parameters in patients with albuminuria at different levels of cystatin C, because the distribution in groups with proteinuria did not analyze women with cystatin C<0.9 and men with cystatin C>0.9 (the number of observations in these groups was only 1 and 3, respectively). For this reason, there is a limited explanation of why between patients with microalbuminuria, at different levels of cystatin C there are higher values of general clinical and anthropometric indicators at lower levels, while these indicators in general groups are characterized by diametrically opposite patterns (the value of these indicators increased as the level of albumin in the urine increases) [12].

Thus, the level of cystatin C may predict the risk of complications of diabetes and signal the "preclinical" phase of renal dysfunction. Terms similar to preclinical kidney disease - prehypertension, preneuropathy, preretinopathy, etc.

## Conclusions

1. In the group of subjects in which cystatin C was taken into account, and in the general group (excluding cystatin C) there were common tendencies to increase the degree of retino-, angio- and neuropathy with increasing albumin levels in the urine. However, the percentage of those in the group with a more severe degree of these complications is higher when using this biochemical indicator, which avoids "blind zones" in the early diagnosis of complications of T1D.

2. Between healthy and patients with T1D with varying degrees of albuminuria found differences in general clinical and anthropometric parameters, and they are greater the higher the level of cystatin C.

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#### ОСОБЛИВОСТІ ЧАСТОТИ АНГІО-, РЕТИНО- ТА НЕЙРОПАТІЇ, ЗАГАЛЬНОКЛІНІЧНИХ ТА АНТРОПОМЕТРИЧНИХ ПОКАЗНИКІВ У ХВОРИХ НА ЦУКРОВИЙ ДІАБЕТ 1 ТИПУ З РІЗНИМ РІВНЕМ АЛЬБУМІНУ В СЕЧІ В ЗАЛЕЖНОСТІ ВІД РІВНЯ ЦИСТАТИНУ С

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*Цистатин С поліпшує стратифікацію ризику серед осіб з діабетичною нефропатією щодо ризику смертності, серцево-судинних захворювань, порушень з боку органу зору і нервової системи на доклінічній і початковій стадії. Мета дослідження - вивчити частоту ангіо-, ретино- та нейропатії, а також відмінності загально-клінічних та антропометричних показників у хворих на цукровий діабет 1 типу (ЦД 1) з різним рівнем альбуміну в сечі в залежності від рівня цистатину С. Вибірка складала 78 чоловіків і 62 жінки віком 22-26 років, хворих на ЦД 1, які проходили стаціонарне лікування в терапевтичних відділеннях №1 і №2 Вінницького обласного високоспеціалізованого ендокринологічного центру. Контрольна група складалась з 8 практично здорових чоловіків і 13 практично здорових жінок аналогічного віку. Усім пацієнтам імуноферментним методом визначали рівень мікроальбумінурії та цистатину С. Проводили оцінку частоти ангіо-, ретино- та нейропатії, загально-клінічних (систолический АТ, діастолічний АТ, пульс) та антропометричних (зріст, вага, площа поверхні тіла, обхват талії, індекс маси тіла) показників. Статистична обробка отриманих результатів проведена в ліцензійному пакеті "Statistica 5.5", з використанням непараметричних методів оцінки. Встановлено, що в групі чоловіків рівень цистатину С у яких <0,9, та у жінок з рівнем цистатину С >0,9 при наростанні альбумінурії зростає відсоткова частка пацієнтів з важчим ступенем мікросудинних ускладнень ЦД 1. У хворих на ЦД 1 порівняно з контрольною групою величина систолічного артеріального тиску достовірно більша та збільшується по мірі зростання рівня альбуміну в сечі (при цистатині С <0,9 - у чоловіків з нормо-, мікроальбумінурією та протеїнурією на 7,14%, 8,1% та 10,8%; у жінок з нормо-, мікроальбумінурією на 7,5% та 10,0%; при цистатині С >0,9 - у чоловіків з нормо-, мікроальбумінурією на 4,9% та 7,2%; у жінок з протеїнурією на 19,5%). Аналогічні зміни встановлені для діастолічного артеріального тиску (при цистатині С <0,9 - у чоловіків з протеїнурією на 13,0%; у жінок з нормо-, мікроальбумінурією на 11,4% та 13,4%; при цистатині С >0,9 - у чоловіків з мікроальбумінурією на 9,0%; у жінок з нормо- та протеїнурією на 9,5% та 21,5%) та величини пульсу (при цистатині С <0,9 - у чоловіків з мікроальбумінурією і протеїнурією на 18,4% та 12,6%; у жінок з мікроальбумінурією на 9,13%; при цистатині С >0,9 - у чоловіків з мікроальбумінурією на 12,0%; у жінок з нормо-, мікроальбумінурією та протеїнурією на 10,1%, 16,3% та 25,3%). У хворих на ЦД 1 порівняно із контрольною групою величина довжини тіла достовірно менша та зменшується в міру збільшення рівня альбуміну в сечі (при цистатині С <0,9 - у чоловіків з нормо-, мікроальбумінурією та протеїнурією на 3,6%, 6,7% та 9,0%; у жінок з мікроальбумінурією на 1,9%; при цистатині С >0,9 - у чоловіків з нормо-, мікроальбумінурією на 5,2% та 7,3%; у жінок з нормоальбумінурією та протеїнурією на 2,6% та 4,3%). Аналогічні зміни встановлені у чоловіків для величини маси тіла (при цистатині С <0,9 - з мікроальбумінурією та протеїнурією на 13,6% і 30,1%; при цистатині С >0,9 - з нормо- і мікроальбумінурією на 10,2% і 25,4%) та величини площі поверхні тіла (при цистатині С <0,9 - з нормо-, мікроальбумінурією та протеїнурією на 5,8%, 10,8% і 18,9%; при цистатині С >0,9 - з нормо- і мікроальбумінурією на 8,2% і 16,2%). Величина*

обхвату талії у хворих чоловіків на ЦД 1 з нормоальбумінурією достовірно більша, а у хворих інших груп достовірно менша порівняно із здоровими досліджуваними (при цистатині  $S < 0,9$  - з нормо-, мікроальбумінурією і протеїнурією на 3,8%, 1,2% та 5,2%; при цистатині  $S > 0,9$  - з мікроальбумінурією на 5,1%). У хворих жінок порівняно із здоровими обхват талії достовірно більший (при цистатині  $S < 0,9$  - з нормо- і мікроальбумінурією на 11,2% та 10,7%; при цистатині  $S > 0,9$  - з нормо- та протеїнурією на 9,7% та 6,0%). У хворих на ЦД 1 чоловіків з протеїнурією порівняно з контрольною групою величина індексу маси тіла достовірно менша на 9,7% (цистатин  $S < 0,9$ ). Величина індексу маси тіла достовірно більша у хворих жінок з нормоальбумінурією та мікроальбумінурією - відповідно на 11,8% та 17,7% (цистатин  $S < 0,9$ ) та у пацієток з протеїнурією на 7,2% (цистатин  $S > 0,9$ ) порівняно із здоровими жінками. Таким чином, між здоровими та хворими на ЦД 1 пацієнтами з різним ступенем альбумінурії встановлені відмінності загальноклінічних та антропометричних показників, причому вони тим більші, чим більший рівень цистатину С.

**Ключові слова:** цукровий діабет 1 типу, рівень цистатину С, рівень альбуміну в сечі, ангіо-, ретино- та нейропатія, загально-клінічні показники, антропометричні показники.

#### **ОСОБЕННОСТИ ЧАСТОТЫ АНГИО-, РЕТИНО- И НЕЙРОПАТИИ, ОБЩЕКЛИНИЧЕСКИХ И АНТРОПОМЕТРИЧЕСКИХ ПОКАЗАТЕЛЕЙ У БОЛЬНЫХ САХАРНЫМ ДИАБЕТОМ 1 ТИПА С РАЗЛИЧНЫМ УРОВНЕМ АЛЬБУМИНА В МОЧЕ В ЗАВИСИМОСТИ ОТ УРОВНЯ ЦИСТАТИНА С**

**Кривовяз Ю.А., Вернигородский В.С., Дзевульская И.В., Шевчук Ю.Г., Жученко П.С.**

Цистатин С улучшает стратификацию риска среди лиц с диабетической нефропатией относительно риска смертности, сердечно-сосудистых заболеваний, нарушений со стороны органа зрения и нервной системы на доклинической и начальной стадии. Цель исследования - изучить частоту ангио-, ретино- и нейропатии, а также различия общеклинических и антропометрических показателей у больных сахарным диабетом 1 типа (СД 1) с различным уровнем альбумина в моче в зависимости от уровня цистатина С. Выборка составила 78 мужчин и 62 женщины в возрасте 22-26 лет, больных СД 1, которые проходили стационарное лечение в терапевтических отделениях №1 и №2 Винницкого областного высокоспециализированного эндокринологического центра. Контрольная группа состояла из 8 практически здоровых мужчин и 13 практически здоровых женщин аналогичного возраста. Всем пациентам иммуноферментным методом определяли уровень микроальбуминурии и цистатина С. Проводили оценку частоты ангио-, ретино- и нейропатии, общеклинических (систолическое АД, диастолическое АД, пульс) и антропометрических (рост, вес, площадь поверхности тела, обхват талии, индекс массы тела) показателей. Статистическая обработка полученных результатов проведена в лицензионном пакете "Statistica 5.5", с использованием непараметрических методов оценки. Установлено, что в группе мужчин уровень цистатина С у которых  $< 0,9$ , и у женщин с уровнем цистатина  $S > 0,9$  при нарастании альбуминурии растет процентная доля пациентов с более тяжелой степенью микрососудистых осложнений СД1. У больных СД1 по сравнению с контрольной группой величина систолического артериального давления достоверно больше и увеличивается по мере роста уровня альбумина в моче (при цистатине  $S < 0,9$  - у мужчин с нормо-, микроальбуминурией и протеинурией на 7,14%, 8,1% и 10,8%, у женщин с нормо-, микроальбуминурией на 7,5% и 10,0%, при цистатине  $S > 0,9$  - у мужчин с нормо-, микроальбуминурией на 4,9% и 7,2%, у женщин с протеинурией на 19,5%). Аналогичные изменения установлены для диастолического артериального давления (при цистатине  $S < 0,9$  - у мужчин с протеинурией на 13,0%, у женщин с нормо-, микроальбуминурией на 11,4% и 13,4%, при цистатине  $S > 0,9$  - у мужчин с микроальбуминурией на 9,0%, у женщин с нормо- и протеинурией на 9,5% и 21,5%) и величины пульса (при цистатине  $S < 0,9$  - у мужчин с микроальбуминурией и протеинурией на 18,4% и 12,6%, у женщин с микроальбуминурией на 9,13%, при цистатине  $S > 0,9$  - у мужчин с микроальбуминурией на 12,0%, у женщин с нормо-, микроальбуминурией и протеинурией на 10,1%, 16,3% и 25,3%). У больных СД1 по сравнению с контрольной группой величина длины тела достоверно меньше и уменьшается по мере увеличения уровня альбумина в моче (при цистатине  $S < 0,9$  - у мужчин с нормо-, микроальбуминурией и протеинурией на 3,6%, 6,7% и 9,0%, у женщин с микроальбуминурией на 1,9%, при цистатине  $S > 0,9$  - у мужчин с нормо-, микроальбуминурией на 5,2% и 7,3%, у женщин с нормоальбуминурией и протеинурией на 2,6% и 4,3%). Аналогичные изменения установлены у мужчин для величины массы тела (при цистатине  $S < 0,9$  - с микроальбуминурией и протеинурией на 13,6% и 30,1%, при цистатине  $S > 0,9$  - с нормо- и микроальбуминурией на 10,2% и 25,4%) и величины площади поверхности тела (при цистатине  $S < 0,9$  - с нормо-, микроальбуминурией и протеинурией на 5,8%, 10,8% и 18,9%, при цистатине  $S > 0,9$  - с нормо- и микроальбуминурией на 8,2% и 16,2%). Величина окружности талии у больных мужчин СД1 с нормоальбуминурией достоверно больше, а у больных других групп достоверно меньше по сравнению со здоровыми испытуемыми (при цистатине  $S < 0,9$  - с нормо-, микроальбуминурией и протеинурией на 3,8%, 1,2% и 5,2%, при цистатине  $S > 0,9$  - с микроальбуминурией на 5,1%). У больных женщин по сравнению со здоровыми обхват талии достоверно больше (при цистатине  $S < 0,9$  - с нормо- и микроальбуминурией на 11,2% и 10,7%, при цистатине  $S > 0,9$  - с нормо- и протеинурией на 9,7% и 6,0%). У больных СД1 мужчин с протеинурией по сравнению с контрольной группой величина индекса массы тела достоверно меньше на 9,7% (цистатин  $S < 0,9$ ). Величина индекса массы тела достоверно больше у больных женщин с нормоальбуминурией и микроальбуминурией - соответственно на 11,8% и 17,7% (цистатин  $S < 0,9$ ) и у пациенток с протеинурией на 7,2% (цистатин  $S > 0,9$ ) по сравнению со здоровыми женщинами. Таким образом, между здоровыми и больными СД1 у пациентов с разной степенью альбуминурии установлены различия общеклинических и антропометрических показателей, причем они тем больше, чем больше уровень цистатина С.

**Ключевые слова:** сахарный диабет 1 типа, уровень цистатина С, уровень альбумина в моче, ангио-, ретино- и нейропатия, общеклинические показатели, антропометрические показатели.

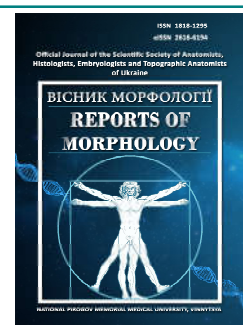




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# The vaginal bacterial dysbiosis severity predicting model according to the normobiota index

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*The local and systemic immunodeficiency is the main mechanism for vaginal bacterial dysbiosis and its extreme manifestation - bacterial vaginosis (BV) development. The complex immune response study and the establishment of the main mechanisms and factors, reflecting it and corresponding to the microbiocenosis severity disorder are relevant. Aim - to develop a neural network model of the severity of vaginal bacterial dysbiosis based on the assessment of normobiota. Divided into the following groups according to the Conditionally pathogenic microflora index (CPMI) and normobiota index (NBI): normocenosis (n=53), dysbiosis I (n=128) and II degree (n=117) among the latter 83 patients with PNB>1 Ig GE/sample were identified, in whom BV was established. Molecular genetic studies of the epithelium scraping from the vagina posterolateral wall were carried out by Polymerase chain reaction ("DNK-Technologija" LLC, RF). Facultative and obligate anaerobes, myco- and ureplasmas, and yeast-like fungi were quantified. The content of immunoglobulins, lysozyme, cytokines, complement, phagocytosis activity of leukocytes, hormones, the number of lymphocytes and their fractions, as well as the vaginal discharge pH (a total of 58 indexes) were identified in blood and vaginal discharge. For statistical and mathematical analysis, the Statistica 10 software (StatSoft, Inc., USA) was used. Using neural network modeling, it was revealed that among all the factorial signs for determining the bacterial dysbiosis degree, the complement component C4 and  $\gamma$ -INF content in the vaginal discharge and circulating immune complexes (CIC) and TNF $\alpha$  in the blood were important. A linear neural network model was built on the selected set of factor signs (the Cohen's kappa coefficient consent index on the training set was  $\kappa=0.87$  (95% CI 0.82-0.91)), for confirming plurality -  $\kappa=0.89$  (95% CI 0.77-1.00). With normocenosis, the complement activation PNB was decreased, and  $\gamma$ -INF and TNF $\alpha$  content was increased. The CIC levels blood increase corresponded to the opportunistic microflora growth and reflected the humoral immune response activation, which suggests that this indicator is an early dysbiosis marker. With I degree dysbiosis all factors had positive relationship with NBI, which reflected the immune system stress state. In case of II degree dysbiosis, NBI had a negative relationship with  $\gamma$ -INF content in the vaginal discharge, and CIC in the blood, while positive - with C<sub>4</sub> content in vaginal discharge, and TNF $\alpha$  in the blood, which proved the immune system dysregulation and caused its further suppression with the BV-association immunodeficiency development. The immune system reaction during the BV development evolved from non-specific resistance reactions to cytokine-induced reactions of specific humoral immunity in response to the BV-associated microbiota growth, which subsequently experienced depletion and loss of immune control.*

**Keywords:** bacterial vaginosis, normobiota index, immune system.

### Introduction

Vaginal bacterial dysbiosis is a nonspecific infectious non-inflammatory syndrome in which the content of obligate- and facultative-anaerobic opportunistic pathogens increases in vaginal discharge with a decrease

in the content or complete absence of normobiota (*Lactobacillus spp.*) [1, 12, 22]. The extreme severity of dysbiosis is bacterial vaginosis (BV), which causes inflammation of the pelvic organs, and during pregnancy



causes the threat of pregnancy, premature birth and postpartum infections of mother and child [19, 21]. BV has been shown to contribute to the incidence of HIV [16] and papillomavirus infection, as well as associated with cervical cancer [2].

The causes of BV are disruption of the vaginal ecosystem due to suppression of natural defense mechanisms - vaginal microbiocenosis, the formation of colonial resistance factors, as well as local nonspecific immune defense (phagocytosis reactions and complement activation, etc.) [3, 18]. Activated by molecular microbial pathogens, macrophages of the vaginal mucosa through the formation of proinflammatory cytokines [15] trigger cellular and humoral immune responses [11]. At the same time, against the background of BV and an increase in the absolute number of BV-associated pathogens (*Gardnerella vaginalis*, *Mycoplasma hominis*, etc.) there is a decrease in systemic and local inflammatory response, local and systemic immunodeficiency [4].

The main mechanism of BV development is local immunodeficiency and reduction of colonization resistance of vaginal discharge [3, 18], but, in addition to local, systemic immunodeficiency is also formed [5]. Antigen-presenting cells of the vagina, primarily macrophages and dendritic cells, are activated by bacterial antigens, release proinflammatory cytokines and involve cellular and humoral links in the immune response [11]. Decreased systemic and local inflammatory response correlates with the degree of bacterial dysbiosis [17].

Thus, a comprehensive study of the local and local immune response, as well as the establishment of the main mechanisms of BV development and the factors that reflect them, seems relevant.

The aim of the study was to develop a neural network model of the severity of vaginal bacterial dysbiosis based on the assessment of normobiota.

## Materials and methods

The study used data from 298 women between the ages of 16 and 64 who consulted a gynecologist for a preventive examination or for complaints of discomfort in the genital area. The exclusion criterion was the presence of unduly pathogenic microorganisms in the scrapings of the vaginal epithelium (*Trichomonas vaginalis*, *Neisseria gonorrhoeae*, *Chlamydia trachomatis* and *Herpes Simplex Virus 1,2*). The presence of more than 15-20 leukocytes in the field of view in vaginal smears, which indicated the presence of an inflammatory reaction, was also an exclusion criterion.

During the examination, a scraping of the epithelium was taken from the posterior lateral wall of the vagina using a urogenital probe. Molecular genetic studies were performed by polymerase chain reaction (PCR). DNA was isolated using a set of reagents "Proba-HS" ("DNA Technology", Russia). Amplification of test tubes with the reaction mixture was performed in the amplifier "DTLite"

("DNA Technology", Russia). To study the state of the vaginal biocenosis, we used the "Femoflor 16" test system, which is designed for real-time PCR. The microbiota was quantified by the following indicators [14]: total bacterial mass (TBM), normobiota (*Lactobacillus spp.*), Obligate anaerobes (*Lactobacillus spp.*), облигатні анаероби (*Atopobium vaginalis*, *Eubacterium spp.*, *Gardnerella vaginalis*, *Prevotella bivia*, *Porphyromonas spp.*, *Lachnobacterium spp.*, *Clostridium spp.*, *Megasphaera spp.*, *Veilonella spp.*, *Dialister spp.*, *Mobiluncus spp.*, *Corynebacterium spp.*, *Peptostreptococ spp.*, *Sneathia spp.*, *Leptotrihia spp.*, *Fusobacterium spp.*), facultative anaerobes (*Enterobacteriaceae spp.*, *Staphylococcus spp.*, *Streptococcus spp.*), Mycoplasma and ureaplasma (*Ureaplasma urealiticum + parvum*, *Mycoplasma hominis + genitalium*) and yeast-like fungi (*Candida spp.*).

The criterion for dividing patients into groups was conditionally pathogenic microflora index (CPMI), which was calculated as the difference between the sum of all opportunistic pathogens and the number of lactobacilli (in lg GE/sample). In the normocenosis CPMI was lower than -3 lg GE/sample (1 group; n=53), in dysbiosis of the first degree CPMI was in the range from -3 to -1 lg GE/sample (group 2; n=128) and in dysbiosis II degree CPMI was more than -1 lg GE/sample (group 3; n=117) [9]. Groups with dysbiosis were divided into subgroups according to the normobiota index (NBI), which was calculated as the difference between the total bacterial mass (TBM) and the number of lactobacilli (in lg GE/sample). In group 2 there are three subgroups: 1 - with  $NBI \leq 0$ , lg GE/sample (n=23), 2 - with NBI from 0.3 to 1.0 lg GE/sample (n=83) and 3 - with  $NBI > 1$  lg GE/sample (n=22). In group 3, two subgroups were distinguished: 1 - with  $NBI \leq 1$  lg GE/sample (n=34) and 2 - with  $NBI > 1$  lg GE/sample (n=83). In the last subgroup, the degree of dysbiosis was maximal and corresponded to the state of BV [8].

According to standard immunological methods [5, 20], the content of immunoglobulins A (IgA), M (IgM) and G (IgG) in blood serum and vaginal discharge was determined ("Granum" test systems, Ukraine); the content of immunoglobulin G<sub>2</sub> (IgG<sub>2</sub>) and secretory IgA (sIgA) ("Hema", Russia); the content of transforming growth factor 1 $\beta$  (TGF-1 $\beta$ ) (DRG, USA); immune complexes (IC, in vaginal discharge) and circulating IC (CIC, in blood) by their selective precipitation in a solution of polyethylene glycol; content of interleukins 1 $\beta$  (IL1 $\beta$ ), 2 (IL2), 4 (IL4), 6 (IL6), 8 (IL8), 10 (IL10), tumor necrosis factor  $\alpha$  (TNF $\alpha$ ) and  $\gamma$ -interferon ( $\gamma$ -INF) ("Vector-Best", Russia); content of complement components C3 and C4 ("PLIVA-Lachema Diagnostica s.r.o", Czech Republic); lysozyme (DRG, USA). Determination of leukocyte phagocytic activity (PAL) was performed using a suspension of yeast cells ("Granum", Ukraine); PAL was calculated as the average number of particles absorbed by one active neutrophil per 100 cells, the PAL index (IPAL) was calculated as the percentage of phagocytes from the number of counted neutrophils. The

**Table 1.** Factor features of the indicators primary analysis of vagina colonial resistance, immune system and hormonal regulation system.

X1	Age	X20	IL10	X39	CD22+
X2	Day of menstrual cycle	X21	TNF $\alpha$	X40	PAL
Indicators of VD:		X22	TGF-1 $\beta$	X41	I <sub>PAL</sub>
X3	IgM	X23	pH	X42	CIC
X4	IgA	Indicators in the blood:		X33	C <sub>3</sub>
X5	IgG	X24	FSH	X44	C <sub>4</sub>
X6	IgG2	X25	LH	X45	$\gamma$ -INF
X7	sIgA	X26	E2	X46	IL1 $\beta$
X8	Lysozyme	X27	PG	X47	IL2
X9	PAL	X28	TS	X48	IL4
X10	IPAL	X29	CR	X49	IL6
X11	IC	X30	PRL	X50	IL8
X12	C <sub>3</sub>	X31	fT <sub>3</sub>	X51	IL10
X13	C <sub>4</sub>	X32	fT <sub>4</sub>	X52	TNF $\alpha$
X14	$\gamma$ -INF	X33	Lc	X53	TGF-1 $\beta$
X15	IL1 $\beta$	X34	CD16+	X54	IgM
X16	IL2	X35	CD3+	X55	IgA
X17	IL4	X36	CD4+	X56	IgG
X18	IL6	X37	CD8+	X57	IgG2
X19	IL8	X38	IPI	X58	sIgA

**Notes:** VD - vaginal discharge.

number of lymphocytes in the blood (Lc) was counted [20]; quantitative determination of CD3+, CD4+, CD8+, CD16+ and CD22+ cells was performed with the use of erythrocyte diagnosticums of "Granum" (Ukraine); calculated the immunoregulatory index (IRI) as the ratio of CD4+/CD8+. The pH of vaginal discharge was determined using test strips "Kolpo-Test pH" manufactured by "Biosensor AN" (RF). Enzyme-linked immunosorbent assay in the serum determined the content of hormones: luteotropic (LH), follicle-stimulating (FSH), prolactin (PRL), cortisol (CR), progesterone (PG), estradiol (E2), testosterone (TSC), free triiodothyronine (T3) and free thyroxine (T4) using reagent kits produced by "Granum" (Ukraine).

Arithmetic mean (M) and mean error (m) were used for descriptive data statistics. Paired independent data samples were compared according to the Mann-Whitney (U) test. The construction of prognostic models was performed using neural network modeling [10]. The method of genetic analysis was used to select the most significant factor traits. In all types of comparative statistical analysis, the significance of the differences was taken at p<0.05. The statistical software package Statistica 10 (StatSoft, Inc., USA) was used for statistical processing of the obtained data.

Prediction of the severity of dysbiosis by NBI was

performed by neural network analysis, which used the results of examination of all 298 patients, 53 of whom were diagnosed with normocenosis, 128 dysbiosis of I degree and 117 - dysbiosis of II degree. At the beginning of the analysis, all the studied factors (58 indicators in total) were presented as factor features, which are shown in Table 1.

To check the quality of model prediction, all observations (using a random number generator) were divided into three sets: training (used to calculate model parameters, 248 cases), control (used to control model retraining, 20 cases), confirmatory (used to check adequacy models when forecasting on new data, 30 cases).

### Results

A linear neural network model was built and trained on a complete set of 58 factor features (see Table 1). Cohen kappa agreement index for this model on a training set was  $\kappa=0.99$  (95% CI 0.97-1.00), on the confirmatory set -  $\kappa=1.00$  (95% CI 0.99-1.00), which testified to the adequacy of the model that was built.

Significant traits were selected using a genetic algorithm method to identify the factors most associated with NBI. As a result, four factor traits were selected: levels in the vaginal discharge of the complement component C4 (X13) and  $\gamma$ -INF (X14), as well as blood levels of CIC (X42) and TNF $\alpha$  (X52).

A linear neural network model was built and trained on a separate set of four factor features. Cohen kappa agreement index for this model on a training set was  $\kappa=0.87$  (95% CI 0.82-0.91), on the confirmatory set  $\kappa=0.89$  (95% CI 0.77-1.00), which testified to the adequacy of the constructed model.

Thus, the model for predicting the severity of dysbiosis for NBI, based on four factor traits, gave a "very good" ( $\kappa>0.81$ ) agreement, which indicated the high significance of the selected factor traits (content in the vaginal discharge of C<sub>4</sub> and  $\gamma$ -INF, as well as the content in the blood of CIC and TNF $\alpha$ ) to predict the severity of dysbiosis by NBI.

The constructed model can be expressed by a system of equations (1):

$$\begin{aligned}
 V0 &= -0,101 \times X13 - 0,091 \times X14 + 0,001 \times X42 - 0,031 \times X52 + 1,726 \\
 V1 &= 0,092 \times X13 - 0,262 \times X14 - 0,010 \times X42 + 0,019 \times X52 - 1,380 \\
 V2 &= 0,009 \times X13 - 0,171 \times X14 - 0,012 \times X42 + 0,012 \times X52 + 0,654
 \end{aligned}
 \tag{1}$$

where V0 corresponds to the diagnosis of "normocenosis", V1 corresponds to the diagnosis of "grade I dysbiosis", V2 corresponds to the diagnosis of "grade II dysbiosis"; the decision is made on the maximum value of the resulting indicator V.

Changes in the content of selected by the results of neural network modeling of the immune system in dysbiosis of varying degrees are given in Table 2.

The content of the complement component C<sub>4</sub> in the vaginal discharge was increased compared to the norm

**Table 2.** Indicators of the immune system, which were selected at a previous stage by the results of neural network modulation (M±m).

Group, subgroup		Content in VD		Blood content	
		C4, µg/ml	γ-INF, pg/ml	CIC, Ex.U.	TNFα, pg/ml
1 (normocenosis), n=53		3.532±0.071	1.871±0,061	46.12±1,32	14.64±0,46
2 (dysbiosis of the I degree), n=128	1, n=23	3.642±0.111	1.931±0.051	44.33±2.41	18.92±0.73*
	2, n=83	4.622±0.081*	2.472±0.041*	54.91±1.03*	31.89±0.59*
	3, n=22	2.281±0.071*	0.841±0.031*	33.63±1.12*	30.76±1.22*
3 (dysbiosis of the II degree), n=117	1, n=34	1.191±0.031*	0.711±0.021*	45.36±1.59	31.29±0.87*
	2, n=83	0.601±0.011*	0.411±0.011*	25.23±0.39*	52.62±0.86*

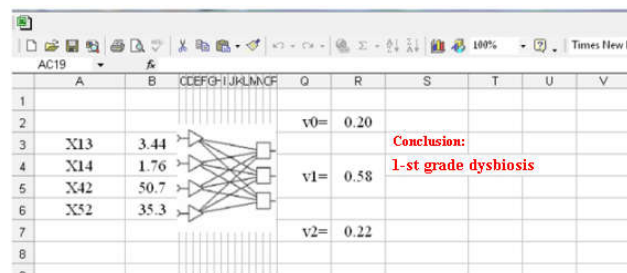
**Notes:** VD - vaginal discharge; \* - probability of discrepancies using the Mann-Whitney U-test compared with the corresponding indicator in group 1 (p<0.001).

for the normocenosis in grade I dysbiosis (2 subgroups - in 1.3 times; p<0.001), decreased with the transition to grade II dysbiosis (up to 50-66% of the level of normocenosis) and decreased sharply on the background of BV (up to 17%; p<0.001). Earlier we showed [6, 7] that the content of immune complexes in vaginal discharge also decreased in grade II dysbiosis and the decrease in complement content was the reason for such a decrease in the formation of immune complexes on the background of BV.

The content of γ-INF did not actually change in subgroup 1 in dysbiosis of the first degree, but reached a maximum in subgroup 2 (increased in 1.3 times; p<0.001). With the deepening of the level of dysbiosis, the content of γ-INF decreased and in BV and was 21.9% of normocinosis (p<0.001). The level of complement changed similarly. γ-IFN is an active proinflammatory factor that is associated with innate and adaptive immunity, along with other proinflammatory factors (such as IL1β and TNFα), it activates T-lymphocytes and monocytes to activate the expression of the chemokine cascade [5]. Therefore, the activation of humoral immunity, a significant indicator of which according to the results of studies was the C4 component of complement and the content of γ-IFN, which involves the cell, reflected in the dysbiosis of the first degree reaction to the local immune response. With the further development of dysbiosis, significant suppression of both indicators was noted.

Confirmation of the important regulatory role of γ-IFN is its negative relationship with NBI, detected during regression analysis (β-regression coefficient was -0.28; p<0.001) in grade I dysbiosis. The same signs of dependence were found in the phagocytic activity of leukocytes (IPAL) and the content of sIgA, which indicates an active reaction of the nonspecific immune system to the factors of colonization resistance of vaginal discharge.

CIC contain antigens, antibodies and components of complement C<sub>3</sub>, C<sub>4</sub>, C<sub>1q</sub> [5]. According to the results of regression analysis, in dysbiosis of the first degree in blood content, the CIC had a negative effect on NBI (β-regression coefficient was -0.138; p=0.027). Therefore, such data suggest that dysbiosis was a provoking factor for the



**Fig. 1.** The interface expert system predicting the severity of dysbiosis according to NBI.

activation of the humoral part of the immune system.

For the practical use of the model for predicting the severity of dysbiosis by NBI in the environment of the Excel spreadsheet implemented an expert system. Figure 1 shows its interface. To work in the program, you must enter the values of the indicators for a particular patient in the appropriate cells of the spreadsheet. The expert system will issue a forecast of the severity of dysbiosis according to NBI. From the three equations (V0, V1 and V2) the maximum value of the calculated NBI is chosen.

Thus, patient P., 40 years old, had the following indicators: levels in vaginal discharge C<sub>4</sub> - 3.44 µg/ml and γ-INF - 1.94 pg/ml; blood levels of CIC - 42.7 Ex.U. and TNFα - 13.8 pg/ml. Predicted NBI - "Normocenosis", the actual value of NBI - 0.05; diagnosis: Normocenosis.

Patient P., 31 years old, had the following indicators: levels in vaginal discharge C<sub>4</sub> - 4.38 µg/ml and γ-INF - 1.76 pg/ml; blood levels of CIC - 50.7 Ex.U. and TNFα - 35.3 pg/ml. Predicted NBI - "Dysbiosis of the I degree"; the actual value of NBI - 0.4, diagnosis: Dysbiosis of the first degree (Fig. 1).

Patient S., 16 years old, had the following indicators: levels in vaginal discharge C<sub>4</sub> - 0.53 µg/ml and γ-INF - 0.46 pg/ml; blood levels of CIC - 23.9 Ex.U. and TNFα - 57.5 pg/ml. Predicted NBI - "Dysbiosis of the II degree", the actual value of NBI - 4.6; diagnosis: Dysbiosis of the II degree.

**Discussion**

As we showed earlier [6, 7, 8], in the process of development of vaginal dysbiosis there was a formation of

a single pathological hormonal-immune system, which causes and supports the development of BV. Such a system included the formation of local and systemic immunodeficiency and a number of hormonal disorders. In this work, the task was to develop and analyze a mathematical model of the pathological process and determine the factors that determine the severity of dysbiosis. As a result, NBI was considered, which objectively reflected the degree of increase in pathogenic microflora and decrease in the number of lactobacilli [8, 14].

Analysis of the system of equations 1 showed some patterns of progression of dysbiosis. In the normocenosis (equation V0) the negative signs of the coefficients (ie those that reduced NBI) had the content in the vaginal discharge of the complement component  $C_4$ ,  $\gamma$ -INF and the content of TNF $\alpha$  in the blood, and positive (increased in parallel with NBI) - the content in the blood CIC. In other words, in the normocenosis, the conditionally pathogenic microflora was directly controlled by the activation of complement ( $C_4$ ) and the increase in the level of cytokines -  $\gamma$ -INF and TNF $\alpha$ . The increase in the content of CIC in the blood reflected the parallel to the growth of opportunistic pathogenic microflora activation of the humoral immune response. Therefore, this state of the immune system can be characterized as controlled in relation to the development of vaginal dysbiosis, and the accumulation in the blood of CIC in the normocenosis can be recommended as an early marker of activation of opportunistic pathogens.

In grade I dysbiosis (equation V1), none of the coefficients had a negative sign of the coefficients, ie they all had a positive relationship, and therefore increased according to the increase in NBI. In other words, all significant factors responded to the activation of opportunistic pathogenic microflora, which, under such conditions, acquired the properties of an uncontrolled process that had the ability to self-sustain and progressive self-stimulation [8, 9]. In our opinion, the change of the sign of all coefficients to positive reflected the maximum stress of immune mechanisms with the progression of dysbiosis.

In grade II dysbiosis (equation V2), negative coefficients appeared for the content of  $\gamma$ -INF in the vaginal discharge and the CIC content in the blood, and positive for the content of  $C_4$  in the vaginal discharge and TNF $\alpha$  in the blood. Therefore, we can assume that under conditions of developed vaginal dysbiosis, dysregulation of the immune system was formed, which changed the state of tension and preceded the pronounced suppression of reactions of both local and systemic immunity in BV. In our opinion, in dysbiosis there is an escape of opportunistic pathogens (first, anaerobic) from the control of the immune system, which causes BV-associated immunodeficiency and the development of BV.

Earlier we showed [8, 9] that against the background of reduced TBM and LB content sharply increased NBI,

detectability and number of opportunistic pathogens (especially anaerobes, which did not occur in the normocenosis - *Sneathia spp.* + *Leptotrihia spp.* + *Fusobacterium spp.*), as well as uncharacteristic for the normocenosis mycoplasmas. It is possible that these antigens activated the response of the immune system, which in the process of developing BV evolved from nonspecific resistance to cytokine-induced reactions of specific humoral immunity. This explains the negative relationship of NBI with the content in the vaginal discharge of the activator of the immune system -  $\gamma$ -INF and the level in the blood of the CIC in grade II dysbiosis.

On the other hand, it also explains the positive relationship with the NBI content in the vaginal discharge of the complement component  $C_4$ . Regarding the positive relationship of TNF $\alpha$  in the blood with NBI, it should be noted that according to [4] the growth of BV-associated microflora corresponds to the activation of cytokine cascades in vivo, and according to [13] in vitro studies showed that proinflammatory cytokines in high concentrations characteristic of BV, stimulate the growth of opportunistic pathogens. Thus, we can assume that in the process of BV development, proinflammatory cytokines are transformed from factors that inhibit the growth of pathogenic microflora into factors that activate it.

Discussing the results presented in table 2, we can establish that the correspondence of shifts in the content of complement  $C_4$  in vaginal discharge and CIC in the blood indicates a single mechanism of involvement of these parts of the immune system in dysbiosis, and mathematical proof of this connection in neural network analysis indicates the possibility of the content of CIC in the blood as a prognostic factor of stress of the humoral part of the immune system in grade I dysbiosis and its depletion in grade II dysbiosis and BV.

In our study, all studied proinflammatory cytokines (IL1 $\beta$ , IL6, IL8, TNF $\alpha$  and IL2) had a similar response - a clear increase according to the degree of bacterial dysbiosis with a maximum at BV: 3.0-6.0 times ( $p < 0.001$ ) in comparable for the level of the normocenosis. The leading position in this list of TNF $\alpha$  confirms its role as an immediate factor that regulates immune inflammation - the cytokine of "first stage" [5]. Other interleukins help to prolong and expand inflammation and recruit immunocompetent cells into the focus, involving the endocrine and other body systems in inflammation.

The prospect of further development is to implement the developed neural network model in health care practice and conduct an expert evaluation of the effectiveness of the proposed method for predicting the severity of vaginal bacterial dysbiosis according to indicator of normobiota.

## Conclusions

1. By neural network modeling, it was found that among all the factor signs for determining the degree of bacterial dysbiosis were important content in the vaginal discharge:

complement component C4 and  $\gamma$ -INF; in the blood - CIC and TNF $\alpha$ . A dedicated set factor variable was constructed linear neural network model (Cohen kappa index for the training set was  $\kappa=0.87$  (0.82-0.91 CI 95%), to supporting the set -  $\kappa=0.89$  (95% CI 0.77-1.00).

2. Complement activation, increased content of  $\gamma$ -INF and TNF $\alpha$  reduced the rate of normobiota in the normocenosis. The increase in the content of CIC in the blood corresponded to the growth of opportunistic pathogenic microflora and reflected the activation of the humoral immune response, which gave reason to consider this indicator as an early marker of dysbiosis.

3. In grade I dysbiosis, all factors had a positive relationship with the rate of normobiota, which, in our opinion, contributed to the uncontrolled growth of opportunistic pathogens (first, anaerobic) microflora and reflected the state of stress of the immune system. In grade II dysbiosis, NBI had a negative relationship with the content of  $\gamma$ -INF in the vaginal discharge and the CIC in the blood, while it was positive for the content of C<sub>4</sub> in the vaginal discharge and TNF $\alpha$  in the blood. Thus, under conditions of advanced vaginal dysbiosis, dysregulation of the immune system was formed, which could lead to its suppression and the development of BV-associated immunodeficiency.

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## МОДЕЛЬ ПРОГНОЗУВАННЯ СТУПЕНЯ ТЯЖКОСТІ ВАГІНАЛЬНОГО БАКТЕРІАЛЬНОГО ДИСБІОЗУ ЗА ПОКАЗНИКОМ НОРМОБІОТИ

Грузевський О.А., Мінухін В.В.

Основним механізмом розвитку вагінального бактеріального дисбіозу та його крайнього прояву - бактеріального вагінозу (БВ) - є локальний та системний імунodefіцит. Актуальним є вивчення комплексної імунної відповіді та встановлення

основних механізмів та чинників, що її відображають та відповідають тяжкості порушення мікробіоценозу. Мета дослідження - розробити нейромережеву модель ступеня тяжкості вагінального бактеріального дисбіозу на підставі оцінки показнику нормобіоти. Були використані дані 298 жінок, які за індексом умовно патогенної мікрофлори (ИУПМ) та показником нормобіоти (ПНБ) були розподілені на групи: нормоценоз (n=53), дисбіоз I (n=128) і II ступеня (n=117); серед останніх виокремлено 83 пацієнтки з ПНБ>1 Іg GE/зразок, в яких був встановлений БВ. Молекулярно-генетичні дослідження зіскрібка епітелію з задньобовкової стінки піхви проводили методом полімеразної ланцюгової реакції ("ДНК-технологія", РФ). Кількісно визначали факультативні і облигатні анаероби, міко- і уреплазми та дріжджоподібні гриби. У крові та вагінальному секреті визначали вміст імуноглобулінів, лізоциму, цитокинів, комплементу, активність фагоцитозу лейкоцитів, гормонів, кількості лімфоцитів та їх фракцій, а також рН вагінального секрету (загалом - 58 показників). Для статистичного та математичного аналізу використовували програму Statistica 10 (StatSoft, Inc., USA). Шляхом нейромережевого моделювання було з'ясовано, що серед усіх факторних ознак для визначення ступеня бактеріального дисбіозу мали значення вміст у вагінальному секреті компоненту комплементу C4 і  $\gamma$ -INF, а у крові - циркулюючих імунних комплексів (ЦІК) і TNF $\alpha$ . На виділеному наборі факторних ознак була побудована лінійна нейромережева модель (показник згоди каппа Коена на навчальній множині склав  $\kappa=0,87$  (95% ВІ 0,82-0,91)), на підтвердженій множині -  $\kappa=0,89$  (95% ВІ 0,77-1,00). Активація комплементу, підвищення вмісту  $\gamma$ -INF та TNF $\alpha$  знижували показник нормобіоти при нормоценозі. Підвищення у крові вмісту ЦІК відповідало росту умовно патогенної мікрофлори та відображало активацію гуморальної імунної відповіді, що дає підставу вважати цей показник раннім маркером дисбіозу. При дисбіозі I ступеня всі чинники мали позитивні зв'язки з ПНБ, що відображало стан напруження імунної системи. При дисбіозі II ступеня ПНБ має від'ємний зв'язок з вмістом у вагінальному секреті  $\gamma$ -INF, а у крові - ЦІК, тоді як позитивний - з вмістом у вагінальному секреті C $_4$ , а у крові - TNF $\alpha$ , що свідчило про дисрегуляцію імунної системи та обумовлювало її подальшу супресію з розвитком БВ-асоційованого імунodefіциту. Реакція імунної системи у процесі розвитку БВ еволюціонувала від реакцій неспецифічної резистентності до індукованих цитокінами реакцій специфічного гуморального імунітету у відповідь на зріст БВ-асоційованої мікробіоти, що згодом зазнавало виснаження та втрату імунного контролю.

**Ключові слова:** бактеріальний вагіноз, показник нормобіоти, імунна система.

#### МОДЕЛЬ ПРОГНОЗИРОВАНИЯ СТЕПЕНИ ТЯЖЕСТИ ВАГИНАЛЬНОГО БАКТЕРИАЛЬНОГО ДИСБИОЗА ПО ПОКАЗАТЕЛЯМ НОРМОБИОТЫ

**Грузевский А.А., Минухин В.В.**

Основным механизмом развития вагинального бактериального дисбиоза и его крайнего проявления - бактериального вагиноза (БВ) - является локальный и системный иммунодефицит. Актуальным является изучение комплексного иммунного ответа и установление основных механизмов и факторов, отражающих его и соответствующих тяжести нарушения микробиоценоза. Цель исследования - разработать нейросетевую модель степени тяжести вагинального бактериального дисбиоза на основании оценки показателя нормобиоты. Были использованы данные 298 женщин, которые по индексу условнопатогенной микрофлоры (ИУПМ) и показателям нормобиоты (ПНБ) были распределены на группы: нормоценоз (n=53), дисбиоз I (n=128) и II степени (n=117); среди последних были выделены 83 пациентки с ПНБ>1 Іg GE/образец, у которых был установлен БВ. Молекулярно-генетические исследования соскоба эпителия с заднебоковой стенки влагалища проводили методом полимеразной цепной реакции ("ДНК-технология", РФ). Количественно определяли факультативные и облигатные анаэробы, мико- и уреплазмы и дрожжеподобные грибы. В крови и вагинальном секрете определяли содержание иммуноглобулинов, лизоцима, цитокинов, комплемента, активность фагоцитоза лейкоцитов, гормонов, количества лимфоцитов и их фракций, а также рН вагинального секрета (всего 58 показателей). Для статистического и математического анализа использовали программу Statistica 10 (StatSoft, Inc., USA). Путем нейросетевого моделирования было установлено, что среди всех факторных признаков для определения степени бактериального дисбиоза имели значение содержание в вагинальном секрете компонента комплемента C4 и  $\gamma$ -INF, а в крови - циркулирующих иммунных комплексов (ЦИК) и TNF $\alpha$ . На выделенном наборе факторных признаков была построена линейная нейросетевая модель (показатель согласия каппа Коена на обучающем множестве составил  $\kappa=0,87$  (95% ВІ 0,82-0,91)), на подтверждающем множестве -  $\kappa=0,89$  (95% ВІ 0,77-1,00). При нормоценозе снижало ПНБ активация комплемента, повышение содержания  $\gamma$ -INF и TNF $\alpha$ . Повышение в крови содержания ЦИК соответствовало росту условнопатогенной микрофлоры и отражало активацию гуморального иммунного ответа, что дает основание считать этот показатель ранним маркером дисбиоза. При дисбиозе I степени все факторы имели положительные связи с ПНБ, что отражало состояние напряжения иммунной системы. При дисбиозе II степени ПНБ имел отрицательную связь с содержанием в вагинальном секрете  $\gamma$ -INF, а в крови - ЦИК, тогда как положительный - с содержанием в вагинальном секрете C $_4$ , а в крови - TNF $\alpha$ , что свидетельствовало о дисрегуляции иммунной системы и обуславливало ее дальнейшую супрессию с развитием БВ-ассоциированного иммунодефицита. Реакция иммунной системы в процессе развития БВ эволюционировала от реакций неспецифической резистентности до индуцированных цитокинами реакций специфического гуморального иммунитета в ответ на рост БВ-ассоциированной микробиоты, что впоследствии приводило к истощению и потере иммунного контроля.

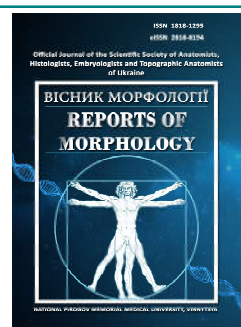
**Ключевые слова:** бактериальный вагиноз, показатель нормобиоты, иммунная система.



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# Morphometric study of angioarchitectonic under the effect of opioid (experimental study)

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*Opioids are potent drugs that are widely used to combat pain in severe wounds and in cancer patients. The professional literature provides a limited amount of data on the morphometric analysis of the links of the hemomicrocirculatory bed of the cerebellar cortex and choroid of the eyeball under the influence of an opioid in the experiment. The aim of the work was to establish the features and conduct morphometric studies of the links of the hemomicrocirculatory bed of the cerebellar cortex and choroid in normal conditions and under conditions of 6-week exposure to an opioid in the experiment. The experimental study was carried out on 24 male white rats, 3 months old and weighing 160-180 g. The material for the study is represented by preparations of the choroid and cerebellum with an injected vascular bed. Compared with the control group of animals, profound destructive changes in the angioarchitectonics of the choroid and cerebellar cortex were found under the influence of Nalbuphine. The capillary component is destroyed, the arterioles are tortuous, sclerosed, their lumen is uneven, the wall is thickened, the venules are dilated and deformed. A decrease in comparison with the norm in the diameter of arterioles, the density of the network of exchange vessels, arterio-venular coefficient, as well as an increase in the diameter of venules and an indicator of trophic activity of the tissue. Expansion of arterio-venular anastomoses indicates the discharge of blood from the arterioles into the venous bed, bypassing the destroyed capillaries. A pronounced relationship was established between the depth of structural transformations of the hemomicrocirculatory bed of the cerebellar cortex, choroid of the white rat's eye and morphometric parameters. It has been confirmed that the triggering mechanism of destructive changes under the influence of narcotic analgesics is the development of angiopathy.*

**Keywords:** morphometry, opioids, experiment, eyeball, cerebellum, vascular tunic.

### Introduction

It is well-recognized that, despite similar pain characteristics, some people with chronic pain recover, whereas others do not [1]. Opioids are potent drugs that are widely used to control cancer pain and are routinely used analgesics in patients with chronic wounds [17, 21]. Increasing evidence suggest that opioids mediate clinically relevant effects that go beyond their classical role as analgesics [5, 16]. However, it is undeniable that the long-term and systematic use of opioids can cause structural changes of organs and systems [11, 24].

The significant metabolic and structural changes, early disability, and mortality in opioid-dependent patients require the study of pathogenetic mechanisms of the development and progression of multiple polyorganic comorbid conditions [9, 10]. Nowadays, a number of unresolved

issues regarding the features of the hemomicrocirculatory bed of cerebellar cortex and eyeball in norm and after long-term opioid influence remain, what makes the current study relevant.

Nalbuphine is a synthetic kappa-receptor (KOR) agonist opioid and partial mu-receptor (MOR) antagonist which has been synthesized in an attempt to provide analgesia without the undesirable side effects of pure agonists. The central and peripheral analgesic action and the lower risk of addiction/dependence potential seems to be related to the agonist action on KOR, while its MOR antagonist action would be responsible for its lower risk of respiratory depression [3, 25].

It is used to treat moderate to severe pain and provide preoperative and postoperative analgesia and sedation



[4, 20]. Endothelial cells (human arterial and rat microvascular) contain a high affinity, saturable opiate binding site [22].

The aim of the work was to establish the features and conduct morphometric studies of the links of the hemomicrocirculatory bed of the cerebellar cortex and choroid in normal conditions and under conditions of 6-week exposure to an opioid in the experiment.

### Material and methods

The study was carried out on 24 mature white male rats aged 3.0 months and body weight 160-180 g. The injection of Nalbuphine hydrochloride (Rusanpharma LTD., India) was done intramuscularly with a gradual weekly increase in dosage: from 8 mg/kg to 35 mg/kg. Sampling of the material was carried out after 6 weeks of drug injection. The control group consisted of 9 white rats to which saline solution (Indar, Kyiv, Ukraine) was injected. The experiments were conducted in compliance with the provisions of the "Guide for the care and use of laboratory animals, 8th edition, 2011". Euthanasia was performed by way of overdosing intraperitoneal anesthesia using Thiopental sodium (Kyivmedpreparat, Kyiv, Ukraine).

The material is represented by the preparations of eyeballs and cerebellum of rats with injected vascular way. An injection mass consisting of a mixture of 20% solution of collargol and glycerol in a 2 : 1 ratio was used for the injection of the bloodstream.

For morphometric analysis of the vessels of hemomicrocirculatory bed of the eyeball and cerebellar cortex, the following quantitative criteria were used: microvascular diameter, arteriolar-venular coefficient, tortuosity coefficient, density of mesh of exchange vessels, index of trophic tissue activity. Statistical analysis of the results of the study was performed by a computer program "InStat" for biomedical and epidemiological studies.

### Results

The layer of veins, the layer of arteries, and the capillary layer are clearly distinguished in the choroid of the rat (Fig. 1). Arterial branches of the annular arteries of the iris are included in the ciliary processes, branching into numerous anastomosing microvessels, which form a thick mesh of broad capillaries. Capillary nets occupy almost the entire volume of ciliary processes. Along the edges of the ciliary processes, veins extend through which the venous blood drains into the venous plexus which is behind the ciliary muscle. The blood supply of the ciliary muscle is provided by the branches of the iris vessels and the choroid, which anastomoses with each other.

In the iris of the white rat clearly visible are annular arteries of the iris, which, arcuately arched, go towards each other and, anastomosing with each other in the anterior and posterior regions of the iris, form a large arterial circle of the iris. From the large arterial circle of the iris, many branches are going both to the ciliary body and the iris.



Fig. 1. The angioarchitectonic of the choroid of the control animal (rat) eye ball. Microphoto. x200. Injection of blood vessels by ink gelatin mass. 1 - arteriole; 2 - capillaries.

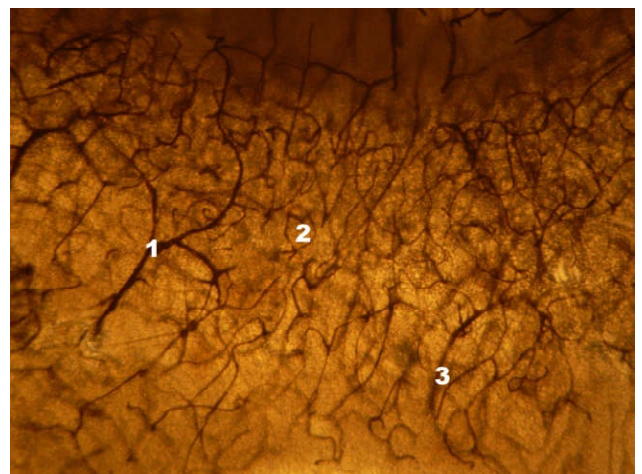


Fig. 2. Vascularization of the cerebellar cortex of the white rat in norm. Microphoto. x160. Injection of blood vessels by ink gelatin mass. 1 - arteriole; 2 - capillary net; 3 - venules.

Arterioles of the iris go radially to the pupil margin, where a capillary loop mesh surrounding the pupil is formed. Capillary loop mesh of the pupillary edge of the iris is gentle, the loops are thin.

Under morphometric study of angioarchitectonic in norm - the diameter of arterioles -  $(21.80 \pm 2.30) \mu\text{m}$ , the diameter of venules -  $(27.09 \pm 1.44) \mu\text{m}$ , capillaries of ciliary processes -  $(6.40 \pm 0.34) \mu\text{m}$ .

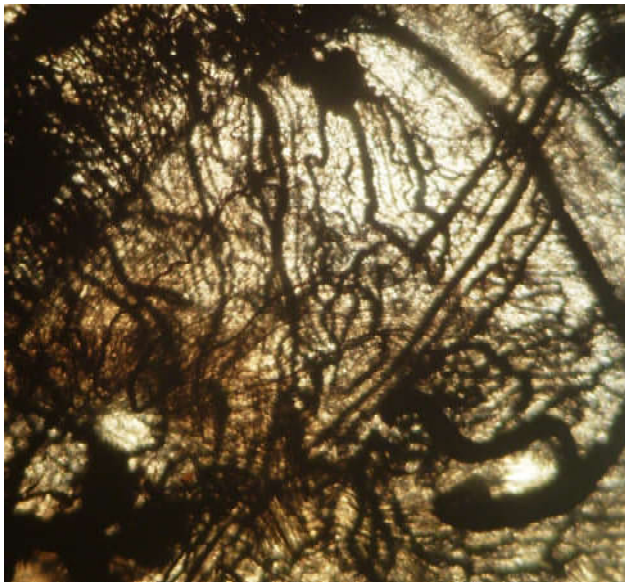
The vascularization of the cerebellum of the white rat is mainly provided by the right and left nasal and right and left caudal ventral arteries, which are the branches of the basilar artery, and the caudal dorsal arteries, which are branches of the vertebral arteries. The cerebellar arteries, penetrating into the cerebellum, separate and form in the cerebellar cortex a hemomicrocirculatory bed containing classically arterioles, precapillary arterioles, capillaries, postcapillary venules and venules. Anastomosing capillaries form a capillary net in the cerebellar cortex (Fig. 2).

According to the results of morphometric studies of the vessels of the hemomicrocirculatory bed of the cerebellar cortex of the white rat, the following values of morphometric





**Fig. 3.** Expansion of the arteriolar component of the cerebellar cortex of the white rat after 6 weeks of injection of Nalbuphine. Injection of blood vessels by ink gelatin mass. Microphoto. x160. 1 - arteriolar component; 2 - cerebellar artery.



**Fig. 4.** Deep destructive changes of the links of the hemomicrocirculatory bed of the iris of the eyeball of white rat under conditions of 6-week administration of Nalbuphine. Microphoto. x100.

parameters were obtained in norm: the diameter of arterioles -  $(20.58 \pm 0.32) \mu\text{m}$ , venules -  $(29.00 \pm 0.20) \mu\text{m}$ , respectively, arteriolar-venular coefficient is  $0.71 \pm 0.01$ . The diameter of the capillaries of the cerebellar cortex of the white rat in norm -  $(5.81 \pm 0.01) \mu\text{m}$ , the density of the mesh of the exchange vessels is  $62.20 \pm 2.19$ , the trophic activity of the cerebellar cortex tissue is  $(45.62 \pm 0.97) \mu\text{m}$ .

After 6 weeks of experimental study, deep destructive changes of vessels of hemomicrocirculatory bed of the vascular tunic of the eyeball and cerebellar cortex are observed.

The arteriolar component of the cerebellar cortex is significantly enlarged (Fig. 3). The diameter of the arterioles at this time of the experiment is  $(35.46 \pm 2.14) \mu\text{m}$ , the control

is  $(20.40 \pm 0.68) \mu\text{m}$ . During this period arteriolar-venular anastomoses widen in the cerebellar cortex. Vascular loops of the cerebellar cortex lose a delicate, tortuous pattern, often breaking off.

The diameter of the cerebellar cortex capillary after 6 weeks of administration of Nalbuphine is  $(8.25 \pm 0.94) \mu\text{m}$ , control -  $(5.80 \pm 0.10) \mu\text{m}$ . Increased vascular permeability, hemorrhage, microaneurysms were detected.

It is quite natural that stagnation and disturbance of outflow of venous blood cause expansion of the venular component, which is confirmed by a significant increase in the diameter of venules to  $(48.34 \pm 2.42) \mu\text{m}$ , control -  $(28.24 \pm 0.19) \mu\text{m}$  in morphometric analysis. The change in the arteriolar-venular coefficient is evident, which at this stage of the study is  $0.74 \pm 0.01$ . There is a change in the density of vessels, violation of their integrity, which is confirmed by the following changes in morphometric parameters. The density of the mesh of the exchanged vessels decreases sharply, this can be explained by the development of significant destruction, mainly of the cerebellar cortex capillaries, and is  $33.80 \pm 4.42$  (control -  $62.10 \pm 0.36$ ). The index of tissue trophic activity increases to  $(67.70 \pm 2.34) \mu\text{m}$  control -  $(47.64 \pm 0.78) \mu\text{m}$ . Responsive destructive changes in the vessels of the hemomicrocirculatory bed are observed in the choroid of the eyeball after 6 weeks of the experiment (Fig. 4).

According to morphometric parameters: the diameter of the arterioles is  $(28.42 \pm 0.41) \mu\text{m}$ , control  $(21.80 \pm 2.30) \mu\text{m}$ ; the diameter of the venules increased to  $(48.74 \pm 2.59) \mu\text{m}$ , the control -  $(27.09 \pm 1.44) \mu\text{m}$ , which led to a probable decrease of the arteriolar-venular coefficient to  $0.58 \pm 0.03$ , the control -  $0.8 \pm 0.07$ . The density of the mesh of the exchange vessels was reduced to  $64.00 \pm 5.82$ , the control was  $120.40 \pm 7.53$ , and the index of tissue trophic activity increased to  $(31.94 \pm 2.61) \mu\text{m}$ , the control -  $(18.15 \pm 0.74)$ . The diameter of the capillaries of the ciliary processes is  $(9.76 \pm 0.51) \mu\text{m}$ , control -  $(6.40 \pm 0.34) \mu\text{m}$ .

In both described organs there is an expansion of arteriolar-venular anastomoses, which indicates the discharge of blood from the arterioles into the venous way, bypassing the destroyed capillaries.

### Discussion

The results of the present work are a fragment of the scientific research project "The structure of organs and their bloodstream in ontogenesis under the effect of laser irradiation and pharmaceutical agents in cases of blood supply disorders, reconstructive surgeries and diabetes mellitus" (State Registration Number 0110U001854), being conducted at the Department of Normal Anatomy, Danylo Halytsky Lviv National Medical University.

The number of people consuming narcotic analgesics does not decrease, but on the contrary tends to increase [5, 16]. Nalbuphine is an agonist of  $\kappa$ -opioid receptors and a partial antagonist of  $\mu$ -opioid receptors, which can stimulate  $\kappa$ -receptors and antagonize the acute rewarding

effects of morphine, has a short duration of action and rapid clearance compared with other opioids and is less likely to cause side effects [15]. It is widely used either as an analgesic or as an adjuvant with morphine [4, 12, 19]. For this reason, the number of requests for opioids in Pubmed has traditionally remained the same. The relevance of the topic of drug use and drug dependence has led to numerous animal experiments (white Wistar rats) to study the macro-, micro- and ultrastructure of organs and systems under the influence of opioids [8, 13]. The study of microscopic and electron microscopic level revealed the pattern of primary lesion of the vascular component. This prompted the study of angioarchitectonics to evaluate the microcirculatory bed [7, 14].

A number of authors describe that vessels of the hemomicrocirculatory bed are most likely to respond to the influence of environmental factors, and therefore one of the first to undergo structural restructuring, which is the basis for the development of the pathological process [2, 6, 18, 23]. Since no numerical values of morphometric indices of white rats of different ages were found in the analysis of professional literature, a model for comparing long-term opioid (Nalbuphine) versus control was selected.

Expansion of arterioles and venules during this study period, compared to control, is due to the inability of these vessels to cope with significant blood flow. In our opinion, the dilation of the veins of the choroid of the eyeball at this time of the experiment contributes to the increasing in the capacity of the venular part of the hemomicrocirculatory bed, tortuosity of arterioles provides a decrease in the force of the pulse impulse in these vessels, the appearance of additional arteriolar-venular anastomoses and its activation leads to increasing of capillar bloodflow. Dilation of arteriolar-venular anastomoses of the vascular membrane of the eyeball and cerebellar cortex indicates the discharge of blood from the arterioles into the venous way, bypassing the ruptured capillaries. Additional anastomoses are opened. We consider this as a compensatory mechanism that enhances the flow and, accordingly, provides the unloading of the destroyed capillary nets of both us and other organs [26]. Therefore, the preparations clearly visualize "empty" areas where there is no capillary

component. Actually, these changes in angioarchitectonics determine the nature and features of clinical manifestations of the pathological process.

The presented experimental study article differs from the one published in this field in that it describes new data on the effect of opioids on the features of angioarchitecture of two organs: the vascular sheath of the eyeball and the cerebellar cortex of the white rat. Mathematical analysis allowed to systematize the obtained experimental data and represent a comparative characteristic of angioarchitectonics of the studied structures in normal and pathological conditions. It is valuable that morphometric data from control animals can be used as a norm in future studies. The findings allow us to broaden our knowledge and address the conflicting issues of the effects of opioids on the eyeball and cerebellum. The structure of the hemomicrocirculatory way creates a morphological basis for understanding the pathogenesis of ophthalmic and neurological diseases of drug users and patients who have to take opioids for a long time and to decide on optimal treatment tactics. The findings are important for both morphologists and clinicians.

### Conclusions

Thus, the morphometric analysis of the vessels of the hemomicrocirculatory bed of the eyeball and the cerebellar cortex of the white rat allowed us to estimate the degree of vascularization in norm and under prolonged exposure to Nalbuphine. There is a clear relationship between the depth of structural transformations of the vessels of hemomicrocirculatory bed of the cerebellar cortex and the choroid of the eyeball and morphometric parameters in the experiment.

The deformation of the arterioles and the irregularity of their lumen, the destruction of the capillary component, significant dilation and deformation of the venules were revealed. Reduction, in comparison with control, diameter of arterioles, density of a net of exchange vessels, arteriolar-venular coefficient. An increasing of the diameter of venules, of trophic activity of the tissue testify to the destructive changes of the hemomicrocirculatory bed of the cerebellar cortex and the choroid of the eyeball under the influence of Nalbuphine.

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#### МОРФОМЕТРИЧНЕ ВИВЧЕННЯ АНГІОАРХІТЕКТОНІКИ ПІД ВПЛИВОМ ОПІОІДУ (ЕКСПЕРИМЕНТАЛЬНЕ ДОСЛІДЖЕННЯ)

**Матешук-Вацеба Л.Р., Підвальна У.Є., Бекесевич А.М.**

Опіоїди - сильноїючі препарати, що широко застосовуються у боротьбі з болем при важких пораненнях та в онкозахворих. Фахова література наводить обмежену кількість даних морфометричного аналізу ланок гемомікроциркуляторного русла кори мозочка та судинної оболонки очного яблука під впливом опіоїду в експерименті. Метою дослідження було встановити особливості та провести морфометричне дослідження ланок гемомікроциркуляторного русла судинної оболонки очного яблука і кори мозочка в нормі за умов 6-тижневого впливу опіоїду в експерименті. Експериментальне дослідження проведено на 24 білих щурах самцях віком 3 міс і вагою 160-180 г. Матеріал для дослідження представлений препаратами судинної оболонки ока і мозочка з ін'єктованим судинним руслом. У порівнянні з контрольною групою тварин виявлено глибокі деструктивні зміни ангіоархітектоніки судинної оболонки очного яблука та кори мозочка під впливом налбуфіну. Капілярний компонент зруйнований, артеріоли звивисті, просвіт їх нерівномірний, стінка їх потовщена, склерозована, венули розширені та деформовані. Встановлено зменшення, порівняно з нормою, діаметра артеріол, щільності сітки обмінних судин, артеріоло-венулярного коефіцієнта, а також збільшення діаметра венул та показника трофічної активності тканини. Розширення артеріоло-венулярних анастомозів свідчить про скидання крові з артеріол до венозного русла, минаючи зруйновані капіляри. Виявлено виражений зв'язок між глибиною морфологічних змін ланок гемомікроциркуляторного русла кори мозочка, судинної оболонки ока білого щура та морфометричними показниками. Підтверджено, що пусковим механізмом деструктивних змін під впливом наркотичних анальгетиків є розвиток ангіопатії.

**Ключові слова:** морфометрія, опіоїди, експеримент, око, мозочок, судинна оболонка.

**МОРФОМЕТРИЧЕСКОЕ ИЗУЧЕНИЕ АНГИОАРХИТЕКТониКИ ПОД ВОЗДЕЙСТВИЕМ ОПИоиДА (ЭКСПЕРИМЕНТАЛЬНОЕ ИССЛЕДОВАНИЕ)**

**Матешук-Вацеба Л.Р., Пидвальна У.Е., Бекесевич А.М.**

*Опиоиды - сильнодействующие препараты, широко применяемые в борьбе с болью при тяжелых ранениях и у онкобольных. Профессиональная литература приводит ограниченное количество данных морфометрического анализа звеньев гемомикроциркуляторного русла коры мозжечка и сосудистой оболочки глазного яблока под влиянием опиоида в эксперименте. Целью работы было установить особенности и провести морфометрические исследования звеньев гемомикроциркуляторного русла коры мозжечка и сосудистой оболочки глазного яблока в норме и в условиях 6-недельного воздействия опиоида в эксперименте. Экспериментальное исследование проведено на 24 белых крысах-самцах возрастом 3 мес и весом 160-180 г. Материал для исследования представлен препаратами сосудистой оболочки глаза и мозжечка с инъецированным сосудистым руслом. По сравнению с контрольной группой животных обнаружены глубокие деструктивные изменения ангиоархитектоники сосудистой оболочки глазного яблока и коры мозжечка под влиянием налбуфина. Капиллярный компонент разрушен, артериолы извилистые, склерозированные, просвет их неравномерный, стенка утолщена, венулы расширенные и деформированные. Установлено уменьшение, по сравнению с нормой, диаметра артериол, плотности сетки обменных сосудов, артериоло-венулярного коэффициента, а также увеличение диаметра венул и показателя трофической активности ткани. Расширение артериоло-венулярных анастомозов свидетельствует о сбросе крови из артериол в венозное русло, минуя разрушенные капилляры. Установлена выраженная связь между глубиной структурных преобразований гемомикроциркуляторного русла коры мозжечка, сосудистой оболочки глаза белой крысы и морфометрическими показателями. Подтверждено, что пусковым механизмом деструктивных изменений под воздействием наркотических анальгетиков является развитие ангиопатии.*

**Ключевые слова:** морфометрия, опиоиды, эксперимент, глаз, мозжечок, сосудистая оболочка.

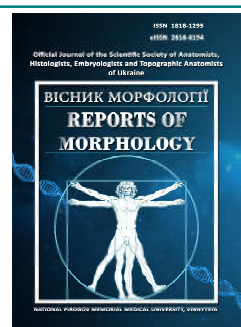
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# Features of cephalometric parameters, which usually do not change during surgery and orthodontic interventions, in Ukrainian young men and women with orthognathic occlusion and different types and profiles of the face according to Schwarz A.M.

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*During the existence of the X-ray method of research, numerous author's methods of cephalometric examination and analysis using the method of lateral teleroentgenography (methods by Schwarz, Down's, Steiner, Tweed's, Harvold, Ricketts, McNamara, Jaraback, Burstone, Bjork, etc.) have been developed. Dentists around the world prefer certain methods, each of which has its advantages and disadvantages. But there is still no single unified methodology for such research and doctors and researchers are constantly trying to improve them and gain new information about their effectiveness. The aim of the study was to establish cephalometric teleroentgenographic parameters and determine their features in Ukrainian young men and women with orthognathic occlusion depending on profiles and facial types according to Schwarz A.M. (hereinafter orthognathic occlusion), divided into separate groups of studies on different profiles and different face types according to Schwarz A.M. (3 groups of different profiles and 3 groups of different face types for people of each sex). Cephalometric teleroentgenographic parameters, which usually do not change during surgery and orthodontic interventions, were studied. Statistical mathematical processing of the research results was performed in the licensed package "Statistica 6.0" using non-parametric methods of evaluation of the obtained results. Numerical reliable and tendencies of differences in certain indicators between groups of young men or women with different profiles or face types have been established. The greatest differences, both in young men and women, were recorded between the indicators determined by the methods of cephalometry by Schwarz A.M. and Bjork A., and the least - by the method of Ricketts R.M. The obtained results testify to the expediency of using the division of young men and women into separate groups by profile and type of face to adhere to the personalized principle in the morphological assessment of cephalometric and gnathometric indicators of lateral teleroentgenography.*

**Keywords:** young men, young women, orthognathic occlusion, lateral teleroentgenography of the head, cephalometric parameters, types and profiles of the face according to Schwarz A.M.

### Introduction

Establishing morphometric parameters of various organs and structures of the human body has always been one of the main tasks of human anatomy as a basic science. This fully applies to craniometry, determination of morphometric parameters of craniofacial structures, morphometric parameters of the human dental system, and so on. Accumulated over hundreds of years of observations and research, a huge amount of factual material needs

constant updating and detailing to determine the characteristics of the values of these indicators in the population of different racial, sex, population, age, ethnic groups, etc. [17, 22, 28].

Conducting cephalometric examinations and taking into account cephalometric parameters is extremely important in practical dental activities, especially in recent decades due to the widespread introduction in diagnostic practice of



radiological methods, one of which is the method of teleroentgenography of the head - obtaining a survey image of the head, skull projections by X-ray examination [20, 21].

The use of teleroentgenography allows to obtain a lifetime image that best corresponds to the actual size of the head and its bone structures and their location and allows to obtain not only qualitative but also important quantitative morphometric parameters. An important advantage of this research method is the ability to display not only bone structures but also the contours of the soft tissues of the face [6, 22, 23].

Lateral teleroentgenography with determination of cephalometric parameters, indicators of the dental system are an extremely important diagnostic method in dentistry, especially in orthodontics, maxillofacial surgery, orthopedic dentistry to determine the structure of the cerebral and facial skull, features of jaw structures, assessment of symmetry determination of congenital or acquired pathological changes of the dental system, etc. [26, 29].

This method of research and its results are important at all stages of patient management, starting from planned or urgent diagnosis and further to plan the nature and extent of possible intervention and evaluate the effectiveness of treatment, both early and long-term [7, 12, 25].

During the existence of the X-ray method of research, numerous author's methods of cephalometric research and analysis using the method of lateral teleroentgenography have been developed. The most famous are the methods of Schwarz, Down's, Steiner, Tweed's, Harvold, Ricketts, McNamara, Jaraback, Burstone, Bjork, which have found their supporters in different countries around the world. Each of the author's methods is important, quite informative, has both its advantages and disadvantages. But the very existence of such a large number of methods of cephalometric analysis, instead of one unified, indicates their certain imperfection, which is the subject of discussion among scientists and practitioners and encourages further development and improvement of the methodological framework in this direction. It is also important that the author's indicators in most cases are obtained on certain contingents of the population of individual countries. At the same time, a number of studies indicate the presence of significant differences in the results depending on racial, ethnic, population, sex and other group characteristics of the studied patients [1-5, 8, 9, 11, 13].

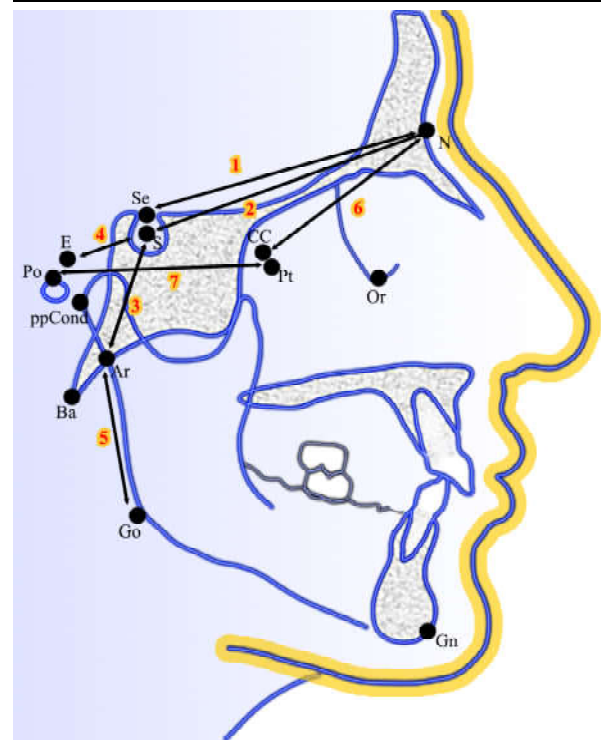
The aim of the work is to establish cephalometric teleroentgenographic indicators and determine their features in Ukrainian young men and young women with orthognathic occlusion depending on the profiles and face types according to Schwarz A.M.

**Materials and methods**

Lateral teleroentgenograms were studied and analyzed in 49 young men (age group from 17 to 21 years) and 76 young women (age group from 16 to 20 years) with

**Table 1.** Quantitative distribution of young men and women depending on the profile of the face or type of face.

Research groups	Young men	Young women
1 profile (back face profile by Schwarz A.M.)	23	37
2 profile (straight face profile by Schwarz A.M.)	9	15
3 profile (front face profile by Schwarz A.M.)	17	24
1 type (back face type by Schwarz A.M.)	13	23
2 type (average face type by Schwarz A.M.)	18	24
3 type (front face type by Schwarz A.M.)	18	29



**Fig. 1.** Cephalometric distances of baseline parameters, which usually do not change during surgery and orthodontic interventions. 1 - distance N-Se; 2 - distance N-S; 3 - distance S-ar; 4 - distance S-E; 5 - ar-Go distance; 6 - distance N-CC; 7 - P-PTV distance.

physiological occlusion as close as possible to orthognathic (hereinafter orthognathic occlusion) (part of primary cephalometric parameters obtained from the bank data of the research center of National Pirogov Memorial Medical University, Vinnytsya). Groups of young men and young women of different profiles and different types of faces were formed according to Schwarz A.M. [27, 28] (Table 1).

Teleroentgenography was performed on a dental cone-beam tomograph Veraviewepocs 3D Morita (Japan).

Cephalometric analysis was performed using licensed medical software OnyxCeph<sup>3</sup>™, version 3DPro (Image Instruments GmbH, Germany), which is designed for image management and analysis in dentistry for diagnosis, treatment and presentation of patients in general dentistry, orthodontics, orthopedics, implantology, facial surgery, etc.

Cephalometric points were determined in accordance



**Fig. 2.** Cephalometric angles of baseline parameters, which usually do not change during surgery and orthodontic interventions. 10 - angle H; 11 - angle N-S-Ar; 12 - angle N-S-Ba; 13 - angle POr-NBa.

with the recommendations of Phulari B.S. [24] and Doroshenko S.I. and Kulginsky E.A. [19].

Figures 1 and 2 show the cephalometric points, lines, distances and angles that were determined.

Cephalometric points (see Fig. 1):

N (nasion) - the most anterior point of the fronto-nasal suture (connection of the frontal bone and nasal bone in the plana sagittalis);

SE (sellia turcica entru) - a constructive point in the middle of the distance between the posterior and anterior inclined processes of the sphenoid bone;

E - structural point is located at the intersection of the perpendicular from the point ppCond (the most dorsally located point of the mandibular head) to the line S-N;

S (sella) - constructive point in the center of the sella turcica;

CC (center of cranium according to Ricketts) - constructive point at the intersection of Ba-N and Pt-Gn lines;

Pt (pterygomaxillare) - the upper distal point of the pterygomandibular cleft, located at the intersection of the round hole with the posterior wall of the pterygomandibular cleft;

Po (porion) - is located on the upper edge of the external auditory canal;

Or (orbitale) - the lowest part of the infraorbital margin, located on the orbital margin of the chin bone;

Ar (articulare) - the intersection of the anterior surface of

the main part of the occipital bone with the posterior surface of the neck of the mandible;

Ba (basion) - the lowest point of the anterior edge of the large occipital foramen in the plana sagittalis;

Go (gonion) - the posterior point on the lower contour of the body of the lower jaw.

In this study, we, according to the author's methods of cephalometric analysis of lateral teleroentgenograms, determined a group of basic, key cephalometric parameters that usually do not change during surgery and orthodontic interventions and in relation to which during the analysis of lateral radiographs determine the inclination, anteroposterior or vertical location (upper and lower jaws, closing plane, individual teeth) (see Fig. 1, Fig. 2):

- according to Schwarz A.M.: distance N-Se - distance from point Se to point N, or length of the front part of the skull base (mm); angle H is the angle formed by the lines Po-Or (Frankfurt plane (Fp)) and Pn (nasal perpendicular, perpendicular line from point N '(skin nasion) to line Se-N)), determines the angle of inclination of the Frankfurt plane to the base skull (°);

- according to Bjork A.: angle N-S-Ar (saddle angle) - the angle between the anterior cranial base and the lateral cranial base, which determines the position of the temporomandibular joint and glenoid fossae and is formed by lines N-S and S-ar (°); angle N-S-Ba - the angle formed by the lines S-N (front of the skull base) and S-Ba (°); N-S index: S-Ar' - indicator of the ratio of distances ar'-S and N-S;

- by Jarabak J. R.: distance N-S - length of the anterior cranial base, the distance from point N to point S (mm); distance S-ar - the length of the lateral cranial base, the distance from point S to point ar (mm); indicator S-ar:ar-Go - indicator of the ratio of S-ar and ar-Go distances;

- according to Steiner C. C.: distance S-E - length of the posterior part of the skull base (according to Steiner), distance from point S to structural point E (mm);

- by Burstone C. J.: distance ar-Go - the length of the branch of the mandible, the distance from the point Ar to the point Go (mm);

- according to Ricketts R.M.: angle POr-NBa - angle of cranial inclination (deflection), formed by lines Po-Or and Ba-N (°); distance N-CC - anterior length of the skull base, distance from point N to point SS (mm); distance P-PTV - the distance from point Po to point Pt, parallel to the Frankfurt plane (mm).

Statistical mathematical processing of the research results was performed in the licensed package "Statistica 6.0" using non-parametric methods of evaluation of the obtained results. The reliability of the difference between the values between the independent quantitative values was determined using the U-test of Mann-Whitney.

## Results

When comparing the values of N-Se distance in young men of different facial profiles, we found statistically significant differences: higher values of this indicator in

**Table 2.** Cephalometric indicators of N-Se, H, S-E and ar-Go in young men and women with different profiles or with different face types.

Groups	Young men			Young women		
	(M±σ)	p		(M±σ)	p	
N-Se (mm)						
Profile 1	67.70±2.18	p <sub>1-2</sub>	<0,05	64.89±6.33	p <sub>1-2</sub>	=0.090
Profile 2	69.78±2.28	p <sub>1-3</sub>	<0,01	65.53±2.10	p <sub>1-3</sub>	>0.05
Profile 3	74.65±15.46	p <sub>2-3</sub>	>0,05	66.04±5.05	p <sub>2-3</sub>	>0.05
Type 1	68.38±2.96	p <sub>1-2</sub>	>0,05	65.27±3.84	p <sub>1-2</sub>	>0.05
Type 2	72.56±15.24	p <sub>1-3</sub>	>0,05	65.67±6.96	p <sub>1-3</sub>	>0.05
Type 3	69.94±3.84	p <sub>2-3</sub>	>0,05	65.24±4.79	p <sub>2-3</sub>	>0.05
H (°)						
Profile 1	95.35±2.85	p <sub>1-2</sub>	>0,05	95.32±2.51	p <sub>1-2</sub>	>0.05
Profile 2	94.44±2.13	p <sub>1-3</sub>	<0,01	94.13±2.33	p <sub>1-3</sub>	<0.01
Profile 3	91.88±3.30	p <sub>2-3</sub>	<0,05	92.96±3.21	p <sub>2-3</sub>	>0.05
Type 1	96.31±2.75	p <sub>1-2</sub>	<0,05	96.17±2.48	p <sub>1-2</sub>	<0.01
Type 2	93.44±3.09	p <sub>1-3</sub>	<0,01	93.88±1.75	p <sub>1-3</sub>	<0.01
Type 3	92.83±3.03	p <sub>2-3</sub>	>0,05	93.28±3.27	p <sub>2-3</sub>	>0.05
S-E (mm)						
Profile 1	21.22±2.65	p <sub>1-2</sub>	>0,05	19.43±2.58	p <sub>1-2</sub>	>0.05
Profile 2	20.11±2.93	p <sub>1-3</sub>	>0,05	18.67±1.59	p <sub>1-3</sub>	>0.05
Profile 3	21.82±5.95	p <sub>2-3</sub>	>0,05	18.13±3.86	p <sub>2-3</sub>	>0.05
Type 1	21.62±2.53	p <sub>1-2</sub>	>0,05	20.30±2.49	p <sub>1-2</sub>	>0.05
Type 2	21.67±5.77	p <sub>1-3</sub>	>0,05	19.00±2.38	p <sub>1-3</sub>	<0.01
Type 3	20.50±2.96	p <sub>2-3</sub>	>0,05	17.62±3.18	p <sub>2-3</sub>	<0.05
ar-Go (mm)						
Profile 1	50.92±4.87	p <sub>1-2</sub>	=0,094	46.45±5.55	p <sub>1-2</sub>	>0.05
Profile 2	54.08±3.46	p <sub>1-3</sub>	<0,05	48.19±4.06	p <sub>1-3</sub>	=0.074
Profile 3	57.59±12.66	p <sub>2-3</sub>	>0,05	48.58±5.97	p <sub>2-3</sub>	>0.05
Type 1	50.29±4.77	p <sub>1-2</sub>	>0,05	46.90±3.63	p <sub>1-2</sub>	>0.05
Type 2	54.79±12.93	p <sub>1-3</sub>	<0,01	47.31±6.33	p <sub>1-3</sub>	>0.05
Type 3	55.38±4.06	p <sub>2-3</sub>	<0,05	48.07±6.02	p <sub>2-3</sub>	>0.05

**Notes:** here and in the following tables, p<sub>1-2</sub>, p<sub>1-3</sub>, p<sub>2-3</sub> - the significance of differences in relevant indicators between young men or women of relevant profiles or face types.

groups of young men with the second and third facial profiles, compared with young men of the first facial profile (respectively, p<0.05 and p<0.01) (Table 2). Among groups of young women with different facial profiles, this indicator showed only a lower value in persons with the first profile, compared with young women with the second profile (p=0.090) (see Table 2).

The values of the angle H in young men with the first and second facial profiles are significantly higher than in young men with the third facial profile (respectively, p<0.01 and p<0.05), and in young men with the first type of face - significantly higher than in young men with the second and with the third face type (respectively, p<0.05 and p<0.01)

(see Table 2). In young women with the first facial profile, this figure was higher than in young women with the third facial profile (p<0.01), and in young women with the first type of face - greater than in young women with the second and third facial types (p<0.01 in both cases) (see Table 2).

The S-E distance in young women with the first and second face types is significantly greater than in young women with the third face type (respectively, p<0.01 and p<0.05) (see Table 2).

The ar-Go distance in young men with the third facial profile was significantly greater than in young men with the first profile (p<0.05), and in young men with the second profile - slightly greater than in young men with the first facial profile (p=0.094). There is only a tendency (p=0.074) to higher values of ar-Go distance in young women with the third facial profile, compared with young women with the first profile (see Table 2).

The values of the angle N-S-Ar, both in young men and young women with the first profile of the face are significantly higher than in young men or young women with the third profile (p<0.05 in both cases); also slightly more important in young women with the first facial profile than in young women with the second profile (p=0.082) (Table 3). Young women with the first type of face have a significantly larger N-S-Ar angle than young women with the second and third

**Table 3.** Cephalometric indicators of N-S-Ar, N-S-Ba and N-S:S-Ar in young men and women with different profiles or with different face types.

Groups	Young men			Young women		
	(M±σ)	p		(M±σ)	p	
N-S-Ar (°)						
Profile 1	126.9±5.1	p <sub>1-2</sub>	>0.05	126.3±4.9	p <sub>1-2</sub>	=0.082
Profile 2	125.8±6.6	p <sub>1-3</sub>	<0.05	124.2±4.2	p <sub>1-3</sub>	<0.05
Profile 3	123.3±5.5	p <sub>2-3</sub>	>0.05	122.2±7.2	p <sub>2-3</sub>	>0.05
Type 1	127.8±5.4	p <sub>1-2</sub>	>0.05	128.3±5.1	p <sub>1-2</sub>	<0.01
Type 2	124.6±4.7	p <sub>1-3</sub>	>0.05	124.4±4.0	p <sub>1-3</sub>	<0.001
Type 3	124.6±6.5	p <sub>2-3</sub>	>0.05	121.9±6.3	p <sub>2-3</sub>	>0.05
N-S-Ba (°)						
Profile 1	130.2±4.6	p <sub>1-2</sub>	>0.05	131.2±5.2	p <sub>1-2</sub>	=0.055
Profile 2	131.0±5.8	p <sub>1-3</sub>	<0.05	128.5±3.8	p <sub>1-3</sub>	<0.05
Profile 3	126.2±5.7	p <sub>2-3</sub>	>0.05	127.6±6.9	p <sub>2-3</sub>	>0.05
Type 1	131.2±4.0	p <sub>1-2</sub>	>0.05	132.2±5.7	p <sub>1-2</sub>	<0.05
Type 2	128.4±6.1	p <sub>1-3</sub>	>0.05	129.5±3.7	p <sub>1-3</sub>	<0.05
Type 3	128.0±5.6	p <sub>2-3</sub>	>0.05	127.4±6.4	p <sub>2-3</sub>	>0.05
N-S:S-Ar						
Profile 1	3.422±0.460	p <sub>1-2</sub>	>0.05	3.589±0.470	p <sub>1-2</sub>	>0.05
Profile 2	3.778±0.618	p <sub>1-3</sub>	>0.05	3.740±0.307	p <sub>1-3</sub>	=0.082
Profile 3	3.694±0.612	p <sub>2-3</sub>	>0.05	4.113±1.153	p <sub>2-3</sub>	>0.05
Type 1	3.431±0.477	p <sub>1-2</sub>	>0.05	3.435±0.473	p <sub>1-2</sub>	<0.05
Type 2	3.578±0.544	p <sub>1-3</sub>	>0.05	3.721±0.461	p <sub>1-3</sub>	<0.01
Type 3	3.694±0.622	p <sub>2-3</sub>	>0.05	4.114±1.000	p <sub>2-3</sub>	>0.05



**Table 4.** Cephalometric indicators of N-S, S-ar and S-ar:ar-Go in young men and women with different profiles or with different face types.

Groups	Young men			Young women		
	(M±σ)	p		(M±σ)	p	
N-S (mm)						
Profile 1	69.26±2.07	p <sub>1-2</sub>	<0.05	66.46±6.34	p <sub>1-2</sub>	=0.092
Profile 2	71.22±1.99	p <sub>1-3</sub>	<0.01	66.93±2.43	p <sub>1-3</sub>	>0.05
Profile 3	75.65±15.68	p <sub>2-3</sub>	>0.05	67.63±4.89	p <sub>2-3</sub>	>0.05
Type 1	69.92±2.56	p <sub>1-2</sub>	>0.05	66.87±3.72	p <sub>1-2</sub>	>0.05
Type 2	73.94±15.38	p <sub>1-3</sub>	>0.05	67.08±7.24	p <sub>1-3</sub>	>0.05
Type 3	71.11±3.64	p <sub>2-3</sub>	>0.05	66.83±4.58	p <sub>2-3</sub>	>0.05
S-ar (mm)						
Profile 1	34.43±2.92	p <sub>1-2</sub>	>0.05	32.00±4.15	p <sub>1-2</sub>	>0.05
Profile 2	33.22±1.30	p <sub>1-3</sub>	=0.061	32.20±1.70	p <sub>1-3</sub>	>0.05
Profile 3	38.59±9.35	p <sub>2-3</sub>	<0.01	32.83±3.47	p <sub>2-3</sub>	>0.05
Type 1	33.85±2.34	p <sub>1-2</sub>	=0.082	32.00±2.92	p <sub>1-2</sub>	>0.05
Type 2	37.33±8.98	p <sub>1-3</sub>	>0.05	32.50±4.43	p <sub>1-3</sub>	>0.05
Type 3	35.28±4.21	p <sub>2-3</sub>	>0.05	32.38±3.30	p <sub>2-3</sub>	>0.05
S-ar:ar-Go						
Profile 1	69.48±9.38	p <sub>1-2</sub>	<0.05	69.14±7.34	p <sub>1-2</sub>	>0.05
Profile 2	61.89±5.82	p <sub>1-3</sub>	>0.05	66.00±6.96	p <sub>1-3</sub>	>0.05
Profile 3	67.12±7.95	p <sub>2-3</sub>	>0.05	67.46±8.80	p <sub>2-3</sub>	>0.05
Type 1	69.69±7.12	p <sub>1-2</sub>	>0.05	68.70±8.66	p <sub>1-2</sub>	>0.05
Type 2	68.83±8.16	p <sub>1-3</sub>	<0.05	69.29±6.82	p <sub>1-3</sub>	>0.05
Type 3	63.94±9.48	p <sub>2-3</sub>	<0.05	66.34±7.71	p <sub>2-3</sub>	>0.05

face types (p<0.01 and p<0.001, respectively) (see Table 3).

The angle of N-S-Ba in both young men and young women with the first facial profile is significantly greater than in the corresponding sex groups with the third profile (p<0.05 in both cases); there is a pronounced tendency to a higher value of this indicator in young women with the first facial profile than in young women with the second profile (p=0.055) (see Table 3). Young women with the first type of face have a significantly larger N-S-Ba angle than young women with the second and third face types (p<0.05 in both cases) (see Table 3).

When comparing the values of the N-S:S-Ar indicator, no significant differences were found between young men with different profiles or with different facial types, as well as between young women with different facial profiles. Significantly higher values of NS:S-Ar in young women with the third and second facial types than in young women with the first type of face (respectively, p<0.01 and p<0.05), as well as higher values in young women with the second type of face, than in young women with the first type (p=0.082) (see Table. 3).

When comparing the values of the distance N-S in young men of different facial profiles found statistically significant differences: higher values of this indicator in groups of

young men with the second and third facial profiles, compared with young men of the first facial profile (respectively, p<0.05 and p<0.01) (Table 4). Between the groups of young women with different facial profiles, only a smaller value of the N-S distance was found in persons with the first profile, compared with young women with the second profile (p=0.092) (see Table 4).

The S-ar distance in young men with the third facial profile is significantly greater than in young men with the second facial profile (p<0.01); there is also a tendency to its higher values in young men with the first profile of the face than in young men with the third profile (p=0.061) (see Table 4). There was also a slightly higher value of S-ar distance in young women with the second type of face than in young women with the first type (p=0.082) (see Table 4).

S-ar:ar-Go values were found to be higher in young men with the first facial profile compared to young men with the second profile (p<0.05) and in young men with the first and second facial types compared to young men with the third type (p<0.05 in all cases) (see Table 4).

Values in young men of different groups by profile or face type did not differ significantly, and in young women recorded greater values of N-CC distance in persons with the second facial profile than with the first (p<0.05), and there were tendencies to higher values in young women

**Table 5.** Cephalometric indicators of POr-NBa, N-CC and P-PTV in young men and women with different profiles or with different face types.

Groups	Young men			Young women		
	(M±σ)	p		(M±σ)	p	
POr-NBa (°)						
Profile 1	26.29±3.35	p <sub>1-2</sub>	>0.05	26.39±2.71	p <sub>1-2</sub>	>0.05
Profile 2	24.84±2.54	p <sub>1-3</sub>	>0.05	25.96±2.12	p <sub>1-3</sub>	>0.05
Profile 3	24.84±2.22	p <sub>2-3</sub>	>0.05	25.39±2.19	p <sub>2-3</sub>	>0.05
Type 1	26.07±3.07	p <sub>1-2</sub>	>0.05	26.20±2.85	p <sub>1-2</sub>	>0.05
Type 2	25.26±3.08	p <sub>1-3</sub>	>0.05	25.42±1.71	p <sub>1-3</sub>	>0.05
Type 3	25.39±2.69	p <sub>2-3</sub>	>0.05	26.30±2.64	p <sub>2-3</sub>	>0.05
N-CC (mm)						
Profile 1	57.82±3.07	p <sub>1-2</sub>	>0.05	54.36±5.53	p <sub>1-2</sub>	<0.05
Profile 2	57.67±3.24	p <sub>1-3</sub>	>0.05	55.49±2.46	p <sub>1-3</sub>	>0.05
Profile 3	61.62±13.95	p <sub>2-3</sub>	>0.05	54.85±4.01	p <sub>2-3</sub>	>0.05
Type 1	58.66±2.39	p <sub>1-2</sub>	>0.05	55.47±3.95	p <sub>1-2</sub>	>0.05
Type 2	61.23±13.66	p <sub>1-3</sub>	>0.05	55.54±6.11	p <sub>1-3</sub>	=0.071
Type 3	57.32±3.33	p <sub>2-3</sub>	>0.05	53.49±3.24	p <sub>2-3</sub>	=0.058
P-PTV (mm)						
Profile 1	-39.46±2.87	p <sub>1-2</sub>	>0.05	-37.91±3.81	p <sub>1-2</sub>	>0.05
Profile 2	-40.59±1.69	p <sub>1-3</sub>	>0.05	-37.09±2.13	p <sub>1-3</sub>	>0.05
Profile 3	-42.23±8.85	p <sub>2-3</sub>	>0.05	-36.96±4.06	p <sub>2-3</sub>	>0.05
Type 1	-39.97±2.55	p <sub>1-2</sub>	>0.05	-37.50±2.72	p <sub>1-2</sub>	>0.05
Type 2	-41.03±8.74	p <sub>1-3</sub>	>0.05	-37.63±3.98	p <sub>1-3</sub>	>0.05
Type 3	-40.70±2.94	p <sub>2-3</sub>	>0.05	-37.27±4.02	p <sub>2-3</sub>	>0.05

with first and second facial types than young women with the third facial type (respectively,  $p=0.071$  and  $p=0.058$ ) (Table 5).

No significant differences were found between POr-NBa angle or P-PTV distance in either young men or women with different facial profiles or with different facial types (see Table 5).

### Discussion

Thus, we have established the following features of the values of the group of cephalometric indicators, which are not subject to correction in the implementation of orthodontic interventions between groups of young men with different facial profiles according to Schwarz A.M.:

- in young men with the first facial profile significantly higher values of S-ar: ar-Go (by 12.3%;  $p<0.05$ ) than in young men with the second profile and angles H (by 3.8%;  $p<0.01$ ), NS-Ar (by 2.9%;  $p<0.05$ ) and NS-Ba (by 3.2%;  $p<0.05$ ) than in young men with the third profile;

- young men with the second facial profile had significantly higher values of N-Se distance (by 3.1%;  $p<0.05$ ) than young men with the first profile and angle H (by 2.8%;  $p<0.01$ ) than young men with the third profile;

- in young men with the third facial profile significantly higher values of distances N-Se (by 10.3%;  $p<0.01$ ), ar-Go (by 13.1%;  $p<0.05$ ) and NS (by 9.2%;  $p<0.01$ ) and a tendency to greater values of S-ar distance (by 12.1%;  $p=0.061$ ) than in young men with the first profile and significantly higher values of S-ar distance (by 16.2%;  $p<0.01$ ) than in young men with the second facial profile.

The following differences in cephalometric parameters of this group were found in young women with different facial profiles:

- young women with the first facial profile had significantly higher values of angles: H (by 2.5%;  $p<0.01$ ), NS-Ar (by 3.4%;  $p<0.05$ ) and NS-Ba (by 2.8%;  $p<0.05$ ) than in young women with a third facial profile. We can also note a pronounced tendency to higher values of the angle N-S-Ba (by 2.1%;  $p=0.055$ ) and higher values of the angle N-S-Ar (by 1.9%;  $p=0.082$ ) compared to young women with the second facial profile;

- young women with the second facial profile had a significantly higher value of the N-CC distance (by 2.1%;  $p<0.05$ ) and a greater value of the N-S distance (by 0.7%;  $p=0.092$ ) compared to young women with the first facial profile;

- young women with the third facial profile had a significantly higher value of the N-CC distance (by 2.1%;  $p<0.05$ ) and a higher value of the N-S distance (by 0.7%;  $p=0.092$ ) compared to young women with the first facial profile.

Summarizing the analysis of the results obtained in groups of young men and women with different facial profiles, we can conclude about the more pronounced variability of the studied indicators in young men than in young women, as the number of established differences

(11 significant differences and 1 tendency to differences between groups of boys against 4 significant differences and 2 tendencies to differences between groups of girls) and the number of indicators that were different (8 indicators out of 13 studied in young men - N-Se, H, ar-Go, NS-Ar, NS-Ba, NS, S-ar, S-ar:ar-Go and 4 indicators out of 13 studied in young women - H, NS-Ar, NS-Ba, N-CC, ar-Go).

The greatest differences between the groups of different profiles of young men or young women were recorded between the indicators determined by the method of cephalometry according to Schwarz A. M. (3 in young men and 1 in young women), Bjork A. (2 in young men and 3 in young women) and Jarabak J. R. (5 in young men). At the same time, the indicators determined by Ricketts R.M. revealed only 1 significant difference.

Summarizing and analyzing the features of the values of the group of cephalometric indicators, which are not subject to correction in the implementation of orthodontic interventions between groups of young men with different facial types according to Schwarz A.M., it was found:

- in young men with the first type of face significantly higher value of the angle H (by 3.1%;  $p<0.05$ ) than in young men with the second type of face and angle H (by 3.7%;  $p<0.01$ ) and the indicator S-ar:ar-Go (by 9.0%;  $p<0.05$ ) than in young men with the third type;

- in young men with the second type of face significantly higher values of S-ar: ar-Go (by 7.6%;  $p<0.05$ ) than in young men with the third type of face and higher values of S-ar (by 10.3%;  $p=0.082$ ) than in young men with the first type;

- in young men with the third type of face significantly higher values of ar-Go (by 10.1%;  $p<0.01$ ) than in young men with the first type and significantly higher values of ar-Go distance (by 1.1%;  $p<0.05$ ) than in young men with the second type of face.

In young women with different face types, the following differences were found in the indicators studied:

- in young women with the first type of face significantly higher values of the angles H (by 2.4%;  $p<0.01$ ), NS-Ar (by 3.1%;  $p<0.01$ ) and NS-Ba (by 2.1%;  $p<0.05$ ), compared with young women with the second type of face and significantly higher values of the angles H (by 2.5%;  $p<0.01$ ), NS-Ar (by 5.3%;  $p<0.001$ ) and NS-Ba (by 3.8%;  $p<0.05$ ) and the distance SE by 15.2%;  $p<0.01$ ), as well as the tendency to higher values of the distance N-CC (by 3.7%;  $p=0.071$ ), compared with young women with the third type of face;

- young women with the second type of face had a significantly higher value of NS: S-Ar (by 8.3%;  $p<0.05$ ) and a greater value of the distance S-ar (by 10.3%;  $p=0.082$ ) than young women with the first type of face and significantly higher values of S-ar:ar-Go (by 7.6%;  $p<0.05$ ), distance S-E (by 7.8%;  $p<0.01$ ) and a tendency to higher values of distance N-CC (by 3.8%;  $p=0.058$ ) than in young women with the third type of face;

- young women with the third type of face had significantly higher values of ar-Go distance (by 10.1%;  $p<0.01$ ) and N-S:S-Ar (by 19.8%;  $p<0.01$ ) than young women with the first

type of face.

Thus, in the groups of young men and young women with different facial types, in contrast to the results of comparison of persons with different facial profiles, more pronounced variability of the studied indicators in young women than in young men as the number of established differences (11 significant differences and 2 tendencies to differences between groups of young women against 6 significant differences between groups of young men) and the number of indicators that were different (6 indicators out of 13 studied in young women - H, SE, NS-Ar, NS-Ba, NS: S-Ar and P- PTV and 3 indicators from 13 studied at young men - H, ar-Go, S-ar: ar-Go).

Most differences between the groups of different types of faces of young men or young women were recorded between the indicators determined by the method of cephalometry by Schwarz A.M. (2 in young men and 2 in young women) and Bjork A. (4 in young women). At the same time, the indicators determined according to Ricketts R.M. revealed only 2 tendencies to differences in young women.

The following indicators did not have significant differences between certain groups of people of different sexes of different profiles or different types of faces: POr-NBa angle and P-PTV distance according to Ricketts R.M.; distance N-Se by Shwars A.M.; distance N-S and S-ar and index S-ar: ar-Go according to Jarabak J.R.; distance S-E by Steiner C.C.; N-S index: S-Ar and N-S-Ar angle by Bjork A.; ar-Go distance by Burstone C.J.

A number of researchers have determined cephalometric parameters in Ukrainian young men and young women, using various generally accepted methods of cephalometry [14-18, 20, 21].

Comparing our results for young men or women with different profiles or different types of faces, with the results of these authors for young men and young women in general, without division into groups by types and profiles of faces, it should be noted that there are a number of differences in our indicators. Thus, in young men with the first and second facial profiles and with the third facial type, the values of N-CC distance (according to the Ricketts R.M. method) were smaller, and in young men with the third facial profile, on the contrary, greater than in young men without division into profiles and types face according to Chernysh A.V. and others. (2018); and in young women with the first profile and in young women with the third type of face - smaller [14]. The P-PTV distance in adolescents with the third facial profile was greater than in adolescents without division on the facial profile according to Chernysh A.V. et al. and approached the author's index of Ricketts R.M., and in young women with the third profile - on the contrary, less than the established results for Ukrainian young women in general [14].

The ar-Go distance (according to the Burstone C.J. method) according to the results of our research in young men with first profile and the first type of face is greater than in young men in general, without division into individual

profiles and face types according to Dmitriev M.O., Chernysh A.V. and Chugu T.V. [16]. It should be noted that both our results (for all profiles and types of faces) and the results of young people in general [16] the value of this indicator in Ukrainian young men is greater than those of Burstone C.J. [10]. At the same time, the results of our studies did not show differences between the values of this indicator in young women of different profiles and face types compared with the results of Dmitriev M.O., Chernysh A.V. and Chugu T.V. [16] and Burstone C.J. [10].

Comparing the distance N-Se and the angle H according to the method of Shwars A.M. with the same indicators determined by Dmitriev M.O. et al. [18] in young men and young women of the Podillia region of Ukraine in general, without division into separate types and profiles of the face, it should be noted that young women of all types and profiles of the face, as well as young men with the second profile or the third type of face coincided with the values defined for young men and young women in general. On the other hand, it is smaller in young men with the first profile or the first type of face, and larger in young men with the third profile or the second type of face than in young men without division into types and profiles of the face. The angle H in young men with the third facial profile is smaller, and in young men with the first type of face and in young women with the first type of face - greater than in young men or young women in general, without division into individual types and profiles of the face. It should also be noted that the distance S-E according to the method of Steiner C.C. in young women with the third type of face have lower values than in Ukrainian young women in general [15].

Thus, the results indicate that for a more individualized approach to the determination of cephalometric parameters in young men and young women, it is advisable to use the definition of their profile and face type.

## Conclusions

1. The values of cephalometric teleroentgenographic parameters, which usually do not change during surgical and orthodontic interventions in Ukrainian young men and young women with orthognathic occlusion with different profiles and face types according to Schwarz A.M. have been established.

2. There are differences in certain indicators between groups of young men or young women with different profiles or types of faces. The greatest differences, both in young men and young women, were recorded between the indicators determined by the methods of cephalometry by Schwarz A. M. and Bjork A., and the least - by the method of Ricketts R.M.

3. The obtained results testify to the expediency of using the division of young men and young women into separate groups by profile and type of face to adhere to the personalized principle in the morphological assessment of cephalometric and gnatometric indicators of lateral teleroentgenograms.

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**ОСОБЛИВОСТІ ЦЕФАЛОМЕТРИЧНИХ ПОКАЗНИКІВ, ЯКІ ЗАЗВИЧАЙ НЕ ЗМІНЮЮТЬСЯ ПІД ЧАС ХІРУРГІЧНИХ ТА ОРТОДОНТИЧНИХ ВТРУЧАНЬ, В УКРАЇНСЬКИХ ЮНАКІВ І ДІВЧАТ З ОРТОГНАТИЧНИМ ПРИКУСОМ І РІЗНИМИ ТИПАМИ ТА ПРОФІЛЯМИ ОБЛИЧЧЯ ЗА SCHWARZ A.M.**

**Прокопенко О.С., Беляєв Е.В., Дмитрієв М.О., Черкасова О.В., Скорук Р.В.**

За час існування рентгенологічного методу дослідження розроблені чисельні авторські методики цефалометричного дослідження та аналізу з застосуванням методу бокової телерентгенографії (методики Schwarz, Down's, Steiner, Tweed's, Harvold, Ricketts, McNamara, Jaraback, Burstone, Bjork тощо). Лікарі-стоматологи різних країн надають перевагу певним окремим методикам, кожна з яких має як свої переваги, так і окремі недоліки. Але досі не існує єдиної уніфікованої методики таких досліджень і постійно лікарі та дослідники намагаються їх удосконалити і отримати нові відомості щодо їх ефективності. Мета дослідження - встановлення цефалометричних телерентгенографічних показників і визначення їх особливостей в українських юнаків і дівчат із ортогнатичним прикусом в залежності від профілів і типів обличчя за Schwarz A.M. Проведено цефалометричне дослідження і аналіз бокових телерентгенограм голови у 49 юнаків та 76 дівчат із фізіологічним прикусом максимально наближеним до ортогнатичного (в подальшому ортогнатичний прикус), розділених на окремі групи досліджень за різними профілями і різними типами обличчя за Schwarz A.M. (по 3 групи різного профілю і по 3 групи різного типу обличчя для осіб кожної статі). Досліджували цефалометричні телерентгенографічні показники, які зазвичай не змінюються під час проведення хірургічних та ортодонтичних втручань. Статистичну математичну обробку результатів дослідження провели в ліцензійному пакеті "Statistica 6.0" з використанням непараметричних методів оцінки отриманих результатів. Встановлені чисельні достовірні та тенденції відмінностей за визначеними показниками між групами юнаків, або дівчат з різними профілями, або типами обличчя. Найбільше відмінностей, як у юнаків, так і у дівчат зафіксовано між показниками, визначеними за методиками цефалометрії за Schwarz A.M. та за Bjork A., а найменше - за методикою Ricketts R.M. Отримані результати свідчать про доцільність використання розподілу юнаків і дівчат на окремі групи за профілем і типом обличчя для дотримання персоналізованого принципу у морфологічній оцінці цефалометричних і гнатометричних показників бокових телерентгенограм.

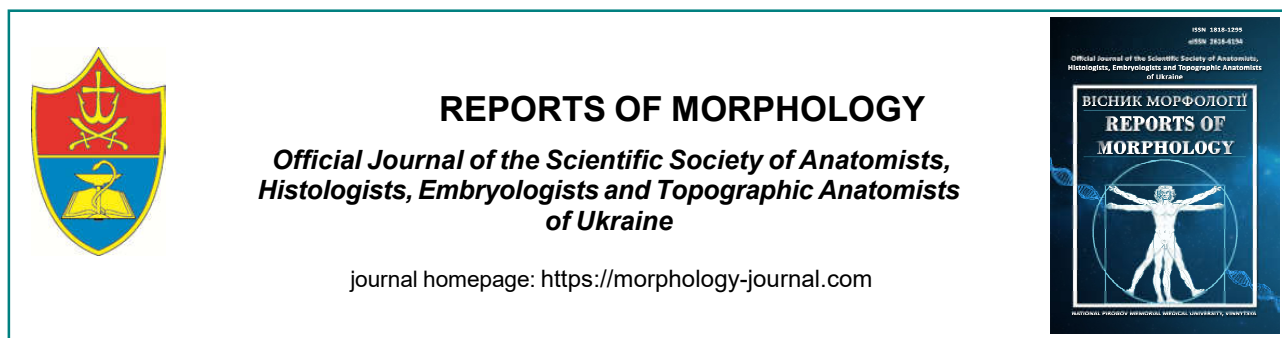
**Ключові слова:** юнаки, дівчата, ортогнатичний прикус, бокова телерентгенографія голови, цефалометричні показники, типи та профілі обличчя за Schwarz A.M.

**ОСОБЕННОСТИ ЦЕФАЛОМЕТРИЧЕСКИХ ПОКАЗАТЕЛЕЙ, КОТОРЫЕ ОБЫЧНО НЕ ИЗМЕНЯЮТСЯ ВО ВРЕМЯ ХИРУРГИЧЕСКИХ И ОРТОДОНТИЧЕСКИХ ВМЕШАТЕЛЬСТВ, У УКРАИНСКИХ ЮНОШЕЙ И ДЕВУШЕК С ОРТОГНАТИЧЕСКОМ ПРИКУСОМ И РАЗЛИЧНЫМИ ТИПАМИ И ПРОФИЛЯМИ ЛИЦА ПО SCHWARZ A.M.**

**Прокопенко А.С., Беляєв Э.В., Дмитриев Н.А., Черкасова Е.В., Скорук Р.В.**

За время существования рентгенологического метода исследования разработаны многочисленные авторские методики цефалометрического исследования и анализа с применением метода боковой телерентгенографии (методики Schwarz, Down's, Steiner, Tweed's, Harvold, Ricketts, McNamara, Jaraback, Burstone, Bjork и т.д.). Врачи-стоматологи разных стран мира отдают предпочтение определенным отдельным методикам, каждая из которых имеет как свои преимущества, так и отдельные недостатки. Но до сих пор не существует единой унифицированной методики таких исследований и постоянно врачи и исследователи пытаются их усовершенствовать и получить новые сведения об их эффективности. Цель исследования - установление цефалометрических телерентгенографических показателей и определение их особенностей у украинских юношей и девушек с ортогнатическим прикусом в зависимости от профилей и типов лица по Schwarz A.M. Проведено цефалометрическое исследование и анализ боковых телерентгенограмм головы у 49 юношей и 76 девушек с физиологическим прикусом максимально приближенным к ортогнатическому (далее ортогнатический прикус), разделенных на отдельные группы исследований по разным профилям и различным типам лица по Schwarz A.M. (по 3 группы разного профиля и по 3 группы разного типа лица для лиц каждого пола). Исследовали цефалометрические телерентгенографические показатели, которые обычно не изменяются во время проведения хирургических и ортодонтических вмешательств. Статистическую математическую обработку результатов исследования провели в лицензионном пакете "Statistica 6.0" с использованием непараметрических методов оценки полученных результатов. Установлены многочисленные достоверные различия и тенденции различий по определенным показателям между группами юношей или девушек с разными профилями или типами лица. Наибольшее количество различий, как у юношей, так и у девушек зафиксировано между показателями, определенными по методикам цефалометрии по Schwarz A.M. и по Bjork A., а меньше всего - по методике Ricketts R.M. Полученные результаты свидетельствуют о целесообразности использования распределения юношей и девушек на отдельные группы по профилям и типам лица для соблюдения персонализированного принципа в морфологической оценке цефалометрических и гнатометрических показателей боковых телерентгенограмм.

**Ключевые слова:** юноши, девушки, ортогнатический прикус, боковая телерентгенография головы, цефалометрические показатели, типы и профили лица по Schwarz A.M.



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# The state of vegetative regulatory systems of pupils with different academic performance

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Sotnikova-Meleshkina Zh.V.

*The transition to primary school requires pupils to adapt to new conditions of the educational environment, accompanied by the use of physiological reserves of the body. School stress can hinder the academic success of children and adolescents, creative solutions to complex and new problems, which, in turn, can lead to underestimation of abilities and underestimation of pupils self-esteem. Chronic exposure to academic stress can lead to school burnout and the formation of psychosomatic disorders. The aim of the study was to compare the functional state of autonomic regulatory systems among pupils with different levels of academic achievement. 60 children (10-13 years old) took part in the longitudinal study. The functional state of the autonomic regulatory systems was determined by analyzing 5-minute recordings of the electrocardiogram in a state of quiet wakefulness with eyes closed in a sitting position. Pupils were divided into 3 groups depending on the level of their academic performance, which was determined by the average score of 8 basic subjects. Statistical data processing was performed using the Mann-Whitney, Kraskell-Wallis, Dunnett, Fisher's  $\varphi$ -test. Significant differences in heart rate variability (HRV) parameters depending on the level of academic achievement were found only in the 5th and 6th years of study, which reflected the different physiological cost of adaptation to primary school. The vegetative profile of pupils with academic performance lower than the average in the 5th year of study indicated overstrain of regulatory systems and fatigue. Among pupils with academic performance higher than the average in the 6th year of study 2.4 times more often than pupils with average academic performance, the optimal state of autonomic regulatory systems was observed (respectively, 46.2% and 19.1%,  $p \leq 0.05$ ), and in the 7th year of study - 2.5 times compared to pupils with academic performance below average (respectively, 46.2% and 18.2%,  $p \leq 0.05$ ), which may indicate a higher physiological price for adaptation to primary school pupils with academic performance below average. Fatigue and overexertion of the mechanisms of autonomic regulation are characteristic of pupils with academic performance below average, while pupils with academic performance above average had a high level of stress adaptive-compensatory mechanisms in the 6th year of study. The category of pupils with a level of academic performance below average should be assigned to the risk group in terms of the development of autonomic dysfunction and psychosomatic diseases.*

**Keywords:** heart rate variability, vegetative profile, academic performance, pupils.

### Introduction

School as a place of socialization and development is of great importance in the lives of adolescents [7]. Increased demands to be successful and productive in modern society can deplete human resources and can lead to stress in adolescents [4, 21]. International studies have shown that adolescents have a significant increase in stress related to school and stress-related health problems [6, 11, 17]. It

is shown that school stress can hinder pupils' academic success [10], including due to the negative impact on memory processes [15, 19]. In addition, stress, by altering the balance between memory systems, can hinder creative solutions to complex and/or new problems, which in turn can lead to underestimation of pupils' abilities and underestimation of their self-esteem [20].



Chronic exposure to academic stress can lead to school burnout, defined as an emotional state of exhaustion, cynicism, and depersonalization, [21] and the formation of psychosomatic disorders.

Therefore, *the aim* of our study was to compare the functional state of autonomic regulatory systems among pupils with different levels of academic achievement.

**Materials and methods**

The study involved 60 middle school pupils (10-13 years). The functional state of vegetative regulatory systems was determined by analyzing *statistical* (Mean, Mo, SDNN, ΔX, AMo, CVr, pNN50), *spectral* (TP, VLF, LF, HF, VLF%, LF%, HF%, LFn, HFn, LF/HF, IC) and *autocorrelation groups of indicators* (CC1, CC0), as well as an integrated indicator of regulatory system activity (IRSA) [3, 13].

HRV measurement was performed at 5, 6 and 7 years of study based on the analysis of 5-minute electrocardiogram recordings in the II standard lead in a state of quiet wakefulness with eyes closed in a sitting position using the hardware-software complex "BrainTest" ("DX-system", Kharkiv, Ukraine). Mathematical analysis of cardio signals was performed using the "Cardio Tension Test" module of the application package "NeuroResearcher@ Innovation Suite, V. 17.5" ("Institute of Medical Informatics and Telemedicine", Kharkiv, Ukraine).

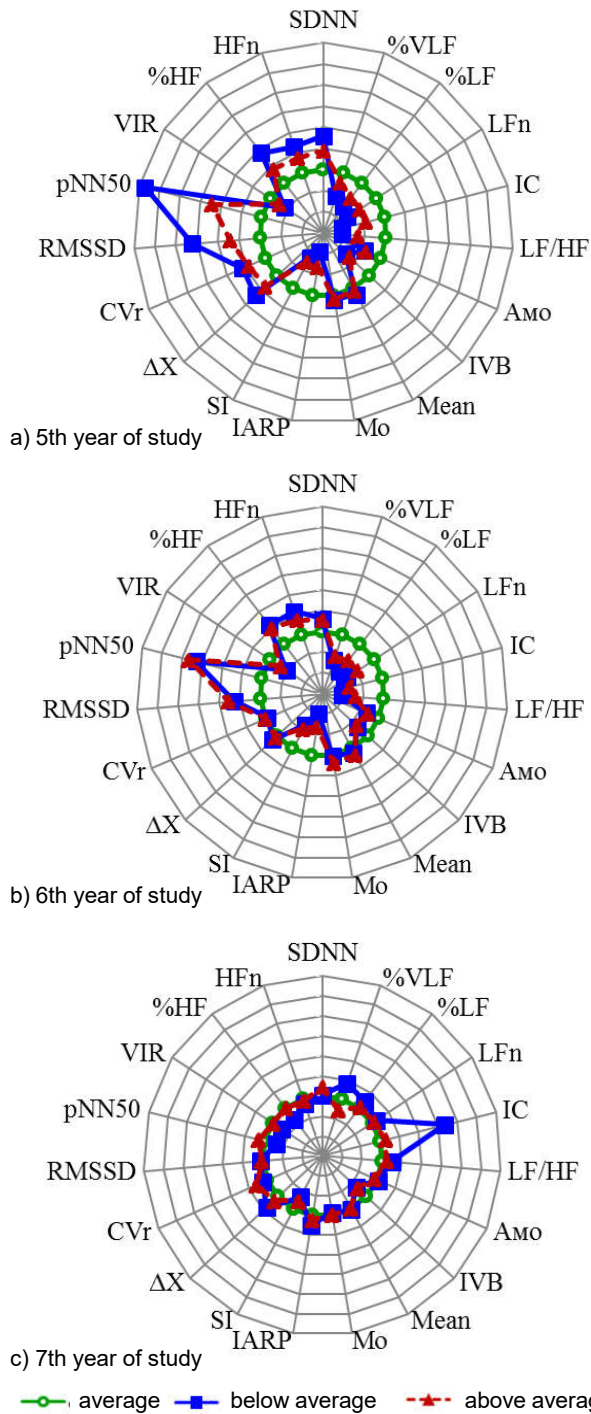
Determining the level of academic performance of pupils involved copying the data of annual academic performance from eight basic subjects (Ukrainian language, Ukrainian literature, algebra, world history, biology, physics, chemistry, foreign language), based on which we calculated the average score with further division of pupils into 3 groups: with a level of academic success below average (up to 7.85 points), with an average level (from 7.85 to 9.19 points inclusive) and above average level (over 9.19 points).

To test the hypothesis of equality of measures of the central tendency in pairwise comparisons, the Mann-Whitney test was used, and for multiple comparisons, the nonparametric ANOVA by the Crackell-Wallis test was used, followed by a posteriori comparison by the Dunnett test. Nominal features were described as a percentage, and their pairwise comparison was carried out by the φ-criterion of Fisher's angular transformation. Differences at  $p \leq 0.05$  were considered significant.

The study was conducted in accordance with the bioethical norms of the Declaration of Helsinki (as amended in 2013) with the informed consent of parents and approved by the Commission on Bioethics of the SI "Institute of Child and Adolescent Health of the National Academy of Medical Sciences of Ukraine".

**Results**

It was found that in the fifth year of study, pupils with higher than average academic performance, compared with pupils with average academic performance, were characterized by probably ( $p \leq 0.05$ ) higher values of SDNN,

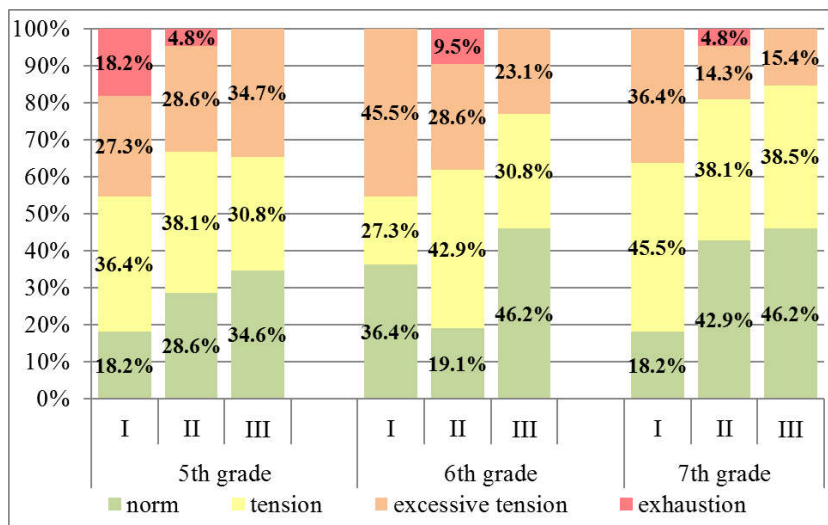


**Fig. 1.** Vegetative profiles of pupils with different levels of academic achievement.

TP, CVr, HF, pNN50 (Fig. 1a).

The same changes in HRV parameters were observed among pupils with below-average academic performance. However, they were also added probably ( $p \leq 0.05$ ) lower values of AMo, SI, IARP, VIR, IVB, CC1, LF%, LFn, LF/HF and probably ( $p \leq 0.05$ ) higher values of Mean, RMSSD, VLF,





**Fig. 2.** Distribution of primary school pupils with lower than average (I), average (II) and higher than average (III) academic performance by levels of activity of regulatory systems.

HF%, HFn, compared with pupils with average academic performance.

At the same time, in the fifth year of study, we did not find significant differences in the values of HRV between pupils with academic performance above and below average.

At year 6, pupils with below-average academic performance were characterized by significantly ( $p \leq 0.05$ ) lower IARP, CC1, and probably ( $p \leq 0.05$ ) higher RMSSD values compared to pupils with average academic performance, whereas among pupils with academic performance higher than the average, only a similar trend was observed ( $0.07 \leq p < 0.10$ ). At the same time, pupils with above-average academic performance were characterized by a significantly ( $p \leq 0.05$ ) lower heart rate and longer cardio cycle, compared with pupils with average academic performance. A similar trend ( $p = 0.06$ ) was observed among pupils with below-average academic performance. Only pupils with below-average academic performance were characterized by probably ( $p \leq 0.05$ ) lower CC0 values compared to pupils with average academic performance. Pupils with above-average academic performance were characterized by probably ( $p \leq 0.05$ ) higher values of RMSSD, pNN50, CC1, CC0.

At year 6, pupils with below-average academic performance were characterized by significantly ( $p \leq 0.05$ ) lower IARP, CC1, and probably ( $p \leq 0.05$ ) higher RMSSD values compared to pupils with average academic performance, whereas among pupils with academic achievement. academic performance was higher than the average, only a similar trend was observed ( $0.07 \leq p < 0.10$ ). At the same time, pupils with above-average academic performance were characterized by a significantly ( $p \leq 0.05$ ) lower heart rate and longer cardio cycle, compared with pupils with average academic performance. A similar trend ( $p = 0.06$ ) was observed among pupils with below-average academic performance. Only pupils with below-average

academic performance were characterized by probably ( $p \leq 0.05$ ) lower CC0 values compared to pupils with average academic performance. Pupils with above-average academic performance were characterized by probably ( $p \leq 0.05$ ) higher values of RMSSD, pNN50, CC1, CC0.

At the 7th year of study, the vegetative profiles of pupils with different academic performance were similar (Fig. 1c).

A comprehensive assessment of the state of autonomic regulatory systems of pupils with different academic performance according to IRSA (Fig. 2) found that among pupils with academic performance higher than the average in the sixth year of study 2.4 times more often than pupils with average academic performance, observed optimal condition

of autonomic regulatory systems (respectively, 46.2% and 19.1%,  $p \leq 0.05$ ), and at the 7th year of study - 2.5 times compared to pupils with academic performance below average (respectively, 46.2% and 18.2%,  $p \leq 0.05$ ).

### Discussion

The transition to primary school requires pupils to adapt to new conditions of the educational environment, accompanied by the use of physiological reserves of the body. The ratio between the achieved level of performance and spent physiological reserves to achieve them speak of the physiological "price" of adaptation [22]. As a performance we used the indicator of academic performance, and physiological resources were evaluated by the parameters of heart rate variability. Lower heart rate variability (HRV) was seen as a sign of limited ability to regulate emotional state, performance, and social functioning [5, 12, 14, 18].

According to the results of one-factor dispersion analysis of Kruskal-Wallis, significant differences in HRV parameters depending on the level of academic performance were found only in 5 and 6 years of study (Fig. 1), which may reflect different physiological costs of adaptation to primary school depending on academic performance.

From the above we can conclude that in the 5th year of study, pupils with above-average academic performance were characterized by an increase in overall heart rate variability due to increased absolute activity of the parasympathetic nervous system, compared with pupils with average academic performance. This state of autonomic regulatory systems in pupils with above-average academic performance can be characterized as a moderate predominance in the state of quiet wakefulness of the activity of the autonomous control circuit over the central while maintaining sympathetic-vagal balance at the level

of the autonomous control circuit.

In contrast to pupils with above-average academic performance, among pupils with below-average academic performance in the 5th year of study, we observed not only an increase in absolute but also specific activity of the parasympathetic nervous system against a decrease in specific activity of the sympathetic nervous system. In addition, there was an increase in the absolute activity of the suprasedgmental structures of the autonomic nervous system against the background of a decrease in the relationship between the central and autonomous circuits of regulation and the lower stress index of regulatory systems. This caused a significant shift in the sympathetic-vagal balance towards vagotonia, which was probably confirmed ( $p \leq 0.05$ ) by a lower heart rate and a longer cardiocycle. Such features of the vegetative profile indicate a pronounced predominance of the autonomous control loop and reflect the overstrain of regulatory systems and, probably, fatigue of pupils.

It is noteworthy that at the 6th year of study the vegetative profile of pupils with academic performance above and below average becomes similar and indicates a pronounced vagotonia against the background of a significant reduction in the effects of the central loop of regulation. Although in both cases of deviation of academic performance from the average level, we can talk about the overstrain of the mechanisms of regulation and fatigue of pupils, but these processes are more pronounced among pupils with academic performance below average. At the same time, compensation mechanisms are still included in pupils with above-average academic performance by strengthening the relationship between the central and autonomous control circuits, but there are insufficient central mobilizing influences. This is probably due to the fact that the 6th year of study has a sensitive period of formation of autonomic regulatory systems, which reduces the adaptive reserves of pupils.

Since in the seventh year of study the vegetative profiles of pupils with different academic performance in the conditions of quiet wakefulness are as similar as possible, we can assume that this is a period of stable adaptation to the educational environment of primary school. Note that among pupils with academic performance above the average 2.5 times more often observed the optimal state of autonomic regulatory systems compared with pupils with academic performance below average (respectively, 46.2% and 18.2%,  $p \leq 0.05$ ) in which stress/overstrain of autonomic regulation was observed 2.5 times more often (respectively, 53.8% and 81.8%,  $p \leq 0.05$ ). This gives grounds to claim that pupils with academic performance below the average pay a higher physiological price for adaptation to primary school.

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We do not currently know any studies of the relationship between academic performance and heart rate variability among elementary school pupils. E.V. Fedorova and co-authors [8] found that academically unsuccessful pupils of 5 years of school had lower heart rates. We also observed this trend, however, pupils with above-average academic performance also had lower heart rates than pupils with average academic performance [8]. In a study by O.B. Gileva (2012) found differences in TP and LF/HF between pupils aged 7-16 with different levels of academic achievement, with more stress on adaptation mechanisms found among excellent pupils [9]. Studies by T.V. Agafonkina et al. (2008, 2015) found that "excellent" pupils have better autonomic nervous system and adaptive abilities than pupils who study satisfactorily and well [1] in terms of heart rate, blood pressure and autonomic index of Kerdo at rest, cold and orthostatic tests. At the same time, in a study by L.A. Alexandrova and co-authors (2014) noted that high school pupils with high academic performance have a higher level of stress of physiological mechanisms, and according to the authors this is due to the fact that current tasks are in the zone of immediate development, ie exceed current abilities but trigger mechanisms of personal self-regulation [2].

These data partially coincide with our results on the stress of adaptive-compensatory mechanisms in pupils with academic performance higher than the average at 6 years of study. However, in our study, depletion of the mechanisms of autonomic regulation was observed among pupils with low academic performance.

Prospects for further development are the formation of an algorithm for pre-nosological diagnosis of school maladaptation and its prevention in middle school students.

## Conclusions

1. Adaptation to the educational environment of primary school was observed in the third year of study (7th year of school).
2. Deviations of academic success from the average level were accompanied by the predominance of the activity of the autonomous circuit of regulation in conditions of quiet wakefulness. Fatigue and overstrain of the mechanisms of autonomic regulation are characteristic of pupils with academic performance below average, while pupils with academic performance above average had a high level of stress of adaptive-compensatory mechanisms in the sixth year of study.
3. The category of pupils with a level of academic achievement below average should be assigned to the risk group in terms of the development of autonomic dysfunction and psychosomatic diseases.

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## СТАН ВЕГЕТАТИВНИХ РЕГУЛЯТОРНИХ СИСТЕМ УЧНІВ З РІЗНОЮ АКАДЕМІЧНОЮ УСПІШНІСТЮ

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Перехід до основної школи вимагає адаптації учнів до нових умов освітнього середовища, що супроводжується використанням фізіологічних резервів організму. Шкільний стрес може перешкоджати академічній успішності дітей та підлітків, творчому вирішенню складних та нових проблем, що, відповідно, може призвести до недооцінки здібностей та заниження самооцінки школярів. Хронічний вплив академічного стресу може призвести до шкільного вигорання та формування психосоматичних розладів. Мета дослідження - порівняти функціональний стан вегетативних регуляторних систем серед учнів з різним рівнем академічної успішності. У лонгітудинальному дослідженні взяли участь 60 дітей (10-13 років). Функціональний стан вегетативних регулюючих систем визначали шляхом аналізу 5-хвилинних записів електрокардіограми у стані спокійного неспання з закритими очима в положенні сидячи. Учні було розподілено на 3 групи у залежності від рівня їх академічної успішності, що визначається за середнім балом із 8 базових предметів. Статистична обробка даних проведена із використанням критеріїв Манна-Уїтні, Краскелла-Уоліса, Даннета,  $\phi$ -критерія Фішера. Достовірні відмінності за параметрами варіабельності серцевого ритму (ВСР) в залежності від рівня академічної успішності виявлені тільки на 5-му та 6-му роках навчання, що відображало різну фізіологічну ціну адаптації до основної школи. Вегетативний профіль учнів з академічною успішністю нижчою за середню на 5 році навчання вказував на перенапруження регуляторних систем та перевтомлення. Серед учнів з академічною успішністю вищою за середню на шостому році навчання у 2,4 рази частіше порівняно з учнями з середньою академічною успішністю спостерігався оптимальний стан вегетативних регуляторних систем (відповідно, 46,2% і 19,1%,  $p \leq 0,05$ ), а на 7-му році навчання - у 2,5 рази порівняно з учнями з академічною успішністю нижчою за середню (відповідно, 46,2% і 18,2%,  $p \leq 0,05$ ), що може свідчити про вищу фізіологічну ціну за адаптацію до основної школи учнів з академічною успішністю нижчою за середню. Перевтомлення та перенапруження механізмів вегетативної регуляції притаманно школярам з академічною успішністю нижчою за середню, тоді як у учнів з академічною успішністю

вищою за середню спостерігався високий рівень напруження адаптаційно-компенсаторних механізмів на 6-му році навчання. Категорія учнів з рівнем академічної успішності нижчою за середню має бути віднесена до групи ризику з точки зору розвитку вегетативної дисфункції та психосоматичних захворювань.

**Ключові слова:** *варіабельність серцевого ритму, вегетативний профіль, академічна успішність, учні.*

#### **СОСТОЯНИЕ ВЕГЕТАТИВНЫХ РЕГУЛЯТОРНЫХ СИСТЕМ УЧАЩИХСЯ С РАЗНОЙ АКАДЕМИЧЕСКОЙ УСПЕВАЕМОСТЬЮ**

**Сотникова-Мелешкина Ж.В., Редька И.В., Михальчук О.Я.**

Переход в основную школу требует адаптации учащихся к новым условиям образовательной среды, сопровождается использованием физиологических резервов организма. Школьный стресс может препятствовать академической успеваемости детей и подростков, творческому решению сложных и новых проблем, что, соответственно, может привести к недооценке способностей и занижению самооценки школьников. Хроническое воздействие академического стресса может привести к школьному выгоранию и формированию психосоматических расстройств. Цель исследования - сравнить функциональное состояние вегетативных регуляторных систем среди учащихся с разным уровнем академической успеваемости. В лонгитудинальном исследовании приняли участие 60 детей (10-13 лет). Функциональное состояние вегетативных регулирующих систем определялось путем анализа 5-минутных записей ЭКГ в состоянии спокойного бодрствования с закрытыми глазами в положении сидя. Учащиеся были разделены на 3 группы в зависимости от уровня их академической успеваемости, который определялся по среднему баллу по восьми базовым предметам. Статистическая обработка данных проведена с использованием критериев Манна-Уитни, Краскелла-Уоллиса, Даннета,  $\phi$ -критерия Фишера. Достоверные различия по параметрам вариабельности сердечного ритма (BCP) в зависимости от уровня академической успеваемости обнаружены только на 5-м и 6-м годах обучения, что отражало различную физиологическую цену адаптации к основной школе. Вегетативный профиль учащихся с академической успеваемостью ниже средней на 5 году обучения указывал на перенапряжение регуляторных систем и переутомление. Среди учащихся с академической успеваемостью выше средней на 6 году обучения в 2,4 раза чаще по сравнению со школьниками со средней успеваемостью наблюдалось оптимальное состояние вегетативных регуляторных систем (соответственно, 46,2% и 19,1%,  $p \leq 0,05$ ), а на 7 году обучения - в 2,5 раза по сравнению с учащимися с успеваемостью ниже средней (соответственно, 46,2% и 18,2%,  $p \leq 0,05$ ), что может свидетельствовать о более высокой физиологической цене адаптации к основной школе учащихся с академической успеваемостью ниже среднего. Переутомление и перенапряжение механизмов вегетативной регуляции характерно для школьников с академической успеваемостью ниже средней, тогда как у учащихся с успеваемостью выше средней наблюдался высокий уровень напряжения адаптационно-компенсаторных механизмов на шестом году обучения. Категория учащихся с уровнем академической успеваемости ниже средней должна быть отнесена к группе риска с точки зрения развития вегетативной дисфункции и психосоматических заболеваний.

**Ключевые слова:** *вариабельность сердечного ритма, вегетативный профиль, академическая успеваемость, учащиеся.*

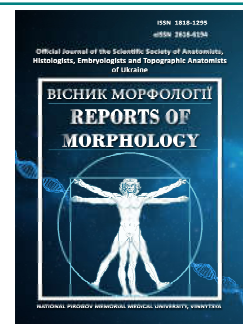
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## Micro- and submicroscopic changes of the cerebellar cortex 21 days after modeling the burn

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*The cerebellum is a complexly organized multifunctional component of the brain and a target in numerous lesions, so the study of its morphofunctional state in various pathological conditions and burns does not lose its relevance. The aim of our study was to establish the features of micro- and submicroscopic changes in the structural components of the cerebellar cortex after 21 days under conditions of experimental thermal trauma. The experimental study was simulated on white laboratory male rats. Grade III burns were applied under thiopental-sodium anesthesia with copper plates heated in boiled water to a temperature of 97-100°C. The size of the affected area was 18-20% of the epilated surface of the body of rats. Histological changes were studied for 21 days from the beginning of the experiment. Semi-thin sections for light microscopy were stained with methylene blue, for electron microscopy the obtained ultra-thin sections were contrasted with uranyl acetate and lead citrate according to the Reynolds method. With the help of micro- and submicroscopic studies, significant alternative changes of both neurons of all layers of the cerebellar cortex and parts of the microcirculatory tract of the organ were revealed. Neurons are disorganized, their shape is changed, there is low functional activity, due to a decrease in the area of the chromatophilic substance. Often there are cell-free areas in the ganglion layer, there is a shift of Purkinje cells deep into the granular layer, and grain cells are pushed sharply into the higher molecular layer. Thus, 21 days after the experimental thermal injury, destructive-degenerative changes of neurons of the cerebellar cortex, paravasal edema and disorders of transendothelial metabolism were established.*

**Keywords:** cerebellar cortex, micro- and submicroscopic changes, neurons, thermal trauma.

### Introduction

It is known that thermal trauma directly causes morphofunctional changes in the skin, and indirectly affects all organs and systems [5, 8, 12, 18]. The burn is a strong painful stimulus, which leads to a violation of neuro-humoral mechanisms. It is established that structural and functional changes can occur in different parts of the central nervous system: vacuolation, displacement of nuclei, pyknosis, disorders of intracellular metabolism of neurons [4, 14, 15, 19]. Structural and functional reorganization of the nervous system under exogenous, stressful influences, in particular, the cerebellar cortex occurs against the background of significant vascular disorders and especially microcirculation disorders, leads to the development of dystrophic and neurodegenerative changes [1, 7, 11, 13, 16].

The study of the morphofunctional state of the cerebellum in various conditions and burns, in particular, does not lose

its relevance, because this organ is one of the structures of the brain that responds quickly to stressors of exogenous origin. The study of micro- and submicroscopic state of the structural components of the cerebellar cortex in severe thermal injuries is an urgent task of theoretical and practical medicine.

Therefore, *the aim* of the work was to establish the histological reorganization of the components of the cerebellar cortex of laboratory animals 21 days after the application of experimental thermal trauma.

### Materials and methods

The experiment was performed on 15 adult white male rats, which were kept in standard vivarium conditions on a balanced diet. Animal care and all manipulations were carried out in compliance with the rules of the Strasbourg



"European Convention for the Protection of Vertebrate Animals Used for Research and Other Scientific Purposes" and the "General Ethical Principles of Animal Experimentation" (Kyiv, 2013).

Grade III burns were applied under thiopental-sodium anesthesia with two copper plates heated in boiled water to a temperature of 97-100°C on the epilated surface of the skin of the animal's back for 30 seconds. The size of the affected area was 18-20% of the body surface. Experimental animals were decapitated on day 21 of the experiment.

For histological examination, pieces of cerebellar tissue were taken and fixed in a 10% solution of neutral formalin; alcohol, dehydrated and poured into paraffin blocks. Sections obtained on a rotary microtome AMR 400 were stained with hematoxylin and eosin [9]. To obtain semi-thin sections (1-2 µm) and for electron microscopic examination, pieces of cerebellar tissue were fixed in 2.5% glutaraldehyde solution, post-fixed with 1% Osmium Tetroxide solution on phosphate buffer. Further processing was performed according to conventional methods. Semi-thin sections made on an LKB-3 ultramicrotome were stained with methylene blue, and ultra-thin sections were contrasted with uranyl acetate and lead citrate according to the Reynolds method [9].

Histological specimens were examined using a MIKROmed SEO SCAN optical microscope and photo-documented using a Vision CCD Camera with a histological specimen image output system. Electron microscopic study was performed in an electron microscope PEM-125K.

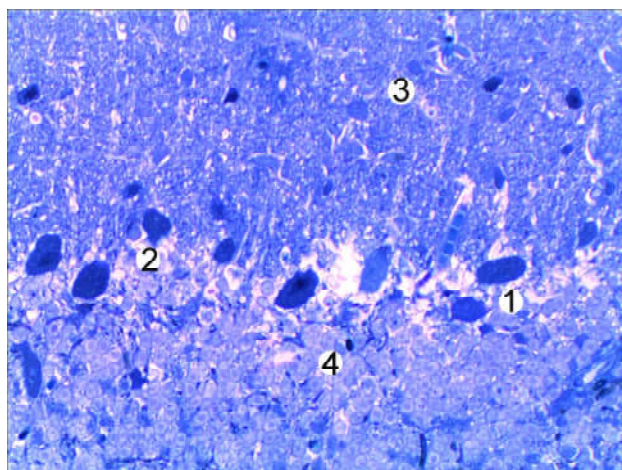
## Results

Conducted microscopic examinations of the cerebellar cortex 21 days after thermal injury revealed a polymorphism of neuronal changes. The clear histoarchitectonics of layers of a cerebellar cortex is lost (Fig. 1).

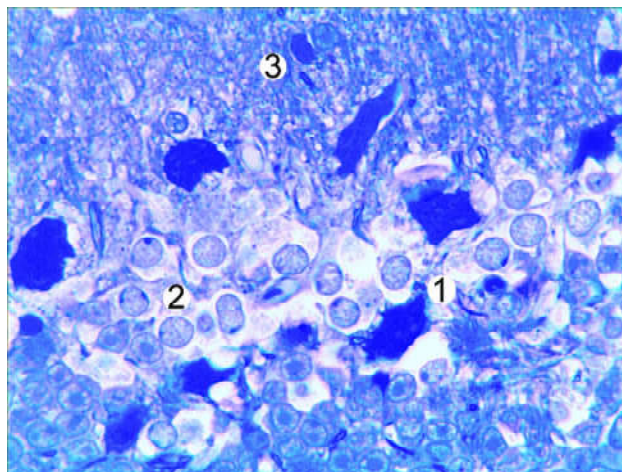
The cells of the molecular layer undergo significant destructive changes, the shape of stellate and basket neurons is changed, they become elongated. Their nuclei are compacted, pyknotically changed. Nucleoli in neurons are rare. Significant intra- and pericellular edema of neurons is observed.

The ganglion layer loses its integrity and order. Purkinje cells are located in separate areas in many rows, or bright, cell-free, neuron-free zones are found, their migration into granular and molecular layers is noted. Neurons are disorganized, have graceful processes. The vast majority of Purkinje cells are sharply hyperchromic, the neuroplasm of which has a characteristic rich color. Individual cells have an elongated shape and are significantly destructively altered. There are also sharply hypochromic neurons with total chromatolysis, the disappearance of chromatophilic matter from the entire neuroplasm of the cell (Fig. 2).

Some Purkinje cells have a spherical shape, there are cavities, "shadow" cells, which indicates a deep irreversible malnutrition processes that occur in the cerebellar cortex



**Fig. 1.** Microscopic changes of the cerebellar cortex 21 days after experimental thermal injury. Disruption of the stratified arrangement of neurons (1), sharply hyperchromic Purkinje cells (2), loss of cell density and destruction of the molecular layer (3), destruction of granular layer neurons (4). Methylene blue. x200.



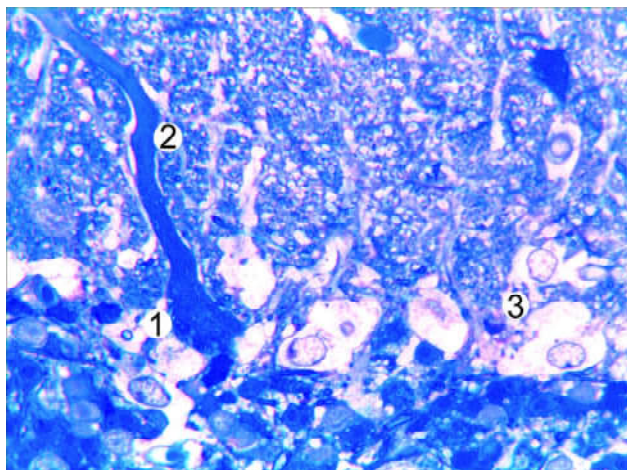
**Fig. 2.** Microscopic changes of the ganglion layer of the cerebellar cortex 21 days after experimental thermal injury. Multi-row arrangement of sharply hyperchromic Purkinje cells (1), migration of grain-cells into the ganglion layer (2), alteration of neurons of the molecular layer (3). Methylene blue. x400.

(Fig. 3). Neurons of the granular layer are also significantly altered, there are cell-free areas.

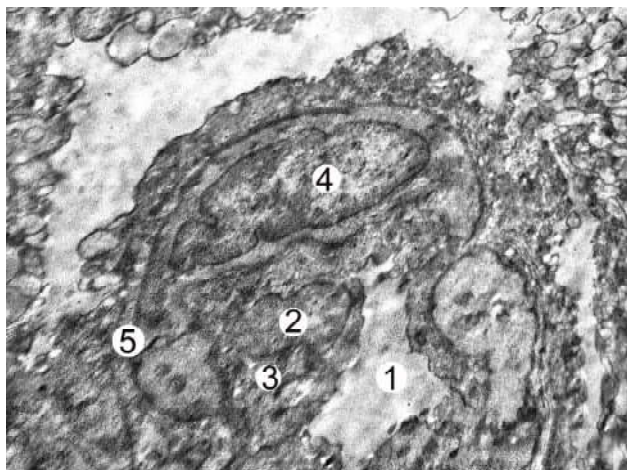
Micro- and submicroscopically, significant remodeling of the components of the microcirculatory tract was also established during this period of the experiment. The wall of the arterioles is compacted, homogeneous, fuzzy, destructively altered. The nuclei of endothelial cells are osmophilic, pyknotically altered with a predominance of heterochromatin in the karyoplasm, there are deep intussusception of the karyolemma. There is a destruction of organelles, which is accompanied by fragmentation of the membranes of the granular endoplasmic reticulum and mitochondria. The basement membrane is uneven, has indistinctly contoured areas (Fig. 4).

The blood supply to the venules is significant, areas of





**Fig. 3.** Histological changes of Purkinje cells of the ganglion layer of the cerebellar cortex 21 days after experimental thermal trauma. Sharply hyperchromic Purkinje cell (1) and significant axon thickening (2), intra- and pericellular neurons edema (3). Methylene blue. x400.



**Fig. 4.** Ultrastructural changes in the arterioles of the cerebellar cortex 21 days after experimental thermal injury. Narrowed lumen (1), nucleus fragment (2), edema and destruction of the endothelial cell cytoplasm (3), altered smooth myocyte (4), basement membrane (5). x9000.

wall thinning and destruction are detected, and endowment of endothelial cells into the lumen of the vessel is also detected.

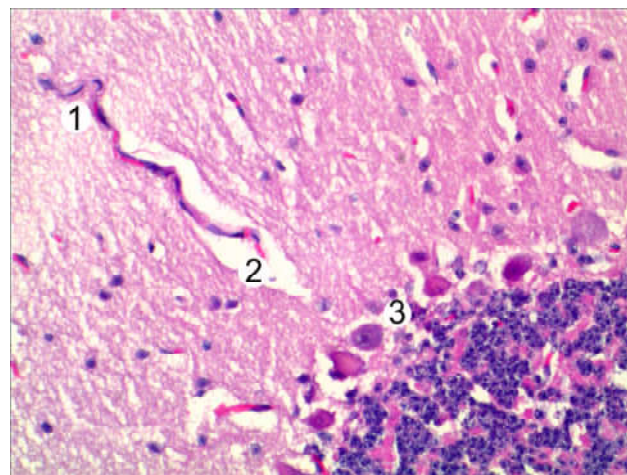
The lumen of hemocapillaries is narrowed both due to edema of the cytoplasm of endothelial cells and significant perivascular edema. Their wall undergoes changes: it becomes thinner, its destruction is observed, there are blood capillaries, the lumens of which are asleep (Fig. 5).

At the ultrastructural level, the nuclei of endothelial cells are compacted, pyknotically altered, in the karyoplasm of which heterochromatin predominates. In the cytoplasm of cells there is significant destruction and damage to the membranes of the granular endoplasmic reticulum, Golgi complex and mitochondria. The tubules of the endoplasmic reticulum are dilated, fragmented, some of them are

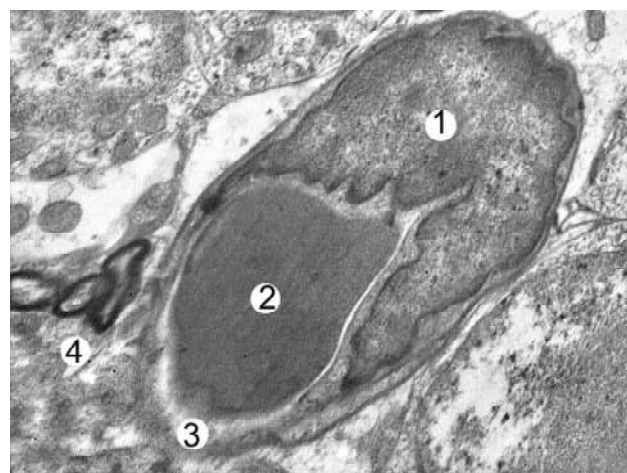
destroyed. The electronic density of the basement membrane is disturbed, in some areas it is thinned or unevenly thickened (Fig. 6).

Submicroscopically found signs of destructive changes of varying degrees in the neurons of all layers of the cerebellar cortex. There are hypochromic neurons with intensely bright karyo- and neuroplasm, which corresponds to total tigrolysis. The vast majority of neurons (especially Purkinje stem cells) were hyper- and sharply hyperchromic, which was manifested by an altered nuclear-cytoplasmic ratio and significant damage to the nucleus and organelles of the neuroplasm (Fig. 7).

On the membranes of the dilated vacuole-like tubules of the granular endoplasmic reticulum there are only single ribosomes, thickened tanks of the Golgi complex, near them there are few vacuoles and bubbles. Irregularly shaped

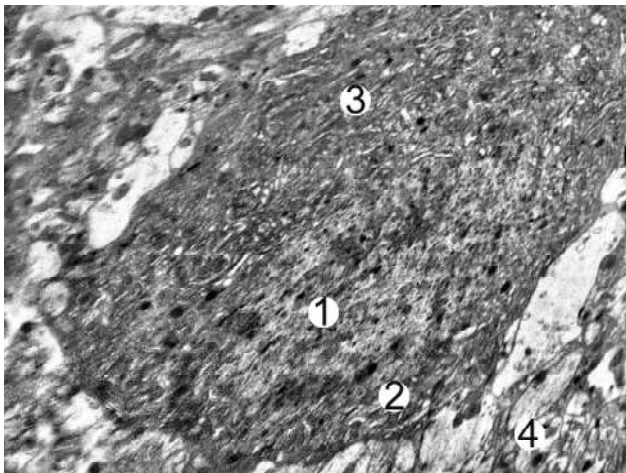


**Fig. 5.** Microscopic changes of the hemocapillary of the molecular layer of the cerebellar cortex 21 days after the experimental thermal injury. The lumen of the dormant capillary (1), significant perivascular edema (2), pericellular neurons edema (3). Hematoxylin-eosin. x200.

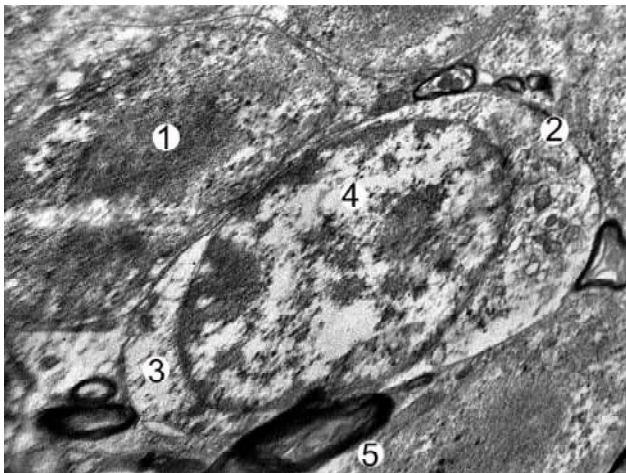


**Fig. 6.** Ultrastructural changes in the blood capillary of the cerebellar cortex 21 days after the experimental thermal injury. Altered nucleus of endothelial cell (1), erythrocyte in a narrow lumen (2), homogeneous, fuzzy basement membrane (3), neuropyle destruction (4). x11000.





**Fig. 7.** Ultrastructural changes of the hyperchromic neuron of the ganglion layer of the cerebellar cortex 21 days after the experimental thermal injury. The osmophilic nucleus of the Purkinje cell (1), the fuzzy karyolemma (2), the electron-dense neuroplasm (3), and the neuropyle (4). x10000.



**Fig. 8.** Submicroscopic organization of neurons of the granular layer of the cerebellar cortex 21 days after experimental thermal trauma. Dark neuron with pyknotic nucleus (1), light neuron (2) with electron light neuro- (3) and karyoplasm (4), myelin nerve fiber (5). x9000.

mitochondria with an enlightened matrix are disorganized and are in the stage of autolysis. Molecular layer neurons are also disorganized. The cytoplasm of cells is poor in organelles and they are destructively altered, fragmented, except for single mitochondria. Nuclei of irregular shape, have indistinct homogeneous nuclear membranes, the karyoplasm is dominated by marginally placed heterochromatin, no nucleoli.

The neurons of the granular layer are also severely damaged. The shape of the cells is changed. Dark neurons with high electron density of nucleoplasm and cytoplasm and light neurons with enlightened organelle-poor karyo- and neuroplasm are determined. Nuclei of irregular shape, osmophilic, compacted, no nucleoli. In the electron-dense neuroplasm of organelle cells there are few, single

mitochondria with reduced cristae and osmophilic matrix are present, deformed and fragmented tubules of the endoplasmic reticulum with single ribosomes are found (Fig. 8).

### Discussion

The results of micro- and submicroscopic study of neurons of the cerebellar cortex in experimental thermal trauma are to some extent consistent with the available data from the literature [1, 3, 17].

In the works of the authors [2, 14] it was found that the action of stressors causes disorganization of microstructures and angioarchitectonics of the cerebellar cortex. We found significant neurodegenerative changes in the neurons of all layers of the cerebellar cortex. 21 days after the experimental thermal injury, the stellate and basket cells of the molecular and grain cells of the granular layers were transformed, the nuclei were pyknotized, and perivascular edema was observed in most of them. Purkinje cells of the ganglion layer are diffusely located, their order is lost, voids are often found in the place of neurons, degenerating neurons were in the stage of apoptosis and shadow cells were detected.

The authors of the study [13] believe that the formation of cell-free gaps in the ganglion layer of the cerebellar cortex is associated with the elimination of some Purkinje cells due to the action of a negative factor. Similar results were also obtained by Yu.E. Morozov with co-authors [14], who observed a complex of morphological features in neurons of the cerebellar cortex, which cause profound destructive changes. Irregularly shaped neurons with blurred contours were detected, and their processes thinned. The authors concluded that the main signs of neuronal changes in thermal trauma to the cerebellum were violations of the structure of all major elements of the cell.

To some extent, our studies are consistent with the data of M. Delion and co-authors [6]. It should be noted that among Purkinje cells there are sharply hyperchromic, which often had a narrowed shape with slender or thickened processes, and hypochromic with total dissolution of chromatophilic substance were detected. In the granular layer, according to researchers [13, 14], most grain-cells had an altered shape. Neurons, according to our research, are relatively dense, but there are areas where cells were located mosaically. Neurons of the granular layer are characterized by light and dark forms, different osmophilicity, which indicates different functional activity of cells.

Thus, the results of histological studies correlate with scientific data of other researchers and found that 21 days after severe thermal injury significantly increases the destruction of membrane structural components of neurons of the cerebellar cortex and components of the microcirculatory tract, accompanied by the development of dystrophic processes in the body.

In further researches it is planned to establish degree

of morphological changes of a cerebellum at a thermal trauma with use of correcting factors.

### Conclusions

Thus, micro- and submicroscopic studies 21 days after the experimental thermal injury, which has a pathogenic effect on the cerebellum of the affected animals, revealed significant changes in the structural rearrangement of the organ. It is established that the neurons of the cerebellar

cortex undergo profound destructive-degenerative changes. Neurons of the molecular and granular layers change their shape, there is pericellular edema. Purkinje cells are dominated by sharply hyperchromic and single hypochromic neurons. Significant transformation of the structural organization of hemocapillaries of the cerebellar cortex, the development of paravasal edema, wall damage, transendothelial metabolism and significant hemato-neural relationships in organ.

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### МІКРО- ТА СУБМІКРОСКОПІЧНІ ЗМІНИ КОРИ МОЗОЧКА ЧЕРЕЗ 21 ДОБУ ПІСЛЯ МОДЕЛЮВАННЯ ОПІКОВОГО УРАЖЕННЯ Огінська Н.В., Небесна З.М., Гетманюк І.Б.

Мозочок є складно організованою поліфункціональною складовою головного мозку та мішенню при численних ураженнях, тому вивчення його морфофункціонального стану при різних патологічних станах та опіках зокрема не втрачає своєї актуальності. Метою нашого дослідження було встановити особливості мікро- та субмікроскопічних змін структурних компонентів кори мозочка через 21 добу за умов експериментальної термічної травми. Експериментальне дослідження моделювали на білих лабораторних щурах-самцях. Опік III ступеня наносили під тіопентал-натрієвим наркозом мідними пластинами, нагрітими у кип'яченій воді до температури 97-100°C. Розміри ділянки ураження складали 18-20% епільованої поверхні тіла щурів. Гістологічні зміни вивчали на 21 добу від початку експерименту. Напівтонкі зрізи для світлової мікроскопії забарвлювали метиленовим синім, для електронної мікроскопії отримані ультратонкі зрізи контрастували уранілацетатом та цитратом свинцю за методом Рейнольдса. За допомогою мікро- та субмікроскопічних досліджень було

виявлено значні альтеративні зміни як нейронів усіх шарів кори мозочка так і ланок мікроциркуляторного русла органу. Нейрони дезорганізовані, форма їх змінена, відзначається низька функціональна активність за рахунок зниження площі хроматофільної речовини. Часто наявні безклітинні ділянки у гангліонарному шарі, відзначається зміщення клітин Пуркіньє глибоко в зернистий шар, а клітини зерна різко виштовхуються до вище розташованого молекулярного шару. Таким чином, через 21 добу після експериментальної термічної травми встановлені деструктивно-дегенеративні зміни нейронів кори мозочка, паравазальний набряк та порушення трансендотеліального обміну.

**Ключові слова:** кора мозочка, мікро- та субмікроскопічні зміни, нейрони, термічна травма.

#### **МИКРО- И СУБМИКРОСКОПИЧЕСКИЕ ИЗМЕНЕНИЯ КОРЫ МОЗЖЕЧКА ЧЕРЕЗ 21 СУТКИ ПОСЛЕ МОДЕЛИРОВАНИЯ ОЖГОВОГО ПОРАЖЕНИЯ**

**Огинская Н.В., Небесна З.М., Гетманюк И.Б.**

Мозжечок является сложной организованной полифункциональной составляющей головного мозга и мишенью при многочисленных поражениях, поэтому изучение его морфофункционального состояния при различных патологических состояниях и ожогах в частности не теряет своей актуальности. Целью нашего исследования было установить особенности микро- и субмикроскопических изменений структурных компонентов коры мозжечка через 21 день в условиях экспериментальной термической травмы. Экспериментальное исследование моделировали на белых лабораторных крысах-самцах. Ожог III степени наносили под тиопентал-натриевым наркозом медными пластинами, нагретыми в кипяченой воде до температуры 97-100°C. Размеры участка поражения составляли 18-20% эпилированной поверхности тела крыс. Гистологические изменения изучали на 21 сутки от начала эксперимента. Полутонкие срезы для световой микроскопии окрашивали метиленовым синим, для электронной микроскопии полученные ультратонкие срезы контрастировали уранилацетатом и цитратом свинца по методу Рейнольдса. С помощью микро- и субмикроскопических исследований были выявлены значительные альтеративные изменения как нейронов всех слоев коры мозжечка, так и звеньев микроциркуляторного русла органа. Нейроны дезорганизованы, форма их изменена, отмечается низкая функциональная активность за счет снижения площади хроматофильного вещества. Часто имеются бесклеточные участки в ганглионарном слое, отмечается смещение клеток Пуркиньє глибоко в зернистый слой, а клетки зерен резко выталкиваются в выше расположенный молекулярный слой. Таким образом, через 21 день после экспериментальной термической травмы установлены деструктивно-дегенеративные изменения нейронов коры мозжечка, паравазальный отек и нарушение трансэндотелиального обмена.

**Ключевые слова:** кора мозжечка, микро- и субмикроскопические изменения, нейроны, термическая травма.

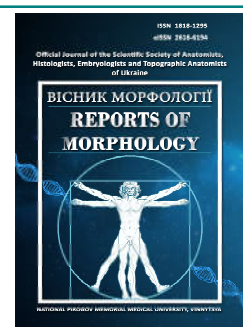
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# Morphometric peculiarities of the cervix uteri in immunodeficiency states

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*Enhancing each other's effects - HIV infection and the changes caused by alcohol abuse, trigger a chain of pathological reactions that sometimes lead to reversible and often irreversible pathological processes in the cervix uteri (CU). Local and general decrease in immunoresistance invariably leads to disturbance of physiological CU microbiome. Given all the above, the aim of our work was to identify pathological changes in CU that occur in HIV-infected women on the background of chronic alcoholism. Section material of 110 women of reproductive age from 20 to 40 years, which were divided into four groups, was studied. After the manufacture of micropreparations, a morphometric study was performed. Significant pathological changes in CU were revealed in HIV-infected women suffering from alcoholism. The maximum thickness of nonkeratinized stratified squamous epithelium ( $734.23 \pm 61.33 \times 10^{-6} \text{m}$ ) was characteristic of a group of HIV-infected women suffering from chronic alcoholism. In HIV-infected women, this figure is 3.14% lower and is  $711.21 \pm 59.28 \times 10^{-6} \text{m}$ . In women suffering from alcoholism without concomitant HIV infection, this figure is  $697.8 \pm 47.88 \times 10^{-6} \text{m}$ , which is 5% less than in the group with combined pathology. As expected, the lowest value was in the comparison group, where it was  $527.23 \pm 44.37 \times 10^{-6} \text{m}$ . There was a significant difference between the comparison group of HIV-infected women with alcoholism and the control group, which is 28.2%. In the studied material of HIV-infected patients, a high degree of distribution of the severity of cervical dysplasia was determined - 9.1%. Moderate and low degree, respectively, 19.42 and 37.12% in this group of subjects. When studying the degree of infiltration of the lamina propria mucosa in the study groups, it was found that the largest number of cases of severe infiltration (44.5%) was found in the group suffering from chronic alcoholism, and in the HIV groups no such case was detected. On the basis of the conducted research it is possible to assume the combined influence of the factors promoting development of pathological processes both in an epithelium, and in a mucous membrane of CU. Thus, HIV infection and alcohol abuse can exacerbate each other's pathological effects and lead to pronounced pathomorphological changes in CU, namely: thickening of the mucosal epithelium due to frequent development of various types of warts, mucosal dysplasia and even severe cellular infiltration of the mucous membrane.*

**Key words:** cervix uteri, HIV, alcoholism, epithelium, histology.

### Introduction

Diseases of the cervix uteri (CU) are widespread in the population, which is associated with low detection rate, imperfection of the medical system, sometimes with asymptomatic or poor symptoms of inflammatory and non-inflammatory processes in this area [3, 8].

Although periodic medical examinations and regular cytological screenings are designed to reduce morbidity and mortality rates among women, the problem of CU pathological processes is extremely acute today [7]. The

prevalence of this group of diseases remains high both in Ukraine and throughout the world [19].

Special attention of medical personnel in terms of the complexity of the clinical course, diagnostic features, individual approach to treatment is occupied by risk groups [12]. The greatest variability and complexity of the course of this group of diseases differs in women with immunosuppression [20, 22]. At the same time, HIV-infected women and women suffering from alcoholism represent a

global problem [16, 18].

Strengthening the effects of each other, both HIV infection and changes caused by alcohol abuse trigger a chain of pathological reactions leading to reversible and often irreversible pathological processes in CU [13, 17].

As it is known, ethanol, which is the main component of all alcoholic beverages, belongs to the 1st category of carcinogens, which means that there are reliable signs of its carcinogenicity for humans.

On the other hand, a local and general decrease in immunoresistance invariably leads to a disruption of the physiological microbiome of CU, which means that it increases the risk of infection with pathogenic microorganisms, fungal flora, viruses, in particular, the human papillomavirus (HPV) [6, 9]. 99% of all precancerous diseases occur against the background of HPV infection [1, 4]. To date, there are many studies devoted to changes in CU due to alcoholism, as well as in HIV infection, however, there are no data on changes in CU when these pathological processes are combined, which is very important, since they are very often combined in this cohort of patients.

Considering all of the above, *the purpose* of our work was to identify pathological changes in the cervix that occur in HIV-infected women against the background of chronic alcoholism.

### Materials and methods

For the study, we took material from 110 women of reproductive age from 20 to 40 years. All the subjects were divided into 4 groups. Group 1 (30 women) consisted of women diagnosed with HIV infection. In this group, both the anamnestic data (interviewing relatives) and the autopsy results (the main symptom was the presence of alcoholic cirrhosis of the liver), alcohol abuse was confirmed. The second group (25 women) consisted of people with confirmed HIV infection without any data on concomitant alcoholism. In patients of the third group, only anamnestic and postmortem signs of alcohol abuse were identified. The control group consisted of women (30) who died from diseases not related to alcohol abuse, pathology of the reproductive system without concomitant HIV infection (deaths as a result of accidents).

The collected material was fixed in 10% neutral buffered formalin and then embedded in paraffin. At the next stage, sections with a thickness of  $5 \times 10^{-6}$  m were made from the prepared paraffin blocks. Subsequently, the sections were stained with hematoxylin and eosin. Microscopic examination was carried out on an "Olympus BX41" microscope with further morphometric examination in the "Olympus DP-soft 3.12" software.

The following indicators were determined: the thickness of the nonkeratinized stratified squamous epithelium (NSSE) in all groups of patients, the relative volumes of warts, expressed as a percentage [5], the distribution of the severity of CIN in the test material as a percentage among the entire test material. Statistical processing of

the obtained results was performed using methods of variation statistics. Correspondence of the distribution to the normal distribution was determined by the Shapiro-Wilk's test, which showed that the samples are close to the normal distribution. Statistical indicators are presented in the format  $M \pm \sigma$ , where M is the arithmetic mean,  $\sigma$  is the standard deviation, Student's t-test. Correlation analysis was carried out using Spearman's rank correlation coefficient. The statistical difference between the studied parameters was considered significant at  $p < 0.05$ .

All studies were conducted in accordance with the Declaration of Helsinki, approved by the Ethics Commission of the Odessa National Medical University (Protocol 3, dated October 17, 2011).

### Results

As a result of the studies carried out, we obtained results, indicating the severity of pathological processes in the CU in HIV-infected women suffering from alcoholism.

At the first stage, the thickness of the epithelial layer of the cervical mucosa was determined. Table 1 shows the numerical values obtained when determining the thickness of the epithelial layer. The maximum thickness of the NSSE was typical for the group of HIV-infected women suffering from chronic alcoholism -  $734.23 \pm 61.33 \times 10^{-6}$  m. In HIV-infected women, this indicator is 3.14% less and is  $711.21 \pm 59.28 \times 10^{-6}$  m. In the group of examined women who suffered from chronic alcoholism without concomitant HIV infection, this indicator was  $697.8 \pm 47.88 \times 10^{-6}$  m, which is 5% less than in the group with combined pathology. The expected minimum indicator was in the comparison group,

**Table 1.** The thickness of the cervical epithelium in the examined groups of women ( $M \pm \sigma$ ).

Morphological sign	Study groups			
	HIV infection	chronic alcoholism	chronic alcoholism and HIV infection	comparison group
Thickness of nonkeratinized stratified squamous epithelium, $10^{-6}$ m	$711.2 \pm 59.3^*$	$697.8 \pm 47.9^*$	$734.2 \pm 61.3^*$	$527.2 \pm 44.4$

**Notes:** \* - the likelihood of differences between comparison groups  $p < 0.05$ .

**Table 2.** Relative volumes of warts in the surveyed groups of women (%).

Types of genital warts	Study groups			
	HIV infectio (n=25)	chronic alcoholism (n=25)	chronic alcoholism and HIV infection (n=30)	comparison group (n=30)
Genital wart	16.00 (4)	8.00(2)	16.67 (5)	3.33 (1)
Flat wart	12.00 (3)	8.00 (2)	26.67 (8)	3.33 (1)
Plantar wart	8.00 (2)	4.00 (1)	10.00 (3)	3.33 (1)
Total	36.00(9)	20.00 (5)	53.33 (16)	10.00 (3)

**Table 3.** Severity of cervical dysplasia in the studied groups of women (%).

	Study groups			
	HIV infection	chronic alcoholism	chronic alcoholism and HIV infection	comparison group
Low degree	23.32*	15.43*	37.12*	6.92
Moderate degree	14.21*	8.67	19.42*	5.99
High degree	8.31*	4.61	9.10*	3.23

**Notes:** \* - the likelihood of differences between comparison groups  $p < 0.05$ .

**Table 4.** The degree of infiltration of the lamina propria by immunocompetent cells in the examined groups of women.

Infiltration rate	Study groups			
	HIV infection	chronic alcoholism	chronic alcoholism and HIV infection	comparison group
Absence	4.7	0	10.3	0
Weak	60.2	12.9	65.6	87.5
Moderate	35.1	42.6	34.1	9.1
Expressed	0	44.5	0	3.4
Total	100	100	100	100

where it was  $527.23 \pm 44.37 \times 10^{-6} \text{m}$ . There was a significant difference between the comparison group of HIV-infected women suffering from alcoholism and the control group, which is 28.2%.

One of the possible reasons for changes in the thickness of the epithelium may be a much more frequent development of condylomatous vegetations in the studied groups, especially often associated with HIV infection. During the histological examination of the cervix, three forms of condylomatous vegetations were morphologically verified: genital, flat and plantar. The relative volumes of distribution of such forms are presented in table 2.

In the group of women surveyed with a combination of HIV infection and alcoholism (see Table 2), attention is drawn to the presence of a greater number of different types of warts: genital warts in this group amounted to 16.67%, flat - 26.67%, plantar - 10%, which in total amounted to 53.33%.

In the group of women with only confirmed HIV infection, similar indicators were 16, 12 and 8%, which amounted to 36%.

It should be noted that in the group of women who abused alcohol, there was also an increase in the relative volume of warts among all selected cases. These figures were 8%, 8% and 4% (only 20%) for genital warts, flat and plantar warts, respectively.

Predictably the best results of the studied indicator can confirm the minimum thickness of the NSSE in the control group of women. The relative volumes of warts were 3.33% for each type of warts, which in total amounted to only 10%.

Another possible change in the thickness of the

epithelium is the significantly more frequent cases of CIN. So, in most cases of the comparison group, no signs of dysplasia of the cervical epithelium were detected, while in the studied groups a significant increase in the specific volume of cases with dysplastic changes was revealed. Mild dysplasia is characterized by preserved anisomorphism and stratification of the surface and intermediate layers, focal basal cell hyperactivity with an increase in the nuclear-cytoplasmic ratio. At the same time, the structuring of the nucleoli in the nuclei of the cells of the basal and parabasal layers, their moderate basophilia are revealed.

Depending on the severity of dysplasia, the examined cervix was characterized by normal, weak, moderate or severely disturbed cytoarchitectonics. The nuclei were of normal size or slightly enlarged, had the same oval shape, with predominantly normal polarization and mild hyperchromicity. Mitotic figures were present in 85% of cases in all layers, in 10% they were observed only in the basal layer, in 5% they were few in both the basal and other layers of the epithelium.

With high CIN, a pronounced violation of cytoarchitectonics was observed (the size and shape of the cells varied significantly). Cells with transparent cytoplasm, as a rule, were absent, or there was a small number of them in certain areas, the size of the nuclei varied markedly with a predominance in the direction of increase, the nuclei had different shapes, polarization in many was absent, there was a pronounced hyperchromicity of the nuclei with the presence of even single pathological mitoses and mitotic figures.

The distribution of the test material depending on the severity of CIN is presented in Table 3. A high degree of distribution of the severity of CIN was determined in the test material of HIV-infected patients, it was 9.1%. Moderate and low degree, respectively, 19.42 and 37.12% in this group of persons. The best result according to this indicator was determined in the comparison group. It was 3.23, 5.99 and 6.92%, respectively, for high, moderate and low degrees. The group of HIV-infected women without signs of chronic alcoholism and the group of women with alcoholism occupy an intermediate position, according to the indicators between the two previous groups. The values that were revealed in these studied groups of women were 23.32, 14.21, 8.31%, respectively.

When assessing the degree of infiltration of the lamina propria by immunocompetent cells (lymphohistiocytes) using the semi-quantitative method from 0 to 3 points (0 - absence, 1 - weak, 2 - moderate, 3 - severe infiltration), it was found that the largest number of cases with weak infiltration belonged to the group of combined chronic alcoholism and HIV infection (65.64%). It should be noted that 10.32% of cases with no infiltration were found in this group, i.e. almost complete absence of lymphocytes.

When studying the degree of infiltration of the lamina propria in the study groups, attention is drawn to the fact



that the largest number of cases of severe infiltration (44.5%) was found in the group with chronic alcoholism, and not a single such case was found in the groups with HIV.

### Discussion

On the basis of the study, we can assume the combined influence of factors contributing to the development of pathological processes both in the epithelium and in the mucous membrane of CU [1]. On the one hand, this is due to the presence of hormonal imbalance, which can be explained by the presence of sclerotic-degenerative changes in the ovaries [7], which is manifested by a progressive decrease in the size of primordial follicles and a decrease in the number of both primary and secondary, and tertiary follicles due to the presence of HIV infection [13] and concomitant chronic alcoholism [2, 14].

An equally important link in the pathogenetic chain of changes is the effects caused by the influence of confirmed HIV infection [11]. It can be assumed that the main link leading to the development of pathological processes of CU in this case is pronounced immunosuppression, which leads to an increased risk of HPV infection, the development of various types of genital warts, and the development of dysplastic changes in CU [6, 18]. These changes, in turn, are the substrate for the thickening of the mucous membrane, which was the maximum thickness in HIV-infected women suffering from chronic alcoholism [14, 15]. It should also be noted that the thickness index was significantly less ( $p < 0.05$ ) in women who had clinical and anamnestic data only on the presence of chronic alcoholism, compared with the group of HIV-infected women. Therefore, it can be assumed that the presence of HIV infection is of greater importance for the emergence of a cascade of pathological reactions leading to changes in the mucous membrane of the CU [19, 21]. This assumption could be confirmed by the data obtained in the study of the prevalence of various types of warts and data on the severity of dysplasia [3].

Thus, in the comparison group, the relative volumes of warts were minimal and equally distributed across all three types (genital, flat and plantar). In the group of HIV-infected patients, genital warts prevailed, with a combination of HIV infection and alcoholism - flat, with alcoholism - genital

and flat. As is known, the presence of warts many times increases the risks of developing CU oncological processes [15]. Thus, having only anamnestic data on alcohol abuse, information about a woman's HIV status, data from a physical examination by a gynecologist, the presence of contributing factors for the development of malignant neoplasms can be suspected, which should cause even greater oncological alertness when working with this cohort of persons [16, 22].

In all four groups of patients, the degree of immunocompetent cell infiltration of the lamina propria was determined. It was revealed that the most pronounced infiltration was determined in women suffering from chronic alcoholism. At the same time, in the control group, as in the group of HIV-infected women and even in the group of HIV-infected against the background of chronic alcoholism, weak infiltration prevailed. These changes can be explained by the pronounced immunosuppression caused by HIV infection. Due to this, inflammatory changes do not develop. Consequently, the patients of these study groups are characterized by more dysplastic rather than inflammatory processes in the CU.

Given the prevalence of severe and moderate infiltration of the lamina propria in patients with alcohol abuse, it is in them that a pronounced picture of cervicitis should be expected.

Thus, the data obtained in this study will help to timely and effectively examine women with risk factors in terms of development pathological processes of CU, which means choosing a treatment for correcting these processes, and therefore increasing the duration and quality of life of HIV-infected patients with clinical and anamnestic data characteristic of the presence of concomitant chronic alcohol abuse.

### Conclusions

HIV infection and alcohol abuse can increase the pathological effects of each other and lead to pronounced pathomorphological changes in CU, which consist in thickening of the epithelium of the mucous membrane due to the frequent development of various types of warts, dysplasia of the mucous membrane and also pronounced cellular infiltration of the lamina propria.

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## МОРФОМЕТРИЧНІ ОСОБЛИВОСТІ ШИЙКИ МАТКИ ПРИ ІМУНОДЕФІЦИТНИХ СТАНАХ

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Посилуючи ефекти один одного ВІЛ-інфікування та зміни, зумовлені зловживанням алкоголю, запускають ланцюжок патологічних реакцій, котрі призводять іноді до зворотних, а часто і незворотніх патологічних процесів у шийці матки (ШМ). Локальне та загальне зниження імунорезистентності незмінно веде до порушення фізіологічного мікробіому ШМ. З огляду на все викладене вище, метою нашої роботи стало виявлення патологічних змін ШМ, що виникають у ВІЛ-інфікованих жінок на тлі хронічного алкоголізму. Досліджено секційний матеріал 110 жінок репродуктивного віку від 20 до 40 років, які були розділені на чотири групи. Після виготовлення мікропрепаратів проводили морфометричне дослідження. Виявлено виражені патологічні зміни в ШМ у ВІЛ-інфікованих жінок, що страждали алкоголізмом. Максимальна товщина багаточарового плоского незроговілого епітелію ( $734,23 \pm 61,33 \times 10^{-6} \text{ м}$ ) характерна для групи ВІЛ-інфікованих жінок, які страждають на хронічний алкоголізм. У ВІЛ-інфікованих жінок цей показник на 3,14% менше і становить  $711,21 \pm 59,28 \times 10^{-6} \text{ м}$ . У жінок, які страждають на алкоголізм без супутньої ВІЛ-інфекції даний показник становить  $697,8 \pm 47,88 \times 10^{-6} \text{ м}$ , що на 5% менше, ніж у групі з поєднаною патологією. Очікувано мінімальним показник виявився в групі порівняння, де він становив  $527,23 \pm 44,37 \times 10^{-6} \text{ м}$ . Встановлена достовірна різниця між групою порівняння ВІЛ-інфікованих жінок, що страждали алкоголізмом, і контрольною групою, яка становить 28,2%. У досліджуваному матеріалі ВІЛ-інфікованих пацієнток визначений високий ступінь розподілу вираженості дисплазії шийки матки - 9,1%. Помірний і низький ступінь відповідно 19,42 і 37,12% у даній групі досліджуваних. При вивченні ступеня інфільтрації власної пластинки слизової у групах дослідження виявлено, що найбільша кількість випадків вираженої інфільтрації (44,5%) виявлена в групі, що страждали на хронічний алкоголізм, а в групах з ВІЛ не було виявлено жодного такого випадку. На основі проведеного дослідження можна припустити поєднаний вплив факторів, що сприяють розвитку патологічних процесів як в епітелії, так і в слизовій оболонці ШМ. Таким чином, ВІЛ-інфікування та зловживання алкоголем можуть посилювати патологічні ефекти один одного і призводити до виражених патоморфологічних змін ШМ, а саме:

потощення епітелію слизової оболонки за рахунок частого розвитку різних видів кондилом, дисплазії слизової оболонки і навіть вираженої клітинної інфільтрації власної пластинки слизової оболонки.

**Ключові слова:** шийка матки, ВІЛ, алкоголізм, епітелій, гістологія.

#### **МОРФОМЕТРИЧЕСКИЕ ОСОБЕННОСТИ ШЕЙКИ МАТКИ ПРИ ИММУНОДЕФИЦИТНЫХ СОСТОЯНИЯХ**

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Усиливая эффекты друг друга ВИЧ-инфицирование и изменения, обусловленные злоупотреблением алкоголя, запускают цепочку патологических реакций, приводящих иногда к обратимым, а часто и к необратимым патологическим процессам в шейке матки (ШМ). Локальное и общее снижение иммунорезистентности неизменно ведет к нарушению физиологического микробиома ШМ. Учитывая все изложенное выше, целью нашей работы было выявление патологических изменений ШМ, возникающих у ВИЧ-инфицированных женщин на фоне хронического алкоголизма. Исследован секционный материал 110 женщин репродуктивного возраста от 20 до 40 лет, которые были разделены на четыре группы. После изготовления микропрепаратов проводили морфометрическое исследование. Выявлены выраженные патологические изменения в ШМ у ВИЧ-инфицированных женщин, страдавших алкоголизмом. Максимальная толщина многослойного плоского неороговевающего эпителия ( $734,23 \pm 61,33 \times 10^{-6} \text{ м}$ ) была характерна для группы ВИЧ-инфицированных женщин, страдающих хроническим алкоголизмом. У ВИЧ-инфицированных женщин этот показатель на 3,14% меньше и составляет  $711,21 \pm 59,28 \times 10^{-6} \text{ м}$ . У женщин, которые страдают алкоголизмом без сопутствующей ВИЧ-инфекции, данный показатель составляет  $697,8 \pm 47,88 \times 10^{-6} \text{ м}$ , что на 5% меньше, чем в группе с сочетанной патологией. Ожидаемо минимальным показатель оказался в группе сравнения, где он составил  $527,23 \pm 44,37 \times 10^{-6} \text{ м}$ . Выявлена достоверная разница между группой сравнения ВИЧ-инфицированных женщин, страдавших алкоголизмом, и контрольной группой, которая составляет 28,2%. В исследуемом материале ВИЧ-инфицированных пациенток определена высокая степень распределения выраженности дисплазии шейки матки - 9,1%. Умеренная и низкая степень соответственно 19,42 и 37,12% в данной группе исследуемых. При изучении степени инфильтрации собственной пластинки слизистой в группах исследования выявлено, что наибольшее количество случаев выраженной инфильтрации (44,5%) выявлено в группе с хроническим алкоголизмом, а в группах с ВИЧ не было выявлено ни одного такого случая. На основе проведенного исследования можно предположить сочетанное влияние факторов, способствующих развитию патологических процессов как в эпителии, так и в слизистой оболочке ШМ. Таким образом, ВИЧ-инфекция и злоупотребление алкоголем могут усиливать патологические эффекты друг друга и приводить к выраженным патоморфологическим изменениям ШМ, а именно: утолщению эпителия слизистой оболочки за счет частого развития различных видов кондилом, дисплазии слизистой оболочки и даже выраженной клеточной инфильтрации собственной пластинки слизистой оболочки.

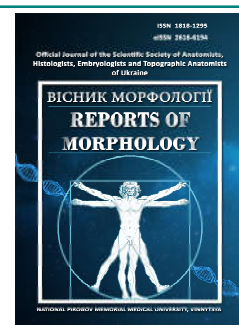
**Ключевые слова:** шейка матки, ВИЧ, алкоголизм, эпителий, гистология.



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# Morphometric evaluation of features of remodelling of the venous bed of the limbs at experimental trophic ulcer and its correction

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*The progressive course of diseases of the veins of the lower extremities leads to the development of complications in more than 80% of patients with post-thrombotic disease. Purpose of the work: to study the morphometric features of remodeling of the venous bed of the extremities in experimental trophic ulcer and its correction. A complex of morphological methods was used to examine the veins of the lower limbs of 18 adult male Vietnamese pigs, which were divided into 3 groups. 1 group consisted of 6 intact practically healthy animals, the 2 group - 6 pigs with simulated trophic ulcer, the 3 group - 6 animals with Lidocaine-corrected ulcer. The area of the trophic ulcer was measured. The trophic ulcer was taken together with the surrounding tissues, from which histological micropreparations were made, which determined the diameters of capillary venules, venules, outer and inner diameters of venous vessels, the height of endothelial cells, the diameter of their nuclei, nuclear-cytoplasmic ratios in these cells, volume of damaged endotheliocytes. A correlation analysis was performed between the ulcer area and morphometric parameters. Quantitative values were processed statistically. Strong positive correlations have been established between nuclear-cytoplasmic relation in endothelial cells, the relative volume of damaged endotheliocytes, and the area of the ulcer defect ( $r=+0,84\pm 0,03$ ), at correction of an ulcer by Lidocaine they weakened a little. It was found that the correction of trophic ulcers with Lidocaine led to a decrease in its area by 25.8%. It is established that the experimental trophic ulcer leads to pronounced remodeling of the venous bed of the lower extremities, which is characterized by significant dilation of venous vessels, venous plethora complicated by hypoxia, atrophic, dystrophic, necrobiotic, infiltrative processes and sclerosis. The use of Lidocaine in experimental trophic ulcer improves the adaptive-compensatory and regenerative processes of the modeled pathology, reduces the relative volume of damaged endothelial cells in the venous vessels of the lower extremity by 18.8% ( $p<0.001$ ).*

**Key words:** trophic ulcer, veins, morphometry, Lidocaine.

### Introduction

Chronic venous insufficiency is a symptom complex that characterizes a condition caused by venous disease, in particular, varicose veins, postthrombotic disease or venous angiodysplasia, caused by the presence of venous hypertension, the clinical signs of which are heaviness, fatigue of the lower extremities, edema, edema, flatulence, pain, telangiectasia, varicose veins, trophic disorders and ulcers [24].

Trophic ulcers of the lower extremities are found in almost every fifth patient with CVI, they often recur and cause a deterioration in quality of life [7]. Trophic ulcers among the world's population are found in 0.2% of patients with varicose

veins under the age of 50 years and in 2.6% - over 50 years.

Valve failure of the venous system of the lower extremities leads to the formation of persistent phlebohypertension, which progresses rapidly, causing pathogenetic and destructive changes in the soft tissues of the lower extremities.

Despite significant advances in diagnosis and treatment in recent years, the choice of pathogenetic treatment in patients with severe chronic venous insufficiency remains controversial and unresolved. The development of irreversible trophic changes in the soft tissues of the lower extremities in most patients leads to no effect from treatment

by known methods, and even with a positive result of treatment there is a high risk of recurrence - 30-80%.

The progressive course of diseases of the veins of the lower extremities leads to the development of complications in 15-25% patients with varicose veins and more than 80% of patients with postthrombotic disease [4, 13, 30]. The complexity and versatility of pathogenetic mechanisms of trophic ulcers of the lower extremities requires different and multifaceted approaches to their treatment. At the same time, clinicians use various methods of treatment, which take into account the stage of chronic venous insufficiency, the presence of complications and the predominant syndrome [31]. It should be noted that to this day the search for timely, complete, adequate diagnosis, correction and prevention of trophic ulcers of the lower extremities [4, 13, 25, 26]. In recent decades, morphologists have widely used morphometric research methods that allow to quantify various physiological and pathological processes and adequately interpret them [1, 9, 18].

The aim of the study was morphometric study of the features of remodeling of the venous bed of the extremities in experimental trophic ulcer and its correction.

### Materials and methods

A set of morphological methods was used to examine the veins of the hind limbs of 18 adult male Vietnamese pigs, which were divided into 3 groups. The first group consisted of 6 intact practically healthy animals, the 2nd group - 6 pigs with simulated trophic ulcer [6], the 3rd group - 6 animals with trophic ulcer corrected by lidocaine at a dose of 1.0 mg/kg.

Euthanasia of experimental animals was performed by bloodletting under conditions of thiopental-sodium anesthesia [10, 11]. The trophic ulcer was removed together with the surrounding tissues, which were fixed in 10% neutral formalin solution, passed through ethyl alcohols of increasing concentration and placed in paraffin. Microtome sections 5-7  $\mu\text{m}$  thick after dewaxing were stained with hematoxylin-eosin, according to Van Gieson, Mallory, toluidine blue [8]. Venous vascular morphometry was performed on histological micropreparations. The diameters of capillary venules (DCV), venules (DV), external (EDVV) and internal (IDVV) diameters of venous vessels, height of endotheliocytes (HE), diameter of their nuclei (DN), nuclear-cytoplasmic relations (NCR) were determined, the relative volume of damaged endotheliocytes (RVDE) [1, 27]. 50 measurements were performed on each micropreparation. The area of the trophic ulcer was determined by the planimetric method [26]. Correlation analysis was performed between the area of the ulcer defect and the studied morphometric parameters to determine the correlation coefficient ( $r$ ). The strength of correlations was assessed by four degrees: strong ( $r=0.7-0.9$ ), significant ( $r=0.5-0.7$ ), moderate ( $r=0.3-0.5$ ), weak ( $r<0.3$ ) [1].

Quantitative morphological parameters were processed statistically. Processing of the latter was performed in the

department of system statistical researches of Ivan Horbachevsky Ternopil National Medical University in the software package STATISTIKA. The difference between comparative morphometric parameters is determined by the Student's and Mann-Whitney test [12, 15]. Experiments and euthanasia of experimental animals were performed in accordance with the "General Ethical Principles of Animal Experiments" adopted by the First National Congress on Bioethics (Kyiv, 2001), in accordance with the "European Convention for the Protection of Vertebrate Animals for Research and Other Scientific Purposes", also according to the Law of Ukraine "On protection of animals from cruel treatment" (from 21.02.2006) [23].

### Results

Studies have shown that the area of the ulcer defect in uncorrected trophic ulcer was equal to  $(22.1\pm 0.3)$   $\text{mm}^2$ , and in the 3rd group of observations (corrected ulcer) -  $(16.4\pm 9.2)$   $\text{mm}^2$ . These morphometric parameters were statistically significant ( $p<0.001$ ) differed from each other and the last quantitative morphological index was lower than the previous one by 25.8%. Therefore, the correction of trophic ulcers with lidocaine led to a marked reduction in the area of the ulcer defect. The obtained morphometric parameters of the venous bed of the lower extremities are presented in table 1.

A comprehensive analysis of the data in Table 1 showed that the studied morphometric parameters of groups 2 and 3 of the observation groups differed from similar controls. Thus, the diameter of the venules in the simulated trophic ulcer was statistically significantly ( $p<0.001$ ) increased from  $(12.85\pm 0.09)$   $\mu\text{m}$  to  $(15.90\pm 0.12)$   $\mu\text{m}$ , ie by 23.7%. With corrective correction of the simulated pathology, this quantitative morphological index decreased by 15.1% compared with the above, but did not reach the level of control  $(13.50\pm 0.09)$   $\mu\text{m}$  ( $p<0.01$ ). The diameter of venules in group 2 of experimental animals with a pronounced statistically significant difference ( $p<0.001$ ) increased by 21.9%, and in group 3 was reduced by 14.9% ( $p<0.001$ ) compared to the same previous parameter.

**Table 1.** Morphometric characteristics of the venous bed of the lower extremities of experimental animals ( $M\pm m$ ).

Indicator	Observation group		
	1 group	2 group	3 group
DCV, $\mu\text{m}$	12,85 $\pm$ 0,09	15,90 $\pm$ 0,12***	13,50 $\pm$ 0,09**
DV, $\mu\text{m}$	26,97 $\pm$ 0,15	32,80 $\pm$ 0,18***	27,90 $\pm$ 0,15**
EDVV, $\mu\text{m}$	40,34 $\pm$ 0,33	47,76 $\pm$ 0,27***	41,80 $\pm$ 0,24**
IDVV, $\mu\text{m}$	28,33 $\pm$ 0,21	34,62 $\pm$ 0,24***	30,10 $\pm$ 0,21***
HE, $\mu\text{m}$	4,821 $\pm$ 0,031	4,501 $\pm$ 0,031***	4,661 $\pm$ 0,031**
DN, $\mu\text{m}$	3,482 $\pm$ 0,021	3,432 $\pm$ 0,021	3,442 $\pm$ 0,031
NCR	0,520 $\pm$ 0,003	0,580 $\pm$ 0,003***	0,545 $\pm$ 0,005**
RVDE,%	2,301 $\pm$ 0,031	39,20 $\pm$ 0,48***	20,40 $\pm$ 0,16***

**Notes:** \*\*- $p<0,01$ ; \*\*\*- $p<0,001$  compared to the first group.

The outer diameter of the venous vessels of the intact lower extremity was equal to  $(40.34 \pm 0.33) \mu\text{m}$ , and in the conditions of trophic ulcer -  $(47.76 \pm 0.27) \mu\text{m}$ . A statistically significant difference ( $p < 0.001$ ) was found between the given morphometric parameters and the last quantitative morphological indicator exceeded the previous one by 18.4%. Correction of trophic ulcers led to a decrease in the outer diameter of the veins of the lower extremity, which reached  $(41.80 \pm 0.24) \mu\text{m}$ . The above morphometric parameter was less than the same indicator in the conditions of uncorrected trophic ulcer by 12.5% ( $p < 0.01$ ), and also exceeded the similar control value by 3.6% ( $p < 0.01$ ).

The inner diameter of the studied vessels in the simulated experiment was also markedly ( $p < 0.001$ ) increased from  $(28.33 \pm 0.21) \mu\text{m}$  to  $(34.62 \pm 0.24) \mu\text{m}$ , ie by 22.2%. In the 3rd group of observations (corrected trophic ulcer) the studied morphometric parameter was statistically significant ( $p < 0.001$ ) decreased by 17.8%. At the same time, this quantitative morphological indicator differed from the similar control by 6.2% ( $p < 0.001$ ).

The simulated pathology (trophic ulcer) led to the structural rearrangement of endothelial cells of the veins of the lower extremities, which was confirmed by changes in their morphometric parameters [19]. Thus, in control observations, the height of endothelial cells was equal to  $(4.821 \pm 0.031) \mu\text{m}$ , and in group 2 animals -  $(4.501 \pm 0.031) \mu\text{m}$ . Between the given quantitative morphological indicators the expressed statistically significant ( $p < 0.001$ ) difference is revealed and the last indicator appeared less than the previous by 6.6%. The nuclei of these cells under the conditions of this experiment decreased by only 1.4% ( $p > 0.05$ ). Uneven disproportionate changes in the spatial characteristics of the nucleus and cytoplasm led to violations of the relationship between them, which was determined by the nuclear-cytoplasmic relationship in the studied cells. In undamaged venous vessels of the lower extremities, this morphometric parameter was equal to  $0.520 \pm 0.003$ , in the 2nd group of observations -  $0.580 \pm 0.003$ . These quantitative morphological parameters were statistically significant ( $p < 0.001$ ) differed. The last morphometric parameter exceeded the previous one by 11.5%.

The height of endothelial cells in the conditions of corrected trophic ulcer was equal to  $(4.661 \pm 0.031) \mu\text{m}$ . This morphometric parameter differed by 2.9% ( $p < 0.01$ ) from the same control, ie to a lesser extent compared with uncorrected trophic ulcer. The sizes of nuclei in the studied group of animals were similarly changed.

The correlation analysis revealed the presence of strong positive correlations in the 2nd group of observations between nuclear-cytoplasmic relations in endotheliocytes, the relative volume of damaged endotheliocytes and the area of the ulcer defect ( $r = +0.84 \pm 0.03$ ). Slightly lower positive correlations were found between these quantitative morphological parameters in group 3 animals, where the correlation coefficient ranged from 0.53 to 0.65 (significant

correlations).

### Discussion

Nuclear-cytoplasmic ratios in endothelial cells in the conditions of corrected trophic ulcer change to a lesser extent (4.8%,  $p < 0.01$ ) compared with group 3 [18, 28]. It should be noted that some researchers believe that changes in nuclear-cytoplasmic relationships in cells are a violation of structural-cellular homeostasis and a sign of damage to the studied structures [1, 27]. It is known that the violation of a significant number of endothelial cells can lead to endothelial dysfunction, which exacerbates and worsens the course of the main pathology [16, 17].

Given the established positive correlation strong relationships in the second group of observations between the nuclear-cytoplasmic ratio in endothelial cells, damaged endothelial relative volume and area of the ulcer and positive correlation between quantitative morphological parameters of the third group of experimental animals, we can say that the size of ulcer defects on the limb depend on the severity of the structural adjustment of the venous bed. Remodeling of the latter significantly disrupts the drainage of venous blood, promotes the development of venous plethora and hypoxia [2, 27].

In the light microscopy examination of micropreparations of tissues of the lower extremity in a simulated trophic ulcer, a pronounced dilation and plethora of venous vessels was observed. Venous vessels of the hemomicrocirculatory tract (capillary venules and venules) are dilated, tortuous, with uneven lumen, varicose and with numerous sacculations [22]. Stasis, sludges and thrombosis foci, plasmorrhagia of the walls and paravasal space were observed in these microvessels. There were foci of diapedetic hemorrhage. In the tissues of the lower extremity were also found foci with a decrease in microvessels due to their reduction and avascular zones [14, 21]. Endothelial cells of venous vessels were edematous, dystrophically and necrobiotically altered, acquired a rounded shape with foci of their desquamation. There were also pronounced structural changes (dystrophy, necrobiosis, infiltration, sclerosis) in the cells and tissues of the lower extremity. In the corrected ulcer, the described morphological changes were similar, but less pronounced [29].

Less pronounced destructive processes and improved regeneration in the tissues of the lower extremity in experimental trophic ulcers corrected by lidocaine can be explained by the fact that the introduction of this drug limits the release of inflammatory mediators by neutrophils and monocytes, reduces the release of interleukins IL-1, IL-8, tumor necrosis factor, prostaglandins, thromboxanes, leukotrienes, lysosomal enzymes, free radicals, degranulation of mast cells with the release of histamine [3, 5, 20].

Further, a comprehensive study of structural changes in the venous bed of the lower extremities in trophic ulcers will take into account its morphological changes by

clinicians in the diagnosis, correction and prevention of this pathology.

### Conclusions

Experimental trophic ulcer leads to a pronounced remodeling of the venous bed of the lower extremities, which

is characterized by pronounced dilation of venous vessels, venous plethora complicated by hypoxia, atrophic, dystrophic, necrobiotic, infiltrative and sclerotic processes.

The use of lidocaine in experimental trophic ulcers improves the adaptive-compensatory and regenerative processes and the course of the simulated pathology.

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#### МОРФОМЕТРИЧНА ОЦІНКА ОСОБЛИВОСТЕЙ РЕМОДЕЛЮВАННЯ ВЕНОЗНОГО РУСЛА КІНЦІВОК ПРИ ЕКСПЕРИМЕНТАЛЬНІЙ ТРОФІЧНІЙ ВИРАЗЦІ ТА ЇЇ КОРЕКЦІЇ

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Прогресуючий перебіг захворювань вен нижніх кінцівок призводить до розвитку ускладнень у понад 80% хворих на посттромботичну хворобу. Мета роботи: вивчити морфометричні особливості ремоделювання венозного русла кінцівок при експериментальній трофічній виразці та її корекції. Комплексом морфологічних методів досліджені вени задніх кінцівок 18 статевозрілих свиней-самців в'єтнамської породи, які були розділені на три групи: 1 група нараховувала 6 інтактних практично здорових тварин, 2 групу становили 6 свиней зі змодельованою трофічною виразкою, 3 група - 6 тварин із виразкою, корегованою лідокаїном. Вимірювали площу трофічної виразки. Забирали трофічну виразку разом з оточуючими тканинами, з яких виготовляли гістологічні мікропрепарати, де визначали діаметри закапілярних венул, венул, зовнішній та внутрішній діаметри венозних судин, висоту ендотеліоцитів, діаметр їх ядер, ядерно-цитоплазматичні відношення у цих клітинах, відносний об'єм пошкоджених ендотеліоцитів. Проводили кореляційний аналіз між площею виразки та морфометричними параметрами. Кількісні величини обробляли статистично. Встановлені сильні позитивні кореляційні взаємозв'язки між ядерно-цитоплазматичними відношеннями в ендотеліоцитах, відносним об'ємом пошкоджених ендотеліоцитів та площею виразкового дефекту ( $r=+0,84\pm 0,03$ ); при корекції виразки лідокаїном вони дещо слабшали. Виявлено, що корекція трофічної виразки лідокаїном призводила до зменшення її площі на 25,8%. Встановлено, що експериментальна трофічна виразка призводить до вираженого ремоделювання венозного русла нижніх кінцівок, яке характеризується значним розширенням венозних судин, венозним повнокров'ям, що ускладнюється гіпоксією, атрофічними, дистрофічними, некробіотичними, інфільтративними та склеротичними процесами. Застосування лідокаїну при експериментальній трофічній виразці покращує адаптаційно-компенсаторні та регенераторні процеси змодельованої патології, зменшує відносний об'єм пошкоджених ендотеліоцитів у венозних судинах нижньої кінцівки на 18,8% ( $p<0,001$ ).

**Ключові слова:** трофічна виразка, вени, морфометрія, лідокаїн.

#### МОРФОМЕТРИЧЕСКАЯ ОЦЕНКА ОСОБЕННОСТЕЙ РЕМОДЕЛИРОВАНИЯ ВЕНОЗНОГО РУСЛА КОНЕЧНОСТЕЙ ПРИ ЭКСПЕРИМЕНТАЛЬНОЙ ТРОФИЧЕСКОЙ ЯЗВЕ И ЕЕ КОРРЕКЦИИ

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Прогрессирующее течение заболеваний вен нижних конечностей приводит к развитию осложнений у более 80% больных посттромботической болезнью. Цель работы: изучить морфометрические особенности ремоделирования венозного русла конечностей при экспериментальной трофической язве и ее коррекции. Комплексом морфологических методов исследованы вены задних конечностей 18 половозрелых свиней-самцов вьетнамской породы, которые были разделены на три группы: 1 группа насчитывала 6 интактных практически здоровых животных, 2 группу составили 6 свиней со смоделированной трофической язвой, 3 группа - 6 животных с язвой, скорректированной лидокаином. Измеряли площадь трофической язвы. Забирали трофическую язву вместе с окружающими тканями, из которых изготавливали гистологические микропрепараты, где определяли диаметры закапиллярных венул, венул, внешний и внутренний диаметры венозных сосудов, высоту эндотелиоцитов, диаметр их ядер, ядерно-цитоплазматические отношения в этих клетках, относительный объем поврежденных эндотелиоцитов. Проводили корреляционный анализ между площадью язвы и морфометрическими параметрами. Количественные величины обрабатывали статистически. Установлены сильные положительные корреляционные взаимосвязи между ядерно-цитоплазматическими отношениями в эндотелиоцитах, относительным объемом поврежденных эндотелиоцитов и площадью язвенного дефекта ( $r=+0,84\pm 0,03$ ); при коррекции язвы лидокаином они незначительно ослабевали. Выведено, что коррекция трофической язвы лидокаином приводила к уменьшению ее площади на 25,8%. Установлено, что экспериментальная трофическая язва приводит к выраженному ремоделированию венозного русла нижних конечностей, которое характеризуется значительным расширением венозных сосудов, венозным полнокровием, что осложняется гипоксией, атрофическими, дистрофическими, некробіотическими, инфильтративными и склеротическими процессами. Применение лидокаина при экспериментальной трофической язве улучшает адаптационно-компенсаторные и регенераторные процессы смоделированной патологии, уменьшает относительный объем поврежденных эндотелиоцитов в венозных сосудах нижней конечности на 18,8% ( $p<0,001$ ).

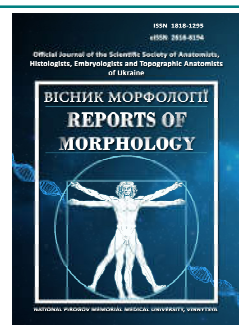
**Ключевые слова:** трофическая язва, вены, морфометрия, лидокаин.



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# Changes in the asymmetric dimethylarginine and endothelial nitric oxide synthase levels in pathogenesis of experimental diabetic retinopathy

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*Endothelial dysfunction associated with impaired nitric oxide excretion plays an important role in the onset and progression of diabetic retinopathy. It has been proven that a decrease in the activity of endothelial NO-synthase (eNO-S), the inhibitor of which is asymmetric dimethylarginine (ADMA), plays an important role in this. Objective: to study the level of asymmetric dimethylarginine and endothelial nitric oxide synthase at different stages of development of diabetic retinopathy in the experiment. The study was conducted in Wistar white rats of 180-200 g weight. According to the tasks, the animals were separated into 2 groups as follows: group 1 - 60 intact animals, group 2 - 60 animals with simulated diabetic retinopathy without further correction. Type 2 diabetes mellitus and diabetic retinopathy were simulated through intraperitoneal administration of Streptozotocin (Sigma, USA) diluted in 0.1M citrate buffer with pH=4.5. Streptozotocin dose of 55 mg per kg of animal weight was divided into two administrations. The streptozotocin intake was preceded by a 28-day high-fat diet. Our study showed impaired endothelial function in diabetic retinopathy, as evidenced by an increased ADMA level ( $p < 0.001$ ). We have determined a stepwise increase of asymmetric dimethylarginine level in blood of rats with simulated pathology which is apparent in its highest at phase 3. Pathogenetic effect of increased ADMA on eNO-S activity was verified at all experimental stages. Impairment of physiological nitric oxide synthesis in simulated pathology has been proved as evidenced by reduced activity of endothelial NO-synthase yet on the 30th day with further negative dynamics up to the 180th day ( $p < 0.001$  compared with the intact group findings).*

**Keywords:** *diabetic retinopathy, endothelial dysfunction, asymmetric dimethylarginine, endothelial NO-synthase, comparative description.*

### Introduction

Endothelial dysfunction which is associated with impaired secretion of nitric oxide is an essential link in pathogenesis of diabetes mellitus and plays the key role in occurrence and progression of diabetic retinopathies [14, 39]. Reduction of endothelium-dependent vasodilatation is also reported in atherosclerosis progression [23]. Depression of the endothelial nitric oxide synthase activity has impact both on essential and secondary hypertension [26]. Impairments in nitric oxide metabolism occur both through lack of substrate in the form of arginine in the endothelial cells and activation of its inhibitors. Hence, depression of endothelial NO-synthase activity in many cases is explained by accumulation of methylated arginine derivatives which are inhibitors to the

above enzyme. Asymmetric dimethylarginine (ADMA) is the most typical among these inhibitors [11]. It should be also noted that symmetric dimethylarginine is not an NO-synthase inhibitor [23, 28, 39]. The major pathophysiological effect of ADMA and homocysteine is depression of the endothelium-dependent vasodilatation. This process depends on some factors but first of all on reduction of nitric oxide synthesis in the endothelial cells and restriction of accessibility to muscular guanylate cyclase for it. It was firstly proved in 1992 that ADMA, in contrast to other methylated arginine derivatives, was an endogenous inhibitor of nitric oxide synthesis [33, 36, 37, 39]. It is proved that ADMA injection causes high hypertension [1].

L-arginine-NO transformation occurs with direct

involvement of endothelial NO-synthase (eNOS). ADMA has been determined to be an endogenous competitive inhibitor of the endothelial NO-S [8]. Considering the above, ADMA level and endothelial nitric oxide synthase in vascular complications of diabetes mellitus, in particular in diabetic retinopathies, appear to be relevant for investigators.

**Study purpose:** to study the level of asymmetric dimethylarginine and endothelial nitric oxide synthase at different stages of development of diabetic retinopathy in the experiment.

### Materials and methods

The study was conducted in Wistar white rats of 180-200 g weight. According to the tasks, the animals were separated into 2 groups as follows: Group 1 - 60 intact animals, and Group 2 - 60 animals with simulated diabetic retinopathy without further correction.

Type 2 diabetes mellitus and diabetic retinopathy were simulated by means of intraperitoneal administration of Streptozotocin (Sigma, USA) diluted in 0.1M citrate buffer with pH 4.5 [22]. Streptozotocin dose of 55 mg per kg of animal weight was divided into two administrations. Streptozotocin administration was preceded by a 28-day diet rich in fats [15].

The animals were withdrawn from the experiment by means of decapitation under brief ether anaesthesia according to the Rules to Perform Works with Use of Experimental Animals approved with Decree of the Ministry of Health of Ukraine No.249 of 01.03.2012 and the Law of Ukraine No.3447-IV, About Animal Protection Against Cruelty (as amended on 15.12.2009 and 16.10.2012).

The animals were withdrawn from the experiment in three stages: Study stage 1: 30th day after commencement of diabetes mellitus simulation; Study stage 2: 60th day after commencement of diabetes mellitus simulation; Study stage 3: 180th day after commencement of diabetes

mellitus simulation. The endothelial synthase ( $\mu\text{mol/L/hour}$ ) and ADMA level ( $\mu\text{mol/L}$ ) were determined with spectrofluorometric technique in blood of the experimental rats.

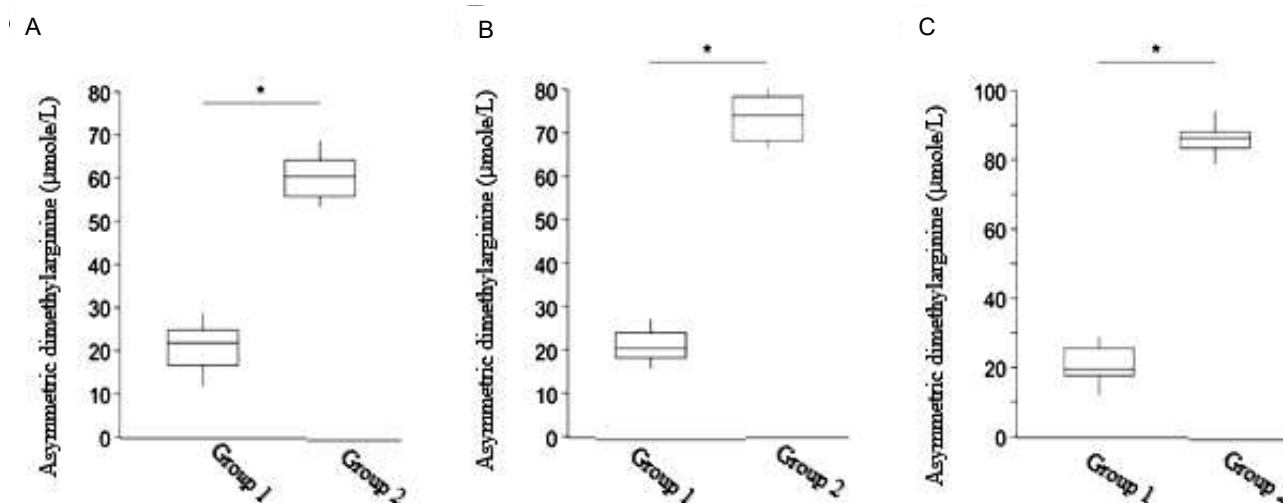
Prior to application of parametric methods that are based on normality of statistical distribution, we have verified the arrays of numeric data being studied for normality with Shapiro-Wilk's W test. As the numeric data in the samples were normally distributed, we have used Student's parametric criterion. The data obtained are presented in the figures in the form of box diagrams.

### Results

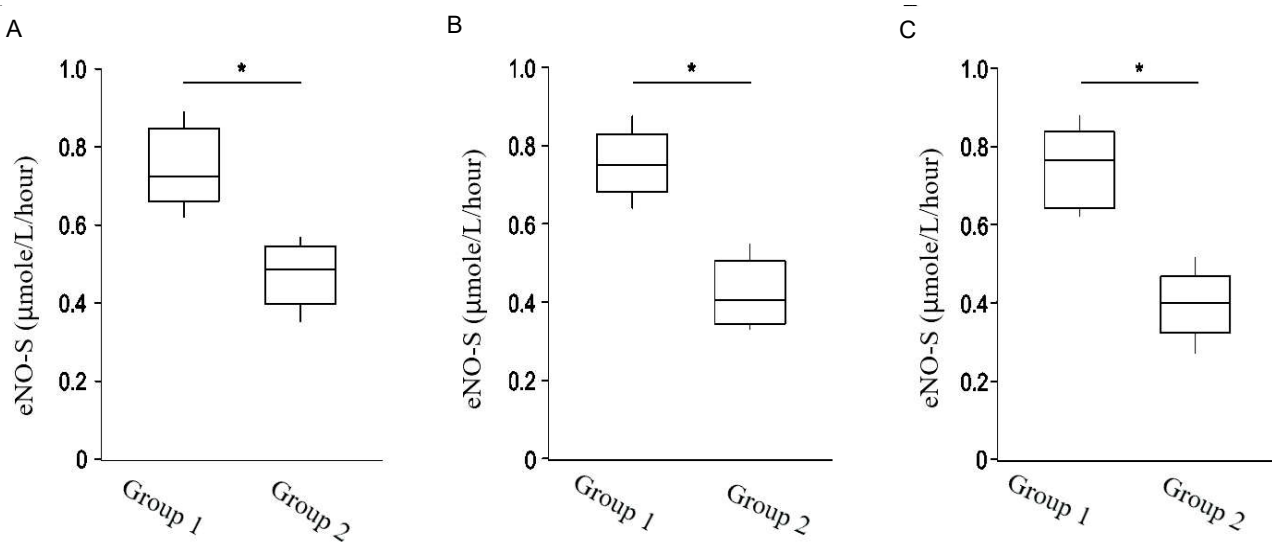
In testing ADMA level at the first stage of our study we have determined its significant (by 65.67%) enhancement in the group of simulated diabetic retinopathy ( $p < 0.001$ ). The following findings were obtained at the second stage: an increased level of the studied endothelial dysfunction marker was registered: increase by 71.97% versus the intact animals ( $p < 0.001$ ) and by 18.6% versus the experimental uncorrected group ( $p < 0.001$ ). On the 180th day, even more apparent increase in ADMA was revealed in the rats with simulated diabetic retinopathy: compared with findings of Group 1, its level was pathologically increased by 75.85% ( $p < 0.001$ ). Compared with Stage I findings, ADMA level was higher by 29.85% ( $p < 0.001$ ), and with Stage II findings, an increase in concentration of endothelial NO-synthase by

**Table 1.** Dynamics of ADMA ( $\mu\text{mol/L}$ ) level in the blood of experimental animals with simulated diabetic retinopathy at various study stages ( $M \pm m$ ).

Groups	Stages		
	I	II	III
Group 1	20.81 $\pm$ 1.28	20.80 $\pm$ 0.86	20.82 $\pm$ 1.25
Group 2	60.40 $\pm$ 1.16	74.21 $\pm$ 1.36	86.13 $\pm$ 1.08



**Fig. 1.** Comparative indicators of the level of ADMA content in the blood of experimental animals of both groups. A - ADMA concentration ( $\mu\text{mol/L}$ ) after 30 days; B - after 60 days and C - 180 days after the induction of diabetic retinopathy. \* - statistically significant difference between the indicators of the control and experimental groups.



**Fig. 2.** Comparative parameters of eNO-S activity ( $\mu\text{mol/L/hour}$ ) in the blood of experimental animals of both groups. A - distribution of eNO-S level values after 30 days; B - after 60 days and C - 180 days after the induction of diabetic retinopathy. \* - statistically significant difference between the indicators of the control and experimental groups.

**Table 2.** Dynamics of eNO-S ( $\mu\text{mol/L/hour}$ ) activity in the blood of experimental animals with simulated diabetic retinopathy at various study stages ( $M\pm m$ ).

Groups	Stages		
	I	II	III
Group 1	0.752±0.030	0.751±0.023	0.750±0.031
Group 2	0.480±0.021	0.433±0.020	0.401±0.022

13.83% ( $p<0.001$ ) was revealed (Table 1, Fig. 1).

As to NO-synthase activity, we have determined on the 30th day its notable decrease (by 56.25%) in the blood of rats with simulated diabetic retinopathy (statistically distinctions at significance level  $p<0.001$ ). On the 60th day (Stage II), we have found out that activity of endothelial nitric oxide synthase lowered even more, by 11.63% compared with findings of the same group at the previous stage ( $p<0.05$ ), and by 56.25% than findings of the intact rats ( $p<0.001$ ). On the 180th day, eNO-S activity decreased by 87.50% compared with findings of Group 1.

Having reviewed the activity dynamics for the studied enzyme, we have found that it was lower by 20.0% ( $p<0.001$ ) compared with the first stage findings and by 7.5% compared with the second stage (Table 2, Fig. 2).

To summarize the above, we can affirm that our experiment has proved the development of endothelial dysfunction subsequent to investigational diabetic retinopathy as evidenced by apparent increase in ADMA level at each of the study stages. Additionally, we have confirmed the inhibiting effect of asymmetric dimethylarginine on the endothelial NO-synthase activity. As mentioned above, eNO-S activity is a marker of the nitric oxide physiological synthase, and decrease in this appears to be another indicator of the endothelial functional condition.

### Discussion

The key role in regulation of the endothelial function is played by nitric oxide the synthesis of which requires for L-arginine amino acid. As mentioned above, asymmetric dimethylarginine is deemed to be an important NO-synthase inhibitor to block connection of L-arginine with the enzyme. Concentration of this inhibitor in blood is variable and depends on multiple reactions both on cellular and tissular levels [13]. Arginine residues in proteins which are catalysed with S-adenosyl methionine-dependent methyltransferases undergo post-translational methylation and are essential for metabolism of the said enzymes. Since the methylation is irreversible, it can be affected only through proteolysis, therefore methylated proteins are highly metabolic [13, 32]. Free ADMA is expressed under proteolysis being induced by intracellular dimethylarginine dimethylaminohydrolase affected by a few factors, mainly the glycated proteins [18].

Inhibition of dimethylarginine dimethylaminohydrolase occurs with depression of NO synthesis and increase in ADMA content [13, 19]. Once in the blood, approximately 10% of the total amount of ADMA are partially hydrolysed in cells and partially excreted by kidneys. ADMA affects cells negatively contributing to oxidising stress, shortening telomeres, inhibiting NO release, increasing secretion of Interleukin-8 and monocyte chemotactic factor 1 [2]. Its effect involves the entire body: increased blood pressure, enhanced pulmonary and total peripheral vascular resistances, decreased cardiac output [13, 16]. Such atherogenesis processes like expression of inflammatory and chemotactic cytokines, monocyte adhesion [6, 9, 13], and accumulation of oxidised low-density lipoproteins activate ADMA in macrophages [21]. Patients with the atherosclerosis risk factors such as diabetes mellitus [21,

34], hypercholesterolemia [5], hyperhomocysteinemia [30, 31], and obesity presented increased ADMA concentrations in blood plasma. This correlates with initial atherosclerosis markers, such as carotid intima-media thickness [13, 38].

ADMA which is a structural analogue of L-arginine depresses activity of all isoforms of NO-synthases thus inhibiting formation of nitric oxide in tissues and blood plasma. It has been proved that ADMA significantly depresses NO synthesis [27, 36]. There is a strong correlation between the levels of nitric oxide in plasma under physiologic conditions which, once broken, result in vascular pathologies [7]. The studies showed that L-arginine activates vasomotor reactions in vivo [3, 4, 10]. And this is despite that endogenous L-arginine is 30 times higher in physiologic concentrations in blood plasma than Michaelis-Menten Constant for L-arginine in a NO-synthase catalysed reaction [10, 24, 27]. At first, the effect of L-arginine on vascular tone appeared to be a little paradoxical since NO-synthase was totally saturated with a substrate, and additional effect of exogenous arginine could not affect intensity of the nitric oxide synthesis [3]. A little later, we have found such endogenous analogues of L-arginine like N-monomethyl-L-arginine (NMMA), asymmetric N-N-dimethyl-L-arginine (ADMA), and symmetric N-N-dimethyl-L-arginine (ADMA) [17, 27]. Two of them, ADMA and NMMA are able to inhibit NO-synthase activity [12, 25]. This allows

to explain the "L-arginine paradox" since higher concentration of the substrate is required for NO-synthase activation with its endogenous inhibitors. ADMA under physiologic conditions is a stronger inhibitor than NMMA as its concentration in blood plasma is 5 times higher [20, 27, 35].

Considering the above, further analysis of the nitric oxide physiological synthesis markers and use of L-arginine for correction of pathological conditions caused by a sharply increased ADMA level in blood is enlightening.

### Conclusion

1. Our study showed impairment of the endothelial functional condition in investigational diabetic retinopathy as evidenced by increased level of ADMA ( $p < 0.001$ ).

2. We have determined a stepwise increase of asymmetric dimethylarginine level in blood of rats with simulated pathology which was apparent in its highest at phase 3.

3. Pathogenetic effect of increased ADMA on eNO-S activity was verified at all experimental stages.

4. Impairment of physiological nitric oxide synthesis in simulated pathology has been proved as evidenced by reduced activity of endothelial NO-synthase yet on the 30th day with further negative dynamics up to the 180th day ( $p < 0.001$  compared with the intact group findings).

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### ЗМІНА РІВНЯ АСИМЕТРИЧНОГО ДИМЕТИЛАРГІНІНА І ЕНДОТЕЛІАЛЬНОЇ СИНТАЗИ ОКСИДУ АЗОТУ В ПАТОГЕНЕЗІ ЕКСПЕРИМЕНТАЛЬНОЇ ДІАБЕТИЧНОЇ РЕТИНОПАТІЇ

Сірман Я.В., Савицький І.В., Прейс Н.І.

У виникненні та прогресуванні діабетичної ретинопатії важливу роль відіграє ендотеліальна дисфункція, пов'язана з порушенням виділення оксиду азоту. Доведено, що важливу роль при цьому відіграє зниження активності ендотеліальної NO-синтази (eNO-S), інгібітором якої є асиметричний диметиларгінін (ADMA). Мета дослідження: вивчити рівень



асиметричного диметиларгініна і ендотеліальної синтази оксиду азоту на різних етапах розвитку діабетичної ретинопатії в експерименті. Дослідження проводили на білих щурах лінії Вістар масою 180-200 г. Відповідно до завдання дослідження тварини були розділені на 2 групи: 1 група - 60 інтактних тварин, 2 група - 60 тварин з моделюванням діабетичної ретинопатії без подальшої корекції. Цукровий діабет 2 типу та діабетичну ретинопатію моделювали внутрішньоочеревинним введенням стрептозотоцину (Sigma, США), розведеного в 0,1М цитратному буфері з рН=4,5. Дозу стрептозотоцину 55 мг на кг ваги тварини поділяли на 2 прийоми. Прийому стрептозотоцину передувала 28-добова дієта, багата жирами. Наше дослідження показало порушення функціонального стану ендотелію при досліджуваній діабетичній ретинопатії, про що свідчить підвищений рівень ADMA ( $p < 0,001$ ). Ми визначили ступеневе підвищення рівня асиметричного диметиларгініна в крові щурів із модульованою патологією, максимальний прояв котрого спостерігалось у третій фазі. Патогенетичний ефект підвищеного ADMA на активність eNO-S підтвержений на всіх етапах експерименту. Порушення фізіологічного синтезу оксиду азоту при патології підтверджується зниженням активності ендотеліальної NO-синтази ще на 30 добу з подальшою негативною динамікою до 180 дня ( $p < 0,001$  у порівнянні з даними інтактної групи).

**Ключові слова:** діабетична ретинопатія, ендотеліальна дисфункція, асиметричний диметиларгінін, ендотеліальна NO-синтаза, порівняльний опис.

### **ИЗМЕНЕНИЕ УРОВНЯ АСИММЕТРИЧНОГО ДИМЕТИЛАРГИНИНА И ЭНДОТЕЛИАЛЬНОЙ СИНТАЗЫ ОКСИДА АЗОТА В ПАТОГЕНЕЗЕ ЭКСПЕРИМЕНТАЛЬНОЙ ДИАБЕТИЧЕСКОЙ РЕТИНОПАТИИ**

**Сирман Я.В., Савицкий И.В., Преис Н.И.**

В возникновении и прогрессировании диабетической ретинопатии важную роль играет эндотелиальная дисфункция, связанная с нарушением выделения оксида азота. Доказано, что важную роль при этом играет снижение активности эндотелиальной NO-синтазы (eNO-S), ингибитором которой является асимметричный диметиларгинин (ADMA). Цель исследования: изучить уровень асимметричного диметиларгинаина и эндотелиальной синтазы оксида азота на различных этапах развития диабетической ретинопатии в эксперименте. Исследование проводили на белых крысах линии Вистар массой 180-200 г. В соответствии с заданием исследования животные были разделены на 2 группы: 1 группа - 60 интактных животных, 2 группа - 60 животных с моделированной диабетической ретинопатией без дальнейшей коррекции. Сахарный диабет 2 типа и диабетическую ретинопатию моделировали внутрибрюшным введением стрептозотоцина (Sigma, США), разведенным в 0,1М цитратном буфере с рН=4,5. Дозу стрептозотоцина 55 мг на кг веса животного разделяли на два приема. Приему стрептозотоцина предшествовала 28-дневная диета, богатая жирами. Наше исследование показало нарушение функционального состояния эндотелия при диабетической ретинопатии, о чем свидетельствовал повышенный уровень ADMA ( $p < 0,001$ ). Мы определили ступенчатое повышение уровня асимметричного диметиларгинаина в крови крыс с моделируемой патологией, максимальное проявление которого наблюдалось в третьей фазе. Патогенетический эффект повышенного ADMA на активность eNO-S подтвержден на всех этапах эксперимента. Нарушение физиологического синтеза оксида азота в моделируемой патологии подтверждается снижением активности эндотелиальной NO-синтазы еще на 30-е сутки с отрицательной динамикой до 180 дня ( $p < 0,001$  по сравнению с данными интактной группы).

**Ключевые слова:** диабетическая ретинопатия, эндотелиальная дисфункция, асимметричный диметиларгинин, эндотелиальная NO-синтаза, сравнительное описание.

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The introduction reflects the state of research and the relevance of the problem according to the world scientific literature (at least 15 references to English articles in international journals over the past 5 years). At the end of the entry, the purpose of the article is formulated (contains no more than 2-3 sentences, in which the problem or hypothesis is addressed, which is solved by the author).

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