International Angiology
EDIZIONI MINERVA MEDICA

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International Angiology 2022 Nov 03
DOI: 10.23736/S0392-9590.22.04940-9

Article type: Original Article

Article first published online: November 3, 2022
Manuscript accepted: October 24, 2022
Manuscript revised: September 29, 2022
Manuscript received: June 28, 2022

Monocyte chemoattractant protein 1 plasma concentration in blood from varicose veins decreases under venoactive drug treatment

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Abstract

Background: Vein-specific inflammation leads to vascular smooth muscle cells proliferation and extracellular matrix degradation of vein wall. This process is known as remodeling and is promoted by "trapped" leukocytes. Monocyte chemoattractant protein 1 (MCP-1) is a chemokine responsible for trafficking of leukocytes from blood to vein wall. The aim of this study was to measure the MCP-1 concentration in varicose veins blood before and after venoactive drug therapy and to compare it with a concentration of blood from varicose veins of subjects who did not receive drug treatment.

Methods: Non-randomized comparative study was conducted on 30 patients with primary varicose veins. 20 patients of the study group received diosmin 900 mg/hesperidin 100 mg once daily. 10 controls received no treatment. MCP-1 level was measured (pg/ml) in the blood from varicose veins twice, at the day of inclusion and after 60 days. Legs discomfort related to chronic venous disease (CVD) symptoms was measured with 10-cm visual analogue scale (VAS) at inclusion and at completion of the study.

Results: Median (interquartile range, IQR) MCP-1 concentrations in treatment and control groups at inclusion were 171.9 (124.4-216.0) and 157.0 (120.1-163.1), resp., p=0.285. After 60 days of treatment MCP-1 level decreased, but non-significantly to 152.3 (124.1-178.3). In patients who did not receive treatment chemokine level slightly increased to 163.0 (134.0-172.9). Median changes over time were -6.6 (-30.9 – 7.4) and 10.6 (-3.7 – 19.2) in the study and control groups, resp. (p=0.048). After 60 days in 12 of 19 and 2 of 9 patients of treatments and control groups MCP-1 decreased (p=0.103). Odds ratio for MCP-1 decreasing was 9.5 (95% CI 1.1-81.5, p=0.043) for those who received venoactive drug. Mean ($\pm$ standard deviation, SD) legs discomfort significantly dropped in the study group from 5.7 ($\pm$2.5) to 1.9($\pm$2.2) (p=0.0003), while in controls no changes were registered: 3.4 ($\pm$1.3) and 3.5 ($\pm$ 1.4), resp., p=0.28). Mean difference of VAS at baseline and at follow-up was -3.5 ($\pm$2.6) and 0.9 ($\pm$2.1), resp. (p<0.0001).

Conclusions: Plasma concentration of MCP-1 in varicose veins blood demonstrates a tendency to decrease under two months treatment with a venoactive drug. Future studies are needed to reveal a possible role of MCP-1 as a target considering its role in varicose veins pathogenesis.

Key words: varicose veins, monocyte chemoattractant protein 1, diosmin, hesperidin, vein-specific inflammation

Introduction

Varicose veins are one of the most common vascular pathologies which affects more that 19% of adults in developed countries [1]. The exact mechanism of varicose veins development is still not understood despite dozens of genetic, molecular, biochemical, and histological studies conducted in the past couple decades [2,3]. Vein-specific inflammation is considered as a possible mechanism that leads to remodeling of lower limbs' superficial vein walls [4]. One theory suggests venous inflammation is caused by the "trapping" of leukocytes. Being recruited from circulation, these inflammatory cells infiltrate the venous wall and express superoxide anions, proteases, growth factors, and cytokines, thus promoting vascular smooth muscle cell proliferation and extracellular matrix degradation. Among the chemokines responsible for the trafficking of leukocytes is the monocyte chemoattractant protein 1 (MCP-1) [5]. It's enhanced expression in varicose veins wall [6] and increased concentration in blood taken from varicose veins [7] has been previously demonstrated. Moreover, experimental data confirmed that statin therapy inhibited MCP-1 expression which has been associated with the diminishing of smooth muscle cells proliferation and suppression of varicose veins development [8]. The nonsignificant reduction in MCP-1 expression in varicose veins wall was also confirmed for patients treated with acetylsalicylic acid (ASA) [6]. Nevertheless, in routine practice statins and ASA are not prescribed for chronic venous disease patients. Venoactive drugs, for many of which anti-inflammatory effect

has been demonstrated, are recommended for CVD patients by recent guidelines [9, 10]. We hypothesized that one of the most frequently used drugs, containing diosmin and hesperidin, may influence the level of MCP-1 in varicose veins blood.

Aim **–** to measure the concentration of MCP-1 in varicose veins blood before and after treatment with diosmin/hesperidin and to compare it with the concentration in blood from varicose veins of subjects who did not receive drug treatment.

Material and Methods

Study design

This was a single-center non-randomized comparative study conducted on 30 patients with primary varicose veins, both symptomatic and asymptomatic. Included patients were referred for invasive treatment in a public hospital. All of them were on a waiting list for venous intervention at the time of enrollment in the study. Patients were enrolled from October, 2020 to February, 2021.

Subjects of both genders aged from 18 to 50 were invited to participate in the study. An upper age limit of 50 was set, taking into account that older subjects are often prescribed with ASA and/or statins due to cardiovascular reasons. Those patients also more often use other drugs with anti-inflammatory action, such as non-steroids. Other inclusion criteria were present of varicose side-branches on one or both legs, classes C2 and C3 according to CEAP classification, great or small saphenous veins reflux confirmed by duplex ultrasound, and signed, informed consent. Exclusion criteria were absence of visible varicose veins, any invasive procedures, i.e., sclerotherapy, open surgery or endovenous ablation on either leg at any time before inclusion, superficial thrombophlebitis at the time of inclusion or recently (not less than 3 months before), any kind of chronic venous disease (CVD) treatment during the study or use of ASA, non-steroid anti-inflammatory drugs and statins, personal history of deep venous thrombosis, post-thrombotic

changes in deep veins on either leg found by duplex ultrasound, inability to come for blood sample collection at the end of the study, and unwillingness to sign an informed consent.

Patients were examined both clinically and by duplex ultrasound. Age, body mass index, personal history of varicose veins, CVD signs and symptoms were recorded. Medical history was taken from all patients. Special attention was paid to the personal history of venous invasive procedures, venous thromboembolism, superficial thrombophlebitis and medications prescribed. Disease description was made using basic CEAP classification. Leg discomfort related to CVD symptoms was measured with a 10-cm visual analogue scale (VAS) at inclusion and after completion of the study. VAS score was registered on the leg from which blood samples were collected.

Duplex ultrasound was performed with patients in a standing position. The great saphenous vein was visualized at the saphenofemoral junction and then scanned distally to the ankle. Small saphenous vein was examined at the popliteal fossa and along the calf. Deep veins were scanned at the groin, the popliteal fossa and above the ankle. We used a muscle compression-release maneuver to provoke reflux. Reverse flow of more than 0.5 sec was considered pathological.

Patients were non-randomly divided into two groups. As nearly all of the patients were referred for surgery and many of them were symptomatic, we expected difficulties with recruiting in the control group because there would be no any treatment for two months. Due to that we decided not to randomize patients. The decision on which patient would receive medication was based on a recruiter's discretion. Twenty patients of the study group were treated with diosmin 900 mg/hesperidin 100 mg (Venarus; JSC Alium, Binnopharmgroup, Russian Federation) once daily for 60 days. No treatment beyond medication was allowed for the study group. 10 controls received no venous treatment for 60 days. The concentrations of MCP-1 were measured in blood samples taken from varicose veins of patients of both groups twice, on the day of inclusion and after 60 days.

Blood Samples

Blood samples were drawn manually from the patient in a lying position. The varicose side branch was punctured using a 21G x 1.5“ BD Vacutainer needle. Blood was collected in a BD Vacutainer EDTA blood collection tube. Blood samples were centrifuged, isolated plasma was placed into an Eppendorf 1.5 ml tube and stored at −20∘C. After all samples were collected, we transferred them in a dry ice protected box to Exacte Labs bioanalytical laboratory (Moscow, Russia). Quantitative measurement of MCP-1 in plasma were performed on all the samples in a batch with an enzyme-linked immunosorbent assay kit purchased from Cloud-Clone Corp., Wuhan, China.

Adherence

Adherence of study group patients was measured by pill counting. Adherence was assumed if a count indicated an intake between 90% and 100% of the prescribed dose.

Approval

Study protocol was approved by the ethical committee of Pirogov Russian National Research Medical University (№198, 29.06.2020) and was registered at clinicaltrials.gov as NCT04933591 and at a Russian Registry of CVD as RRT CVD 7.010.

Statistical analysis

Descriptive statistics are presented as numbers and percentages for qualitative variables, with mean and standard deviation (SD) for quantitative variables if data were normally distributed. As plasma levels of MCP-1 were not normally distributed we presented these data with median and interquartile range (IQR). Correlations were measured by Spearman and points-biserial correlation coefficients. Nominal variables were tested by the two-tailed Fisher’s exact test. Differences between two groups were analyzed by Mann-Whitney test or by t-test depending on data distribution. Differences between two matched sets of data were analyzed by Wilcoxon test.

Multiple logistic regression with binary dependent variable for decrease in MCP-1 (decreased or not decreased) and treatment and VAS score of leg discomfort at inclusion as independent variables was performed. Multiple linear regression for change in MCP-1 plasma concentration on treatment and change in VAS score of leg discomfort were estimated. The Statistics Kingdom open online project (www.statskingdom.com) was used for calculations. Significance level was set at 0.05.

Results

Blood samples from varicose veins were successfully collected from all 30 patients at inclusion. Relationships of MCP-1 plasma concentration and age, varicose veins duration, BMI and VAS score at inclusion are presented in Figure 1. No correlations were revealed for these parameters as well as for gender (female/male) with r=-0.0155, p=0.413 and C-class (C2/C3) with r=-0.0893, p=0.639.

Figure 1. - *Correlations of MCP-1 plasma concentration at inclusion and age (A), history of varicose veins (B), BMI (C) and VAS score of leg discomfort (D) at inclusion.*

Two patients, one from each group, were unable to come for blood sample collection at 60th day. Thus, data on 19 patients from the study group and on 9 patients from the control group were included in the comparative analysis. All patients from the study group were adherent to VAD treatment. Demographics of both groups' patients and their clinical characteristics are presented in Table I.

Table I. - *Demographics and clinical characteristics of both groups' patients*

	Study group (venoactive drug), n=19	**Control group (no treatment) n=9**	**p**
Age, years Mean (±SD)	38 (±9.1)	36.8 (±4.9)	0.541
Men/women	2/17	4/5	0.064

Varicose veins duration, years mean (± SD)	9.4 (±5.5)	9 (±3.5)	0.881
BMI, kg/m^2 mean (±SD)	24.6 (±3.9)	25.2 (±2.6)	0.826
CEAP, n C2 C3	 12 7	 8 1	 0.214
Leg discomfort, VAS, cm mean (±SD)	5.7 (±2.5)	3.4 (±1.3)	0.02

Plasma levels of MCP-1 in both groups' patients at inclusion and after 60 days are presented in Table II. We considered any numerical reduction of concentration as decrease. In the study group median MCP-1 concentration decreased from 171.9 (124.4-216.0) to 152.3 (124.1-178.3) after two months of VAD treatment, but the difference did not reach the statistical significance (p=0.144). At the same time, in the control group median MCP-1 level increased from 157.0 (120.1-163.1) to 163.0 (134.0-172.9), but not significantly (p=0.203). We compared changes of MCP-1 concentrations between groups. Medians were -6.6 (-30.9 – 7.4) and 10.6 (-3.7 – 19.2) in study and control groups, resp., with statistically significant difference (p=0.048).

Table II. - *Plasma levels of MCP-1 at inclusion and after 60 days*

MCP-1, pg/ml	**Study group (venoactive drug treated), n=20**	**Control group (no treatment) n=10**	**p**
Before treatment Me (IQR)	171.9 (124.4-216.0)	157.0 (120.1-163.1)	0.285
After treatment Me (IQR)	152.3 (124.1-178.3)	163.0 (134.0-172.9)	0.885
p	0.144	0.203	
Difference at baseline and at follow up Me (IQR)	-6.6 (-30.9; 7.4)	10.6 (-3.7; 19.2)	0.048

We found lower concentration of MCP-1 after 60 days in 12 out of 19 and 2 out of 9 patients from the treatment and control groups, respectively (p=0.103). We performed multiple logistic regression for the binary variable of the decrease in MCP-1 (decreased or not decreased) on treatment and VAS score of leg's discomfort at inclusion, and linear regression for the change in MCP-1 on treatment and change in VAS score of leg's discomfort. In logistic regression odds ratio for VAD treatment compared to no treatment was 9.5 (95% CI 1.1-81.5, p=0.043), for VAS score it was 0.98 (95% CI 0.94-1.02, p=0.405).

Mean leg discomfort significantly dropped in the study group from 5.7 (±2.5) to 1.9 (±2.2) (p=0.0003) after two months, while in the control group no change was found (3.4±1.3 and 3.5±1.4, resp., p=0.28). Changes in mean VAS scores were -3.5 (±2.6) and 0.9 (±2.1), resp. (p<0.0001).

Discussion

In this study we investigated the level of chemokine MCP-1 in varicose veins patients and compared its changes in subjects who were treated with a VAD containing diosmin and hesperidin and in those who did not receive medical treatment. Blood samples were taken from lower legs varicose veins of all the included patients. The measurement of MCP-1 plasma concentration was performed twice, at inclusion and 60 days after. During this period the study group patients received VAD treatment alone, while controls received no therapy of CVD.

We found no correlations of MCP-1 plasma concentration with age, history of varicose veins, BMI, VAS score of leg discomfort, gender and C-class of the disease. After two months of VAD therapy the MCP-1 level in blood from varicose veins decreased, but the difference did not reach statistical significance. In those who did not receive any treatment during follow-up, a tendency of MCP-1 levels to increase was registered with no statistical significance. We compared differences in MCP-1 plasmatic levels at baseline and after completion of the study. After VAD treatment MCP-1 concentration decreased in 12 of 19 patients from the treatment group, while the

same change was observed in only 2 of 9 subjects from the control group. Odds ratio calculated in a logistic regression analysis for MCP-1 decreasing in those who received VAD was 9.5 (95% CI 1.1-81.5, p=0.043). We also compared between-group difference in changes over time and confirmed the tendency of MCP-1 concentration to decrease under VAD treatment (p=0.048).

MCP-1 is a chemokine that is involved in the pathogenesis of a number of conditions, including cardiovascular diseases, brain, bone and joint disorders, respiratory infections, cancer, etc.. MCP-1 plays an active role in inflammation by recruiting monocytes/macrophages at the site of inflammation [5]. Primary chronic inflammation is generally considered a cause of remodeling the venous wall that leads to varicose veins development [3, 4, 9-12]. It has been confirmed that vein specimens taken from varicose veins patients demonstrate increased monocytes/macrophages infiltration [13, 14]. These assumptions were used as a background for studies aimed to investigate an implication of MCP-1 in varicose veins disease.

Shadrina et al. demonstrated that polymorphism rs1024611 in the *MCP-1* gene was associated with varicose veins [15]. Del Rio Sola et al. studied some of the pro-inflammatories for chemokine's expression using ribonuclease protection assays of 46 varicose veins sections removed during phlebectomy and of 7 non-varicose saphenous veins from legs amputated after trauma. The MCP-1 expression was significantly enhanced in varicose veins [6]. In a recent study Arasi et al. confirmed increased expression of MCP-1 by immunohistochemical staining of 12 varicose veins wall in comparison with 6 healthy veins harvested for coronary artery bypass grafting. As for MCP-1 serum concentration in arm blood, it was significantly higher in venous patients than in control subjects [16]. Elevated plasmatic level of MCP-1 in arm blood samples from CVD patients was also observed by Tisato et al. [17] and Zamboni et al. [18]. Lattimer et al. compared plasma concentrations of inflammatory biomarkers including MCP-1 in blood samples taken both from leg and antecubital veins in 24 varicose veins patients and 24 healthy subjects. MCP-1 concentration was significantly increased in blood drawn from leg veins when compared to blood from arm veins in varicose veins patients. In healthy controls MCP-1 level was similar in

both leg and arm samples [7]. On the contrary, Sachdev et al. found a lower concentration of MCP-1 in blood drawn from saphenous veins of patients when compared to antecubital blood of healthy subjects [19]. Grudzińska et al. revealed that lymphocytes taken from incompetent saphenous veins expressed less MCP-1 after stimulation with phytohemagglutinin than the same cells from an arm blood of healthy volunteers [20].

Some authors studied changes of MCP-1 after medical treatment or invasive procedures. Del Rio Sola et al. observed the non-significant reduction of MCP-1 expression in a human's varicose veins wall after 15-day treatment with ASA [6]. Eschrich et al. demonstrated successful inhibition of varicose veins development in a mouse auricle vein ligation model by atorvastatin and rosuvastatin [8]. Both statins led to a decreased of MCP-1 expression and diminishing of smooth muscle cells proliferation with a result of suppressed varicose veins emergence in animal model by about 80%. Surgical removal or laser ablation of GSV resulted in a slight, but statistically significant decrease in MCP-1 serum level in Arase et al. observations [16]. At the same time, Zamboni et al. could not confirm a positive impact of intervention on MCP-1 plasma concentration. After vein-sparing procedure performed according to CHIVA principles they found increased levels of this chemokine comparing with preoperative assessment [18].

We did not include healthy subjects as controls in our study. We also did not drawn blood from the veins in the arms of the patients. So, we could not confirm if MCP-1 plasma levels of patients are increased in comparison with healthy subjects. But we confirmed a possibility of decreased a serum level of MCP-1 under medical treatment. ASA or statins also showed an impact on this chemokine expression. But, these drugs are not used in managing patients with varicose veins as there is no evidence on their effect. VADs are actively prescribed in CVD patients. The most recent guidelines recommend VADs for patients with symptomatic patients (class IIa, level A) [9, 10]. It has been confirmed that VADs suppress vein-specific inflammation as they prevent leukocyte adhesion to the microvalves and to the vein wall [21]. Our data supports possible benefits of medical treatment by confirming MCP-1 level decreasing after therapy with

diosmin/hesperidin that may impact on vein-specific inflammation in venous patients. Further investigations are needed to reveal the exact mechanism of VAD treatment.

Limitations. The main limitations of our study are of a small sample size and non-comparative design. It also needs to be mentioned that in the study group there were more symptomatic patients than in the control group as well as the study group included relatively less male and C3 patients. Those who had more discomfort, venous edema and female patients were less willing to have no treatment for two months. At the same time, we believe that the possible bias related to that is not crucial. Venous signs and symptoms are not related only to the presence of reflux and varicose veins [22, 23]. Patients with other kind of chronic venous disease, like spider veins and telangiectasias, phlebopathy, don't have reflux and varicose veins but often present with symptoms. Another limitation is related to the absence of a normal range of MCP-1 plasma concentration that is not established yet. So, we can only discuss the dynamics of this biomarker level.

Due to study limitations the revealed tendency of MCP-1 serum level decreasing under VAD treatment has to be taken into account with caution. Meanwhile, our results may provide future directions on the influence of drug treatment on vein-specific inflammation.

Conclusions

MCP-1 concentration in blood from varicose veins in patients with primary disease demonstrates a tendency to decrease under treatment with the venoactive drug containing diosmin/hesperidin.

Conflict of Interest

IZ reports honoraria from Alium, Servier, Innothera. OG, OE, VG, ES report no conflicts of interest.

Funding

The study was supported by JSC Alium, Binnopharmgroup. The company did not have any impact on the design, conducting the study, data analysis and presentation, writing of the paper.

Consent to participate

All participants have agreed to be included in this clinical study and provided a written informed consent.

Authors' contributions

Conception and Design - Zolotukhin I, Golovanova O, Efremova O, Seliverstov E.

Analysis and Interpretation - Zolotukhin I, Golovina V.

Data Collection - Zolotukhin I, Golovanova O, Efremova O, Golovina V, Seliverstov E.

Writing the Manuscript - Zolotukhin I, Golovina V.

Critical Revision - Zolotukhin I, Golovanova O, Efremova O, Seliverstov E.

Approval of the Manuscript - Zolotukhin I, Golovanova O, Efremova O, Golovina V, Seliverstov E.

Agreement to be Accountable - Zolotukhin I, Golovanova O, Efremova O, Golovina V, Seliverstov E.

Statistical Analysis - Zolotukhin I, Golovina V.

Obtaining Funding - Zolotukhin I.

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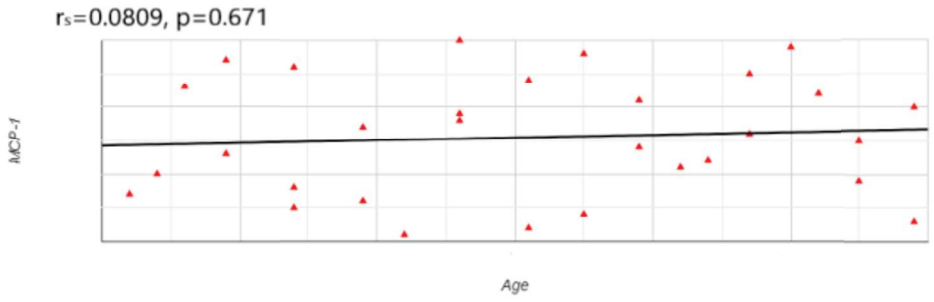
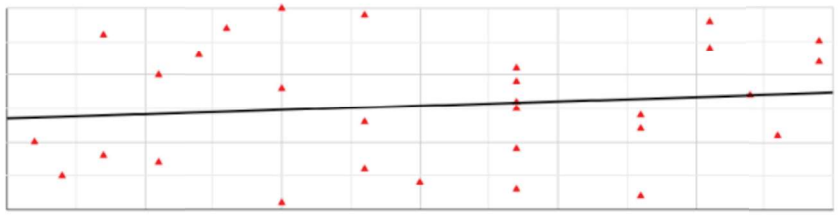
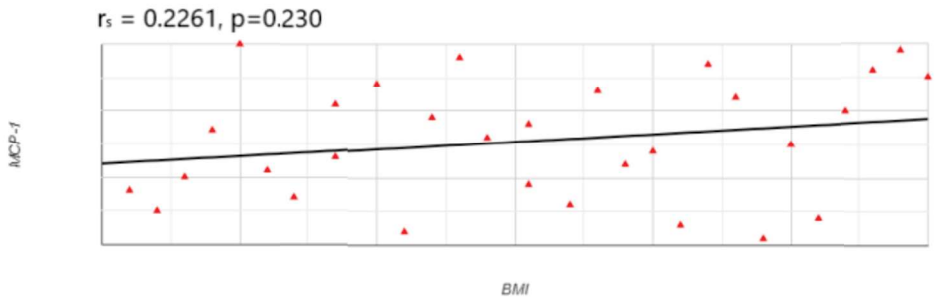

rs=0.0809, p=0.671
MCP-1
Age

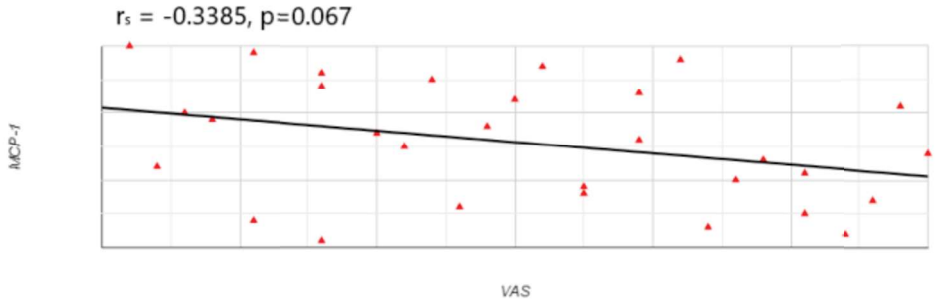
r_s = 0.1298, p=0.494



MCP-1

Varicose veins duration


r_s = 0.2261, p=0.230
MCP-1
BMI


r_s = -0.3385, p=0.067
MCP-1
VAS