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Research article

Cytokine profile and oxidative stress parameters in women with initial manifestations of pelvic venous insufficiency

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Abstract: Pelvic venous insufficiency (PVI) in women is widespread and is closely associated with the risk of reproductive disorders (in 15–25% of patients) and a high rate of the disease recurrence after treatment. The factors involved in venous wall damage include atherogenic stimuli and chronic endotoxin aggression due to inflammatory processes. The changes in the initial stages of the disease are usually minor and selective. There is currently an urgent need to identify initial markers of these changes to develop preventive measures for their correction. Therefore, the aim of this study was to determine the cytokine profile parameters' levels, as well as the activity of lipid peroxidation (LPO) and antioxidant defense (AOD) reactions in women with initial manifestations of PVI. Thirty-nine female patients with PVI (mean age 37.4 ± 9.1 years old) were the subjects of the study. The diagnosis was verified by clinical and instrumental examination including ultrasound angioscanning of the pelvic veins and therapeutic and diagnostic laparoscopy, and it was finally confirmed histologically. The control group included 30 nearly healthy women (mean age 33.5 ± 6.3 years old) who underwent surgical sterilization by laparoscopic access. Spectrophotometric, fluorometric and immunoassay methods were used in the study. The cytokine profile in female patients with PVI, as compared to the control group, was characterized by an increased concentration of proinflammatory (interleukin (IL) (IL-6) and IL-8) and anti-inflammatory cytokines (IL-4 and IL-10) and higher ratio values (IL-6/IL-10). The level of primary LPO products, conjugated dienes, was significantly increased and level of final products TBARs values was decreased in comparison to the control. The AOD system main enzyme activity, superoxide dismutase (SOD), was decreased, while the catalase activity increased. In patients with PVI, the glutathione reduced form concentration was lower than in the control group. The results of the study in women with PVI suggest negative changes in the

cytokine profile and multidirectional changes in the indicators of the LPO system state in the initial stages of the disease. The control of these changes in patients with PVI should probably be an important component of preventive measures in the initial stages of the disease.

Keywords: inflammation; lipid peroxidation; antioxidant defense; pelvic venous insufficiency

Abbreviations: PVI: Pelvic venous insufficiency; LPO: Lipid peroxidation; AOD: Antioxidant defense; TNF: Tumor necrosis factor-alpha; IL: Interleukin; LH: Lipid hydroperoxides; CDs: Conjugated dienes; TBARs: Thiobarbituric acid reactants; SOD: Superoxide dismutase; GSH: Reduced glutathione; GR: Glutathione reductase; GPO: Glutathione peroxidase; G-S-T: Glutathione-S-transferase; SEM: Standard error of the mean

1. Introduction

Pelvic venous insufficiency (PVI) in women is widespread and is closely associated with the risk of reproductive disorders (15–25% of patients) and a high rate of the disease recurrence after treatment [1]. There are hemodynamic disorders, pelvic varicose vein transformation, the presence of chronic pelvic pain and bleeding in this disease. The risk factors of the disease include heredity, gender, age, professional activity, sedentary lifestyle, multiple pregnancies and bad habits [2]. The main defect in PVI is reflux through incompetent ovarian and pelvic vein valves [3,4]. Until now, there are no clear ideas about the mechanisms that lead to valve failure. On the one hand, it can be primary changes in the valve structure that lead to their leakiness and progression of reflux [5]. On the other hand, there can be structural abnormalities in the vein wall, leading to their dilation and, consequently, to valve deformation [4,6]. Regardless of the triggering events, prolonged venous dilatation causes inflammation, which destroys the valve structure further, leading to significant reflux [6]. It is known that endothelial cells are a key link in the chain of reactions of venous wall remodeling. It is known they encounter first to the free radicals, oxidized low-density lipoproteins, chemical agents [7]. An important role is assigned to various biosubstrates, free-radical oxidation, in particular to lipid peroxidation (LPO)-antioxidant defense (AOD) processes [8–11]. It was established that the free radicals' biological effects are realized both through their direct effects on proteins, amino groups, phospholipids and nucleic acids and through LPO products of the chain reaction [12]. Biomembrane deformation, ion transport dynamics changes, enzyme activity changes and other pathological phenomena are possible [12,13].

The factors involved in endothelial damage include atherogenic stimuli and chronic endotoxin aggression due to inflammation [14]. All these factors lead to vascular endothelial damage and dysfunction development. Endothelial dysfunction is understood as an imbalance between production of vasodilative, angioprotective and antiproliferative factors, on the one hand, and vasoconstrictor, prothrombic and proliferative endothelial progenitors, on the other [6,7,15]. Endothelial changes in initial the disease stages are usually minor and selective. At the same time, there is an urgent need to identify initial markers of these changes, which will allow to take initial preventive measures for their correction. There are still insufficient data on cytokine profile changes and nonspecific lipid peroxidation system activity in the PVI initial stages. Presented by these studies mechanisms of LPO influence on varicose veins formation is fragmentary and does not reflect their association with inflammatory reactions [13,16].

Therefore, the aim of this study was to determine the level of cytokine profile parameters, as well as the activity of lipid peroxidation and antioxidant defense reactions in women with PVI initial manifestations.

2. Materials and methods

2.1. Design of study

The subjects were 39 patients with PVI (mean age 37.4 ± 9.1 years old). The diagnosis was verified by clinical and instrumental examination and included pelvic veins ultrasound angioscanning and therapeutic and diagnostic laparoscopy, and it was finally confirmed histologically [17]. The control group included 30 healthy women (mean age 33.5 ± 6.3 years old) who underwent surgical sterilization with laparoscopic access.

Inclusion criteria were as follows: female; reproductive age; primary PVI confirmed diagnosis by ultrasound examination with duplex angioscanning; absence of concomitant gynecological pathology, gynecological diseases and organic lesions in the pelvis; informed consent to participate in the study.

Exclusion criteria were the following: acute gynecological and somatic pathology; oncological diseases; history of pelvic surgeries (not earlier than 6 months); postpartum period (less than 6 months); varicose veins of the lower extremities at the time of study inclusion; refusal to participate in the study.

Inclusion criteria for the control group were as follows: female, reproductive age, absence of acute disease or exacerbation of chronic diseases at the time of the study, absence of pathology of the venous system.

Exclusion criteria for both groups: pregnancy, intake of venotonics, angioprotective drugs, antioxidant drugs or synthetic analogues of female sex hormones (hormonal contraceptives) during the last 6 months.

2.2. Ethics approval

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of the Scientific Centre for Family Health and Human Reproduction Problems, Irkutsk, Russia (protocol No. 3.1 and date of approval 26 October 2012).

2.3. Instrumental measurements

The pelvic varicose veins assessment was performed on a Voluson (GE10 Healthcare, Austria) using a 4–8 MHz convex transducer and 7 MHz vaginal transducer. The ultrasound criterion for PVI was pelvic venous plexus ectasia over 5 mm combined with retrograde blood flow lasting more than 0.5 s recorded during Valsalva test in color Doppler mapping.

All the patients underwent laparoscopy under 3D-video imaging using Cooper surgical (USA) and Laser optic system (Laser Components USA Inc., USA-Germany) equipment to estimate the pelvic varicose veins, severity degree using retrograde hemodynamic testing (patent for an invention) [18]. The technique allowed one to register venectasia in the pelvic venous plexuses and perform biopsy of varicose vein sections for histological verification of the diagnosis.

2.4. Biochemical measurements

Vein blood sampling in patients with PVI and the control group was performed during laparoscopy equipment (Karl Storz, Germany and Cooper Surgical, USA) and a 3D Laser Optic System equipment (Laser Components USA Inc., USA, Germany). Plasma and erythrocyte hemolysate were used as test material. Blood sampling was performed from the ulnar vein in accordance with the generally accepted requirements.

The concentrations of Th1-inflammatory cytokines TNF-a, IL-1β, IL-2, IL-6, IL-8 and Th2-inflammatory interleukins IL-4, IL-10 were assessed by enzyme immunoassay using monoclonal antibody panels (JSC "Vector-Best," Russia). In addition, we calculated the proinflammatory index (PI), reflecting the balance of pro- and anti-inflammatory cytokines, as the ratio IL-6/IL-10.

Venous blood was sampled from the cubital vein between 8:00 and 9:00 a.m. after a 12-h overnight fasting period and collected into two tubes containing EDTA (ethylenediaminetetraacetic acid) anticoagulant. Immediately after collection, the blood was centrifuged at 1500 g for 10 min at 4 °C to separate the plasma from erythrocytes. The plasma was removed, and the erythrocytes were washed three times in cold saline solution (0.9% w/v). The erythrocytes were subsequently hemolyzed by adding 9 volumes (v/v) of cold phosphate buffer (50 mM, pH 7.4). Samples were kept frozen at –40 °C for no more than one month for LPO products and antioxidant enzyme parameter determination.

The LPO processes' intensity was determined: Lipid hydroperoxides (LH) and conjugated dienes (CDs) were determined by the spectrophotometric method, and the level of TBA-reactive products (TBARs) of LPO were determined by fluorometric method [19,20]. Levels of LPO products in blood plasma were evaluated. The concentrations of CDs were detected using the absorbance of plasma heptane extracts at 232 nm. The molar absorption coefficient ($K = 2.2 \times 10^5$ M^{-1} C^{-1}) was used for conversion of absorption. The levels of TBARs were determined by reaction with thiobarbituric acid followed by fluorescence intensity measurements at 515 nm (excitation) and 554 nm (emission).

The AOD system state—activity of superoxide dismutase (SOD) [21] and reduced glutathione (GSH) content [22], as well as catalase, glutathione reductase (GR) (GLUTATHIONE REDUCTASE, Randox Laboratories Ltd., UK), glutathione peroxidase (GPO) (GLUTATHIONE PEROXIDASE, Randox Laboratories Ltd., UK), and glutathione-S-transferase (G-S-T) (GLUTATHIONE-S-TRANSFERASE-π, Immunodiagnostik, Germany) activities was determined

using by commercial kits. Blood plasma was used to determine catalase, GPO and GR levels, whilst erythrocytes were used to determine GSH, G-S-T and SOD levels. The measurements were made by the spectrofluorophotometer "Shimadzu RF-1501" (Shimadzu Corporation, Japan) and spectrophotometer "Shimadzu RF-1650" (Shimadzu Corporation, Japan). Enzyme immunoassay was performed on a MultiSkan ELX808 microplate reader (Biotek, USA).

This work was carried out using the equipment of the Centre of Collective Usage "Center for the Development of Progressive Personalized Health Technologies," Scientific Centre for Family Health and Human Reproduction Problems, Irkutsk.

2.5. Statistical procedure

Statistical processing was performed using Statistica 10.0 software (Statsoft Inc., USA). Descriptive statistics of the findings were presented as medians (Me) and 25 and 75 quartiles (25%, 75%). Comparison of intergroup differences was performed using the nonparametric Mann-Whitney test with Bonferroni correction. The level of statistical significance was taken as $p < 0.05$.

3. Results

The basic characteristics of the patients with PVI and the control group are shown in Table 1. There were no statistically significant differences in the main groups' characteristics ($p > 0.05$).

Table 1. Characteristics of the patients with PVI and the control group (%).

Characteristics	Control group	Patients with PVI	p
Age mean (SEM)	38.6 (3.7)	32.5 (0.8)	$p > 0.05$
Irregular menstruation	26%	23%	$p > 0.05$
Pelvic pain	16%	53%	$p > 0.05$
Dyspareunia	10%	25%	$p > 0.05$
Dysuria	10%	7%	$p > 0.05$
Psycho-emotional disorders	13%	15%	$p > 0.05$
Disorders of pelvic organs' function	16%	25%	$p > 0.05$

Note: $p < 0.05$, statistically significant differences with the control group; PVI, pelvic venous insufficiency; SEM, standard error of the mean.

The cytokine profiles in patients with PVI and women in the control group were characterized by the following statistically significant differences: increases in the concentrations of proinflammatory cytokines, IL-6 and IL-8, against a background of rising anti-inflammatory cytokine values (IL-4 and IL-10) (Table 2). There were also significant differences in the IL-6/IL-10 ratio in the form of higher values in patients with PVI.

Table 2. The levels of cytokines in the blood of patients with PVI (Me, 25–75%).

Parameters	Control	Patients with PVI	p
IL-1β, pg/mL	124.70 (113.88; 131.15)	135.10 (125.14; 158.26)	p > 0.05
IL-2, pg/mL	39.63 (33.25; 45.74)	42.57 (38.83; 54.71)	p > 0.05
IL-4, pg/mL	793.76 (762.58; 825.38)	1205.69 (1050.39; 1390.21)	p = 0.038
IL-6, pg/mL	2241 (3147; 3380)	5162 (4968; 5230)	p < 0.0001
IL-8, pg/mL	1151 (1075; 1217)	2149 (1905; 2389)	p = 0.042
IL-10, pg/mL	1145.72 (1067.43; 1271.58)	1603.81 (1420.83; 1849.45) *	p = 0.046
IL-6/IL-10, units	2.85	3.20 *	p = 0.041

Note: p < 0.05, statistically significant differences with the control group, IL, interleukin; PVI, pelvic venous insufficiency.

The results of the LPO-AOD processes intensity study showed statistically significant differences in the lipid peroxidation products content in patients with PVI (Table 3). Thus, the primary lipid peroxidation products (CDs) level statistically significantly increased relative to control values. Changes in the content of final TBARs revealed an other difference: a decrease of average TBARs values relative to controls.

The content of antioxidant defense parameters in women with PVI also differed statistically significantly (Table 3). The activity of the AOD system main enzyme, SOD, decreased. Catalase activity showed increased values. The glutathione reductase and glutathione peroxidase levels were not statistically significantly different in the studied groups. The reduced form of glutathione (GSH) concentration in patients with PVI differed in showing lower values compared to controls.

Table 3. The level of LPO-AOD components in the blood of patients with PVI (Me, 25–75%).

Parameter	Control	Patients with PVI	p
LH, units	5.19 (5.07–5.3)	5.59 (5.38–5.75)	
CDs, μmol/L	1.85 (1.82–1.86)	5.14 (5.09–5.21)	p = 0.041
TBARs, μmol/L	2.63 (2.56–2.76)	1.11 (1.06–1.17)	p = 0.038
Catalase, μmol/L	41.86 (39.75–44.17)	49.26 (47.63–52.85)	p = 0.041
SOD, units	62.51 (55.20–68.39)	51.89 (50.83–53.97)	p = 0.032
GPO, μmol /g Hb	34.17 (33.95–35.56)	48.95 (48.13–49.38)	p > 0.05
GR, μmol/mL	4.12 (3.94–4.21)	3.04 (3.99–3.05)	p > 0.05
G-S-T, mmol/g Hb	5.21 (4.97–5.38)	5.55 (5.20–6.88)	
GSH, mmol/mL	3.54 (3.35–4.72)	2.91 (2.84–3.06)	p = 0.044

Note: *, p < 0.05, statistically significant differences with the control group; CDs, conjugated dienes; GR, glutathione reductase; GPO, glutathione peroxidase; G-S-T, glutathione-S-transferase; GSH, reduced glutathione; LH, lipid hydroperoxides; PVI, pelvic venous insufficiency; SOD, superoxide dismutase; TBARs, thiobarbituric acid reactants.

4. Discussion

PVI has a chronic course and is difficult to treat, which is due to the variety of factors affecting the venous wall [2,3,23]. In the blood of patients with PVI initial manifestations, we noted cytokine profile changes: increased concentrations of both proinflammatory and anti-inflammatory interleukins. In the blood of patients with PVI, the IL-6/IL-10 ratio was characterized by the prevalence of pro-inflammatory over anti-inflammatory factors.

Venous vessels, unlike arterial ones, are characterized by lower blood flow and tone indices [1]. Under physiological conditions, functioning veins, blood consumes 2 times more oxygen than arteries, while under conditions of venous insufficiency, this requirement increases by 3 times [4]. All this leads to functional restructuring of vascular endothelium, which develops by several stages. The initial stages, as a rule, are characterized by increased endothelial cells synthetic activity, while there are no barrier permeability disturbances [24]. Venous stasis and microcirculation disorders cause local ischemia, and hypoxia triggers the mechanism of leukocyte activation with inflammatory cytokines production, which results in increased inflammation in the affected area [7,14,25,26]. Cytokines are also able to induce acute-phase reactions both at the local and at the systemic level [27]. Cytokines are also involved in leukocyte activation, which leads to the release of free radicals, activation of proteases and subsequent smooth muscle cell degradation [6,7]. Cytokines activate specific receptors and modulate the functions of various cells and tissues. Proinflammatory factors can exert their influence on cells by regulating the transcription factor activation of proinflammatory genes [10]. This leads to further endothelial damage, in the form of connective tissue disorganization and thickening of the venous wall media [11]. In our study, a parallel increase of anti-inflammatory cytokines was found in patients with initial manifestations of PVI, which can be regarded as a compensatory reaction. However, the proinflammatory to anti-inflammatory factors ratio index was increased in patients with PVI.

It was found out that venous insufficiency is accompanied by intensification of lipid peroxidation processes [10,11]. Changes in the lipid peroxidation system may be an important factor in the development and progression of this pathological condition [13]. Studies of PVI pathogenesis proved that decreased oxygen saturation and reactive oxygen species production can also be inductive factors of pelvic veins, pathological changes in women [16,28]. Developing tissue ischemia and endothelial dysfunction can also contribute to further intensification of free radical reactions that lead to a decrease of regenerative capabilities [29]. We have found an increase of CDs (primary LPO products) values combined with reduced final products values, catalase activity compensatory increase and reduced activity of SOD and GSH. The biological effect of free radicals is realized both through their direct effect on cellular biostructures and through LPO products (lipid hydroperoxides, conjugated dienes, aldehydes) formed at different stages of the chain reaction [30]. Likely negative effects may be deformation of biomembranes, changes in the dynamics of ion transport, changes in enzyme activity and other pathological phenomena [29,30,31,32]. Higher values of catalase activity in PVI may indicate the presence of compensatory reactions in response to the LPO products increase. There are studies indicating increased catalase and malonic dialdehyde levels in patients with varicose veins, while the activities of SOD, GPO and G-S-T enzymes showed no statistically significant differences, which was explained by a compensatory response [32,33]. GSH is the main antioxidant of erythrocytes, serves as a coenzyme in methemoglobin reduction into functionally active hemoglobin and detoxifies peroxides and hydroperoxides that are formed during

the reactive oxygen species reaction with membranes, unsaturated fatty acids [34,35]. The apparent deficiency of this antioxidant may indicate the presence of negative changes in the activity of the AOD system, which should be taken into account.

5. Conclusions

The results of the women pelvic venous insufficiency study indicate simultaneous changes in the cytokine profile and multidirectional changes in the indicators of the LPO system state in the initial stages of the disease. These changes involved increased fractions of proinflammatory, anti-inflammatory cytokines and caused an increased IL-6/IL-10 ratio. Primary lipid peroxidation products showed increase values accompanied by a compensatory increase in catalase activity, along with decreased SOD activity and reduced glutathione. Probably, the control of these changes in patients with PVI should be an important component of preventive measures in the early stages of the disease.

Acknowledgments

None.

Conflict of interest

All authors declare no conflicts of interest in this paper.

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