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Original Article

Biochemistry Section

Comparison of Blood Biomarkers in Systemic Blood and Varicose Veins: A Cross-sectional Study

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ABSTRACT

Introduction: Varicose Veins (VV) are enlarged, convoluted, and elongated veins that primarily affect the superficial veins in the lower limbs and are one of the most prevalent indications of vascular diseases. The reasons for elevated venous pressure are understood, but the inflammatory cytokines that initiate the ultimate pathways of tissue destruction and are in charge of the clinical characteristics of Chronic Venous Insufficiency (CVI) are yet unknown. Although inflammation plays a crucial role in the process of tissue apoptosis, it also plays a crucial role in tissue repair and regeneration.

Aim: To investigate changes in blood markers of varicose vein inflammation and endothelial damage and compare them with systemic markers in VV patients and conclude that they are increased in VV blood.

Materials and Methods: The comparative cross-sectional study was conducted in the Department of Biochemistry on 70 patients with primary VV who were scheduled for Outpatient Sclerotherapy at Nootan Medical College and Research Centre, Sankalcahnd Patel Vidyadham in Visnagar, Gujarat, India from April 2021 to June 2023. Chronic lower extremity Venous Disease (CVD) was categorised using the Clinical Aetiology Anatomy Pathophysiology (CEAP) classification method. Blood samples were obtained above the knee from the tortuous and dilated varicose tributaries of the great saphenous vein (local) and from the antecubital (systemic) vein by standard venipuncture. Erythrocytes, leukocytes, platelets, haemoglobin, and haematocrit were determined using an automatic haematology analyser. D-dimer and High sensitivity C-reactive Protein (hsCRP) were determined by an immune turbidimetric assay. IL-6 and von Willebrand factor (vWF) were measured by Enzyme Linked Immunosorbent Assay (ELISA) using commercially available kits according to the manufacturers' instructions. An Independent samples t-test was used to compare group difference, and p-value ≤0.05 were considered significant in all two-sided statistical tests.

Results: Basic haematologic test results {systemic versus (vs) varicose blood samples} were comparable. In VV, the following parameters were significantly increased compared to systemic blood: Haemoglobin (12.85±1.81 g/dL vs. 15.82±1.57 g/dL, p<0.001), hsCRP (1.34±1.01 mg/L vs. 3.78±1.67 mg/L, p<0.001), IL-6 (2.65±1.07 pg/mL vs. 4.17±1.51 pg/mL, p<0.001), vWF (90.73±16.72% vs. 127.30±19.92, p<0.001). D-dimer was also substantially higher in samples extracted from leg VV than in systemic blood (105.87±17.72 ng/mL vs. 85.61±18.18 ng/mL, p<0.001).

Conclusion: Blood from VV has shown a higher level of several inflammatory markers and signs of endothelial dysfunction. This is most likely the result of worsening venous pressure and dilated, convoluted superficial veins that restrict blood flow. The procoagulant qualities of the local blood and damage to the venous wall, leading to a chronic inflammatory response, may accelerate the disease's progression and thrombotic consequences.

Keywords: Chronic venous insufficiency, Endothelial damage, Inflammatory markers

INTRODUCTION

Alterations in the components of blood contribute to the advancement of the disease and are likely accountable for its consequences, such as CVI and superficial vein thrombosis. VV are enlarged, convoluted, and elongated veins that primarily affect the superficial veins in the lower limbs. This condition is one of the most prevalent indications of vascular diseases [1].

Primary VV are a key clinical indicator that may reveal significantly impeded venous drainage from the leg. A wide range of symptoms and signs, from mild clinical manifestations like telangiectasia, reticular veins, and VV to more severe forms like skin changes and Chronic Venous Leg Ulcers (CVLUs), are a result of pathological and haemodynamic changes in the veins of the lower limbs, known as CVD [2,3]. In the Western world, it affects 10-20% of the population; in India, the prevalence is only 5% [4]. Primary and secondary forms should be distinguished based on aetiology, with the latter typically arising as a result of DVT.

The majority of patients with pathological CVI or healthy individuals experiencing physiological CVI from prolonged standing may encounter oedema, leg pain, and subsequent skin abnormalities in the gaiter region, characterised by hyperpigmentation, fibrosis, and even venous ulcers [5,6]. This suggests that inflammatory processes are at play, mediating tissue damage. While the reasons for elevated venous pressure are understood, the inflammatory cytokines initiating the final pathways of tissue destruction and driving the clinical features of CVI remain unknown. Although inflammation plays a critical role in tissue apoptosis, it also contributes significantly to tissue repair and regeneration [7-11].

In this scenario, ineffective perforating veins and saphenous trunks hinder antegrade flow towards the heart, redirecting venous flow back towards the foot due to gravitational forces. This creates a situation where venous pressure in the lower leg veins rises and is sustained during dependency, owing to a continuous hydrostatic column of blood. In a healthy state, the leg's muscle pumps interrupt the hydrostatic column and propel blood upward to counteract the increased pressure [12,13].

Sir William Harvey proposed in the 17th century that the development of VV stems from central valvular incompetence due to valve atrophy. This notion has been supplanted by the major vein wall weakness theories, suggesting that VV result from a hereditary deficiency in vein wall integrity [14]. Collagen matrix proliferation and

disruption and distortion of muscle fibers occur in VV. Both venous hypertension and valvular incompetence can arise from primary vein wall weakening [15].

The CVD has been associated with increased oxidative stress, likely the primary cause of vessel wall damage [16]. Treatment for Reactive Oxygen Species (ROS) hyperproduction in varicose lower limbs can involve removing the great saphenous vein [17]. Various conditions can lead to persistent venous insufficiency. According to one theory, venous hypertension causes red blood cell extravasation and localised iron overload, both of which could generate free radicals. Studies also suggest that the pathophysiology of VV, primarily attributed to decreased Nitric Oxide (NO) bioavailability, may contribute to the deterioration of venous endothelial function [18]. NO, a potent vasodilator and crucial cellular signaling molecule, inhibits platelet adhesion and aggregation, reduces leukocyte adhesion to the endothelium, and has been shown to halt vascular smooth muscle cell proliferation. Therefore, changes in vascular tone and platelet adhesion ensue from the disruption of vessel wall homeostasis caused by decreased NO bioavailability, leading to morphological abnormalities in the vascular wall.

A study identified a correlation between iron deposition in varicose veins and tissue oxidative state, as determined by proton-induced X-ray emission spectroscopy. This correlation is linked to oxidative stress, which may influence the progression of chronic venous disease [19]. The roles of regional variations in blood components in the development of varicose vein issues and the progression of the disease have not been fully elucidated. The aim of present study was to compare the levels of inflammatory and coagulation/fibrinolysis markers in varicose veins with those in blood drawn from cubital veins (systemic blood). The objective of present study was to investigate blood alterations in varicose veins and correlate them with systemic indicators of endothelial damage and inflammation.

MATERIALS AND METHODS

The present comparative cross-sectional study was conducted to compare the blood biomarkers in individuals with VV with those found in systemic blood. The study population consisted of 70 patients conducted in the Department of Biochemistry with primary VV scheduled for Outpatient Sclerotherapy at Gujarat's Nootan Medical College and Research Centre in Visnagar, Gujarat, India from April 2021 to June 2023. Informed written consent was obtained from all patients meeting the inclusion criteria. The study protocol was approved by the State Ethical Committee of the Institute (Ref: NMCRC: HREC/21/SESSION 4/12).

Inclusion criteria: All the patients with primary VV who were scheduled for Outpatient Sclerotherapy at Study Institute and who were willing to participate were included in the study.

Exclusion criteria: History of DVT, prior venous interventions, use of medications affecting coagulation, known coagulation disorders, inability to understand study requirements, active cancer, and co-morbidities were excluded from the study.

Study Procedure

Bilateral venous duplex ultrasound scanning and clinical examinations were performed to identify primary varicose veins. Primary varicosities were observed in all cases, with subsequent varicosities not included. CVD was classified using the CEAP classification method, which considers clinical, aetiological, anatomical, and pathological factors [20]. The majority, 51 (73%), were classified as C2, while the remaining 19 (27%) patients were classified as C3.

Patients underwent examination using ultrasound Logiq p9 machine technology in the supine position with their heads elevated 15 to 30 degrees, utilising a 10 MHz linear array probe (Linear 9L Probe) for transverse and longitudinal imaging. The inguinal ligament was selected as the starting point for inspection.

Standing position was employed to investigate the superficial venous system as it allowed for full vein dilation and accurate detection of reflux. Various transducer placements were used for both longitudinal and transverse views. The examination began in the groin area, noting compressibility, reflux, and tributaries of the saphenofemoral junction. If reflux was present, the diameter of the great saphenous vein in the thigh was measured. Additionally, a B mode scan of the branches and perforating veins was conducted along the length of the great and small saphenous veins from the knee to the ankle. Compressibility, flow, and reflux measurements were taken at the sapheno-popliteal junction and the posterior tibial perforating vein.

Ultrasound of the deep veins of the lower limbs was performed to rule out obstruction, locate and determine the sources of tributaries of the GSV, and detect reflux and its source.

The patient was positioned in a supine position while blood samples were collected from the upper and lower extremities. Blood samples from the leg were obtained above the knee from the tortuous and dilated varicose tributaries of the great saphenous vein (local) and from the antecubital vein (systemic) through standard venipuncture. Citrated venous blood was centrifuged at 5000 rpm for five minutes to separate the plasma, which was then immediately frozen at -70°C for further analysis. The measurements were conducted over a period of three months.

Dyslipidemia was defined as total cholesterol >200 mg/dL, Low Density Lipid (LDL) cholesterol >100 mg/dL, and/or triglycerides >150 mg/dL, and High Density Lipid (HDL) cholesterol <40 mg/dL [21]. Erythrocytes, leukocytes, platelets, haemoglobin, and haematocrit levels were determined using an automatic haematology analyser. D-dimer and hsCRP levels were measured using an immunoturbidimetric assay. IL-6 and vWF levels were assessed by ELISA using commercially available kits following the manufacturers' instructions.

STATISTICAL ANALYSIS

The data was analysed by Statistical Package for Social Sciences (SPSS) version 21.0 software. The mean and Standard Deviation (SD) and MedCalc were utilised for all data analysis. The mean and standard deviation were used to represent parametric values. An independent Samples t-test was employed to compare group differences. A p-value ≤0.05 were considered significant in all two-sided statistical tests.

RESULTS

The key clinical traits and anthropometric information has been depicted in the [Table/Fig-1]. Seventy patients with VV, predominantly females 57 (81%), were included in the study. According to the Clinical, Etiological, Anatomical and Pathophysiological (CEAP) classification, the patients were classified as C2-51 (73%) and C3-19 (27%), with the exception of a positive family history of VV, which was present in most patients 50 (72%). Total 14 (20%) patients with diabetes mellitus were treated with hypoglycemic drugs. The inflammatory markers, fibrinolytic markers, and markers of endothelial damage in systemic and local blood are presented in [Table/Fig-2]. Except for haemoglobin 12.85±1.81 (systemic blood) and 15.82±1.57 (VV), the majority of haematological measures fell within the normal range, with no significant variations between cubital and VV blood samples. However, some circulating inflammatory markers (hsCRP) and Interleukin-6 (IL-6), as well as D-dimer and vWF, were significantly increased in the blood samples taken from VV. Compared to systemic blood samples from the cubital vein, the levels of circulating inflammatory markers like hsCRP (3.78±1.67, p<0.001) and IL-6 (4.17±1.51, p-value <0.001), and fibrinolytic indicators like D-dimer (105.87±17.72, p-value <0.001) and vWF (127.30±19.92, p-value <0.001) were significantly higher in VV blood.

Parameters		Results
Age (years)		56.2±10.2
Gender	Male	13 (19%)
	Female	57 (81%)
Arterial hypertension		30 (43%)
Mean systolic BP (mmHg)		140±13
Mean diastolic BP (mmHg)		85±7
BMI, (kg/m^2)		28.7±5.2
Diabetes mellitus		14 (20%)
Positive family history of Varicose Veins (VV)		50 (72%)
Hyperlipidemia		22 (32%)
Smoking		9 (13%)

[Table/Fig-1]: Clinical characteristics and the presence of risk factors in 70 subjects of Varicose Veins (VV).
Data are expressed as mean-Standard Deviation (SD) for continuous variables and as numbers (N) and percentages (%) or categorical variables

Parameters	Systemic blood	Varicose Vein (VV) Local blood	p-value
Erythrocytes (10^{12}/L)	4.49±0.33	4.42±0.34	0.45
Haemoglobin (g/dL)	12.85±1.81	15.82±1.57	<0.0001
Haematocrit (L)	0.43±0.03	0.41±0.03	0.449
Leukocytes (10^9/L)	6.86±1.61	6.96±1.54	0.086
Thrombocytes (10^9/L)	240±62.62	236±63.80	0.632
hsCRP (mg/L)	1.34±1.01	3.78±1.67	<0.001
D-dimer (ng/mL)	85.61±18.18	105.87±17.72	<0.001
vWF (%)	90.73±16.72	127.30±19.92	<0.001
IL-6 (pg/mL)	2.65±1.07	4.17±1.51	<0.001

[Table/Fig-2]: Serum levels of inflammatory and fibrinolytic markers.
From the blood samples taken from Varicose Veins (VV) (local) and anticubital veins (systemic) Data are expressed as mean±SD. hsCRP=high sensitive C-reactive protein; *Independent t-test p<0.05, statistically significant. vWF=von Willebrand factor; IL-6

DISCUSSION

Numerous factors are believed to be involved in the pathogenesis of VV in the primary veins, leading to further complications. The pathophysiology of these disorders is often associated with oxidative stress and endothelial dysfunction, which exacerbate the inflammatory response. The current study investigated the blood markers of inflammation and endothelial damage, fibrinolysis, and examined any discernible variations between cubital and VV blood. It was believed that alterations in regional haemodynamics due to turbulence in the dilated and tortuous veins lead to the release of various inflammatory chemicals.

In the current investigation, the VV samples showed higher levels of certain inflammatory markers such as hsCRP, D-dimer, vWF, and IL-6 compared to systemic blood.

In the present study, females were more likely to experience VV cases than males. The present finding is supported by a study by Asciutto G et al., where estradiol levels were significantly higher in VV arising from the great saphenous vein than in veins in the upper extremities [22].

The aetiology of these conditions is often associated with oxidative stress and endothelial dysfunction, both of which exacerbate the inflammatory response. Although the continuous increase in venous pressure due to the effects of gravity causes the haemodynamic imbalance that leads to the clinical symptoms of CVI, the molecular mechanisms that result in cellular damage are not fully understood. Hypoxia [23], haemosiderin deposition, fibrinolysis [24], and endothelial dysfunction [25] are known to be significant factors affecting the condition. Inflammation is a key component of the damage process, and leukocytes are the primary cells responsible for generating most cytokines [26,27].

Furthermore, when compared to systemic samples from the same patient, D-dimer concentrations in blood drawn from VV were significantly higher. Some of the present findings are supported by a previous study related to this topic [28]. Specifically, D-dimer, IL-6, and von Willebrand factor concentrations were shown to be higher in VV blood compared to comparable systemic samples.

Both the processes of cellular damage and healing and recovery involve inflammation. Although patients' legs have increased IL-6 concentrations, it may not necessarily be harmful to them. These cytokines could be crucial for tissue healing. For example, IL-6 has been described as both a pro- and an anti-inflammatory molecule [29]. Additionally, IL-6 may promote the regeneration of intestinal cells [30] and hepatocytes [31] in experimental studies. Matrix Metalloproteinases (MMPs), which were not investigated in present study, also play significant roles in both venous injury and repair [32,33].

It is widely accepted that low shear stress, turbulent flow, and stasis promote the production of inflammatory and thrombotic mediators, while laminar shear stress encourages the release of factors that suppress inflammation and reactive free radical production [34].

According to Saharay M et al., venous hypertension causes endothelial activation, which could facilitate endothelial-leukocyte adhesion [35]. Vascular Cell Adhesion Molecule (VCAM)-1, a counter ligand for receptors expressed by monocytes and lymphocytes, is increased in patients with Lower Limb Dermatitis Syndrome (LDS), suggesting that these cells may play a more significant role in the development of skin changes [36]. Therefore, it was anticipated that present study would find an increased local inflammatory response and hypercoagulability in the convolutions of VV. This is most likely the result of increased venous pressure and turbulent venous flow, damaging the vessel wall over time. Systemic inflammation in individuals with VV has only been the subject of a small number of investigations, and data on inflammatory markers in VV are quite uncommon. Monocyte chemoattractant protein-1, macrophage inflammatory protein, interferon-inducible protein-10, and interleukin-8 were all shown to be strongly expressed in VV in one investigation [37]. By attracting leukocytes to the vein wall and causing inflammation, the scientists hypothesised that these chemokines might be crucial in the pathophysiology of VV and their consequences [37].

Neuron-specific Enolase (NSE) showed a marginal increase in one study. It serves as a tumour marker for neuroendocrine malignancies and is produced by neuronal and neuroendocrine cells [38].

In accordance with Virchow's triad, endothelial dysfunction and damage to the venous wall represent one of the fundamental pathways for the development of VV, DVT, and most likely other consequences (thrombosis). In VV, endothelial injury and damage brought on by shear stress from venous hypertension (endothelial dysfunction) are predicted. In patients with VV, endothelial dysfunction is likely the first sign of venous wall cell injury [39].

Since endothelial cells produce von Willebrand factor, its levels were examined in the present study to assess endothelial function and/or damage. In healthy circumstances, it is continuously produced and released into the blood because it aids in coagulation and the development of platelet plugs at the sites of endothelial injury. Subjects with atherosclerotic risk factors and circumstances characterised by vascular injury have higher amounts of it. In present study, von Willebrand factor in VV was substantially higher than in systemic blood.

In the current investigation, VV samples had elevated D-dimer levels, and there was a clear positive association between the varicose and cubital vein samples. These findings imply higher tissue factor release with enhanced procoagulant potential, which can lead to thrombus development and superficial venous thrombosis and increased local fibrin production, likely as a result of injury to the vessel wall.

Limitation(s)

One of the limitations of the present study was that the sample size was not statistically calculated. Additionally, numerous genetic changes and polymorphisms can be found, but none of them alone can cause CVD susceptibility. Nevertheless, they are helpful in expanding the understanding of the risk factors for this illness. There are many additional markers that may be studied, but we were unable to incorporate them all. The present type of study can be made more advanced and in-depth with additional research involving all CVI grades.

CONCLUSION(S)

The findings of present study suggest that several inflammatory markers and markers of endothelial damage are elevated in VV blood compared to systemic blood. Most likely, stasis of venous blood and elevated venous pressure result in chronic local inflammation of the vein wall and endothelial dysfunction. Another factor that contributes to inflammation and coagulation is possibly the turbulent blood flow that occurs in tortuous and dilated veins. All of these haemodynamic parameters, changes in blood components, and illness development are likely the causes of problems such as CVI and superficial vein thrombosis. Inflammatory cytokine levels are elevated in the legs of CVI patients. This could be a minor development in the search for a biomarker particular to CVI. When such biomarkers become accessible, it may be useful for tracking the outcomes of therapy and for better understanding of pressure-mediated cellular damage and repair work.

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