CORRECTION OF THE METABOLOMIC PROFILE AND REDOX POTENTIAL IN PATIENTS WITH LOWER LIMB VARICOSE VEINS (CLASS C4 - C6) IN THE POSTOPERATIVE PERIOD

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ABSTRACT

The present study evaluates the effectiveness of the use of sulodexin (SDX) and ozone therapy in the treatment of lower limb varicose veins (classes C4 to C6) based on the study of the dynamics of plasma biomarkers of oxidative homeostasis and metabolomic profile. In patients of the comparison group, changes in the TAC index in blood plasma tended to increase, however, we didn't established any statistical significance for the entire period of the study: initially - 305.5 ± 18.6 nmol/ml, then 322.3±17.7 nmol/ml on the day 20 after SI (surgical intervention), and 341.6±18.2 nmol/ml (p=0.672) on day 45 after SI. On the contrary, patients of the main group, by the day 20 after SI, had an increase in TAC level from 305.5±18.6 nmol/ml to 365.9±18.1 nmol/ml (by 19.8% of the baseline, p=0.046). By the day 20 after SI, in patients of the comparison group, the SOD activity in blood plasma tended to increase from 10.8±1.8 U/ml to 13.1±1.5 U/ml (by 21.3% relative to the baseline), but no statistical significance was found (p=0.733). The proposed method of complex treatment proved to be effective in correcting oxidative homeostasis and cellular metabolomic profile in patients with LLVV (classes C4 to C6).

Keywords: lower limb varicose veins, oxidative homeostasis, metabolomic profile, redox potential, endovenous laser ablation

INTRODUCTION

Lower limb varicose veins are one of the most common diseases that affect millions of people around the world. In developed countries, the disease affects up to 20% of people, and the incidence increases with age - about 65% of women and 50% of men over 45 [1].

Currently, it is reasonable to assume that oxidative stress increases with varicose veins [2]. Increased production of reactive oxygen species is involved in the endothelial dysfunction genesis through oxidation of lipid membranes and proteins, and activation of inflammation [3].

Increased venous pressure and dilatation in the lower limbs contribute to the development of a complex

vascular reaction leading to obstruction and/or reflux, two main pathogenetic mechanisms that provoke progression of varicose veins. Moreover, these changes result in a hypoxic environment [4]. An imbalance in the complex of cytokines, chemokines, growth factors, proteinases, and endopeptidases produced by activated migrant leukocytes leads to chronic inflammation and the appearance of clinical signs of varicose veins, up to severe manifestations in the form of skin changes and venous ulcers [5].

In recent years, the focus of scientific interest has been the study of oxidative stress as a cellular and molecular basis in the cascade of pathological reactions that determine the formation and progression of LLVV, due to its important role in the activation of endothelial dysfunction processes [6–9]. The validity of this statement is confirmed by a significant pool of studies indicating an important increase in the level of oxidative stress markers in the blood plasma and the walls of pathologically altered veins in patients with LLVV. There is evidence that oxidative stress, leading to activation of signaling pathways, defective gene expression, and modificational transformation of DNA, proteins, and lipids, causes accelerated structural remodeling of the venous wall and disruption of the integrity of smooth muscle cells. Some studies have shown that intensified oxidative stress is a predictor of the progression of valvular insufficiency and complications of LLVV [10].

Given the role of oxidative stress in the pathogenesis of varicose veins, it seems appropriate to include sulodexide in the scheme of complex treatment. The fibrinolytic and lipolytic properties of sulodexide are already known and have become the basis for its use in the treatment of vascular diseases (peripheral arterial disease, chronic lower limb ischemia, post-thrombotic syndrome, venous thromboembolism, diabetic microangiopathy) [11]. However, the modulating effects of sulodexide on oxidative stress have been demonstrated only in experimental studies (decrease, modulation of growth factors, decrease in the expression of matrix metalloproteinases, inflammation reduction, antiangiogenic effects and protection of endothelial cells) [12, 13].

The purpose of this work is to evaluate the effectiveness of sulodexin and ozone therapy in the treatment of lower limb varicose veins (classes C4 to C6), based on the study of the dynamics of plasma biomarkers of oxidative homeostasis and metabolomic profile.

MATERIALS AND METHODS

Our study included 198 patients who underwent minimally invasive surgical interventions (SI) for LLVV of classes C4 to C6. All patients were divided into two groups by independent randomization, depending on the characteristics of postoperative management. The 1st group (comparison group, CG) included 102 patients who had the standard protocol for postoperative management according to the Russian Clinical Guidelines for the Diagnosis and Treatment of CVeD of 2018. In the 2nd group (main group, MG), which included 96 patients, the standard protocol for the postoperative period was supplemented with the use of ozone therapy and sulodexide.

As criteria for the effectiveness of the proposed complex treatment of patients with LLVV of classes C4 to C6 in the early postoperative period, we considered the dynamics of changes in plasma biomarkers of oxidative homeostasis and metabolomic profile, which, according to the results of our multivariate logistic regression analysis at the 1st stage of the study, showed an association with the disease development and progression: TAC, SOD, taurine and sphingomyelin.

The average duration of LLVV at the moment of inclusion of patients in the study was 12.9 [8.8; 14.8] years: in the CG - 12.5 [8.4; 14.3] years, in the MG - 13.3 [9.1; 15.2] years (p=0.852). Patients with CVeD of class C4 predominated - 100 (50.5%): 49 (64.5%) in the CG, 51 (63.0%) in the MG. Comorbid somatic pathology was detected in 110 (55.6%) patients (54 (56.2%) in the CG, 56 (54.9%) in the MG) with arterial hypertension prevailing in its structure - 46 (23.2%): 22 (22.9%) and 24 (23.5%), respectively (Table 2). Three main surgical interventions were performed. These were EVLA of GSV, EVLA of GSV and perforating veins, EVLA of GSV and perforating veins + miniphlebectomy of varicose GSV tributaries. Table 1.

Table 1. Clinical and anamnestic characteristics of patients in study groups

Characteristics	Comparison group n=96	Main group n=102	p
Disease duration 1–5 years, n (%)	14 (14.6)	14 (13.7)	1.000
Disease duration 6 –10 years, n (%)	31 (32.3)	30 (29.4)	1.000
Disease duration >10 years, n (%)	51 (53.1)	58 (56.9)	0.886

CVI clinical class according to CEAP – 4, n (%)	49 (64.5)	51 (63.0)	0.887
CVI clinical class according to CEAP – 5, n (%)	16 (21.1)	18 (22.2)	0.924
CVI clinical class according to CEAP – 6, n (%)	11 (14.4)	12 (14.8)	1.000
Arterial hypertension, n (%)	22 (22.9)	24 (23.5)	0.876
Ischemic heart disease, n (%)	13 (13.5)	11 (10.7)	0.783
COPD, asthma, n (%)	5 (5.2)	7 (6.9)	0.860
Diabetes mellitus, n (%)	5 (5.2)	6 (5.9)	1.000
Chronic gastritis, n (%)	13 (13.5)	14 (13.7)	0.886
Urolithiasis, n (%)	4 (4.2)	5 (4.9)	1.000
EVLA of GSV	22 (22.9)	21 (20.7)	0.832
EVLA of GSV and perforating veins	22 (22.9)	22 (21.5)	1.000
EVLA of GSV and perforating veins + miniphlebectomy of varicose GSV tributaries	52 (54.2)	59 (57.8)	0.812

Note: CVI - chronic venous insufficiency, COPD - chronic obstructive pulmonary disease, GSV - great saphenous vein, EVLA - endovenous laser ablation

RESULTS AND DISCUSSION

Baseline biochemical characteristics of all 198 patients (96 from the CG and 102 from the MG) are presented in Table. 3. In both CG and MG patients, TAC, SOD, and taurine values in the blood serum were statistically significantly lower, and the sphingomyelin value was statistically significantly higher compared to the standard values. Table 2.

Table 2. Baseline biochemical characteristics of patients in the comparison group and the main group (m±CD)

Biochemical characteristics	Standard values	Comparison group n=96	Main group n=102	p
TAC, nmol/ml	401.8±17.4	305.5±18.6	305.9±18.5	1.000
SOD U/ml	22.8±3.1	10.8±1.8	10.6±1.7	1.000
Taurine, μmol/l	64.2±10.2	35.9±6.1	36.2±6.0	0.878
Sphingomyelin, mg/dl	44.9±6.2	61.0±8.3	61.4±8.2	0.886

Note: TAC - total antioxidant capacity, SOD - superoxide dismutase

In patients of the CG, changes in the blood plasma TAC tended to increase, however, no statistical significance was established for the entire period of the study: initially – 305.5 ± 18.6 nmol/ml, on the day 20 after SI (surgical intervention) – 322.3±17.7 nmol/ml, and on the day 45 after SI – 341.6±18.2 nmol/ml (p=0.672). On the contrary, in the MG patients, by the day 20 after SI, a statistically significant increase in TAC was observed, from 305.5±18.6 nmol/ml to 365.9±18.1 nmol/ml (by 19.8% of the baseline, p =0.046). Intergroup differences in the TAC dynamics were close to the level of statistical significance (p=0.579). By the day 45 after SI, in patients of the MG, the TAC in blood plasma increased to 398.9±19.1 nmol/ml. It was statistically significantly higher than the value of the CG patients by 16.8% (p=0.042) and

had no statistically significant differences with the standard values (p=0.867).

By the day 20 after SI in patients of the MG, the SOD activity value in the blood plasma tended to increase from 10.8±1.8 U/ml to 13.1±1.5 U/ml (by 21.3% relative to the baseline), but no statistical significance was detected (p=0.733). In the CG patients, on the day 20 after SI, there was a more pronounced statistically significant increase in the SOD activity in the blood plasma from 10.6±1.7 U/ml to 16.9±1.6 U/ml (by 59.4%, p=0.033 relative to the baseline). Intergroup differences in the dynamics of the SOD activity on the day 20 after SI turned out to be statistically significant (p = 0.046). By the day 45 after SI, patients in both groups had a further increase in the SOD activity in the blood plasma relative to the values recorded on the day 20 after SI. At the same time, in the MG patients, the SOD activity was still statistically significantly higher than in the CG patients – 21.4±1.6 U/ml versus 14.2±1.7 U/ml (by 26.4%, p=0.038) and did not have statistically significant differences with the standard values (p=0.844).

By the day 20 after SI in patients of the CG, the level of taurine in the blood plasma increased slightly from 35.9±6.1 μmol/l to 44.2±8.9 μmol/l (by 23.1% relative to the baseline, p = 0.712), while in patients of the MG, the increase was statistically significant from 36.2±6.0 μmol/l to 60.1±9.1 μmol/l (by 39.8% relative to the baseline, p=0.032). The differences between the CG and the MG in the dynamics of the taurine value reached the level of statistical significance (p=0.036). In addition, in patients of the MG, the level of taurine in the blood plasma did not have statistically significant differences with the standard value (p = 0.867). By the day 45 after SI, in patients of both groups, there was a slight tendency to increase in the level of taurine in the blood plasma relative to the values recorded on the day 15 after SI. Intergroup differences remained statistically significant (48.8±9.1 μmol/l vs. 63.9±9.4 μmol/l, p=0.038).

By the day 20 after SI in patients of the CG, the level of sphingomyelin in the blood plasma decreased slightly from 61.0±8.3 mg/dl to 58.2±7.8 mg/dl (by 4.6% relative to the baseline, p= 0.871), while in patients of the MG the decrease was statistically significant from 61.4±8.2 mg/dl to 52.8±8.1 mg/dl (14.0% relative to the baseline, p=0.048). The differences between the CG and the MG in the dynamics of the sphingomyelin value did not reach the level of statistical significance, but there was a clear tendency in favor of the proposed complex treatment (p = 0.647). By the day 45 after SI, patients of the MG showed a further trend towards a decrease in the sphingomyelin level in the blood plasma relative to the baseline and the level recorded at the previous assessment stage, which did not reach the level of statistical significance (p=0.871 and p=0.624, respectively). In patients of the MG, the level of sphingomyelin in the blood plasma was statistically significantly lower relative to the baseline (by 24.9%, p = 0.002) and the value in the CG (46.1 ± 8.0 mg/dl versus 54.3 ± 7.9 mg/dl, p=0.038) and did not have statistically significant differences with the standard values (p=0.887).

The dynamics of sphingomyelin content in the blood that we discovered in patients of the main group indicates the lipolytic effect of the therapy, which may be associated with both the pharmacodynamics of sulodexide and the effect of increased concentrations of taurine.

CONCLUSION

Thus, the proposed method of complex treatment has proven to be effective in correcting oxidative homeostasis and the cellular metabolomic profile in patients with LLVV of classes C4 to C6, which was confirmed by the earlier restoration of plasma markers of the activity of the antioxidant system and cellular metabolism. The modulating effect of sulodexide and ozone therapy on metabolites (taurine and sphingolipids), accompanied by an antioxidant and lipolytic effect, has been shown. Plasma indicators of TAC, SOD and taurine are informative predictors of treatment effectiveness (regardless of the characteristics of perioperative management).

REFERENCES

1. Gloviczki P., Lawrence P.F., Wasan S.M., Meissner M.H., Almeida J., Brown K.R., Bush R.L., Di Iorio M., Fish J., Fukaya E., Gloviczki M.L., Hingorani A., Jayaraj A., Kolluri R., Murad M.H., Obi A.T., Ozsvath K.J., Singh M.J., Vayuvegula S., Welch H.J., The 2023 Society for Vascular Surgery, American Venous Forum, and American Vein and Lymphatic Society Clinical Practice Guidelines for the Management of Varicose Veins of the Lower Extremities. *Part II, Journal of Vascular Surgery: Venous and Lymphatic Disorders* (2023), DOI: 10.1016/j.jvsv.2023.08.011

2. Guzik B., Chwala M., Matusik P., et al. Mechanisms of increased vascular superoxide production in human varicose veins. *Pol Arch Med Wewn*. 2011;121(9):279–286. DOI: 10.1161/01.cir.0000012748.58444.08

3. Raffetto J.D., Khalil R. A. Mechanisms of Lower Extremity Vein Dysfunction in Chronic Venous Disease and Implications in Management of Varicose Veins. Vessel Plus. *Vessel Plus*. 2021;5:36. DOI: 10.20517/2574-1209.2021.16

4. Ortega M. A., Fraile-Martínez O., García-Montero C., et al. Understanding Chronic Venous Disease: A

Critical Overview of Its Pathophysiology and Medical Management. *J Clin Med.* 2021;10(15):3239. DOI: 10.3390/jcm10153239

5. Raffetto J.D., Mannello F. Pathophysiology of chronic venous disease. *Int Angiol*. 2014;33(3):212-21. PMID: 24755829.

6. Saribal D., Kanber E.M., Hocaoglu-Emre F.S., Akyolcu M.C. Effects of the oxidative stress and genetic changes in varicose vein patients. *Phlebology.* 2019;34(6):406-413. DOI: 10.1177/0268355518814124

7. Akar İ., İnce İ., Aslan C. Oxidative Stress And Prolidase Enzyme Activity In The Pathogenesis Of Primary Varicose Veins. *Vascular.* 2018;26(3);315-321. DOI: 10.1177/1708538117741764

8. Horecka A., Biernacka J., Hordyjewska A., Dąbrowski W., Terlecki P., Zubilewicz T., Musik I., Kurzepa J. Antioxidative mechanism in the course of varicose veins. *Phlebology*. 2018;33(7):464-469. DOI: 10.1177/0268355517721055

9. Agrawal A., Rathor R., Suryakumar G. Oxidative protein modification alters proteostasis under acute hypobaric hypoxia in skeletal muscles: a comprehensive in vivo study. *Cell Stress Chaperones*. 2017;22(3):429-443. DOI: 10.1007/s12192-017-0795-8

10. Herrick S.E., Sloan P., McGurk M., Freak L., McCollum C.N., Ferguson M.W. Sequential changes in histologic pattern and extracellular matrix deposition during the healing of chronic venous ulcers. *Am J Pathol*. 1992;141(5):1085-95.

11. Bignamini A. A., Matuška J. Sulodexide for the Symptoms and Signs of Chronic Venous Disease: A Systematic Review and Meta-analysis. *Adv Ther.* 2020;37(3):1013–1033. DOI: 10.1007/s12325-020-01232-1

12. Shen D., Chen R., Zhang L. Sulodexide attenuates endoplasmic reticulum stress induced by myocardial ischaemia/reperfusion by activating the PI3K/Akt pathway. *J Cell Mol Med.* 2019;23(8):5063–5075. DOI: 10.1111/jcmm.14367

13. *Khizriev S., Mikhailichenko V. Yu., Zhadko S. I., Parshin D. S. Features of expression of growth mediators and matrix metalloproteinases at the local level in patients with lower limb varicose veins, clinical class C4 - C6. Archiv EuroMedica.2022;12(5):el.* DOI 10.35630/2199-885X/2022/12/5.3