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Original Article

Appropriate Surgical Treatment of Symptomatic Primary Varicose Veins Decreases Systemic Inflammatory Biomarkers

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Objective: To evaluate the relationship between systemic inflammatory biomarkers and efficacy of surgical treatment of primary varicose veins of the lower extremities.
Methods: Total 12 patients who underwent endovenous laser ablation or stripping of varicose veins and six healthy subjects were enrolled. Structural and molecular changes of varices were assessed by immunohistochemical staining with anti-monocyte chemotactic protein-1 (MCP-1). MCP-1 and interleukin-6 (IL-6) levels in systemic antecubital blood were measured before and at 12 weeks after treatment.
Results: Immunohistochemical staining revealed prominent manifestation of MCP-1-positive endothelial cells in the walls of varices. Preoperative serum MCP-1 and IL-6 levels in the patients were significantly higher than those in the control (166±12 pg/mL vs 99±10 pg/mL, p=0.003; 5.1±0.95 pg/mL vs 0.0±0.0 pg/mL, p=0.001, respectively). The values were significantly correlated with the severity of chronic venous insufficiency (CVI). Postoperative serum MCP-1 level significantly decreased compared with the preoperative level (152±10 pg/mL vs 166±12 pg/mL, p=0.048). The values after endovenous laser ablation did not significantly decrease compared with those after stripping.
Conclusion: Varicose veins with CVI increase inflammatory biomarker levels in the local tissue and systemic blood. Appropriate treatment of symptomatic varicose veins decreases inflammatory biomarker levels.

Keywords: varicose vein, inflammatory biomarkers, IL-6, MCP-1, endovenous laser ablation

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Introduction

Varicose veins are one of the most common chronic manifestations of vascular pathology and associated with various levels of chronic venous insufficiency (CVI).[1] The risk factors for varicose veins with CVI include heredity, age, female gender, obesity, pregnancy, prolonged standing (job), and height. Although in the general population, varicose veins manifest in the old age, these are not always restricted to the elderly but also involve young people. In chronic venous disease, the relationship between the severity of varicose veins and chronic inflammation plays a key role in the skin changes.[2–5] Venous hypertension, low shear stress, turbulent flow, and stasis caused by varicose veins increase oxidative stress and inflammation, thereby promoting the development of complications, including CVI.[6]

Monocyte chemotactic protein-1 (MCP-1) is a chemokine responsible for monocyte, basophil, and T-cell attraction. The involvement of MCP-1 in the development of primary varicose veins and inflammation could be implicated in the pathogenesis of CVI.[7,8] Interleukin-6 (IL-6) is secreted by T cells and macrophages to stimulate immune response to trauma or other tissue damage thus leading to inflammation.

This study evaluated whether surgical treatment of varicose veins present in the lower extremities decreases inflammatory cytokines in systemic blood. This study is one of the first to compare inflammatory cytokine levels in systemic blood before and after the surgical treatment of varicose veins.

Methods

Informed consent was obtained from all study participants. The study conformed to the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of Tokushima University (#2692-1).

Total 12 patients with primary varicose veins of the lower extremities and who underwent surgical treatment (Group V), particularly, endovenous laser ablation with a 980-nm diode laser (six patients) or surgical stripping (six patients) of the saphenous vein, and six healthy subjects without varicosis (Group C) were enrolled. The surgical indications of all 12 patients were symptomatic with CVI. Patient characteristics are shown in **Table 1**. Age at surgery ranged from 44 to 82 years. There were eight females (66.7%), and CVI grades in Group V were C2 (nine patients) and C3, C5, and C6 (one patient each) according to the revised clinical, etiological, anatomical, pathophysiological (CEAP) classification.

Samples were obtained from patients who underwent surgery for lower extremity varicose veins and coronary artery bypass grafting. Blood samples were collected from the systemic antecubital vein in the supine position before and in the sitting position at 12 weeks after surgery. The blood samples were centrifuged, and plasma was stored at −80°C. In addition, vein samples were harvested during surgery, fixed in 4% paraformaldehyde, and embedded in paraffin.

Paraffin sections were subjected to immunohistochemical staining with human CCL2/JE/MCP-1 antibody (MAB2791, 1:400, R&D Systems, Minneapolis, MN, USA). This was followed by staining using the avidin–biotin complex technique and Vector Red Substrate Kit (Vector Laboratories, Burlingame, CA, USA). The levels of inflammatory cytokines MCP-1 and IL-6 were measured with an ELISA kit (DCP00 and D6050, respectively, R&D Systems) according to the manufacturer's instructions.

Statistical analysis was performed by parametric (paired t test and Student's t test) and nonparametric analyses (Wilcoxon signed rank test, Spearman's rank test, and Mann–Whitney U test). Probability values less than 0.05 were considered significant. Values are expressed as median (interquartile range) ± standard deviation in pg/mL.

Results

Immunohistochemical staining revealed the local manifestation of MCP-1-positive endothelial cells in the wall of excised varicose veins, which differed from the normal saphenous veins harvested for coronary artery bypass grafting (**Fig. 1**).

Preoperative serum MCP-1 and IL-6 levels were significantly higher in Group V than in Group C (MCP-1: 166 ± 12 pg/mL vs 99 ± 10 pg/mL, $p = 0.003$; IL-6: 5.1 ± 2.7 pg/mL vs 0.00 ± 0.00 pg/mL, $p = 0.001$; **Fig. 2A**). Serum MCP-1 and IL-6 levels in all participants were correlated with the severity of CVI as described in CEAP classification (MCP-1: $rs = 0.67$, $p = 0.006$; IL-6: $rs = 0.88$, $p = 0.003$; **Fig. 2B**). The postoperative MCP-1 levels in Group V significantly decreased compared with the

Table 1 Demographics of patients and controls

C-CEAP	Sex	Age	BMI	Comorbidity	Reflux	Intervention	Symptoms
C6	Male	69	23	None	Rt. GSV	Stripping	Ulceration
C5	Female	56	24	None	Rt. SSV	Stripping	Healing ulceration
C3	Female	64	32	Hypothyroidism	Bi. GSV	Laser	Leg aching, edema
C2	Female	69	27	None	Rt. SSV	Stripping	Leg cramps
C2	Male	61	23	Hypertension	Rt. GSV	Laser	Leg malaise
C2	Female	67	20	None	Lt. GSV	Stripping	Leg aching
C2	Female	82	28	COPD	Lt. GSV	Laser	Cosmetic
C2	Female	69	23	None	Rt. GSV	Laser	Leg aching
C2	Female	46	24	None	Rt. GSV	Stripping	Leg malaise
C2	Male	65	29	CKD	Rt. GSV	Laser	Cosmetic
C2	Female	65	20	PAD	Lt. SSV	Stripping	Leg cramps
C2	Male	68	22	None	Bi. GSV	Laser	Leg aching
C0	Male	31	22	None	None		
C0	Male	32	25	None	None		
C0	Male	29	22	None	None		
C0	Female	41	20	None	None		
C0	Female	25	21	None	None		
C0	Female	39	20	None	None		

C-CEAP: clinical-clinical etiological anatomical pathophysiological; BMI: body mass index; GSV: giant saphenous vein; SSV: small saphenous vein; COPD: chronic obstructive pulmonary disease; CKD: chronic kidney disease; PAD: peripheral arterial disease

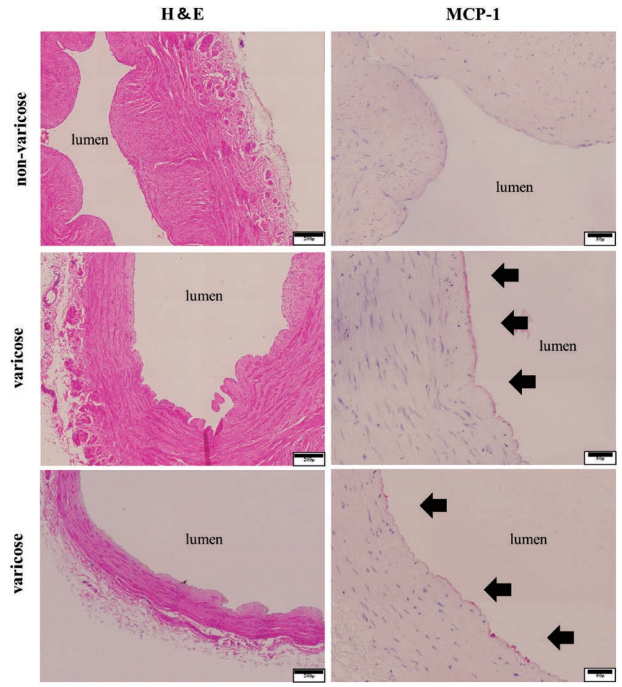

Fig. 1 Morphological analysis and anti-monocyte chemotactic protein-1 (MCP-1)-positive cell. Hematoxylin and eosin staining (bar: 200 µm; left) and immunohistochemical staining with anti-MCP-1 antibody (bar: 50 µm; right) of varicose vein and healthy saphenous vein.

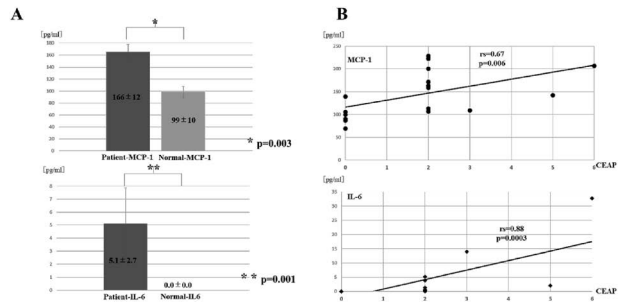

Fig. 2 Values of monocyte chemotactic protein-1 and IL-6 in the blood of healthy subjects and patients with varicose veins before surgical treatment. Blood specimens were obtained from the antecubital vein.
(**A**) Comparison of serum monocyte chemotactic protein-1 (MCP-1) and IL-6 levels between patients before surgical treatment and control. (**B**) Relationship between the MCP-1 and IL-6 levels and severity of chronic venous insufficiency according to clinical, etiological, anatomical, pathophysiological classification before surgical treatment.

preoperative levels (152 ± 10 pg/mL vs 166 ± 12 pg/mL, p = 0.048; **Fig. 3**). In addition, the postoperative IL-6 levels in Group V decreased compared with the preoperative levels (2.1 ± 1.1 pg/mL vs 5.1 ± 2.7 pg/mL, p = 0.16). However, in Group V patients, MCP-1 and IL-6 values after endovenous laser ablation did not significantly decrease compared with those after surgical stripping (**Fig. 4**).

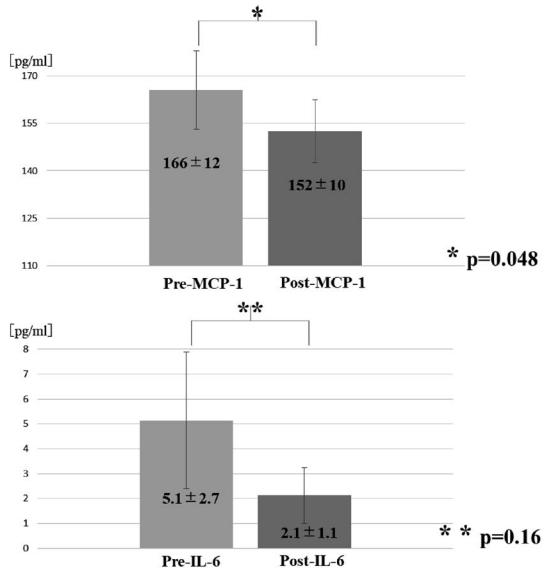

Fig. 3 Comparison of monocyte chemotactic protein-1 and IL-6 levels before and after surgical treatments. Blood samples were collected from the antecubital vein before and at 12 weeks after surgical treatment.

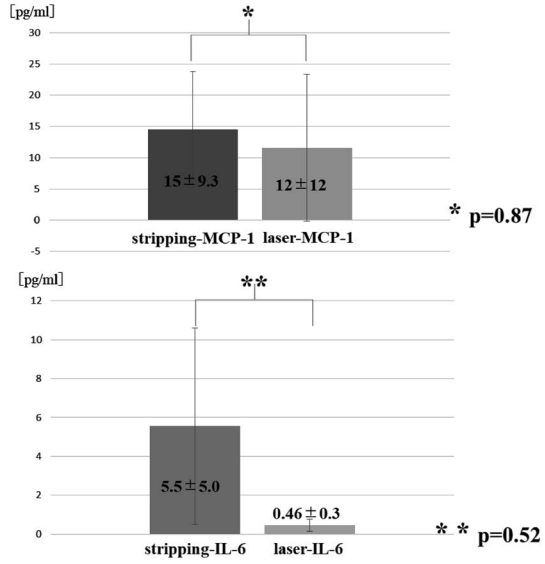

Fig. 4 Comparison of monocyte chemotactic protein-1 and IL-6 levels before and after stripping and those before and after endovenous laser ablation. Blood samples were collected from the antecubital vein before and at 12 weeks after surgical treatment.

Discussion

In the present study, we focused on the levels of inflammatory biomarkers before and after the surgical treatment of varicose veins present in the lower extremities and clarified whether the biomarkers would be potentially useful to monitor the recurrence of varicose veins with CVI and the effects of treatment.

Most patients with CVI develop leg pain, edema, and skin changes with hyperpigmentation, fibrosis, and even venous ulceration. Our data revealed that MCP-1 and IL-6 levels in blood samples collected from the antecubital veins of patients and control significantly correlated with clinical severity based on CEAP classification. The correlation coefficients of MCP-1 and IL-6 levels of all blood samples obtained from the systemic antecubital vein and the clinical severity of venous diseases resemble those in a study by Lattimer et al. study.[4] This study indicated that some inflammatory biomarkers, including MCP-1, and the indicators of endothelial dysfunction were upregulated in the blood sampled from varicose veins compared with that in the systemic blood sampled from the cubital vein. It supports the hypothesis that inflammation is activated in the tissues drained by the varicose veins. However, our data showed that MCP-1 and IL-6 levels in the antecubital veins were higher than the reported data in varicose veins, and the difference in the levels was significant between Group V and Group C patients. It is expected that higher the blood concentration of the biomarker, greater is the probability of binding to the receptor. The systemic increase in inflammatory biomarkers as a result of local consistent production by damaged endothelial cells in varicose veins may be involved in disease progression. Our results showed that MCP-1 clearly accumulated in endothelial cells in the walls of varicose veins compared with that in the control. A previous report demonstrated that MCP-1 is associated with the risk of varicose veins in the lower extremities, and the abundance of MCP-1 protein suppresses the development of varicose veins in an animal model.[8] Our results indicate damage to the vessel walls of varicose veins because of hemodynamic causes, including venous hypertension, low shear stress, turbulent flow, stasis, and increase in inflammatory biomarker levels.

The most notable result in this study is that the appropriate surgical treatment of symptomatic varicose veins, i.e., endovenous laser ablation as well as surgical stripping, promotes decrease in serum inflammatory biomarker levels. Our results demonstrate a significant reduction in systemic inflammatory biomarker levels following both surgical treatments. Therefore, the serum levels of systemic inflammatory biomarkers are capable to evaluate the effects and monitor recurrence after surgical treatment.

However, it remains unclear whether systemic inflammatory biomarker activation by varicose veins with CVI eventually leads to atherosclerotic lesions. The current consensus is that monocytes infiltrate the arterial wall and their activation is the central event in atherosclerosis. Thus, MCP-1 might be a novel therapeutic target for CVI as well as for atherogenesis. The blockade or abrogation of the MCP-1 pathway attenuates the initiation of atheroma formation in an animal model, and MCP-1 is the central mediator in the progression and destabilization of established atheroma.[9,10]

Late vein graft failure after coronary artery bypass grafting is thought to be driven by inflammation.[11] The role of the MCP-1 pathway in the development of vein graft disease might be same as that in varicose veins with CVI. Thus, we might be able to assess the severity of varicose veins with CVI as well as of atherosclerotic lesions in graft by measuring the systemic MCP-1 or IL-6 levels in the cubital vein.

This study offers encouraging results and new insights for targeted therapy or for possible prevention of chronic inflammatory disease. This is a small but significant step toward the discovery of biomarker-specific medicine.

Limitations

Potential limitations of the study include small sample size and overlapping. For the choice of surgical procedure, there is a tendency to choose stripping, such as large saphenous venous dilation. We selected CEAP classification for chronic venous disorders. However, the revised Venous Clinical Severity Score (VCSS) responds with great sensitivity to symptom changes. Therefore, we will further increase the number of cases and use the revised VCSS to examine the effectiveness of the surgical treatment of primary varicose veins.

Conclusion

Varicose veins with CVI show high levels of inflammatory biomarkers in the local tissue as well as in systemic blood from the antecubital vein. Appropriate surgical treatment combined with endovenous laser ablation of symptomatic varicose veins decreases serum inflammatory biomarker levels. Further investigation is necessary to evaluate its potential effect on the entire body.

Disclosure Statement

We have no conflict of interest to disclose.

Author Contributions

Study conception: HA
Data collection: HA, YK
Analysis: NS
Investigation: HA, YK, MS
Writing: HA
Funding acquisition: HA, TK
Critical review and revision: all authors
Final approval of the article: all authors
Accountability for all aspects of the work: all authors

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