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Study On The Levels Of Inflammatory Markers In Venous Blood From Varicose Vein In-Situ And From Peripheral Vein In Patients With Varicose Veins

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ABSTRACT- **Background/objectives:** To evaluate the role, variations and correlate the inflammatory markers such as C-reactive Protein (CRP), Neutrophil to Lymphocyte ratio (NLR) and Erythrocyte Sedimentation Rate (ESR) as well as to analyze the changes in hematological parameters at the site of varicose veins (in-situ). **Materials and methods**: The study enrolled Thirty adult patients (both male and female, aged 18-64 years) with varicose veins. The study groups were divided into patients with varicose veins and a control group. Blood samples were collected from the antecubital vein of the control group and the patient group, and from the varicose vein in-situ of the patient group. Parameters assessed included Erythrocyte count, Leukocyte count, Hemoglobin concentration, Neutrophil and Lymphocyte count, ESR and serum high sensitivity CRP levels. **Results:** Significant increase was found in Erythrocyte count, Leukocyte count, Neutrophil count, Lymphocyte count, NLR, ESR, and CRP levels in patients compared to healthy controls. There was also a significant increase in these parameters including Haemoglobin concentration in blood samples taken from varicose veins compared to systemic blood in patients. The study also found a strong correlation between the Erythrocyte count, Haemoglobin concentration, ESR, and CRP levels in systemic blood and varicose vein samples in patients. **Conclusion**: Levels of inflammatory markers is significantly higher in blood samples collected from patients with varicose veins when compared to those from healthy controls, particularly at the site of varicose vein. This study confirms the hypothesis that inflammation is involved in the pathogenesis and progression of the disease. Therefore, our findings may have implications for the management of patients with varicose veins. Identifying and monitoring inflammatory markers in patients with varicose veins could help in early detection of potential complications and timely intervention along with finding targeted anti-inflammatory therapy for the management of the same. **Key words:** Anti-inflammatory therapy, Anti-leukocyte therapy, Endothelial damage, Inflammation, Inflammatory markers, Varicose veins

1. Introduction

Veins in the extremities can be classified as superficial and deep system of veins. In the legs, these two systems of veins are connected by the perforating veins that allow blood to flow from the superficial system to the deep system at multiple locations. Veins contain bicuspid valves to direct the venous blood centrally. [1]

Varicose veins are dilated, bulging, tortuous superficial veins, measuring at least 3 mm in diameter. Primary varicose veins arise from the superficial system due to defective valves of the saphenous veins, intrinsic weakness of the vein wall, and high intraluminal pressure along with various other contributory factors. Secondary varicose veins result from venous hypertension, deep-venous insufficiency or deep-venous obstruction, and incompetent perforating veins that cause enlargement of superficial veins.[1]

Varicose veins are one of the most frequent manifestations of vascular pathology. Yet, the exact cause of the disease and its pathogenesis are not well understood. Recent studies suggest that besides venous hypertension, there are various potential

inflammatory components that triggers the progression of the disease.[2] Inflammation plays a crucial role in various physiological processes and is typically associated with the body's response to injury or infection.[1] However, chronic inflammation can lead to tissue damage and contribute to the development of various diseases. In varicose veins there are elevated levels of inflammatory markers and indicators of endothelial damage.[2]

It is known that the laminar shear stress positively enhances the release of factors that suppress inflammation, whereas low shear stress, turbulent flow, and stasis enhances the production of inflammation and thrombotic mediators.[3] Therefore, venous stasis promotes the inflammation and deterioration of the venous wall.

The inflammatory response is of two types, acute and chronic inflammation. An acute inflammatory response is a controlled process, with a short time window of minutes up to a few hours and is characterized by the abundant presence of specific type of immune competent cells (Neutrophils), responsible for clearing invading pathogens and promote tissue repair, thus restoring the homeostasis. If an accumulation of lymphocytes in the inflamed tissue predominates, both immune and physiological homeostasis are disrupted, thereby the inflammation can progress to a chronic condition.[4]

Studies show that in the blood collected from the varicose vein, there is a significant increase in the levels of serum C-Reactive Protein (CRP). Serum CRP is a marker for chronic inflammation and typically used to assess the presence and severity of systemic inflammation.[5] However, the role of CRP in varicose veins is not well-established.

Erythrocyte Sedimentation Rate (ESR) is a non-specific marker of inflammation in the body. Although varicose veins themselves do not directly cause an increase in ESR, they can be associated with chronic inflammation and venous insufficiency. Venous insufficiency can lead to blood pooling and increased venous pressure in the affected veins.[5] Therefore, chronic inflammation can develop in the surrounding tissues due to the abnormal blood flow. Inflammation triggers the release of certain proteins, including fibrinogen and immunoglobulins, into the bloodstream.[6] These proteins cause red blood cells to stick together, leading to an increase in the sedimentation rate.[5] Consequently, an elevated ESR level may be seen in individuals with chronic venous insufficiency, including those with varicose veins.

The aim of the study was to evaluate the role and variations in the inflammatory markers such as hsCRP, Neutrophil to Lymphocyte ratio (NLR) and ESR as well as to analyze the changes in hematological parameters at the site of varicose veins. Also, to correlate the levels of these constituents in the blood collected from the varicose veins in situ to the levels in the normal peripheral vein of the same individual. Understanding these could provide valuable insights into the underlying mechanisms in the disease progression, development of complications, recurrence after treatment in patients with varicose veins and may alter the potential implications for patient management with targeted anti-inflammatory interventions.

2. Materials and Methods

This study was carried out in the Department of Physiology, Karnataka Institute of Medical Sciences (KIMS), Hubballi, Karnataka. Thirty patients were enrolled in this study. The patients, both male and female adults (Age: 18 - 64 years) [6] with an established diagnosis of varicose veins, who visited Surgery OPDs and IPDs of KIMS, Hubballi, was selected for this study. The patients with varicose veins were selected for the study groups upon confirmed diagnosis by the

combination of clinical examination and bilateral venous duplex ultrasound scanning.

The exclusion criteria for the study were history of deep vein thrombosis, post-thrombotic syndrome, reflux in the deep venous system, history of recent acute infections, allergy episode or known chronic infection, active skin ulcers or local skin inflammatory changes, history of any disorders known to affect the blood vessel wall such as Klippel-Trenaunay syndrome, May-Turner syndrome etc., Pregnant and lactating women, history of smoking and/or alcohol, history of any disease/ disorder, known to alter levels of inflammatory markers like rheumatoid arthritis, systemic lupus erythematosus, Chronic Kidney Disease, COPD, Cirrhosis, Hepatitis etc. and patients on medications like steroids, NSAIDs, antihistamines, antidepressants, beta blockers and contraceptives.

After considering the inclusion and exclusion criteria, the study groups were selected. Details of the test procedure were explained to the subjects in their vernacular language and informed consent was obtained. Institutional Ethical committee permission was obtained (No. KIMS/PGS/SYN/79/2023-24).

Study groups

GROUP - 1 and GROUP - 2 (N = 30) – Patients with varicose veins (18 - 64 years of age).

GROUP-3 (N = 30) – Control (18 - 64 years of age).

Study design

Cross- sectional study

Period of study

March 2024 to August 2024

Collection of samples

Under strict aseptic precautions, blood samples were collected from the upper extremity (antecubital vein) of the control group and patient group. Blood sample from the lower extremity having varicose vein was obtained above knee, from the tortuous and dilated tributary of the great saphenous vein by standard venepuncture, with the patient lying in supine position to eliminate release of circulating markers due to the influence of hydrostatic pressure. The citrated blood samples were subjected to centrifugation at 5000 r/minute for 5 minutes and was frozen at □ 20□C for further testing.

Lab Investigations

The following parameters were assessed: Erythrocyte count, Total Leukocyte count, Haemoglobin levels, Absolute Neutrophil count, Absolute Lymphocyte count, Erythrocyte Sedimentation Rate (ESR) and Serum high sensitivity CRP levels (hsCRP). Blood samples taken for hematological parameters was assessed by the Auto analyser (Sysmex XNL series) in the Pathology department and CRP kits in the Biochemistry department of KIMS, Hubballi.

Statistical analysis

The statistical analysis was done using statistical software SPSS for mac (Version 29.0.2.0). Mean values were reported with their standard deviations (SD) or/and standard error mean (SEM). The significant differences between Means were determined by the Student's t test for independent samples. For paired samples, Student's t test was used. Statistical analysis was considered significant by fixing the P-value at $P < 0.05$.

3. Results

In this study, we assessed the hematological parameters and inflammatory markers in patients with varicose veins. Erythrocyte count, leukocyte count, haemoglobin concentration, neutrophil count, lymphocyte count, ESR and CRP levels in blood samples taken from antecubital vein of the control and

patient as well as from varicose vein in-situ of the patient were compared. NLR for each of the study group was calculated from the Absolute Neutrophil Count (ANC) and Absolute Leukocyte Count (ALC) obtained.

On comparison of concentration of these parameters in the systemic blood of healthy controls and patients with varicose veins, a significant increase with p value of 0.001 in the erythrocyte count and $p < 0.001$ in leukocyte count, neutrophil count, lymphocyte count, NLR, ESR and CRP levels was found, respectively. Also, an insignificant increase in concentration of haemoglobin was found with p value of 0.094. [Table 1]

On comparison of concentration of the same parameters in the systemic blood (antecubital vein) and local blood (varicose vein in-situ) in patients with varicose veins, a highly significant increase in the erythrocyte count, leukocyte count, haemoglobin concentration [Table 2], neutrophil count, lymphocyte count [Figure 1], NLR [Figure 2], ESR [Figure 3] and CRP levels [Figure 4] was found with p value of < 0.001 . A significant correlation [Table 3] was observed between systemic and local blood of patients with varicose vein in regard to erythrocytes, haemoglobin, ESR and CRP at $r = 0.763$ ($p < 0.001$), $r = 0.978$ ($p < 0.001$), $r = 0.815$ ($p < 0.001$) and $r = 0.831$ ($p < 0.001$), respectively.

4. Discussion

In our study, we analysed the hematological parameters, ESR, NLR and serum C-reactive protein (CRP) levels in venous blood from the varicose vein in-situ (local blood) and from the antecubital vein (systemic blood) of the patient to identify any significant changes in the levels of these parameters when compared to the systemic blood of healthy controls. A significant increase was observed in the erythrocyte count, leukocyte count, haemoglobin concentration, neutrophil count, lymphocyte count, ESR and CRP levels in the venous blood from varicose vein in-situ compared to normal peripheral veins of the same patient. This finding suggests that varicose veins may be associated with a higher level of inflammation in the body.

Increase in the erythrocyte count and haemoglobin may be attributed to the erythrocyte aggregation in chronic venous disease (CVD).[11] As a compensatory mechanism to the increased resistance in the microcirculation of varicose veins, deformability of RBC might increase leading to the higher tendency of aggregation of erythrocytes in the varicose vein, thereby increasing the overall and local count. Although it is understood that erythrocyte aggregation could be the reason for significant change (increase) in haemoglobin levels within the patient, it is only an insignificant increase when compared to the healthy controls.

Elevation in leukocyte count along with relative increase in neutrophils and decrease in lymphocytes was observed in our study. However in a previous study, there was no significant difference in these levels in the systemic and local blood of the patient.2 It is suggested that leukocytes with their ability to roll along the vessel wall causes leukocyte adhesion due to the already existing venous hypertension responsible for the varicose vein. This increased adhesion may also be associated with the increased capillary permeability that leads to edema.[12] The pathological changes in the vascular endothelium which correlates with increasing severity of skin changes as the disease progress could thus be due to the release of cytokines from the leukocytes which is the backbone of an inflammatory process.

Elevated levels of inflammatory markers such as NLR, ESR and CRP have been linked to various chronic diseases such as cardiovascular disease, arthritis and diabetes. NLR and ESR are non-specific markers for

inflammation. CRP is considered as an early marker of inflammation. In a study by Matei et al., it was shown that as CRP is associated with the development of atherosclerotic lesions, it could be possible for it to have an impact on the vasa vasorum of the venous adventitia.[13] The present results is in par with this study revealing a direct involvement of NLR, ESR and CRP in the progression of the disease, thereby confirming the inflammatory nature of the disease despite other studies[14] considering inflammation to not be an important component in the pathogenesis of varicose veins.

Further research is needed to investigate the underlying mechanisms of inflammation in varicose veins and its impact on overall health. It is also required to focus on the dual roles of inflammation in mediating tissue destruction as well as tissue repair. Future studies could also explore the potential role of targeted anti-inflammatory interventions in the management and associated complication of varicose veins.

We conclude our present study with the findings that the level of inflammatory markers is significantly higher in blood samples collected from patients with varicose veins when compared to those from healthy controls. The overall parameters have significantly increased in local blood (varicose vein in-situ) when compared to the systemic blood of the same patient with varicose vein. This study also confirms the hypothesis that inflammation is involved in the pathogenesis and progression of varicose veins. Therefore, our findings may have implications for the management of patients with varicose veins.

Identifying and monitoring inflammatory markers in patients with varicose veins could help in early detection of potential complications and timely intervention along with finding targeted anti-inflammatory therapy for the management of the same. Anti-leukocyte therapies including postural rest and venotonics which alone or in combination with compression have been shown to unstick and inhibit leukocytes.

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Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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TABLE 1. Comparison of systemic blood parameters in patients and control.

	Mean ± SEM N = 30			
	Control	Systemic blood	**t**	**p Value**

Erythrocytes ($\times 10^6/\mu L$)	4.46 ± 0.13	4.99 ± 0.09	– 3.40	0.001
Leukocytes ($\times 10^3/\mu L$)	4.70 ± 0.15	10.07 ± 0.27	–17.38	<0.001
Haemoglobin (G/dL)	13.21 ± 0.21	13.69 ± 0.19	– 1.70	0.094
Neutrophils (%)	56.68 ± 1.19	69.09 ± 0.70	– 8.95	<0.001
Lymphocytes (%)	28.92 ± 0.97	19.86 ± 0.63	7.85	<0.001
NLR	2.06 ± 0.11	3.56 ± 0.11	– 9.51	<0.001
ESR (in mm at 1st hr)	10.73 ± 0.18	15.80 ± 0.72	– 6.83	<0.001
CRP (mg/L)	0.34 ± 0.03	3.07 ± 0.18	–14.61	<0.001

TABLE 2. Comparison of blood parameters in Antecubital vein (Systemic blood) and varicose vein in-situ (Local Blood) in patients with Varicose veins.

	Mean ± SEM N = 30			
	Systemic blood	In-situ Blood	**t**	**p Value**
Erythrocytes ($\times 10^6/\mu L$)	4.99 ± 0.09	5.37 ± 0.09	– 5.67	<0.001
Leukocytes ($\times 10^3/\mu L$)	10.07 ± 0.27	12.29 ± 1.48	–8.52	<0.001
Haemoglobin (g/dL)	13.69 ± 0.19	14.13 ± 0.18	– 11.42	<0.001

TABLE 3. Correlation between blood parameters taken from Antecubital vein (Systemic) and varicose vein in-situ (Local Blood) in patients with Varicose veins.

N = 30		
	r	p Value
Erythrocytes	0.76	< 0.001
Leukocyte	0.35	0.060
Haemoglobin	0.98	< 0.001
Neutrophils	0.29	0.114
Lymphocytes	–0.18	0.348
NLR	–0.25	0.190
ESR	0.82	< 0.001

CRP	0.83	< 0.001

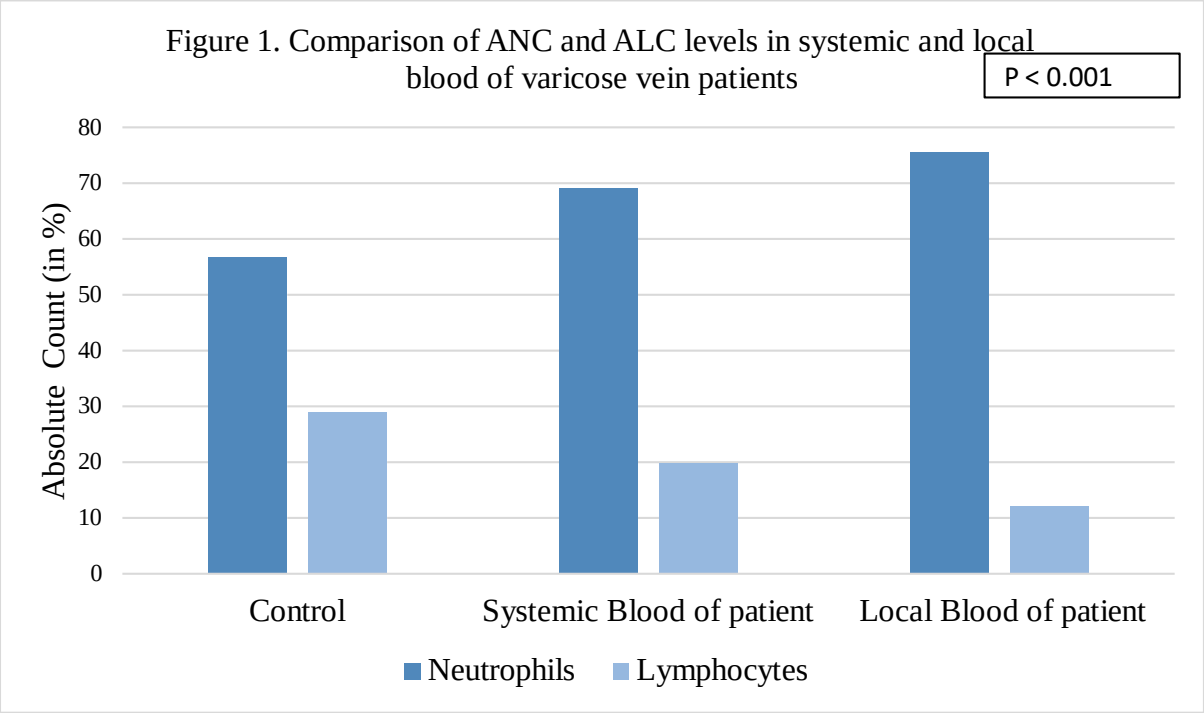


Figure 1. Comparison of ANC and ALC levels in systemic and local blood of varicose vein patients
P < 0.001
Absolute Count (in %)
80
70
60
50
40
30
20
10
0
Control
Systemic Blood of patient
Local Blood of patient
Neutrophils
Lymphocytes

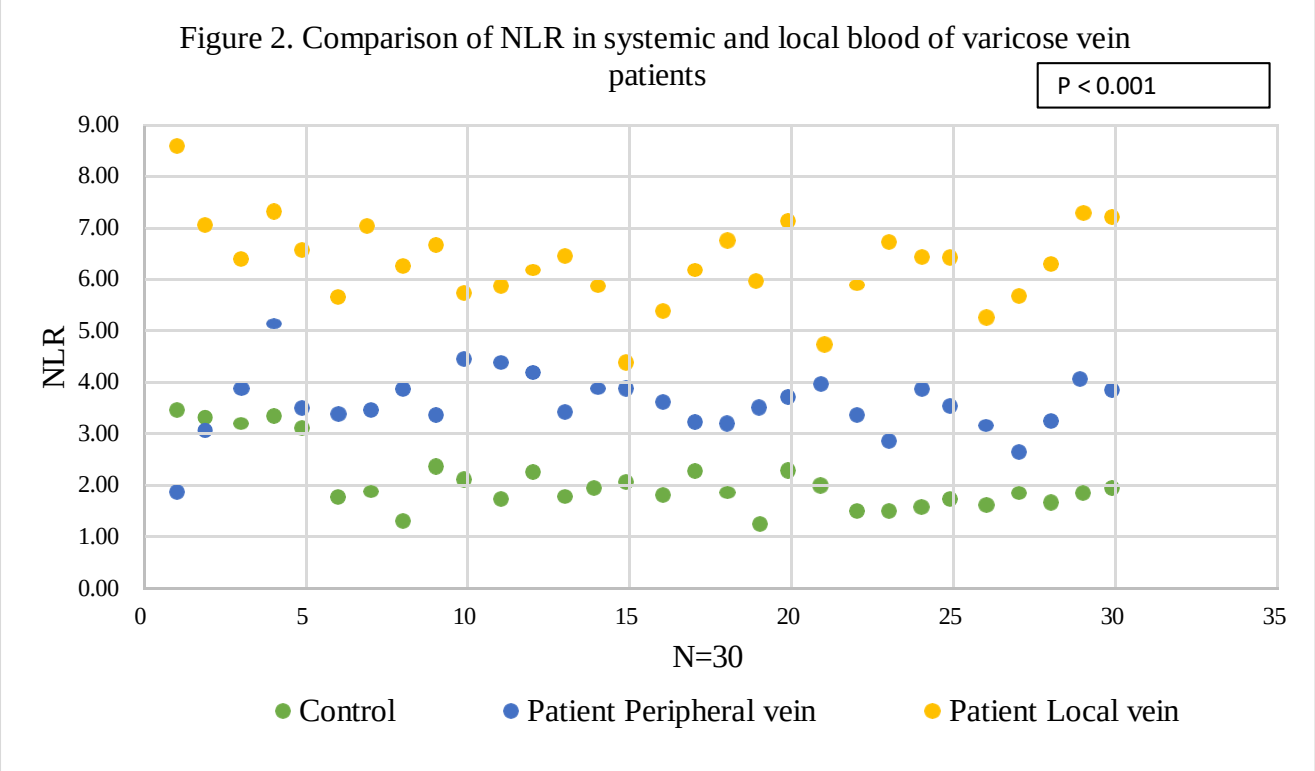
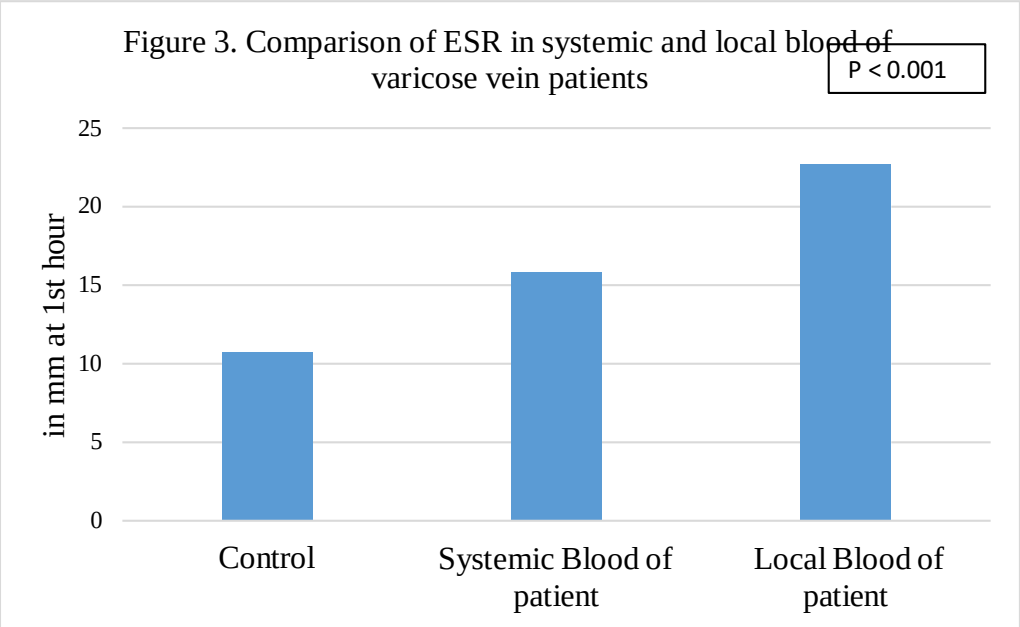
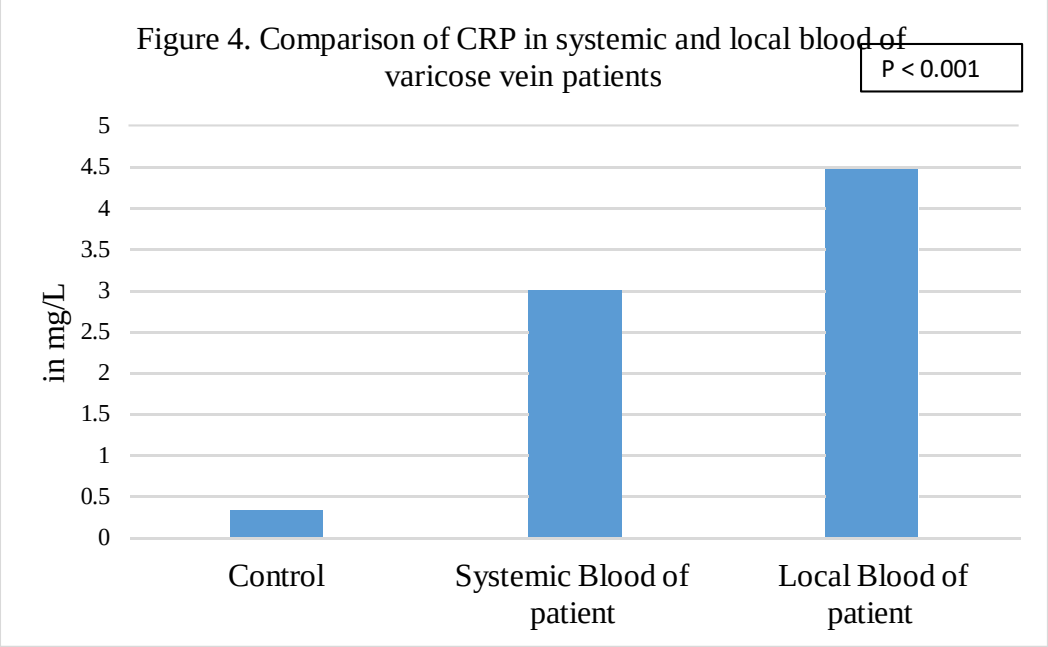


Figure 2. Comparison of NLR in systemic and local blood of varicose vein patients
P < 0.001
NLR
9.00
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
0
5
10
15
20
25
30
35
N=30
Control
Patient Peripheral vein
Patient Local vein





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