UDC: 617.58-007.64:611.018.74:616-008-089-059-089.168.1

Dynamics of endothelial dysfunction indices before and after operation in patients with varicose disease of the lower extremities

D. Yu. Riazanov*[A–F], O. V. Mamunchak[A–D]

SI "Zaporizhzhia Medical Academy of Postgraduate Education, MOH of Ukraine"

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of the article

The aim of the study is to examine the correlation between the endothelial dysfunction indices and the clinical class of the disease before and after the operation in patients with varicose disease of the lower extremities.

Materials and methods. In total, 69 patients with varicose disease of the lower extremities were examined, with preoperative and long-term postoperative measurements of homocysteine, nitrotyrosine and eNOS by the immunoassay analysis.

Results. The results of dynamic changes in endothelial dysfunction indices in 69 patients with varicose disease of the lower extremities, examined before and after an operation, were analyzed. A significant correlation between the mean values of homocysteine, nitrotyrosine and eNOS with the clinical class of lower extremity varicose vein disease (LEVVD) has been determined.

Conclusions. A significant decrease in the indices of homocysteine, nitrotyrosine and increased eNOS was observed long term following operation compared to the initial values before it. It has been established that the homocysteine values were relatively stronger correlated with the patient's age than with the LEVVD clinical class, and nitrotyrosine and eNOS were relatively higher correlated with the LEVVD class than with the patients' age.

Key words: lower extremity, varicose vein, endothelium vascular.

Zaporozhye medical journal 2019; 21 (4), 499–503

DOI: 10.14739/2310-1210.2019.4.173349

***E-mail:** riazanovzm@gmail.com

Динаміка показників ендотеліальної дисфункції до та після операції у хворих на варикозну хворобу нижніх кінцівок

Д. Ю. Рязанов, О. В. Мамунчак

Мета роботи – вивчити у хворих на варикозну хворобу нижніх кінцівок (ВХНК) взаємозв'язок показників ендотеліальної дисфункції з клінічним класом захворювання до та після операції.

Матеріали та методи. Обстежили 69 пацієнтів із варикозною хворобою нижніх кінцівок.

Методом імуноферментного аналізу визначили рівні гомоцистеїну, нітротирозину та eNOS до та у віддалені терміни після операції.

Результати. Вивчили динамічні зміни показників ендотеліальної дисфункції у хворих на варикозну хворобу нижніх кінцівок, яких обстежили до та після операції. Виявили вірогідний взаємозв'язок середніх значень показників гомоцистеїну, нітротирозину та eNOS із клінічним класом ВХНК.

Висновки. Встановили вірогідне зниження показників гомоцистеїну та нітротирозину, підвищення eNOS у віддалені терміни після операції порівняно з вихідними значеннями. Значення гомоцистеїну відносно вище корелює з віком пацієнта, ніж із клінічним класом ВХНК, а нітротирозину та eNOS – відносно вище корелюють із класом ВХНК, ніж із віком пацієнтів.

Ключові слова: нижні кінцівки, варикозна хвороба, ендотелій судинний.

Запорізький медичний журнал. – 2019. – Т. 21, № 4(115). – С. 499–503

Динамика показателей эндотелиальной дисфункции до и после операции у больных варикозной болезнью нижних конечностей

Д. Ю. Рязанов, О. В. Мамунчак

Цель работы – изучить у больных варикозной болезнью нижних конечностей (ВБНК) взаимосвязь показателей эндотелиальной дисфункции с клиническим классом заболевания до и после операции.

Материалы и методы. Обследовали 69 больных варикозной болезнью нижних конечностей.

Методом иммуноферментного анализа определяли уровни гомоцистеина, нитротирозина и eNOS до и в отдаленные сроки после операции.

Результаты. Изучили динамические изменения показателей эндотелиальной дисфункции у 69 больных варикозной болезнью нижних конечностей, обследованных до и после операции. Определена достоверная взаимосвязь средних значений показателей гомоцистеина, нитротирозина и eNOS с клиническим классом ВБНК.

Выводы. Отмечено достоверное снижение показателей гомоцистеина и нитротирозина и повышение eNOS в отдаленные сроки после операции в сравнении с исходными значениями. Установлено, что значения гомоцистеина относительно выше коррелируют с возрастом пациента, чем с клиническим классом ВБНК, а нитротирозина и eNOS – относительно выше коррелируют с классом ВБНК, чем с возрастом пациентов.

Ключевые слова: нижние конечности, варикозная болезнь, эндотелий сосудистый.

Запорожский медицинский журнал. – 2019. – Т. 21, № 4(115). – С. 499–503

A modern study of the venous system pathology has permitted to reconsider a number of provisions concerning aetiology and pathogenesis that may directly affect the diagnosis and therapeutic tactics and, in particular, the concept of endothelial dysfunction (ED) in patients with lower extremity varicose vein disease). In particular, the role of ED as a link of LEVVD pathogenesis has not yet been properly defined [1].

It is known that endothelial cells synthesize and secrete biologically active substances that are vasoconstrictors, vasodilators, involved in the processes of inflammation, thrombosis, proliferation and the vascular wall remodeling. ED is defined as an imbalance between relaxing and constrictive factors, between anti-and procoagulant mediators or growth factors and their inhibitors [2].

The method of assessing the ED severity is evaluation of these substances or endothelium-damaging factors blood content, levels of which are correlated with ED. [3].

Accurate mechanisms of ED development in chronic venous insufficiency of the lower extremities remain understudied and venous hypertension, stasis, ischemia / hypoxia of tissues and vascular walls, free radical damage, cytokine action, hyperhomocysteinemia, endogenous and exogenous intoxication are of key importance [4–6].

Of note, nitric oxide (NO) plays a key role in the mechanisms of ED. A decrease in NO concentration leads to vasoconstriction in veins, stimulation of platelet aggregation, platelet and leukocyte adhesion. [7]

NO is a product of the NO synthases (NOS), which convert L-arginine to NO and L-citrulline. In terms of effects on vascular venous dysfunction, the most important one is endothelial nitric oxide synthase (eNOS), which is an enzyme of endothelial cells and cardiomyocytes. Therefore, the role of eNOS is decisive in the pathophysiological effects of NO on the vascular bed, including the venous one [8,9].

One more significant biological marker of oxidative stress is 3-nitrotyrosine, which is identified as a marker of inflammation and NO production. Assessment of its plasma concentration is a marker of NO-dependent affections in vivo [10–12].

Possible pathogenetic mechanisms of the homocysteine influence on the vascular wall, including the venous one, are under discussion: disorder of the endothelium dependent vasodilatation, oxidative stress, contributing to proteins and lipids peroxidation due to increased superoxide dismutase production, as well as to the increased thrombogenesis and coagulation. It is generally recognized that homocysteine is an atherogenic factor that plays an important role in the early stages of atherogenesis: inhibition of endothelial cell growth, prooxidative effects, mitogenic effects on smooth muscle cells, stimulation of proteins accumulation and collagen biosynthesis.

The result of these processes is the development of ED, and then structural and geometric changes in the vascular wall [13]. However, the effect of homocysteine on the venous wall endothelium functional change in LEVVD is understudied.

These substances are involved in the biochemical processes occurring in the venous endothelium, and can be the trigger point of damage, resulting in endothelial and leukocyte activation, which is the starting point of venous inflammation. Repetitive episodes of inflammation in the endothelium lead to chronic recurrent injury in the venous wall, which in its turn leads to the vein wall remodeling [14,15].

The purpose

The purpose of the study is to examine the correlation between the endothelial dysfunction indices and the clinical class of the disease before and after the operation in patients with varicose disease of the lower extremities.

Material and methods

A total of 69 patients were examined. They were divided into three clinical groups:

1) 23 patients with C3 clinical class of the disease;
2) 23 patients with C4 clinical class of the disease;
3) 23 patients with C5–C6 clinical class of the disease.

Criteria for inclusion in the study: patients with primary LEVVD of C3–C6 clinical classes classified by CEAP, non-operated previously.

Exclusion criteria of the study: patients with primary LEVVD of C0–C2 clinical classes according to CEAP classification; patients with congenital, secondary or unidentified LEVVD etiology; previously operated patients.

Additionally, 15 clinically healthy persons were examined.

Examination of healthy persons and patients with LEVVD included preoperative and long-term postoperative measurements of homocysteine, nitrotyrosine and eNOS, by sandwich enzyme immunoassay technology following the manufacturer's instructions, using standard test systems and photometer "Sunrise TS" (Tecan, Austria) at the Department of Laboratory Diagnostics and General Pathology of SI "Zaporizhzhia Medical Academy of Postgraduate Education of the Ministry of Health of Ukraine".

For measuring these markers, blood plasma sampling was performed in patients using the following procedure: 1 ml of sodium citrate was collected into the test tube, added with 9 ml of whole blood on the wall of the test tube. Then it was shaken and mixed. The blood was centrifuged (no later than 30 minutes after the collection) at 3.000 rpm for 15 minutes. Plasma was drawn into two 1.5 ml eppendorf tubes and stored in a freezer at -20 °C.

Commercially available enzyme-linked immunosorbent assay (ELISA) system kit (Axis-Shield, Norway) was used to determine the total serum homocysteine concentration; nitrotyrosine was measured by ELISA using a kit "Hbt nitrotyrosine" (HyCult Biotechnology, Netherlands) and levels of eNOS – an ELISA Kit for Nitric Oxide Synthase 3 Endothelial (NOS3) (Cloud-Clone Corp., USA).

Statistical data processing was performed taking into account the principles of evidence-based medicine. The database was compiled by means of the Excel software, according to the original documentation with patients' medical records. Statistical calculations were performed using the Statgraphics Plus for Windows 7.0 software package for data statistical analysis.

To describe the selective normal distribution of quantitative characteristics, the mean values and the standard errors of the mean as M ± m were calculated.

Consistency of the values characterized by the normal distribution was verified. The results were considered sta-

Table 1. Results of endothelial dysfunction indices determination before surgery depending on the LEVVD clinical class

ED indices, units	Clinical group			
	Healthy individuals, n = 15	C3, n = 23	C4, n = 23	C5–C6, n = 23
Homocysteine, mmol/l	8.01 ± 0.28*.**.***	21.28 ± 2.36$^{\nabla\nabla\nabla}$	27.97 ± 1.90$^{\nabla\nabla\nabla}$	36.47 ± 3.21
Nitrotyrosine, pg/ml	13.71 ± 0.82*.**.***	34.07 ± 3.29$^{\nabla\nabla\nabla}$	49.03 ± 2.43$^{\nabla\nabla\nabla}$	80.85 ± 10.5
eNOS, ng/ml	96.4 ± 0.81*.**.***	63.07 ± 7.04$^{\nabla\nabla\nabla}$	41.51 ± 2.34$^{\nabla\nabla\nabla}$	33.46 ± 2.91

*: the difference is statistically significant between the healthy individuals and the C3 clinical group patients, P < 0.05; **: the difference is statistically significant between the healthy individuals and the C4 clinical group patients, P < 0.05; ***: the difference is statistically significant between the healthy individuals and the C5–C6 clinical group patients, P < 0.05; $^{\nabla}$: the difference is statistically significant between the patients of the clinical groups C3 and C4, P < 0.05; $^{\nabla\nabla}$: the difference is statistically significant between the patients of the clinical groups C3 and C5–C6, P < 0.05; $^{\nabla\nabla\nabla}$: the difference is statistically significant between the patients of the clinical groups C4 and C5–C6, P < 0.05.

Table 2. Results of endothelial dysfunction indices determination in the long-term after the operation depending on the clinical class of LEVVD

ED indices, units	Clinical group			
	Healthy individuals, n=15	C3, n = 23	C4, n = 23	C5–C6, n = 23
Homocysteine, mmol/l	8.01 ± 0.28* ** ***	15.24 ± 0.98$^{\nabla\nabla\nabla}$	11.16 ± 1.21	10.01 ± 0.56
Nitrotyrosine, pg/ml	13.71 ± 0.82* ** ***	28.56 ± 1.08$^{\nabla\nabla\nabla}$	24.30 ± 0.71$^{\nabla\nabla\nabla}$	18.30 ± 1.12
eNOS, ng/ml	96.4 ± 0.81* ** ***	68.66 ± 1.23$^{\nabla\nabla\nabla}$	79.14 ± 1.69$^{\nabla\nabla\nabla}$	84.39 ± 0.92

*: the difference is statistically significant between the healthy individuals and the C3 clinical group patients, P < 0.05; **: the difference is statistically significant between the healthy individuals and the C4 clinical group patients, P < 0.05; ***: the difference is statistically significant between the healthy individuals and the C5–C6 clinical group patients, P < 0.05; $^{\nabla}$: the difference is statistically significant between the patients of the clinical groups C3 and C4, P < 0.05; $^{\nabla\nabla}$: the difference is statistically significant between the patients of the clinical groups C3 and C5-C6, P < 0.05; $^{\nabla\nabla\nabla}$: the difference is statistically significant between the patients of the clinical groups C4 and C5–C6, P < 0.05.

tistically significant, if the probability level of the test was lower than the predetermined significance level P < 0.05.

Data comparison for quantitative values was performed with the help of the lognormal approximation to a normal distribution (two-tailored T-student test) for small samples.

Results

The obtained results are presented in *Tables 1, 2*.

As it follows from *Table 1*, all the studied ED indices significantly differed between groups of healthy persons and LEVVD patients. It was found that the mean values of homocysteine in patients with class C3 of the disease were significantly higher as compared to those in healthy persons by 2.7 times (t = 4.5; P < 0.05), to patients with class C4 – by 3.5 times (t = 8.4; P < 0.05) and with class C5–C6 – by 4.6 times (t = 7.1; P < 0.05).

A similar dynamics of changes was observed when comparing the mean values of nitrotyrosine: its value was higher by 2.5 times (t = 4.9; P < 0.05) in patients with class C3, by 3.6 times (t = 8.5; P < 0.05) in patients with class C4 and 5.9 times (t = 5.1; P < 0.05) in patients with class C5–C6 than in healthy individuals. The mean eNOS values also significantly differed between the patients and the healthy individuals, but had the opposite correlation nature: values were higher in healthy persons than in patients with C3 by 1.5 times (t = 3.8; P < 0.05), with C4 – by 2.3 (t = 18.4; P < 0.05) and with C5–C6 – by 2.9 times (t = 17.1; P < 0.05).

A comparative analysis of mean values between groups of patients with different LEVVD classes also revealed significant differences differed reliably. Thus, the mean values of homocysteine between groups of patients with class C3 and C4 (t = 2.2; P < 0.05), as well as C3 and C5–C6 (t = 3.8; P < 0.05) significantly differed from the mean values of nitrotyrosine: C3 and C4 (t = 3.6; P < 0.05), as well as C3 and C5–C6 (t = 4.2; P < 0.05). The mean values of eNOS between groups of patients with class C3 and C4 (t = 2.9; P < 0.05), as well as C3 and C5–C6 (t = 3.9; P < 0.05) also differed significantly, but had opposite dynamics of changes. The difference between the groups of patients with class C4 and C5–C6 was: for homocysteine – t = 2.3; P < 0.05; for nitrotyrosine – t = 2.9; P < 0.05; for eNOS – t = 2.2; P < 0.05.

Consequently, the higher LEVVD clinical class was the higher mean values of homocysteine and nitrotyrosine and lower mean value of eNOS were.

In order to establish the nature and strength of the correlation between LEVVD clinical lass, the age of patients and the studied substances concentrations, the correlation analysis was performed. It was found that there was a positive moderate correlation between the class of LEVVD and homocysteine (r = 0.468; P < 0.05) and nitrotyrosine (r = 0.593; P < 0.05), and a moderate negative correlation with eNOS (r = -0.582; P < 0.05).

The same correlation pattern was found for the factors of age and homocysteine (r = 0.600; P < 0.05), nitrotyrosine (r = 0.511; P < 0.05) and eNOS (r = -0.329; P < 0.05). Moreover, the stronger correlation was revealed for the studied values of "age – homocysteine" if compared to "LEVVD class – homocysteine" and the weaker correlation – for the values "age – nitrotyrosine" and "age – eNOS", if compared to the values "LEVVD class – nitrotyrosine" and "LEVVD class – eNOS", respectively.

After 12 months, the operated patients were re-examined (*Table 2*).

The analysis of the results showed that in the long term after the operation, the absolute values of homocysteine were reduced in 23 (100 %) of patients, nitrotyrosine – in 16 (69.5 %) and an increase in eNOS was noted in 21 (91.3 %) of the examined patient.

As shown in table 2, all the studied ED indices significantly differed between the groups of healthy persons and LEVVD patients. It was found that the mean values of homocysteine in patients with class C3 were significantly higher than those in healthy persons (t = 5.8; P < 0.05), as well as in patients with C4 (t = 2.1; P < 0.05) and C5–C6 classes (t = 2.7; P < 0.05). A similar dynamics of changes was observed when comparing the mean values of nitrotyrosine between healthy individuals and patients with C3 class (t = 9.9; P < 0.05) as well as between C4 (t = 9.6; P < 0.05) and C5-C6 classes (t = 2.9; P < 0.05). eNOS mean

values also significantly differed between the patients and the healthy persons, but had the opposite correlation: in patients with C3 (t = 16.7; P < 0.05), C4 (t = 7.8; P < 0.05) and C5-C6 (t = 9.1; P < 0.05).

A comparative analysis of the mean values between groups of patients with different LEVVD classes also demonstrated significant differences. Thus, the mean values of homocysteine between the groups of patients with classes C3 and C4 (t = 2.6; P < 0.05), as well as C3 and C5–C6 (t = 4.6; P < 0.05) significantly differed from the mean values of nitrotyrosine: C3 and C4 (t = 3.3; P < 0.05), as well as C3 and C5-C6 (t = 6.5; P < 0.05). The mean values of eNOS between the groups of patients with classes C3 and C4 (t = 5.0; P < 0.05), as well as C3 and C5–C6 (t = 10.2; P < 0.05) also differed significantly, but with opposite dynamics of changes. The difference between the groups of patients with classes C4 and C5–C6 was: for homocysteine – t = 0.8; P > 0.05, for nitrotyrosine – t = 4.5; P < 0.05, for eNOS – t = 2.7; P < 0.05.

Discussion

Consequently, in the long term after surgery, the higher the initial class of the disease was, the significantly lower mean values of homocysteine and nitrotirosin were, while the mean eNOS values were higher.

Thus, in patients with LEVVD before surgery, there was the significant increase in the mean values of homocysteine and nitrotyrosine, as well as the reduction in eNOS with an increase in the clinical class of the disease. In the long term after the operation, an opposite correlation was noted: the decrease in the mean values of homocysteine and nitrotyrosine, and the increase in eNOS with the initial class of the disease increasing.

A comparative analysis of the changes dynamics of the studied ED indices before and after the operation, depending on the respective clinical class, showed a significant decrease in the mean values of homocysteine (for class C3, t = 2.4, P < 0.05, for C4 – t = 7.5, P < 0.05, for C5 P = 0.05, for C4 – t = 9.8, P < 0.05, for C5–C6 – t = 5.9; P < 0.05), for C4 – t = 8.1; P < 0.05) and nitrotyrosine (for class C3 – t = 2.1, P< 0,05, for C4 – t = 9.8, P < 0.05, for C5–C6 – t = 5.9; P < 0.05) as well as an increase in eNOS (for C4 – t = 13.0; P < 0.05; for C5–C6 – t = 16.7; P < 0.05), except for class C3 (for class C3 – t = 0.78; P < 0.05).

Comparison of the indices in the long-term after the operation with the values in healthy persons showed that the content of homocysteine and nitrotyrosine remained significantly 1.9 (t = 5.8; P < 0.05) and 2.1 times (t = 9.9; P < 0.05) higher in patients with the primary C3 class, respectively, while eNOS was 1.4 times (t = 16.7; P < 0.05) lower, for class C4 they were 1.4 (t = 2.1; P < 0.05) and 1.8 times (t = 9.6; P < 0.05) higher, respectively, while eNOS was 1.2 times (t = 7.8; P < 0.05) lower, and for class C6 these indicators were 1.2 (t = 3.5; P < 0.05) and 1.3 times (t = 3.4; P < 0.05) higher, respectively, but eNOS was 1.1 times (t = 9.6; P < 0.05) lower.

Thus, pronounced chronic ischemia (hypoxia) contributes to eNOS suppression and, consequently, reduction in NO concentration. However, the concentrations of homocysteine and nitrotyrosine are significantly increased. In the long term after the operation, more significant changes in the reduction in homocysteine and nitrotyrosine concentrations as well as eNOS activity enhancement with the initial class of the disease increasing are due to a more pronounced reduction in chronic ischemia (hypoxia) compared with its initial severity.

Conclusions

1) Endothelial dysfunction in patients with LEVVD is characterized by a significant increase in the mean absolute values of homocysteine, nitrotyrosine and a decrease in eNOS values compared to healthy persons, and after the operation, a significant decrease in the mean absolute values of homocysteine, nitrotyrosine and an increase in eNOS values compared to the preoperative indices of the corresponding clinical class of the disease.

2) Patients with LEVVD before the operation demonstrated a significant increase in the mean values of homocysteine, nitrotyrosine and a decrease in eNOS with increasing in the clinical class of the disease. In the long term after the operation, it was found that the higher the initial clinical class of LEVVD was, the significantly lower the mean values of homocysteine and nitrotyrosine and higher the mean values of eNOS were.

3) In the long term after the operation, the mean values of homocysteine and nitrotyrosine remained significantly higher, while those of eNOS were lower than the same indices in healthy persons.

4) The absolute values of homocysteine are relatively stronger correlated with the patient's age than with the clinical class of LEVVD, while nitrotyrosine and eNOS are relatively stronger correlated with the LEVVD class than with the patients' age.

Funding
The study was carried out within the framework of the research work at the SI "Zaporizhzhia Medical Academy of Postgraduate Education Ministry of Health of Ukraine": "Perfecting instrumental methods of diagnosis and surgical treatment of varicose veins of the lower extremities" (state registration number 0116U005142).

Conflicts of interest: authors have no conflict of interest to declare.
Конфлікт інтересів: відсутній.

Надійшла до редакції / Received: 09.11.2018
Після доопрацювання / Revised: 20.12.2018
Прийнято до друку / Accepted: 09.01.2019

Information about authors:
Riazanov D. Yu., DM, PhD, DSc, Professor, Department of Surgery and Low-invasive Technologies, SI "Zaporizhzhia Medical Academy of Postgraduate Education, MOH of Ukraine".
Mamunchak O. V., MD, Postgraduate student, Department of Surgery and Low-invasive Technologies, SI "Zaporizhzhia Medical Academy of Postgraduate Education, MOH of Ukraine

Відомості про авторів:
Рязанов Д. Ю., д-р мед. наук, професор каф. хірургії та малоінвазивних технологій, ДЗ «Запорізька медична академія післядипломної освіти МОЗ України».
Мамунчак О. В., аспірант каф. хірургії та малоінвазивних технологій, ДЗ «Запорізька медична академія післядипломної освіти МОЗ України».

Сведения об авторах:
Рязанов Д. Ю., д-р мед. наук, профессор каф. хирургии и малоинвазивных технологий, ГУ «Запорожская медицинская академия последипломного образования МЗ Украины».
Мамунчак О. В., аспирант каф. хирургии и малоинвазивных технологий, ГУ «Запорожская медицинская академия последипломного образования МЗ Украины».

References

[1] Shevchenko, Y. L., Stoyko, Y. M., Gudymovich, C. G., & Nikitina A. M. (2014). Disfunkcij e'ndoteliya v razvitii varikoznoj bolezni ven nizhnikh konechnostej i vozmozhnosti eyo korrekcii [Endothelial dysfunction in development of varicose veins of lower extremities and possibilities of it's correction]. *Medicinstkij vestnik Yuga Rossii*, 4, 113–119. [in Russian]. https://doi.org/10.21886/2219-8075-2014-4-113-119
[2] Bulayeva, N. I., & Golukhova, E. Z. (2013) E'ndotelial'naya disfunkciya I oksidantnyj stress: rol' v razvitii kardiovaskulyarnoj patologii [Endothelial dysfunction and oxidant stress: the role in cardiovascular pathology]. *Kreativnaya kardiologiya,* 1, 14–22. [in Russian].
[3] Kravchun, N. A., & Chernyavskaya, I. V. (2016) E'ndotelial'naya disfunkciya pri sakharnom diabete: teoreticheskie i prakticheskie aspekty [Endothelial dysfunction in diabetes mellitus: theoretical and practical aspects]. *Zdorov'ya Ukrainy*, 1(33), 15–17. [in Russian].
[4] Balatsky, A. V., Andreenko, E. Yu., Samokhodskaya, L. M., Boitsov, S. A., & Tkachuk, V. A. (2013) Polimorfizm genov e'ndotelial'noj NO-sintazy i konneksina-37 kak faktor riska razvitiya infarkta miokarda u lic bez koronarnogo anamneza [Endothelial NO synthase and connexin 37 gene polymorphisms as a risk factor for myocardial infarction in subjects without a history of coronary artery disease]. *Terapevticheskij arkhiv*, 9, 18–22. [in Russian].
[5] Simonova, O. G., Kolobova, O. I., & Leshchenko, V. A. (2014) Regionarnye ortostaticheskie mekhanizmy e'ndotelial'noj disfunkcii pri varikoznoj bolezni [The regional orthostatic mechanisms of endothelial dysfunction associated with primary varicose veins]. *Flebologiya*, 4, 25–28. [in Russian].
[6] Malakhova, S. M. (2016). Klinichni aspekty polimorfizmu hena endotelialnoi NO-syntazy u profesiinykh sportsmeniv [Clinical aspects of endothelial NO-synthase gene polymorphism in professional sportsmen]. *Pathologia*, 2(37), 98–104. [in Ukrainian]. doi: 10.14739/2310-1237.2016.2.80896 [in Ukrainian].
[7] Okrut, I. E., Shakerova, D. A., & Veselova, T. A. (2012) E'ndotelial'naya disfunkciya i marker sosudisto-trombocitarnogo gemostaza pri metabolicheskom sindrome [Endothelial dysfunction and markers of vascular-platelet hemostasis in the metabolic syndrome]. *Vestnik Nizhnegorodskogo universiteta im. N. I. Lobachevskogo,* 2(3), 216–221. [in Russian].
[8] Nikitina, A. M. (2014) *E'ndotelial'naya disfunkciya v razvitii varikoznoj bolezni venn nizhnikh konechnostej i vozmozhnosti eyo korrekcii* (Avtoref. dis…kand. med. nauk). [Endothelial dysfunction in the development of varicose veins of the lower extremities and the possibility of its correction] (Extended abstract of candidate's thesis). Moscow. [in Russian].
[9] Pfenniger, A., Derouette, J. P., Verma, V., Lin, X., Foglia, B., Coombs, W., et al. (2010) Gap junction protein Cx37 interacts with endothelial nitric oxide synthase in endothelial cells. *Arterioscler Thromb Vasc Biol.*, 30(4), 827–834. doi: 10.1161/ATVBAHA.109.200816
[10] Azizova, G., Dadashova, A., & Amirova, M. (2014) Biomarkery oksidativnogo stressa i sostoyanie antioksidantnoj sistemy pri sakharnom diabete tipa 2 [Biomarkers of oxidative stress and the state of antioxidant system in the present of type 2 diabetes mellitus]. *Universum: Medicina i farmakologiya*, 6(7), 1–9. [in Russian].
[11] Förstermann, U., & Sessa, W. C. (2012) Nitric oxide synthases: regulation and function. *Eur. Heart J.*, 33(7), 829–37. doi: 10.1093/eurheartj/ehr304
[12] Okrut I.Ye., Dautova D.A. (2015) Oksid azota kak pokazatel' aktivnosti svobodnoradikal'nogo okisleniya pri metabolicheskom sindrome. [Nitric oxide as an indicator of the activity of free radical oxidation in metabolic syndrome]. *Innovacionnaya nauka*, 8–2(8), 132–135. [in Russian].
[13] Skvortsov, Yu. I., & Korolkova, A. S. (2011) Gomocistein kak factor riska razvitiya IBS [Homocysteine as a risk factor of ischemic heart disease development (review)]. *Saratovskij nauchno-medicinskij zhurnal*, 7(3), 619–624. [in Russian].
[14] Lazuko, S. S. (2016) *Disfunkciya e'ndoteliya: e'ksperimental'nye i klinicheskie issledovaniya.* Proceedings of the 9th International Scientific and Praktical Conference. Vitebsk. [in Russian].
[15] Grebenchikov, O. A., Philippovskaya, Zh. S., Zabelina, T. S., Skripkin, Y. V., Ulitkina, O. N., & Likhvantsev, V. V. (2017) Opredelennye nitrotirozina ne pozvolyaet ocenit' stepen' vyrazhennosti oksidantnogo stressa i prognozirovat' veroyatnost' razvitiya rannikh oslozhnenij posleoperacionnogo perioda [Nitrotyrosine and its role in assessing the severity of oxidative stress and predicting the incidence of early postoperative complications]. *Patologiya krovoobrashcheniya i kardiokhirurgiya*, 21(2), 77–84 [in Russian]. doi: 10.21688-1681-3472-2017-2-77-84