ScientificScholar®
Knowledge is power
Publisher of Scientific Journals

https://ijrsms.com

International Journal of Recent Surgical and Medical Sciences

Original Article

A Study of Blood Viscosity and Inflammatory Biomarkers' Levels in Bilateral Primary Varicose Veins/Reticular Veins as Predictive Markers

Mukul Urvendra Dahiya[1], Sanjay Singh[1], Saurabh Singh[1], Santosh Kumar Singh[1], Varsha Kumar[2]

[1]Department of General Surgery, [2]Department of Pathology, Moti Lal Nehru Medical College, Prayagraj, India.

***Corresponding author:**
Dr. Mukul Urvendra Dahiya, Department of General Surgery, Moti Lal Nehru Medical College, Prayagraj, India.

dr.mukuldahiya@gmail.com

Received: 11 May 2023

Accepted: 07 August 2023

EPub Ahead of Print: 16 February 2024

Published: 08 August 2024

DOI
10.25259/IJRSMS_4_2023

Quick Response Code:

ABSTRACT

Objectives: Cross-sectional data was collected for a period of 1 year to assess the biomarkers of inflammation, and blood viscosity as predictive markers in patients with primary bilateral varicose veins or reticular veins that might progress to varicose veins.

Material and Methods: A sample of 40 participants, 20 controls and 20 patients aged 18–65 years falling under the reticular veins and symptomatic varicose veins (C1, 2s), primary etiology (Ep), superficial veins (As), reflux pathology (Pr) categories of Clinical, etiologic, anatomic, pathophysiologic (CEAP) classification with the exception of telangiectatic veins, were included in the study. Blood viscosity was measured using a Capillary Viscometer. Evaluated inflammatory markers were tissue Plasminogen Activator (tPA), Plasminogen Activator Inhibitor-1 (PAI-1), and fibrinogen. Analysis was done using Statistical Package for the Social Sciences (SPSS) software and Chi-square, Fisher's, Bonferroni, and Analysis of Variance (ANOVA) tests were applied.

Results: There was a statistically insignificant relationship between occupation and different study groups. Standing hours and serum fibrinogen levels had a significant difference in different study groups. Serum tPA, Serum PAI-1, and blood viscosity had an insignificant difference between different study groups. It was revealed that the pairwise group comparisons of study group C2 vs. control group C0, study group C1 vs. control group C0 and study group C2 vs. study group C1, and study group C2 vs. control group C0 each were significantly different pairwise.

Conclusion: Blood viscosity, tPA and PAI-1 cannot be used as predictive markers in patients with bilateral (B/L) primary varicose veins/reticular veins. Serum fibrinogen levels can be used as predictive markers for the development of B/L primary varicose veins and in our particular study with a predictive range of 189.4–327.9 mg/dL but not for B/L reticular veins. Prolonged standing, irrespective of the occupation of the patient, is associated with the development of B/L reticular veins and B/L varicose veins.

Keywords: Varicose veins, Inflammatory biomarkers, Fibrinogen, Serum tPA, Serum PAI-1

INTRODUCTION

Varicose veins are the most common form of chronic venous diseases of the lower limbs, affecting up to 30% of the population with a slightly higher prevalence in women than in men (25–33% and 10–20%, respectively).[1–4] Incidence of varicose veins in the adult population has been shown to vary among populations (between 10% and 60%) and to increase with age in various studies.[5–10] Enhanced oxidative stress has been implicated as the causative mechanism behind vessel wall injury in recent studies.[11] Reactive oxygen species (ROS) are involved in the blood coagulation cascade.[12] Experimental data have demonstrated that ROS can modify the fibrinolytic response,

affecting the production of tissue Plasminogen Activator (tPA) and its inhibitor Plasminogen Activator Inhibitor-1 (PAI-1), increasing PAI-1 expression by endothelial cells.[13–15] Several hematological abnormalities, such as increased blood cell count and aggregation, increased low-shear rate viscosity, and elevation in plasma fibrinogen, have been identified in venous blood samples collected from the feet of patients with chronic venous diseases.[16,17] Therefore, this study aims to assess the biomarkers of inflammation and blood viscosity as predictive markers in patients with primary bilateral varicose veins or reticular veins that might progress to varicose veins.

MATERIAL AND METHODS

The cross-sectional study with a sample size of 40 patients from 9 different occupations was conducted in the Post Graduate Department of General Surgery, Moti Lal Nehru (MLN) Medical College, Prayagraj, for a period of 1 year from October 2021 to September 2022. The Institutional Ethics Committee (IEC) of MLN Medical College, Prayagraj, has approved this study; approval reference number is -(ECR/922/inst/UP/2017). Patients, including policemen, students, farmers, teachers, surgeons, housewives, army officers, tea stall owners, and guards, aged between 18 and 65 years and admitted to the unit with bilateral primary varicose veins/reticular veins falling under reticular veins and symptomatic varicose veins (C1, 2s), primary etiology (Ep), superficial veins (As), reflux pathology (Pr) categories of Clinical, Etiologic, Anatomic, Pathophysiologic (CEAP) classification classifying chronic venous diseases with the exception of those with telangiectatic veins, were included in the study.[18] This includes patients with clinically present symptomatic bilateral reticular veins and varicose veins of primary etiology in superficial venous system with reflux pathophysiology. Blood samples were taken from the involved varicose veins in the study group and in the control group and sent for evaluation, and blood viscosity was measured using Capillary Viscometer. Evaluated inflammatory markers were:

- tPA
- PAI-1
- Fibrinogen

Serum tPA levels (ng/mL), serum PAI-1 levels (ng/mL) and serum fibrinogen levels (mg/dL) were measured using quantitative enzyme-linked immunosorbent assay (ELISA), which followed the Sandwich-ELISA principle. The concentration of inflammatory biomarkers in the samples was calculated by comparing the optical density (OD) of the samples to the standard curve. Blood viscosity of venous blood samples was measured using Capillary Viscometer based on Poiseuille's Law using the following formula:

$$n_{rel} = \frac{\eta}{\eta_0} = \frac{\rho t}{\rho_0 t_0}$$

where ρ is the blood density, t is the time of outflow of the sample, ρ_0 and t_0 are density and the time of outflow of the reference liquid (water) respectively. The final analysis was done with the help of Microsoft EXCEL and Statistical Package for the Social Sciences (SPSS) software. For statistical significance, p-value less than 0.05 was considered.

RESULTS

The control group included 20 participants with CEAP grade C0. Study group of 20 participants was divided into the C1 study group, which included 6 patients who had C1, Ep, As and Pr with bilateral reticular veins, and the C2 study group, which included 14 patients who had symptomatic bilateral varicose veins (C2s, Ep, As, and Pr) [Figure 1].

- The highest number of cases was in the age group of 31–40 years [Figure 2].
- Mean age of male and female patients was 38.67 ± 13.31 and 33.3 ± 6.88 years, respectively.

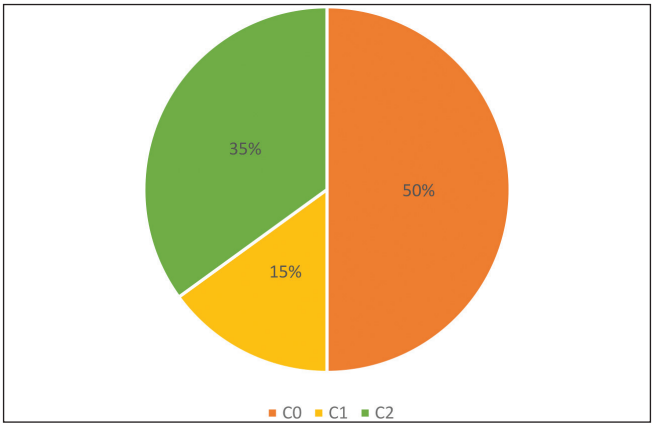

Figure 1: Distribution of cases vs. control; C0: Control group, C1: Study group, C2: Study group.

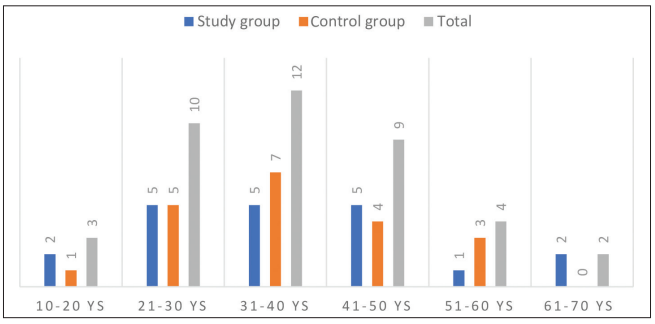

Figure 2: Distribution of patients according to age intervals.

- Mean ages of male patients in the study group and control group were 40 ± 15.65 and 37.5 ± 11.28 years, respectively, while that of female patient in the study group, and the control group were 30.67 ± 5.79 and 37.25 ± 7.18 years, respectively.
- The male–female ratio was 3:1. The ratio in the study group was 7:3 and the ratio in the control group was 4:1. Both the groups contained mainly male patients for the study (70% in the study group and 80% in the control group).

Occupation and standing hours

- There was a statistically insignificant relationship between occupation and different study groups, $\chi^2(16) = 8.2$, p-value = 0.943, Cramér's V = 0.32.
- Mean standing hours value was 5.9 ± 2.12 hours [Table 1].
- Mean value of standing hours showed an increasing trend with the lowest value in the control group C0, followed by the study group C1, and the highest value in the study group C2 [Figure 3].
- Standing hours had a significant difference in different study groups. F = 24.33, p-value = <0.001.
- The Analysis of Variance (ANOVA) showed that there was a significant difference.
- The Bonferroni post-hoc test revealed that the pairwise group comparisons of C2–C0 and C1–C0 had a p-value less than 0.05, and thus, these groups were each significantly different pairwise [Tables 2 and 3].

Table 1: Standing hours versus different groups.

	N	Mean	Std. Deviation
C0	20	4.4	1.05
C1	6	6.33	1.97
C2	14	7.86	1.66
Total	40	5.9	2.12

C0: Control group, C1: Study group, C2: Study group, N: number of participants in each group

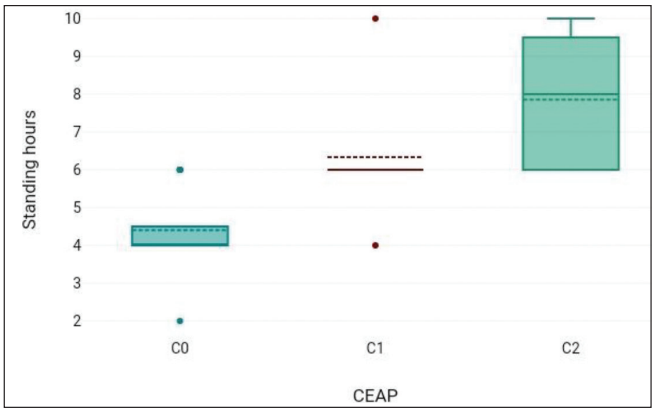

Figure 3: Standing hours in different groups; C0: Control group, C1: Study group, C2: Study group, CEAP: Clinical, etiological, anatomical, and pathophysiologic.

Table 2: Fisher's test: Standing hours versus different groups.

Variables	Average difference	*t*	p-value
C2–C1	1.52	2.18	0.036
C2–C0	3.46	6.93	<0.001
C1–C0	1.93	2.9	0.006

C0: Control group, C1: Study group, C2: Study group; t-test: compares the means between two groups

Table 3: Bonferroni test: Standing hours versus different groups.

	Mean diff.	Std. Error	p-value	95% CI lower limit	95% CI upper limit
C2–C1	1.52	0.699	0.107	−0.24	3.29
C2–C0	3.46	0.499	<0.001	2.2	4.72
C1–C0	1.93	0.666	0.019	0.25	3.62

C0: Control group, C1: Study group, C2: Study group, CI: Confidence Interval

Blood viscosity

- Mean blood viscosity was 1.49 ± 0.07.
- Blood viscosity showed an insignificant difference between different study groups. F = 0.12, p-value = 0.885.

Serum tPA

- Mean serum tPA levels were 53.06 ± 8.46 ng/mL.
- Mean value of serum tPA was the highest in the C2 study group, followed by C0 control group. Lowest mean value of serum tPA was seen in the C1 study group.
- Serum tPA had an insignificant difference between different study groups. F = 0.66, p-value = 0.523.

Serum PAI-1

- Mean serum PAI-1 level was 12.79 ± 6.39 ng/mL.
- Mean value of serum PAI-1 was the highest in the C1 study group, followed by the C2 study group. Lowest mean value of serum PAI-1 was seen in the C0 control group.
- Serum PAI-1 showed no significant difference between different study groups. F = 0.3, p-value = 0.741.

Serum fibrinogen

- Mean serum fibrinogen value was 173.29 ± 55.34 mg/dL [Table 4].
- Mean value of serum fibrinogen levels showed an increasing trend with the lowest value in the control group C0, followed by the study group C1, and the highest value in the study group C2 [Figure 4].
- Serum fibrinogen levels showed a significant difference between different study groups. F = 52.47, p-value = <0.001.

Table 4: Fibrinogen versus CEAP.

	N	Mean	Std. Deviation
C2	14	237.26	48.09
C1	6	143.37	11.11
C0	20	137.48	4.92
Total	40	173.29	55.34

CEAP: Clinical, etiological, anatomical, and pathophysiologic, C0: Control group, C1: Study group, C2: Study group; N: number of participants in each group

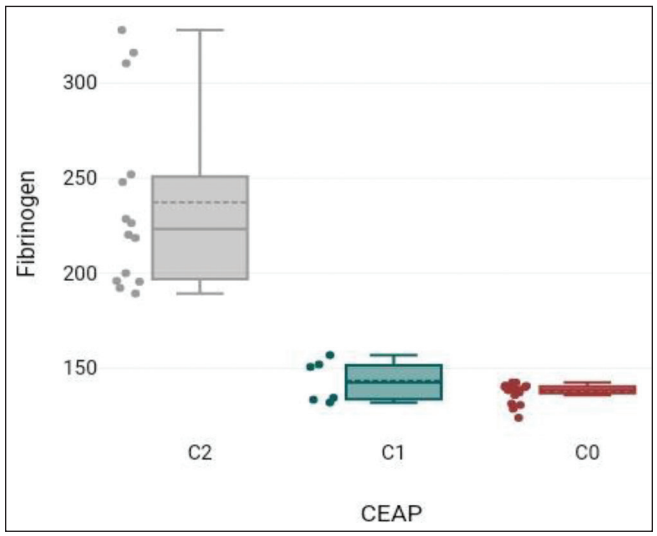

Figure 4: Fibrinogen versus CEAP: Clinical, etiological, anatomical, and pathophysiologic; C0: Control group, C1: Study group, C2: Study group.

Table 5: Fisher's test: Fibrinogen versus CEAP.

Variables	Average difference	*t*	p-value
C2–C1	93.89	6.63	<0.001
C2–C0	99.78	9.87	<0.001
C1–C0	5.89	0.44	0.665

CEAP: Clinical, etiological, anatomical, and pathophysiologic, C0: Control group, C1: Study group, C2: Study group; t-test: compares the means between two groups

- The ANOVA showed that there was a significant difference between different groups.
- The Bonferroni post-hoc test revealed that the pairwise group comparisons of C2–C1 and C2–C0 had a p-value less than 0.05 and thus these groups were each significantly different pairwise [Table 5 and 6].

Table 6: Bonferroni test Fibrinogen versus CEAP.

	Mean diff.	Std. Error	p-value	95% CI lower limit	95% CI upper limit
C2–C1	93.89	14.155	<0.001	58.1	129.67
C2–C0	99.78	10.109	<0.001	74.22	125.33
C1–C0	5.89	13.503	1	−28.25	40.03

CEAP: Clinical, etiological, anatomical, and pathophysiologic, C0: Control group, C1: Study group, C2: Study group, CI: Confidence Interval

DISCUSSION

In the present study, the mean age of patients with bilateral (B/L) varicose veins, reticular veins, and the control group was 35.14 ± 11.9, 42 ± 18.33 and 37.33 ± 12.18 years, respectively. Maximum patients belonged to the age group of 31–40 years. The difference between the different groups and the age of patients was statistically insignificant in contrast with Shafiuddin M *et al.* (2017) where most of the patients belonged to the age group of 21–30 years.[19] The incidence of disease was found to be more in males as compared to females in B/L reticular veins patients and in B/L varicose veins patients. The lower incidence in females was probably due to less cosmetic concern in the middle and lower-class strata.

The relationship between occupation and different study groups was statistically insignificant. However, an increasing trend of mean value of standing hours was the lowest in the control group, followed by B/L reticular veins patients, and the highest in patients of B/L varicose veins. The relationship between the mean duration of standing hours in different study groups is statistically significant. In pairwise group comparisons, B/L varicose veins versus control group and B/L reticular veins versus control group, both groups have shown statistically significant difference. The difference between patients of B/L reticular veins and B/L varicose veins was statistically insignificant. Hence, the result that prolonged standing hours are associated with the development of B/L reticular or varicose veins irrespective of the occupation is in line with Shafiuddin M *et al.* (2017), Mirji *et al.* (2011) and Nagraj *et al.* (2014).[19–21]

The highest mean blood viscosity values are found in patients of B/L reticular veins, followed by patients of B/L varicose veins and the control group. The relation between different study groups and blood viscosity was statistically insignificant. The results are in sync with Flore *et al.* (2015)[22] and contrary to Androulakis & Panoysis *et al.* (1989).[23]

The mean serum tPA values were in patients of B/L varicose veins, followed by the control group. The lowest mean value of serum tPA was seen in patients of B/L reticular veins. The relation between different study groups and serum tPA levels was statistically insignificant and in line with Shireman *et al.* (1996)[8] and Yasim & Kilinç *et al.* (2007)[24] and in contrast with Blomgren *et al.* (2001).[15]

The mean value of serum PAI-1 in patients of B/L reticular veins was followed by patients of B/L varicose veins. The lowest mean value of serum PAI-1 was seen in the control group. The relationship between mean serum PAI-1 values, and different study groups was statistically insignificant and consistent with Shireman *et al.* (1996)[8] and in contrast to Blomgren *et al.* (2001).[15]

There is an increasing trend of mean value of serum fibrinogen levels with the lowest value in the control group followed by B/L reticular veins patients, and the highest value in patients of B/L varicose veins. The relationship between the mean serum fibrinogen levels in different study groups is statistically significant. In pairwise group comparisons, B/L varicose veins versus the control group, and B/L varicose veins versus B/L reticular veins, both groups have shown statistically significant difference. The difference between patients of B/L reticular veins and the control group was statistically insignificant. Hence, it is concluded that raised fibrinogen levels are associated with the development of B/L varicose veins, and not with B/L reticular veins. The results are consistent with Pola *et al.* (1994),[17] Flore *et al.* (2015),[22] Karahan *et al.* (2016),[25] Tiwary & Kumar *et al.* (2020)[26] and contrary to Yasim & Kilinç *et al.* (2007)[24] and Gomez *et al.* (2012).[27]

LIMITATIONS OF THE STUDY

- Due to time constraints and the limited availability of specific patients during the study period, the sample size was small and the results cannot be generalized to the whole population and so a predictive range of fibrinogen for the general population cannot be decided upon. Therefore, further extensive study with a large sample size is required to generate standard cut-offs.
- The results cannot be extended to cases of unilateral (U/L) primary varicose veins.
- The number of male patients was significantly higher than female cases. Therefore, gender bias, if any, has not been fully accounted for.

CONCLUSION

On the basis of our study, we conclude that blood viscosity and biomarkers of inflammation, that is, tPA and PAI-1 cannot be used as predictive markers in patients with bilateral primary varicose veins/reticular veins. Serum fibrinogen levels cannot be used as predictive markers for B/L reticular veins but they can be used as predictive markers for the development of B/L primary varicose veins and in our particular study with a predictive range of 189.4–327.9 mg/dL. The study also concludes that prolonged standing, irrespective of the occupation of the patient, is associated with the development of B/L reticular veins as well as B/L varicose veins.

Ethical approval

The Institutional Ethics Committee (IEC) of MLN Medical College, Prayagraj, has approved this study; approval reference number is - (ECR/922/inst/UP/2017).

Declaration of patients consent

Patient's consent not required as patient's identity is not disclosed or compromised.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

Use of Artificial Intelligence (AI)-Assisted Technology for manuscript preparation

The authors confirm that there was no use of Artificial Intelligence (AI)-Assisted Technology for assisting in the writing or editing of the manuscript and no images were manipulated using the AI.

REFERENCES

1. Evans CJ, Fowkes FGR, Ruckley CV, Lee AJ. Prevalence of Varicose Veins and Chronic Venous Insufficiency in Men and Women in the General Population: Edinburgh Vein Study. J Epidemiol Community Health 1999;53:149–53.
2. Beebe-Dimmer JL, Pfeifer JR, Engle JS, Schottenfeld D. The Epidemiology of Chronic Venous Insufficiency and Varicose Veins. Ann Epidemiol 2005;15:175–84.
3. Robertson L, Evans CA, Fowkes FGR. Epidemiology of Chronic Venous Disease. Phlebology 2008;23:103–11.
4. Rabe E, Guex JJ, Puskas A, Scuderi A. Epidemiology of Chronic Venous Disorders in Geographically Diverse Populations: Results from the Cein Consult Program. Int Angiol 2012;31:105–15.
5. Rizzi A, Quaglio D, Vasquez G, Mascoli F, Amadesi S, Calò G. Effects of Vasoactive Agents in Healthy and Diseased Human Saphenous Veins. J Vasc Surg 1998;28:855–61.
6. Meghdadi S, Rodrigues M, Oguogho A, Santler R, Sinzinger H. 8-epi-PGF 2α and 6-oxo-PGFα in Human (Varicose) Veins: Influence of Age, Sex, and Risk Factors. Angiology 2003;54:317–24.
7. Nicolaides AN. Investigation of Chronic Venous Insufficiency: A Consensus Statement. Circulation 2000;102:e126–e163.
8. Shireman PK, McCarthy WJ, Pearce WH, Shively VP, Cipollone M, Kwaan HC. Plasminogen Activator Levels are Influenced by Location and Varicosity in Greater Saphenous Vein. J Vasc Surg 1996;24:719–24.
9. Golledge J, Quigley FG. Pathogenesis of Varicose Veins. Eur J Vasc Endovasc Surg 2003;25:319–24.
10. Howlader MH, Smith PDC. Symptoms of Chronic Venous Disease and Association with Systemic Inflammatory Markers. J Vasc Surg 2003;38:950–4.

11. Condezo-Hoyos L, Rubio M, Arribas SM, España-Caparrós G, Rodríguez-Rodríguez P, Mujica-Pacheco E. A Plasma Oxidative Stress Global Index in Early Stages of Chronic Venous Insufficiency. J Vasc Surg 2013;57:205–13.
12. Görlach A. Redox Regulation of the Coagulation Cascade. Antioxid Redox Signal 2005;7:1398–404.
13. Seok SJ, Kim SJ, Gil HW, Yang JO, Lee EY, Hong SY. Tissue Plasminogen Activator and Plasminogen Activator Inhibitor-1 Levels in Patients with Acute Paraquat Intoxication. J Korean Med Sci 2011;26:474–81.
14. Jaulmes A, Sansilvestri-Morel P, Rolland-Valognes G, Bernhardt F, Gaertner R, Lockhart BP. Nox4 Mediates the Expression of Plasminogen Activator Inhibitor-1 Via P38 Mapk Pathway in Cultured Human Endothelial Cells. Thromb Res 2009;124:439–46.
15. Blomgren L, Johansson G, Siegbahn A, Bergqvist D. Coagulation and Fibrinolysis in Chronic Venous Insufficiency. Vasa 2001;30:184–7.
16. Khodabandehlou T, Boisseau MR, Le Devehat, C. Blood Rheology as a Marker of Venous Hypertension in Patients with Venous Disease. Clin Hemorheol Microcirc 2004;30:307–12.
17. Pola P, Tondi P, De Martini D, Gerardino L. Influence of Stasis in Fibrinogen Values. Int J Angiol 1994;3:180–2.
18. Lurie F, Passman M, Meisner M, Dalsing M, Masuda E, Welch H. The 2020 Update of the CEAP Classification System and Reporting Standards. J Vasc Surg 2020;8:342–52.
19. Shafiuddin M, Bhavanishankar TP. Study of Risk Factors and Clinical Assessment of Lower Limb Varicose Vein in a Tertiary Care Hospital. Int Surg J 2017;4:2480–3.
20. Mirji P, Emmi S, Joshi C. Study of Clinical Features and Management of Varicose Veins of Lower Limb. J Clin Diagn Res 2012;5:1416–20.
21. Nagaraj H, Hebbar AK, Rajaput AS, Kumar BV. Prospective Clinical Study of Surgical Management of Varicose Veins of Lower Limb and its Complications 2014. VHL Regional Portal IMSEAR | ID: sea-150620
22. Flore R, Ponziani FR, Gerardino L, Santoliquido A, Di Giorgio A, Lupascu A. Biomarkers of Low-Grade Inflammation in Primary Varicose Veins of the Lower Limbs. Eur Rev Med Pharmacol Sci 2015;19:557–62.
23. Androulakis G, Panoysis PA. Plethysmographic Confirmation of the Beneficial Effect of Calcium Dobesilate in Primary Varicose Veins. Angiology 1989;40:1–4.
24. Yasim A, Kilinc M, Aral M, Oksuz H, Kabalci M, Eroglu E. Serum Concentration of Procoagulant, Endothelial and Oxidative Stress Markers in Early Primary Varicose Veins. Phlebology 2008;23:15–20.
25. Karahan O, Yavuz C, Kankilic N, Demirtas S, Tezcan O, Caliskan A. Simple Blood Tests as Predictive Markers of Disease Severity and Clinical Condition in Patients with Venous Insufficiency. Blood Coagul Fibrinolysis 2016;27:684–90.
26. Tiwary SK, Kumar A, Mishra SP, Kumar P, Khanna AK. Study of Association of Varicose Veins and Inflammation by Inflammatory Markers. Phlebology 2020;35:679–85.
27. Gomez I, Benyahia C, Le Dall J, Payré C, Louedec L, Leséche G. Absence of Inflammatory Conditions in Human Varicose Saphenous Veins. Inflamm Res 2013;62:299–308.

How to cite this article: Dahiya MU, Singh S, Singh S, Singh SK, Kumar V. A Study of Blood Viscosity and Inflammatory Biomarkers' Levels in Bilateral Primary Varicose Veins/Reticular Veins as Predictive Markers. Int J Recent Sur Med Sci. 2024;10:57-62. doi: 10.25259/IJRSMS_4_2023