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DYNAMIC PARAMETERS OF ENDOTHELIAL DYSFUNCTION AFTER DIRECT METHODS OF REVASCULARIZATION IN THE PATIENTS WITH CHRONIC LOWER LIMB ISCHEMIA

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Abstract. Introduction. In surgical practice, angiogenesis is very important for predicting the results of surgical intervention on vessels. In the human body, endothelial cells form new capillaries in those localizations where they are in need. Such a phenomenon is observed in healing of wounds in the area adjacent to the damaged tissue, there is a short-term «outbreak» of capillary formation. Local irritation and local infection also cause proliferation of new capillaries, and when inflammation passes, many of the newly formed capillaries undergo reverse development and gradually disappear.

Purpose. To investigate the dynamics of endothelial dysfunction after direct methods of revascularization in chronic lower limb ischemia.

Material and methods. Endothelial damage was evaluated in the presence of desquamated endothelial cells (DEC) in the blood plasma of patients with an indicator that exceeds 2.77×10^5 v1 litreplasma. The method has high sensitivity and allows diagnosis of latent endothelial dysfunction

Results. The results of the complex examination of the treatment of 30 patients, which are operated in the department of vascular surgery of the Zakarpattia Regional Clinical Hospital named after A.V. Andriy Novak from 2012 to 2018 on chronic ischemia in the case of obliterating atherosclerosis of the vessels of the lower extremities. All patients performed femoral and hypodermic bypass grafting. On the basis of the obtained results there is a decrease in the number of desquamated endothelial cells, indicating a significant reduction of endothelial dysfunction.

Conclusions. Reducing the number of desquamated endothelial cells (from $6.12 \pm 0.21 \times 10^5$ to $3.9 \pm 0.08 \times 10^5$ in 1 liter of plasma) after direct methods of revascularization in chronic lower limb ischemia suggests a significant decrease in the level of endothelial dysfunction.

The predominance of regeneration processes over the detection of apoptotic endothelial cells in intima of vessels and the number of desquamated epithelial cells indicates a rational choice of surgical intervention. The obtained results confirm the decrease in the degree of vascular damage, positive dynamic angiogenesis and the effectiveness of the therapy.

Key words: obliteration atherosclerosis of the vessels of the lower extremities, chronic lower limb ischemia, endothelial dysfunction, desquamation of endothelial cells, femoral arteries bypass grafting.

Динамічні параметри ендотеліальної дисфункції після проведення прямих методів ревазуляризації у пацієнтів із хронічною ішемією нижніх кінцівок

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Резюме. Вступ. У хірургічній практиці ангиогенез дуже важливий для прогнозування результатів хірургічного втручання на судинах. У тілі людини ендотеліальні клітини утворюють нові капіляри в тих локалізаціях, де вони потребують. Таке явище спостерігається при загоєнні ран у ділянці, що прилягає до пошкодженої тканини, відбувається короткочасний «спалах» капілярного утворення. Місцеве подразнення і місцева інфекція також викликають проліферацію нових капілярів, і коли запалення проходить, багато новостворених капілярів зазнають зворотного розвитку і поступово зникають.

Мета дослідження: проаналізувати динаміку ендотеліальної дисфункції після прямих методів ревазуляризації при хронічній ішемії нижніх кінцівок.

Матеріали і методи. Ураження ендотелію оцінювали в присутності десквамованих ендотеліальних клітин (DEC) у плазмі крові хворих із показником, що перевищує $2,77 \times 10^5$ в 1 л плазми. Спосіб має високу чутливість і дозволяє діагностувати латентну ендотеліальну дисфункцію.

Результати досліджень. Результати комплексного обстеження лікування 30 хворих, які були прооперовані у відділі судинної хірургії Закарпатської обласної клінічної лікарні ім. Андрія Новака у період 2012–2018 рр. із приводу хронічної ішемії при облітеруючому атеросклерозі судин нижніх кінцівок. У всіх хворих виконували стегове і підшкірне шунтування. На підставі отриманих результатів



спостерігається зменшення кількості десквамованих ендотеліальних клітин, що свідчить про значне зниження ендотеліальної дисфункції.

Висновки. Зниження кількості десквамованих ендотеліальних клітин (з $6,12 \pm 0,21 \times 10^5$ до $3,9 \pm 0,08 \times 10^5$ в 1 л плазми) після прямих методів ревазуляризації при хронічній ішемії нижньої кінцівки свідчить про значне зниження рівня ендотеліальної дисфункції. Переважання процесів регенерації над виявленням апоптозних ендотеліальних клітин в інтимі судин і кількістю десквамованих епітеліальних клітин свідчить про раціональний вибір хірургічного втручання. Отримані результати підтверджують зниження ступеня судинного ушкодження, позитивного динамічного ангиогенезу та ефективності терапії.

Ключові слова: облітераторний атеросклероз судин нижніх кінцівок, хронічна ішемія нижніх кінцівок, ендотеліальна дисфункція, десквамація ендотеліальних клітин, шунтування суглобових артерій.

Introduction

In 1883, the clinic was introduced in the practice of the term «atherosclerosis» by Jean Lobstein, and only in the middle of 1970, Russell Ross proposed the theory of the development of atherosclerosis as a local inflammatory process in the endothelial layer of arteries [1, 2]. One of the most popular theories of development of atherosclerosis is now considered as a pathological process, the damage reaction on the endothelium. Damage is considered not by as mechanical trauma endothelium, but by as dysfunction [3].

According to literary sources, in our time, the attention of scientists around the world is concentrated in the direction of research, the object of which is the endothelium - as the target organ, which is the earliest damaged, and as the key link pathogenesis of diseases [4]. Endothelium - an active endocrine organ, one of the largest in the body, which is diffusely dissipated along with blood vessels in all tissues. Endothelium, distributed throughout the blood, is essentially a body with a huge surface. In general, it is a differentiated structure. Endothelial cells, components of it, perform certain specific functions in the body. It is part and object of control of one of the systems of the body - the circulatory system. Endothelium, by histologists definition a single-layered layer of specialized cells, which all forms a cardiovascular tree inside, the weight of which is 1,8 kg. Quantitatively - this is one trillion cells with diverse biochemical functions, which include systems for the synthesis of proteins and low molecular weight substances, receptors, ion channels [5].

Among the numerous functions of the endothelium, the following can be distinguished:

1. Participation in the implementation of transport functions of the blood circulation system.

2. Participation in the modification of bioactive substances, in particular, the conversion of prohormones into hormones.

3. Participation in maintaining the required blood viscosity and in bleeding stopping (hemostasis). The endothelium secretes and removes a number of substances that interfere with blood coagulation and provide low blood viscosity, which is necessary for unobstructed blood.

4. Participation in regulation of vascular tone and, consequently, in regulation of blood flow. The endothelium secretes and displays a number of conditions, such as narrowing (vasoconstrictors), and widening (vasodilators) the lumen of the blood vessels, and, thus, with other components, change the volume velocity of blood flow in these vessels.

5. Participation in the implementation of protective functions blood system - in the organization of inflammation, allergies and immunity. Endothelium secretes and displays a number of substances that activate the output of leukocytes from the bloodstream, their migration to the of a potential pathological focus site and their participation in the organization of inflammation [6].

In surgical practice, angiogenesis is very important for predicting the results of surgical intervention on vessels. In the human body, endothelial cells form new capillaries in those localizations where they are in need. Such a phenomenon is observed in healing of wounds in the area adjacent to the damaged tissue, there is a short-term «outbreak» of capillary formation. Local irritation and local infection also cause proliferation of new capillaries, and when inflammation passes, many of the newly formed capillaries undergo reverse development and gradually disappear.

Violation of the functional state of the endothelium is manifested in violation of the balance between vasoconstrictor and vasodilator factors of vascular tone regulation, violation of the trophic of the vascular wall, increased platelet



aggregation and increased platelet proliferation. Vasoconstriction is due to the activation of sympatho-adrenal and renin-angiotensin-aldosterone systems. The leading role is played by oxidant stress, hyperproduction of free radicals [7, 8].

Purpose

To investigate the dynamics of endothelial dysfunction after direct methods of revascularization in chronic lower limb ischemia.

Materials and methods

The complex examination results of 30 patients who were operated in the department of vascular surgery of the Zakarpattia Regional Clinical Hospital named after M.Sc. Andriy Novak from 2012 to 2018 on chronic lower limb ischemia at obliterating atherosclerosis was made.

All patients before and after surgical intervention (after 30 days) a venous blood sampling was performed with the subsequent determination of the number of desquamated endothelial cells (taking into account the morphometric characteristics), which determine the degree of endothelial dysfunction. Methods of diagnosis of latent endothelial dysfunction are carried out as follows: on the shoulder region a cuff is applied, in which, using the air pressure is created by positive pressure that exceeds systolic blood pressure by 40-50 mm Hg. Art. After 4 minutes decompression is performed, after which blood collection from the subcutaneous elbow vein is carried out and the number of desquamated endothelial cells is determined by Hladovec J. method. in terms of 1 liter of plasma. In the case of detecting fragments of the endothelium, the cells that make up this fragment are calculated. The obtained results are compared with the initial amount of endothelial cells. Endothelial damage was evaluated in the presence of desquamated endothelial cells (DEC) in the blood plasma of patients, which is an indicator that exceeds 2.77×10^5 /l plasma. The method has a high sensitivity and allows to diagnose latent endothelial dysfunction (patent RU (11) 2234094 (13) C2). The microscope MicrosMCX-100 Daffodil was used for the work.

Results and discussion

Endothelial dysfunction is determined by the following method: in patients in the shoulder area we created a positive pressure that exceeds systolic blood pressure on 40-50 mm Hg. The criterion is the number of desquamated

endothelial cells more than $2,77 \times 10^5$ in 1 L plasma. The method has a high sensitivity and allows diagnosis of latent endothelial dysfunction.

Endothelium of the vessels regulates local processes of hemostasis, proliferation, cell migration into the vascular wall and vascular tone. The concept of endothelial dysfunction, which is understood as an imbalance between the factors that provide all these processes, is formed. Endothelial cell fission to the basement membrane is carried out with the help of vitronectin, fibronectin, cadherins, and more effective in young cells. The process of desquamation reflects the renewal of the endothelium, which has lost the ability to perform its inherent functions as a result of aging or the impact of harmful factors. The basis of desquamation of the endothelium is the activation of proteinases, necrosis and / or apoptosis of the endotheliocytes.

In the case of damage and apoptosis, there is a violation of the functioning of proteins that provide the connection of endothelial cells with the basement membrane, which leads to desquamation of the endothelium. The duration of finding DEC in the blood is about 24-42 hours, during which their seizure and destruction of the macrophages of the liver, lungs and spleen occurs. Apoptosis and endothelial cell necrosis, increased proteinuria, breaking the connection of endothelial cells underlying intima, contribute to provocative cytokines, free radicals and active forms of oxygen. The source of these biologically active substances can be leukocytes, especially adhered to endothelial cells, which is confirmation of the inflammatory response of the organism. Risk factors for the development of atherosclerosis along with cardiovascular factors are infectious agents. In the course of chronic inflammatory reaction, migration and proliferation of smooth muscle cells occurs, which contributes to the further progression of atherosclerosis [9, 10]. Endothelium is characterized by high stability, which is confirmed by the rare detection of apoptotic endothelial cells in the intima of vessels in the normal, with some other pathological processes, reflects the degree of damage to the vessels and allows you to judge the severity of the course of the disease, the effectiveness of the therapy. Adhesion of circulating leukocytes to the wall of blood vessels, as well as migration into subendothelial space, are carried out by adhesion molecules. During the proteolytic cleavage, soluble forms of adhesion molecules appear, which is a sensitive indicator of the prevalence



of atherosclerotic artery defeat. The processes for the activation of endothelial dysfunction in patients with atherosclerotic obliterans of the vessels of the lower extremities are reflected due to increased selectin concentration, type 1

intercellular adhesion molecules, and adhesion molecules of vascular endothelium type 1. A direct correlation is between the degree of chronic arterial ischemia and the level of sP- and sE-selectin [11].

Table 1

Calculation of DEC in patients (n = 30) before and after surgical intervention

| Parameters | Age (years) | number DEC Before test | number DEC After test | P |
|---------------------|-------------|---------------------------|---------------------------|--------|
| Before surgery | 56,32±1,09 | 3,22±0,39x10 ⁵ | 6,12±0,21x10 ⁵ | <0,001 |
| After the operation | 56,32±1,09 | 2,81±0,18x10 ⁵ | 3,9±0,08x10 ⁵ | <0,001 |

The table 1 presents the quantitative characteristics of the level of DEC in plasma before and after the test with compression of the shoulder vessels. At the beginning of the study, a high level of DEC in patients also increased after a testing, which confirmed the presence of endothelial dysfunction in the studied subjects, the number of DEC in patients exceeded the reference values ($3.22 \pm 0.39 \times 10^5$), and after compressing - the number of DEC was doubled ($6.12 \pm 0.21 \times 10^5$) in 1 liter plasma.

After surgery and conducting trial with compression of the shoulder vessel, the number of DEC significantly decreased (to a sample of $2.81 \pm 0.18 \times 10^5$ and after a test - $3.9 \pm 0.08 \times 10^5$ in 1 liter plasma). The levels of DEC did not reach

the reference values but had a significant tendency to decrease.

Based on the performed studies it should be considered that the increase of the number of DEC above 2.77×10^5 in 1 liter of plasma after a short-term test of compression of the shoulder vessels is a diagnostic criterion for endothelial dysfunction, which was confirmed in our investigation.

Based on the complex of general clinical and special research methods performed in patients in the study group, endothelial dysfunction was confirmed.

We also conducted a morphometric study of endothelial cells before and after surgical intervention (Fig. 1, 2).

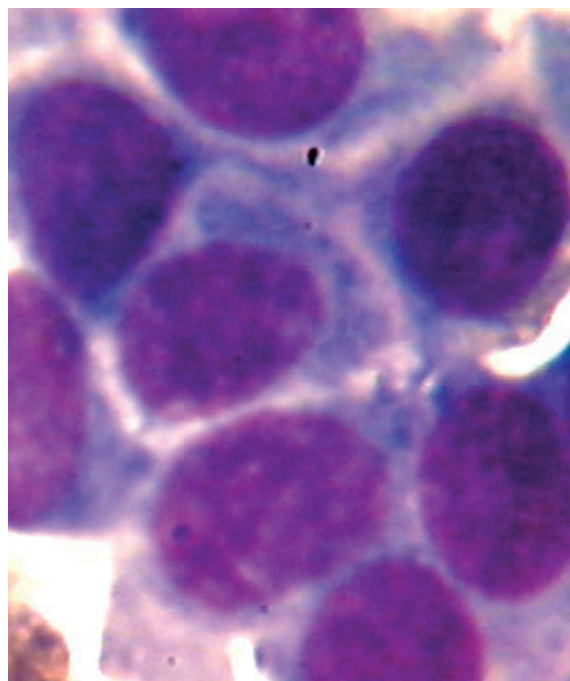


Fig. 1. Cells plast before treatment: there are pronounced polymorphism and proliferation, visualizing nuclides, coarse-grained chromatin, the nucleus occupies virtually the entire cytoplasm.

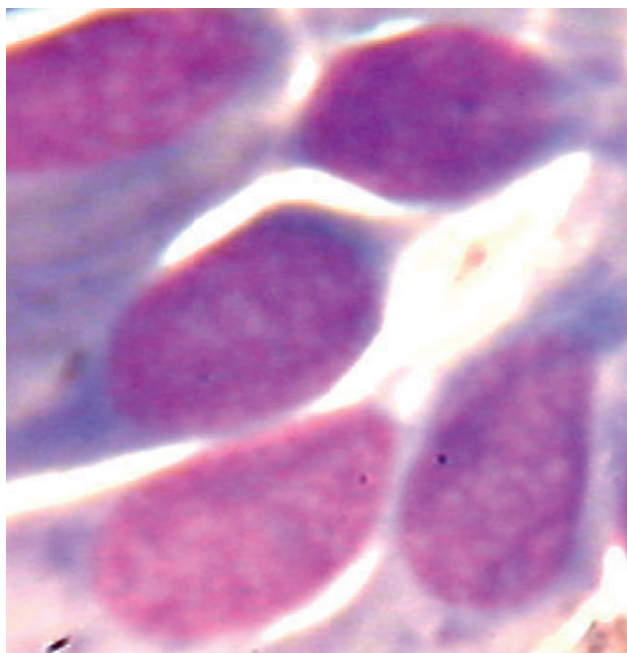


Fig. 2. Cell plasm after treatment: Cells with ovoid and elongated nuclei are more advanced cytoplasm, nucleotides, practically not visualized, delicate chromatin.

There is a positive dynamics under the influence of performed surgical intervention at the cellular level.

Conclusions

Reducing the number of desquamated endothelial cells (from $6.12 \pm 0.21 \times 10^5$ to $3.9 \pm 0.08 \times 10^5$ in 1 liter of plasma) after direct methods of revascularization in chronic lower limb ischemia suggests a significant decrease in the level of endothelial dysfunction.

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