

Comparative characteristics of endothelial dysfunction after endovascular laser vein ablation and classical saphenectomy for varicose veins of the lower extremities

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The objective: to assess the endothelial dysfunction after classical saphenectomy and endovenous laser ablation.

Materials and methods. 100 patients (68 women and 32 men) who were operated on for varicose veins of the lower extremities C2-C4 according to the CEAP classification were under observation. According to the age classification of the World Health Organization, the age of the patients was from 25 to 44 years (young age).

Patients with diseases that could affect the level of endothelial dysfunction were excluded from the study, in particular: coronary heart disease, metabolic cardiomyopathy, cerebrovascular diseases, diffuse connective tissue diseases, diabetes, rheumatoid arthritis. Depending on the type of operation, all patients were divided into two groups: 1st group – 60 patients who underwent endovenous laser ablation (EVLA), 2nd group – 40 patients who underwent classic saphenectomy. 30 healthy persons are included in the control group. By age and clinical classification the patients in the groups were comparable.

Dysfunction was evaluated by determining the level of markers of endothelial dysfunction: P-selectin, E-selectin, tissue plasminogen activator, endothelin-1, vascular endothelium type 1 adhesion molecule, circulating endothelial cells. These indicators were studied in 100 patients (1 day before surgery, 10 and 60 days after surgery).

Results. According to the results of the study, in the early postoperative period (the 10th day) the levels of markers of endothelial dysfunction, namely P-selectin, E-selectin, tissue plasminogen activator, endothelin-1, sVCAM, circulating endothelial cells were higher than before surgery. In the late postoperative period (the 60th day) the levels of the above-mentioned markers decrease, but remain somewhat higher than before the operation. The fact of an increased markers of endothelial dysfunction in different periods of the postoperative period after classic saphenectomy compared to EVLA was established.

Conclusions. 1. The level of endothelial dysfunction is higher after classic saphenectomy, and the inflammatory response after EVLA was less compared to classic saphenectomy, which is also evidenced by statistically significant lower values of sVCAM-1.

2. Existing endothelial dysfunction in the postoperative period requires correction with the help of multicomponent drug therapy.

Keywords: endothelial dysfunction, classic saphenectomy, endovenous laser ablation.

Порівняльна характеристика ендотеліальної дисфункції після операцій ендоваскулярної лазерної абляції вен та класичної сафенектомії при варикозному розширенні вен нижніх кінцівок

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Мета дослідження: оцінювання ендотеліальної дисфункції після проведення класичної сафенектомії та ендовенозної лазерної абляції.

Матеріали та методи. Під спостереженням перебували 100 пацієнтів (68 жінок та 32 чоловіки), які були прооперовані з приводу варикозної хвороби нижніх кінцівок C2–C4 за класифікацією CEAP. Згідно з віковою класифікацією Всесвітньої організації охорони здоров'я, вік хворих становив від 25 до 44 років (молодий вік).

Із дослідження були виключені пацієнти з хворобами, що могли вплинути на рівень ендотеліальної дисфункції, зокрема: ішемічна хвороба серця, метаболічна кардіоміопатія, цереброваскулярні захворювання, дифузні захворювання сполучної тканини, цукровий діабет, ревматоїдний артрит. Залежно від виду операції всі хворі були розподілені на дві групи: 1-а група – 60 пацієнтів, які перенесли ендовенозну лазерну абляцію (ЕВЛА), 2-а група – 40 пацієнтів, яким була виконана класична сафенектомія. До групи контролю включено 30 здорових осіб. За віком та клінічною класифікацією хворі у групах були співставні.

Дисфункцію оцінювали шляхом визначення рівня маркерів ендотеліальної дисфункції: Р-селектину, Е-селектину, тканинного активатора плазміногену, ендотеліну-1, молекули адгезії 1-го типу ендотелію судин, циркулюючих ендотеліальних клітин. Ці показники досліджено у 100 хворих (за 1 добу до операції, через 10 і 60 діб після операції).

Результати. Згідно з результатами дослідження, у ранній післяопераційний період (10-та доба) рівень маркерів ендотеліальної дисфункції, а саме Р-селектину, Е-селектину, тканинного активатора плазміногену, ендотеліну-1, sVCAM, циркулюючих ендотеліальних клітин був вищий, ніж перед операцією. У пізній післяопераційний період

(60-та доба) рівень зазначених вище маркерів знижується, але залишається дещо вищим, ніж до операції. Встановлено факт підвищення маркерів ендотеліальної дисфункції в різні терміни післяопераційного періоду після класичної сафенектомії порівняно з ЕВЛА.

Висновки. 1. Рівень ендотеліальної дисфункції вищий після класичної сафенектомії, а запальна відповідь після ЕВЛА була меншою порівняно з класичною сафенектомією, про що також свідчать статистично значущі нижчі значення sVCAM-1.

2. Найважлива ендотеліальна дисфункція у післяопераційний період потребує корекції за допомогою багатокомпонентної медикаментозної терапії.

Ключові слова: ендотеліальна дисфункція, класична сафенектомія, ендовенозна лазерна абляція.

It is generally accepted that varicose disease is a multifactorial disease whose pathophysiology is characterized by inflammation caused by persistent venous hypertension and valvular insufficiency [1–4]. Changes in shear stress are directly perceived by endothelial cells, which leads to their activation and subsequent recruitment of leukocytes and the release of pro-inflammatory agents [4–6]. Dysfunctional endothelium plays a key role in maintaining the inflammatory cascade with subsequent pathological venous changes and worsening of chronic venous disease [3, 7–10].

Damage to the endothelium caused by mechanical, metabolic, or chemical factors contributes to the development of a disease characterized by inflammation, vasoconstriction, excessive thrombus formation, and adhesion of leukocytes to the vessel wall [8, 11–14].

Matrix metalloproteinase (MMP) activators also play an important role in the emergence of endothelial dysfunction [10, 15–17]. The activity of matrix metalloproteinases causes inflammation and damage to the endothelial cells covering the lumen of the vessel, and the appearance of structural and functional changes in the vein wall [17, 18]. MMP have also been shown to affect the degradation of the extracellular matrix, which in turn causes significant remodeling of the venous tissue, leading to structural and degenerative changes in the vein wall and valve dysfunction [19–22].

In recent years, scientific publications have appeared that claim the presence of endothelial dysfunction after surgery for varicose veins of the lower extremities [20, 23–26].

However, there is no clear answer about the level of ED after various operations on the superficial venous system.

The objective: to assess endothelial dysfunction after classical saphenectomy and intravenous laser ablation.

MATERIAL AND METHODS

100 patients who were operated on for varicose veins of the lower extremities C2-C4 according to the CEAP classification were under observation. Of them, 68 are women, 32 are men. By age, according to the age classification of the World Health Organization, the patients were 25–44 years old (young age). Patients with diseases that could affect the level of endothelial dysfunction, in particular: coronary heart disease, hypertensive disease, metabolic cardiomyopathy, cerebrovascular diseases, diffuse connective tissue diseases, diabetes, rheumatoid arthritis, were excluded from the study.

Depending on the type of operation, all patients were divided into two subgroups. Group 1 (n=60) included patients who underwent endovenous laser ablation (EVLA), group 2 (n=40) included patients who underwent classical saphenectomy. The control was 30 healthy people. By age and clinical classification, the patients in the groups were the same. Endothelial dysfunction (ED) was assessed by

determining the level of ED markers: P-selectin, E-selectin, tissue plasminogen activator, endothelin-1, vascular endothelial adhesion molecule type 1, circulating endothelial cells (CEC). These indicators were studied in 100 patients (1 day before surgery, 10 and 60 days after surgery).

Blood sampling was carried out intraoperatively by puncturing the ulnar vein of the forearm and the trunk of the varicose-dilated large saphenous vein, departing from the saphenous-femoral junction distally by 2 cm.

Concentrations of P-selectin, E-selectin, tissue plasminogen activator, type 1 vascular endothelium adhesion molecule (sVCAM-1-soluble vascular cellular molecule) were determined using kits from Bender MedSystems (Austria) for enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions. The concentration of endothelin-1 was determined using kits from the company «Biomedica» (Canada) for ELISA according to the manufacturer's instructions. Reaction evaluation was performed on a SUNRISE microplate semi-automatic photometer (Tecan, Austria) using a Hydroflex wash station (Tecan, Austria), allowing for standardization of these methods.

To determine the circulating endothelial cells (CEC), we used the method of J. Hladovec and N.N. Petrishcheva et al. (2001). Also, blood for research in the postoperative period was collected from the femoral vein of the limb by its puncture and from the ulnar vein of the forearm. The obtained results were analyzed using the STATISTISA 12.0 program package. The significance of the obtained differences between the results (minimum level of significance $p < 0.05$) was evaluated using the Kruskal-Wallis and Newman-Keuls tests (BioStat program, AnalystSoft Inc.)

RESULTS AND DISCUSSION

The analysis of biochemical markers of ED (Table 1) indicates the presence of clear signs of it in patients with varicose disease. Thus, the number of circulating endothelial cells in venous blood affected by varicose veins of the lower limb was found to be almost 1.7 times higher compared to venous blood taken from the elbow vein and 1.8 times more than in the control group of patients. This indicates the locality of pathological processes in varicose veins and proves the high significance of this marker of endothelial dysfunction in this pathology.

There was also a difference in the sVCAM-1 indicator towards its increase, determined in venous blood taken from the large saphenous vein and from the ulnar vein, respectively (384.2 ± 66.3 ng/ml and 339.1 ± 58.7 ng/ml ($p < 0.05$)).

This indicates a violation of the interaction of endotheliocytes with peripheral blood cells. Thus, the increased expression of the adhesion molecule sVCAM-1 in the vein wall is a sign of the migration of leukocytes through the en-

Table 1

Biochemical markers of endothelial dysfunction before surgery in blood samples from great saphenous vein and ulnar veins in patients with varicose disease (n=100)

Indicator	Control	Great saphenous veins	Ulnar veins
CEC, cells ×104/l	4,3±1,2	7,67±2,3*	4,6±1,1*
sVCAM-1, ng/ml	234,2±57,6	384,2±66,3*	339,1±58,7*
P-selectin, ng/ml	161,9±22,7	201,7±29,4*	176,7±22,5*
E-selectin, ng/ml	39,6±9,6	47,9±11,2*	43,7±8,6*
t-PA, ng/ml	3,4±1,07	2,7±0,6*	2,0±0,9*
Endothelin-1, pmol/ml	2,2±0,4	3,0±1,2*	2,3±0,1*

Note. * – p<0,05.

dothelium, the emergence and maintenance of the inflammatory process in it, with its transition into a chronic one.

We also ascertained an increase in the level of P-selectin, E-selectin in the blood taken from the IVC in comparison with blood samples from the main ulnar vein and in the control group of patients. An increase in the level of selectins P and E in the venous blood indicates a partial delay of leukocytes with their incomplete stop on the surface of the endothelium – rolling.

According to literature data, P-selectin provides the initial stage (rapid rolling of leukocytes), the speed of which slows down when E-selectin is expressed [3–5].

The increase in the level of endothelin-1 in comparison with the control group of patients is also noteworthy – 3.0±1.2 pmol/ml versus 2.2±0.4 pmol/ml (p<0.05). This may be the reason for the proliferation of vascular smooth muscle cells with subsequent hypertrophy of the smooth muscle layer of the venous wall. What we discovered during histological and electron microscopic examination of the venous system of the lower extremities [9]. Thus, the occurrence of ED in varicose disease is characterized by an increase in the number of CECs and the level of biochemical markers VCAM-1, P- and E-selectins.

The obtained results are a confirmation of the scientific opinion about the possible participation of the leukocyte unit in the pathogenesis of varicose transformation of the venous wall in patients with varicose disease and the formation of the so-called «leukocyte unit of inflammation» at the level of varicosely altered venous segments. The above indicates that in the development of varicose veins of the lower extremities, one of the key roles is played by inflammation on the background of functionally changed and activated endothelium.

In our opinion, the determination of ED markers, especially CEC, can be a diagnostic sign of the initial stage of varicose disease, as well as a prognostic sign of postoperative recurrence of varicose disease.

In terms of predicting a possible postoperative recurrence of varicose veins, we were interested in the answer to the question – what changes occur in the functional state of the endothelial system after EVLA and classic saphenectomy. Thus, after EVLA, on the 10th day we ascertained (Table 2) an increase in CEC from (7.13±1.72) cells 104/l to (9.53±1.96) cells 104/l (p< 0.05). At the same time, after the classic (Table 3) saphenectomy, this indicator, in comparison with EVLA, increases by 0.84 times and amounts to (11.25±0.66) cells 104/l (p<0.05).

Also, on the 10th day, an increase in sVCAM-1 was also noted from (356.76±11.78) ng/ml to (477.4±18.69) ng/ml, which is 1.93 times higher than the indicator of the control group and 1.34 times the initial indicator, while with traditional saphenectomy the blood concentration of sVCAM-1 is even higher and is (479.13±17.86) ng/ml (p<0.05). After EVLA, the inflammatory reaction was less than after classic saphenectomy, which is also indicated by a statistically significantly lower sVCAM-1 index.

The increased concentration of sVCAM-1 after surgery also indicates increased adhesion of leukocytes to endothelial cells, as a reaction to major traumatization of the venous wall. When analyzing the level of P-selectin, a slight increase in its concentration is noted from (188.73±8.89) ng/ml before surgery and to (178.7±4.54) ng/ml on the 10th day after minimally invasive intervention, compared to (183.75±3.77) ng/ml (p<0.05) after classical saphenectomy (Fig. 3).

E-selectin before surgery is (47.47±4.93) ng/ml (p<0.05), on the 10th day after EVLA – (33.4±4.0) ng/ml,

Table 2

Comparative characteristics of ED indicators before surgery and in the postoperative period during endovenous laser coagulation (n=60)

Indicator	Control (n=30)	Before surgery	10 days after surgery	60 days after surgery
CEC, cells ×104/l	4,3±1,2	7,13±1,72*	9,53±1,96*	6,13±1,09*
sVCAM-1, ng/ml	234,2±57,6	356,76±11,78*	477,4±18,69*	292,57±24,13*
P-selectin, ng/ml	161,9±22,7	201,7±29,4*	178,7±4,54*	157,57±7,35*
E-selectin, ng/ml	39,6±9,6	47,9±11,2*	33,4±4,0*	25,26±3,0*
Endothelin-1, pmol/ml	2,2±0,4	3,0±1,2*	2,78±0,28*	2,46±0,24*

Note. * – p<0,05.

Comparative characteristics of ED indicators before surgery and in the postoperative period during classic saphenectomy (n=40)

Indicator	Control (n=40)	Before surgery	10 days after surgery	60 days after surgery
CEC, cells ×104/l	4,3±1,2	7,13±1,72*	11,25±0,66*	6,49±1,67*
sVCAM-1, ng/ml	234,2±57,6	356,76±11,78*	479,13±17,86*	301,5±3,97*
P-selectin, ng/ml	161,9±22,7	201,7±29,4*	183,75±3,77*	164,75±4,12*
E-selectin, ng/ml	39,6±9,6	47,9±11,2*	39±3,08*	27,5±2,5*
Endothelin-1, pmol/ml	2,2±0,4	3,0±1,2*	2,83±0,34*	2,75±0,18*

Note. * – p<0,05.

and after classic saphenectomy – 39±3.08 ng/ml (p<0.05) (Fig. 4).

The concentration of endothelin-1 on the tenth day after EVLA and classic saphenectomy is (2.78±0.28) ng/ml and (2.83±0.34) ng/ml (p<0.05), at (2.24±0.016) ng/ml (p<0.05) in the control group. After 60 days after surgi-

cal interventions, signs of ED remain, although according to the analysis of indicators, it is lower than in the early postoperative period.

Thus, after EVLA, the number of CECs remains higher than the control indicators – (6.13±1.09) cells 104/l, with controls – (4.3±1.2) cells 104/l (p<0.05), with the

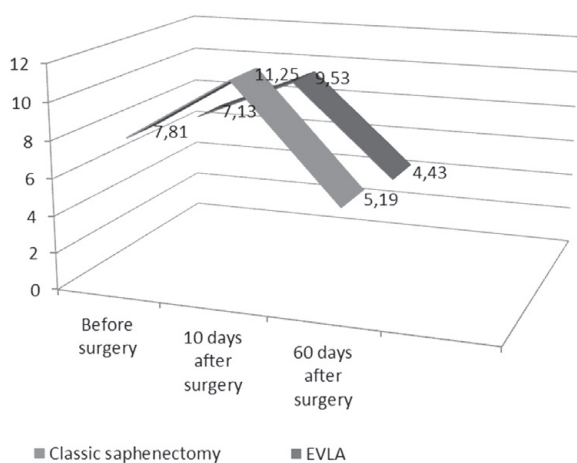


Fig. 1. Comparative characteristics of circulating endothelial cells in EVLA and classic saphenectomy

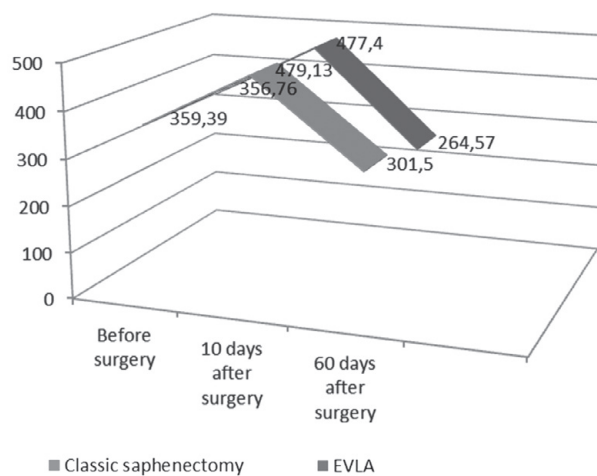


Fig. 2. Comparative characteristics of sVCAM-1 in patients undergoing endovenous laser coagulation and classical phlebectomy

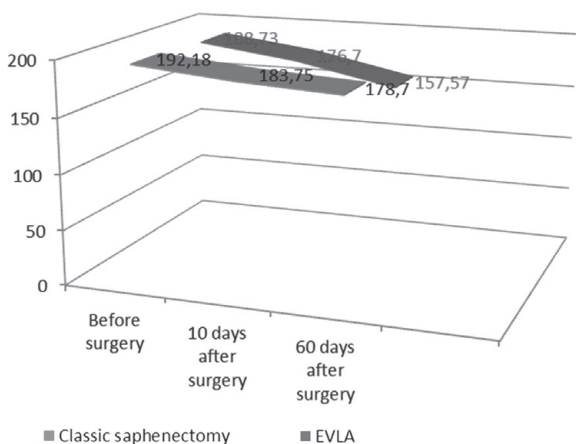


Fig. 3. Comparative characteristics of P-selectin in patients undergoing endovenous laser coagulation and classical phlebectomy

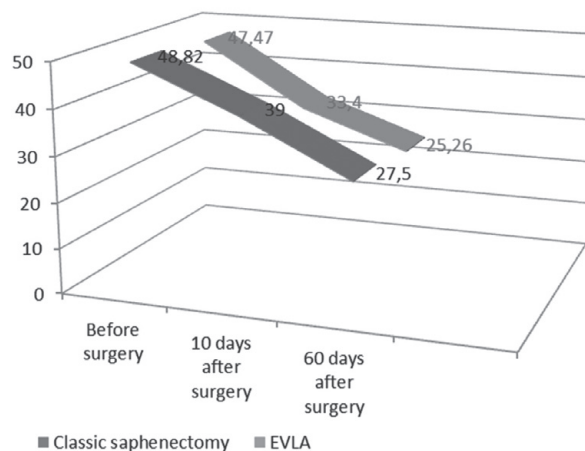


Fig. 4. Comparative characteristics of E-selectin in patients undergoing endovenous laser coagulation and classical phlebectomy

indicator of the control group (3.67 ± 1.44) cells $10^4/l$. After classical saphenectomy, the number of CECs is slightly higher than with EVLA – (6.49 ± 1.67) cells $10^4/l$.

A similar pattern was observed in both groups with sVCAM-1 concentration. On the 60th day after minimally invasive surgical intervention, the concentration of this marker is (292.57 ± 24.13) ng/ml, in the control (234.2 ± 57.6) ng/ml, which is 1.8 times lower than the 10-day indicator of this group. With classical phlebectomy, a less rapid drop is noted – 301.5 ± 3.97 ng/ml, compared to 10 days by 1.6 times (479.13 ± 17.86) ng/ml ($p < 0.05$).

RESULTS AND DISCUSSION

Taken together, our results suggest that EVLA is less damaging to the vascular endothelium and produces lower levels of pro-inflammatory mediators than classical saphenectomy. This may suggest that cellular and systemic changes after EVLA are safer for patients with varicose veins in terms of the development of subsequent ED and inflammation. This may suggest that cellular and systemic changes after EVLA are safer for patients with

varicose veins in terms of the development of subsequent ED and inflammation. During stripping, the entire vein is removed, side branches are disrupted and bleeding into the stripping channel occurs.

During the laser technique, the vessel remains in place and is damaged by heat from the inside without concomitant damage to the surrounding tissues and bleeding. For this reason, either factors associated with altered cell functionality or technical features should be taken into account in the final choice of surgical intervention method for varicose veins.

CONCLUSIONS

1. The level of endothelium is higher after classic saphenectomy, and the inflammatory response after EVLA was less compared to classic saphenectomy, which is also indicated by statistically significantly lower values of sVCAM-1.

2. Existing endothelial dysfunction in the postoperative period requires correction with multicomponent drug therapy.

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