International Journal of Biomedicine 13(1) (2023) 54-57
http://dx.doi.org/10.21103/Article13(1)_OA5

ORIGINAL ARTICLE

Vascular Diseases

INTERNATIONAL JOURNAL OF BIOMEDICINE

Pro- and Anti-Inflammatory Blood Cytokines Levels in Women with Moderate and Severe Pelvic Venous Insufficiency

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Abstract

The aim of this study was to determine the blood levels of pro- and anti-inflammatory cytokines in patients with moderate and severe pelvic venous insufficiency (PVI).

Methods and Results: One hundred and four women with PVI and 30 healthy women (control group [CG]) of reproductive age were examined. The patients with PVI (main group [MG]) were divided into 2 subgroups according to the severity of the disease: MG-moderate (n=63) and MG-severe (n=41). The concentration of pro-inflammatory cytokines (IL-1β, IL-2, IL-6, IL-8), and anti-inflammatory interleukins IL-4 and IL-10 were assessed by enzyme immunoassay using monoclonal antibody panels. Measurements were performed on a microplate photometer. Data analysis showed higher values of pro-inflammatory factors (IL-1β, IL-6, and IL-8) in the MG-moderate than in the CG. The MG-severe, compared with the CG, had high levels of IL-1β, IL-2, IL-6, IL-8, and low IL-10 concentration. In addition, patients of the MG-severe had higher levels of IL-2 than patients of the MG-moderate (P=0.045). The IL-6/IL-10 ratio was characterized by higher values in the MG-moderate and MG-severe than in the CG (P<0.0001). The patients of the MG-severe also had higher levels of the IL-6/IL-10 ratio than patients of the MG-moderate (P=0.020).

Conclusion: In patients with PVI with increasing severity of varicose veins, the pro- and anti-inflammatory cytokine balance is progressively disturbed toward the dominance of pro-inflammatory components. The control of these changes in patients is an important component of the design of therapeutic measures and prevention of morphofunctional disorders occurring with the progression of the disease.(**International Journal of Biomedicine. 2023;13(1):54-57.**)

Keywords: pelvic venous insufficiency • women • cytokines

For citation: Darenskaya MA, Semendyaev AA, Stupin DA, Kolesnikov SI, Semenova NV, Pesterev KV, Kolesnikova LI. Pro- and Anti-Inflammatory Blood Cytokines Levels in Women with Moderate and Severe Pelvic Venous Insufficiency. International Journal of Biomedicine. 2023;13(1):54-57. doi:10.21103/Article13(1)_OA5

Abbreviations

AP-1, activating protein-1; **HIF**, hypoxia-inducible factor 1; **IL,** interleukins; **MCP**, monocyte chemoattractant protein-1; **NADPH**, nicotinamide adenine dinucleotide phosphate; **NF-κB**, nuclear factor kappa-light-chain-enhancer of activated B cells ; **PVI,** pelvic venous insufficiency; **TGF-β**, Transforming growth factor beta; **VCAM-1**, vascular cell adhesion molecule 1.

Introduction

Pelvic venous insufficiency (PVI) in women is characterized by a high prevalence (25%-60% of cases), the risk of reproductive disorders (15%-25%), and the ineffectiveness of treatment measures (recurrence in 5%-100%).[1,2] Diagnosis of primary PVI in women is complicated due to the lack of specific clinical symptoms and laboratory criteria typical of the initial manifestations of the pathological process.[3,4] Even severe forms of the disease can be characterized by an asymptomatic course or the presence of "acute abdomen" syndrome.[4] The clinical manifestations of this pathological condition include the following: the presence of chronic pelvic pain, dyspareunia, cyclic and acyclic bleeding, infertility, and other symptoms.[5-7] The disease onset is characterized by venous hypertension,

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venous insufficiency valve, retrograde blood flow through the ovarian veins, and venous-venous discharge of the gonadal vein pool.[2,8] The formation of venous insufficiency is associated with regional hemodynamic disorders, excessive formation of cellular metabolic products, and inflammatory phenomena in the venous wall.[4,9,10,11] An important component of inflammatory reactions in this pathology is the insufficiency of the immune system.[2,12,13] Immunological dysfunction can promote varicose vein transformation and ultimately determine the course of the disease. Evaluation of immune reactivity changes in patients with PVI at different stages of the disease is relevant.

In this regard, the aim of our work was to determine the blood levels of pro- and anti-inflammatory cytokines in patients with moderate and severe PVI.

Materials and Methods

Design of study

One hundred and four women with PVI and 30 healthy women (control group [CG]) of reproductive age were examined. The patients with PVI (main group [MG]) were divided into 2 subgroups according to the severity of the disease: MG-moderate (n=63) and MG-severe (n=41).

Common criteria for inclusion in the MG and CG: reproductive age (20-45 years), signed informed consent. Main criteria for inclusion in the MG: a confirmed diagnosis of primary PVI based on the results of ultrasound examination with duplex angioscanning; exclusion criteria: the presence of concomitant somatic pathology, gynecological diseases, and organic lesions in the pelvis. Main criteria for inclusion in the CG are the absence of acute, or exacerbation of, chronic diseases and the absence of pathological changes in the venous system. Common exclusion criteria for both groups: pregnancy and intake of venotonic, angioprotective antioxidant drugs or synthetic analogs of female sex hormones (hormonal contraceptives) during the last 6 months

Biochemical measurements

The concentration of pro-inflammatory cytokines (IL-1β, IL-2, IL-6, IL-8), and anti-inflammatory interleukins IL-4 and IL-10 were assessed by enzyme immunoassay using monoclonal antibody panels (JSC "Vector-Best", Novosibirsk, Russia). Measurements were performed on a microplate photometer (MultiskanAscent, Finland). The IL-6/IL-10 ratio, reflecting the balance of pro- and anti-inflammatory cytokines, was also calculated.

Statistical analysis was performed using STATISTICA 10.0 software package (Stat-Soft Inc, USA). The normality of distribution of continuous variables was tested by the Kolmogorov-Smirnov test with the Lilliefors correction and Shapiro-Wilk test. The F-test for equality of two variances was applied. For descriptive analysis, results are presented as median (Me), interquartile range (IQR; 25th to 75th percentiles). Differences of continuous variables departing from the normal distribution, even after transformation, were tested by the Mann-Whitney U-test. A probability value of $P<0.05$ was considered statistically significant.

The study was conducted in accordance with ethical principles of the WMA Declaration of Helsinki (1964, ed. 2013) and approved by the Ethics Committee at the Scientific Centre for Family Health and Human Reproduction Problems (Irkutsk, Russia). Written informed consent was obtained from all participants.

Results and Discussion

Data analysis showed higher values of pro-inflammatory factors (IL-1β, IL-6, and IL-8) in the MG-moderate than in the CG (Table 1). The MG-severe, compared with the CG, had high levels of IL-1β, IL-2, IL-6, IL-8, and low IL-10 concentration (Table 1). In addition, patients of the MG-severe had higher levels of IL-2 than patients of the MG-moderate (P=0.045). The IL-6/IL-10 ratio was characterized by higher values in the MG-moderate and MG-severe than in the CG (P<0.0001) (Fig. 1). The patients of the MG-severe also had higher levels of the IL-6/IL-10 ratio than patients of the MG-moderate (P=0.020) (Fig. 1).

Table 1.

Pro- and anti-inflammatory cytokines levels (pg/ml) in female patients with moderate and severe PVI.

Parameters	CG (n=30) (1)	MG-moderate (n=63) (2)	MG-severe (n=41) (3)	*P*-value
IL-1β	124.70 (113.88;131.15)	157.00 (149.26;166.20)	168.00 (151.34;175.91)	1-2 (0.040) 1-3 (0.032) 2-3 (>0.05)
IL-2	39.63 (33.25;45.74)	47.78 (42.13;58.45)	59.80 (53.42;68.62)	1-3 (0.039) 2-3 (0.045) 1-2 (>0.05)
IL-6	2241 (3147;3380)	5358 (5249;5541)	5574 (5495;5780)	1-2 (<0.001) 1-3 (0.009) 2-3 (>0.05)
IL-8	1151 (1075;1217)	2417 (2210;2726)	3792 (3561;4053)	1-2 (0.034) 1-3 (0.011) 2-3 (>0.05)
IL-4	793.76 (762.58;825.38)	789.57 (750.63;831.42)	751.44 (738.16;788.29)	1-2 (>0.05) 1-3 (>0.05) 2-3 (>0.05)
IL-10	1145.72 (1067.43;1271.58)	762.19 (735.17;801.94)	522.43 (475.26;560.81)	1-3 (0.037) 1-2 (>0.05) 2-3 (>0.05)

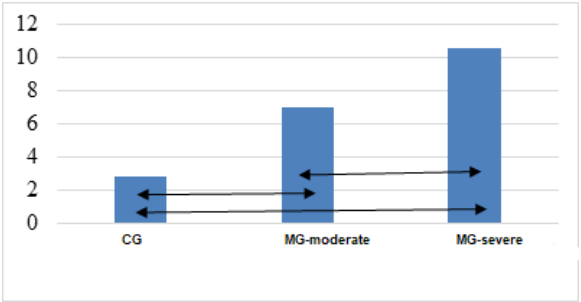

Fig. 1. *Values of the IL-6/IL-10 ratio in the groups* ↔ *(statistically significant differences).*

The findings indicated a pronounced imbalance of pro- and anti-inflammatory factors in patients with increasing degrees of PVI. Thus, the moderate form was characterized by increased values of IL-1β, IL-6, and IL-8, compared to controls.

It was established that the pathological changes in pelvic veins in women are induced by the presence of congestive venous hemodynamics arising due to disturbances of venous outflow.[13] Reduced laminar flow velocity and blood stasis reduce tangential tension and contribute to overstretching of the vascular wall.[4] These changes, as well as associated hypoxia, can trigger a cascade of biochemical processes contributing to variceal transformation.[14-16] It is difficult to find the initiating event in the chain of events since the vein wall may already be altered by the time of clinical manifestations. Undoubtedly, the decisive influence is exerted by the combined effect of external provoking factors and genetic features, which, over time, lead to a condition in which hemodynamic disorders are already chronic.[1,3,4]

Thus, it was found that in response to chronic vascular wall deformation, there is an induction of expression of a wide range of genes responsible for cell proliferation, apoptosis, and migration, regulation of vascular tone, degradation and reorganization of extracellular matrix, inflammation, angiogenesis, and other processes.[2,17] The induction pathways include activating the NADPH-oxidase enzyme complex on the membranes of endothelial and smooth muscle cells. This leads to the generation of reactive oxygen species, which are also inducers of intracellular signal transduction pathways.[18] The developing hypoxia activates specific transcription factors.

These events trigger the work of the key transcription factors AP-1, HIF-1α, HIF-2α, NF-κB, which control dozens of genes.[19] In particular, such genes include genes of matrix metalloproteinases responsible for proteolysis of extracellular matrix components, as well as genes of pro-inflammatory cytokines and chemokines, that stimulate the proliferation and differentiation of B- and T-lymphocytes (IL-6 and IL-12) and attract monocytes/macrophages, neutrophils, eosinophils, basophils and lymphocytes (IL-8 and MCP-1) to the inflammation focus.[20] The accession of the inflammatory process can aggravate venous wall damage due to leukocytic aggression, which leads to the progression of venous framework integrity disorders.[12]

Cytokines, low-mass proteins synthesized mainly by leukocytes, as well as by mononuclear phagocytes and other tissue cells in picomolar and nanomolar concentrations, are also actively involved in this process.[21] Pro-inflammatory cytokines are produced predominantly by activated macrophages and are involved in enhancing inflammatory reactions.[20] In our study, further aggravation of the pathological process (severe form of the PVI) was characterized by more pronounced manifestations of cytokine imbalance. In this case, we noted a greater involvement of pro-inflammatory factors (IL-1β, IL-2, IL-6, IL-8), as well as a decreased concentration of the anti-inflammatory component—IL-10. In this case, we can suggest a cytokine-pronounced damaging effect on the veins. Pro-inflammatory cytokines, as well as adhesion molecules, especially TGF-β, IL-6, IL-8, and VCAM-1 are the cause of venous valve insufficiency, which has been shown in several studies.[12,22-24]

High levels of IL-6/IL-10 ratio with increasing severity of PVI should also be noted. This ratio characterizes the prevalence of pro-inflammatory reactions in the body over anti-inflammatory ones at the systemic level. It is assumed that high cytokine activity mediates impaired regulation of extracellular matrix degradation processes, modification of its components (collagen and elastin), and reduction of smooth muscle cells, which aggravates the pathological process.[25,26]

Conclusion

Thus, in patients with PVI with increasing severity of varicose veins, the pro- and anti-inflammatory cytokine balance is progressively disturbed toward the dominance of pro-inflammatory components. The control of these changes in patients is an important component of the design of therapeutic measures and prevention of morphofunctional disorders occurring with the progression of the disease.

This work was performed with the use of equipment of the Collective Research Center "Centre for the development of progressive personalized health technologies" SC FHHRP, Irkutsk.

Competing Interests

The authors declare that they have no competing interests.

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