Section: General Surgery

Original Research Article

STUDY ON SIGNIFICANCE OF ENDOTHELIAL DYSFUNCTION IN VARICOSE VEINS USING VASCULAR ENDOTHELIAL GROWTH FACTOR AND NITRIC OXIDE

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ABSTRACT

Background: Varicose veins are one of the most prevalent chronic venous disorders, characterized by elongated, tortuous, and dilated superficial veins, most commonly affecting the lower extremities. And are increasingly recognized as a consequence of endothelial dysfunction. Vascular endothelial growth factor (VEGF) and nitric oxide (NO) are key endothelial mediators involved in angiogenesis, vascular homeostasis, and venous wall remodeling. Alterations in these biomarkers may reflect the severity and progression of venous disease. The objective is to evaluate endothelial dysfunction in patients with varicose veins using serum VEGF and NO levels and to correlate these biomarkers with disease severity and Clinical-Etiological-Anatomical-Pathophysiological (CEAP) classification. **Materials and Methods:** The prospective observational study was conducted at a tertiary care teaching centre, over a period of 2 years, which included 100 patients with primary lower-limb varicose veins undergoing surgical treatment. Serum VEGF levels was measured using enzyme-linked immunosorbent assay (ELISA), while serum NO levels were estimated by assessing nitrite/nitrate metabolites. Clinical severity was graded as mild, moderate, or severe, and patients were categorized according to CEAP classification. Statistical analysis included ANOVA and Pearson's correlation, with $p<0.05$ considered significant. **Result:** Mean serum VEGF was 191.01 ± 65.14 pg/mL, whereas mean serum NO was 24.15 ± 9.31 µmol/L. VEGF increased significantly with disease severity, from 118.83 ± 16.90 pg/mL in mild disease to 257.33 ± 23.83 pg/mL in severe disease ($p<0.001$). Conversely, NO decreased from 34.82 ± 4.74 µmol/L to 15.49 ± 3.24 µmol/L across the same severity spectrum ($p<0.001$). Similar pattern was observed across CEAP classes, with VEGF increasing and NO decreasing progressively from C2 to C5 ($p<0.001$). A strong negative correlation was identified between VEGF and NO levels ($r = -0.847$, $p<0.001$). **Conclusion:** Endothelial dysfunction plays a pivotal role in the pathogenesis and progression of varicose veins. Elevated VEGF and reduced NO levels are strongly associated with increasing clinical severity and CEAP stage. These biomarkers may serve as objective indicators of endothelial dysfunction and disease progression in chronic venous disease.

INTRODUCTION

Varicose veins are one of the most prevalent chronic venous disorders, characterized by elongated, tortuous, and dilated superficial veins, most commonly affecting the lower extremities. They represent not only a cosmetic concern but also a pathological condition reflecting underlying structural and functional abnormalities of the venous

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wall and valves. The pathogenesis of varicose veins is multifactorial, involving genetic predisposition, hemodynamic alterations, hormonal influences, and importantly, endothelial dysfunction. In recent years, increasing attention has been given to the role of the endothelium and its mediators, particularly vascular endothelial growth factor (VEGF) and nitric oxide (NO), in the development and progression of varicose veins.[1,2]

The venous endothelium maintains vascular homeostasis through a delicate balance between vasodilator and vasoconstrictor substances, antithrombotic and prothrombotic factors, and pro inflammatory and anti-inflammatory mediators. Among these, nitric oxide and vascular endothelial growth factor play pivotal roles. Nitric oxide, synthesized by endothelial nitric oxide synthase (eNOS), acts as a potent vasodilator and inhibitor of platelet aggregation, leukocyte adhesion, and smooth muscle proliferation. Its continuous production ensures low venous tone and adequate blood flow. VEGF, on the other hand, is a key regulator of angiogenesis and vascular permeability, promoting endothelial proliferation and the formation of new vessels under both physiological and pathological conditions. Dysregulation of these molecules leads to an imbalance in vascular homeostasis, which is a hallmark of endothelial dysfunction and contributes to venous wall remodeling in varicose veins.[2,3]

Inflammation is another important consequence of endothelial dysfunction in varicose veins. The combined effects of inflammation, oxidative stress, and hypoxia perpetuate endothelial injury, creating a vicious cycle of structural degeneration and functional impairment. The degradation of elastin and collagen fibers within the tunica media and adventitia weakens the vein wall, leading to dilation, tortuosity, and valve incompetence—hallmark features of varicose veins.[4,5]

Reduced nitric oxide (NO) bioavailability promotes varicose vein progression by impairing venous relaxation, reducing compliance, and increasing venous pressure, leading to dilation and reflux. The resulting hemodynamic stress enhances VEGF expression, inflammation, and venous wall remodeling. Oxidative stress further decreases NO levels through peroxynitrite formation, causing additional endothelial and smooth muscle damage.[5,6]

MATERIALS AND METHODS

The prospective observational study was conducted within the Department of General Surgery at Dr. D.Y. Patil Medical College Hospital and Research Institute, Kolhapur, for a period of 24 months from April 2024 to February 2026. A consecutive sapling technique was employed and a total of 81 participants were selected for the study. Ethical Clearance was obtained from the Institutional Ethics Committee before starting the study.

Inclusion Criteria

Patients who were clinically diagnosed with primary varicose veins of the lower limbs and were scheduled for elective surgical intervention, who were 18 years of age or older, ensuring the legal capacity to provide informed consent, who provided written, informed consent.

Exclusion Criteria

Patients with any known immunodeficiency status, such as those with active malignancy, HIV/AIDS, or those on long-term immunosuppressive therapy like corticosteroids, presenting with active or healed varicose ulcers, deep vein thrombosis, or significant systemic comorbidities such as advanced liver disease, chronic kidney disease, or uncontrolled diabetes mellitus, pregnant or lactating, with a history of previous venous surgery or sclerotherapy on the affected limb.

The study procedure was systematically executed in a stepwise manner for every enrolled participant. Following admission to the surgical ward, a comprehensive clinical evaluation was performed, which included taking a detailed medical history and conducting a thorough physical examination focused on the venous system of the lower limbs. Pre-operatively, under aseptic conditions, 5 ml of venous blood was drawn from a peripheral vein in the antecubital fossa. The blood sample was allowed to clot and then centrifuged to separate the serum. The serum was aliquoted into sterile cryovials and stored at -80°C until batch analysis was performed to ensure uniformity in the assay conditions. All patients subsequently underwent their planned surgical procedure for varicose veins (e.g., saphenofemoral junction ligation with stripping or phlebectomies) as per the standard surgical protocol of the department. Intraoperatively, a small segment of the excised varicose vein (approximately 1-2 cm) was also collected during the procedure, washed in saline, and preserved in 10% formalin for potential future histopathological examination to correlate tissue changes with serum biomarkers. The post-operative hospital stay was monitored for any immediate complications.

Data was collected using a pre-designed structured proforma and entered into Microsoft excel. The proforma captured all demographic details, clinical findings (including CEAP classification), and results of routine pre-operative laboratory investigations.

Statistical analysis was conducted using the SPSS 26.0 version. Descriptive statistics were computed for all variables; continuous variables like age, BMI, VEGF, and NO levels were expressed as mean ± standard deviation. The Shapiro-Wilk test was used to assess the normality of data distribution. Since the primary analysis involved comparing the study group's biomarker levels to known normative values, a one-sample t-test (for normally distributed data) or a one-sample Wilcoxon signed-rank test (for non-normal data) was utilized. A p-value of less than 0.05 was considered statistically significant for all tests.

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RESULTS

The study included 100 patients with varicose veins. The majority of patients belonged to the 56–60 year's age group (17%), followed by 51–55 years and 66–70 years (14% each), indicating a predominance of middle-aged and elderly individuals. Females constituted 54% of the study population, showing a slight female predominance. Most patients reported symptom duration between 3 and 7 years, reflecting the chronic nature of varicose vein disease and its progressive course. This demographic profile suggests that chronic venous disease is more prevalent in older adults and tends to persist for several years before presentation.

Table 1: Demographic and Clinical Characteristics of the Study Population (N = 100)

Variable	Category	Frequency (n)	Percentage (%)
Age Group (years)	25–30	7	7.0
	31–35	8	8.0
	36–40	8	8.0
	41–45	9	9.0
	46–50	11	11.0
	51–55	14	14.0
	56–60	17	17.0
	61–65	12	12.0
	66–70	14	14.0
Gender	Male	46	46.0
	Female	54	54.0
Duration of Symptoms (Years)	1	8	8.0
	2	8	8.0
	3	17	17.0
	4	14	14.0
	5	11	11.0
	6	14	14.0
	7	16	16.0
	8	12	12.0

Table 2: Distribution of patients according to CEAP classification

CEAP Class	Frequency (n)	Percentage (%)
C2	34	34.0
C3	23	23.0
C4	25	25.0
C5	18	18.0

The largest proportion of patients belonged to C2 (34%), followed by C4 (25%), C3 (23%), and C5 (18%).

A significant number of patients presented with advanced CEAP classes, indicating delayed clinical presentation.

Increasing CEAP class reflects worsening venous pathology and endothelial damage.

CEAP distribution provides a strong basis for severity-based biomarker analysis.

Table 3: Severity grading of varicose veins

Severity	Frequency (n)	Percentage (%)
Mild	34	34.0
Moderate	23	23.0
Severe	43	43.0
Total	100	100.0

Severe disease constituted 43%, followed by mild (34%) and moderate (23%) cases. A higher proportion of severe cases highlights the burden of advanced venous disease in the study population. Severity grading allows meaningful comparison of VEGF and nitric oxide levels across disease stages.

Table 4: Descriptive statistics of Serum VEGF levels

Statistic	Serum VEGF (pg/ml)
N	100
Minimum	81.60
Maximum	316.00
Mean	191.015
Std. Deviation	65.142

Serum VEGF levels showed a wide range from 81.60 pg/ml to 316.00 pg/ml. The mean serum VEGF level was 191.02 ± 65.14 pg/ml, indicating elevated angiogenic activity. The variability suggests heterogeneity in endothelial dysfunction among

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patients. These findings support the role of VEGF as a marker of disease severity in varicose veins.

Table 5: Descriptive statistics of Serum Nitric Oxide levels

Statistic	Serum Nitric Oxide (µmol/L)
N	100
Minimum	9.10
Maximum	42.60
Mean	24.152
Std. Deviation	9.312

Table 6: Comparison of Serum VEGF levels across severity groups

	N	Mean and sd	Std. Error	95% Confidence Interval for Mean	Minimum	Maximum	F - Value	P-Value
Mild	34	118.83 ± 16.90	2.89958	112.93 to 124.73	81.60	157.70	384.90	0.000
Moderate	23	173.73 ± 24.96	5.20629	162.93 to 184.52	114.50	216.90		
Severe	43	257.33 ± 23.83	3.63499	249.99 to 264.66	208.30	316.00		

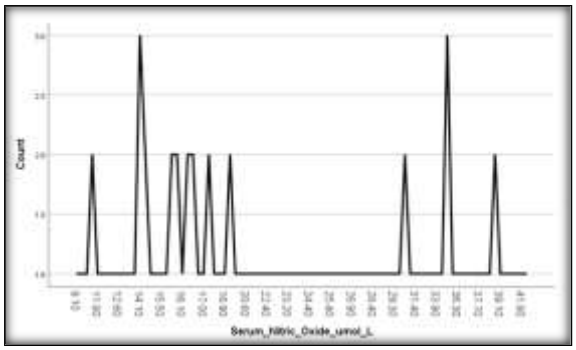

Figure 1

Serum nitric oxide levels ranged from 9.10 µmol/L to 42.60 µmol/L. The mean nitric oxide level was 24.15 ± 9.31 µmol/L, indicating reduced nitric oxide bioavailability. Lower nitric oxide levels reflect impaired endothelial vasodilatory function. This supports the presence of endothelial dysfunction in varicose vein patients.

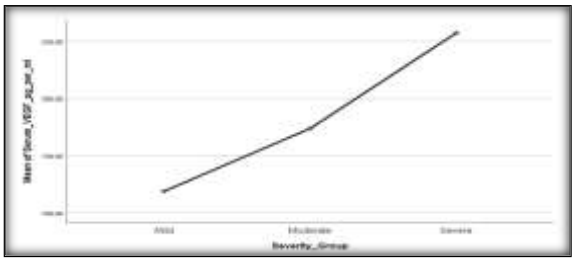

Figure 2

Mean serum VEGF levels increased progressively from mild (118.83 ± 16.90 pg/ml) to moderate (173.73 ± 24.96 pg/ml) and severe disease (257.33 ± 23.83 pg/ml). The difference in VEGF levels across severity groups was statistically significant ($F = 384.907$, $p < 0.001$). This demonstrates a strong association between disease severity and increased angiogenic activity.

Serum VEGF serves as a reliable indicator of worsening endothelial dysfunction.

Table 7: Comparison of Serum Nitric Oxide levels across severity groups

	N	Mean and sd	Std. Error	95% Confidence Interval for Mean	Minimum	Maximum	F -Value	P-Value
Mild	34	34.82 ± 4.74	0.813	33.17 to 36.48	25.10	42.60	231.916	0.000
Moderate	23	24.55 ± 3.68	0.769	22.96 to 26.15	17.30	31.30		
Severe	43	15.49 ± 3.24	0.495	14.49 to 16.49	9.10	23.20		

Table 8: Association between CEAP class and Serum VEGF levels

	N	Mean and sd	Std. Error	95% Confidence Interval for Mean	Minimum	Maximum	F -Value	P-Value
C2	34	118.83 ± 16.90	2.89	112.93 to 124.73	81.60	157.70	253.99	0.000
C3	23	173.73 ± 24.96	5.20	162.93 to 184.52	114.50	216.90		
C4	25	257.03 ± 24.90	4.98	246.75 to 267.31	222.90	316.00		
C5	18	257.74 ± 22.96	5.41	246.32 to 269.16	208.30	288.90		

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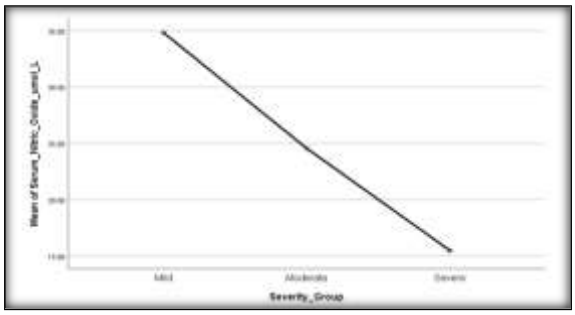

Figure 3

Mean nitric oxide levels decreased significantly from mild (34.82 ± 4.74 µmol/L) to moderate (24.55 ± 3.68 µmol/L) and severe disease (15.49 ± 3.24 µmol/L). The difference across severity groups was highly statistically significant ($F = 231.916$, $p < 0.001$). This inverse trend indicates progressive impairment of endothelial nitric oxide production. Reduced nitric oxide levels correlate with increasing severity of varicose veins.

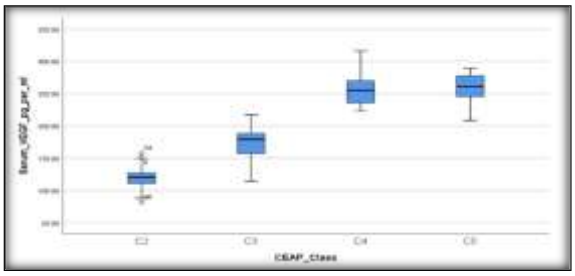

Figure 4

Mean serum VEGF levels increased steadily with advancing CEAP class from C2 to C5. Patients in higher CEAP classes (C4 and C5) demonstrated markedly elevated VEGF levels. The association between CEAP classification and serum VEGF was statistically significant ($p < 0.001$). This confirms that angiogenic activity increases with progression of chronic venous disease.

Table 9: Association between CEAP class and Serum Nitric Oxide levels

	N	Mean and sd	Std. Error	95% Confidence Interval for Mean	Minimum	Maximum	F -Value	P-Value
C2	34	34.82 ± 4.74	0.81	33.17 to 36.48	25.10	42.60	153.03	0.000
C3	23	24.55 ± 3.68	0.76	22.96 to 26.15	17.30	31.30		
C4	25	15.44 ± 3.40	0.68	14.03 to 16.85	9.10	23.20		
C5	18	15.56 ± 3.10	0.73	14.02 to 17.11	11.30	22.40		

Table 10: Correlation between Serum VEGF and Nitric Oxide levels

Variables	Mean ± SD	Pearson Correlation (r)	p-value
Serum VEGF (pg/ml)	191.02 ± 65.14	1	—
Serum Nitric Oxide (µmol/L)	24.15 ± 9.31	−0.847	<0.001*

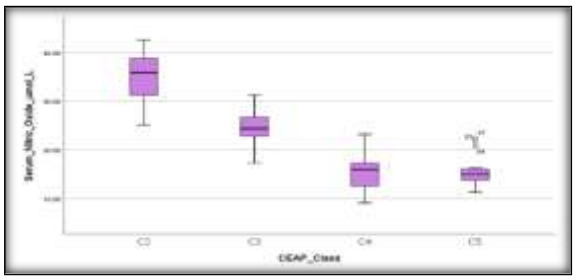

Figure 5

Mean serum nitric oxide levels showed a progressive decline with increasing CEAP class, from C2 (34.82 ± 4.74 µmol/L) to C5 (15.56 ± 3.10 µmol/L). Patients with advanced CEAP classes (C4 and C5) demonstrated markedly reduced nitric oxide levels, reflecting worsening endothelial dysfunction. The difference in nitric oxide levels across CEAP classes was statistically significant ($F = 153.03$, $p < 0.001$). This finding confirms that endothelial nitric oxide impairment increases with disease progression in varicose veins.

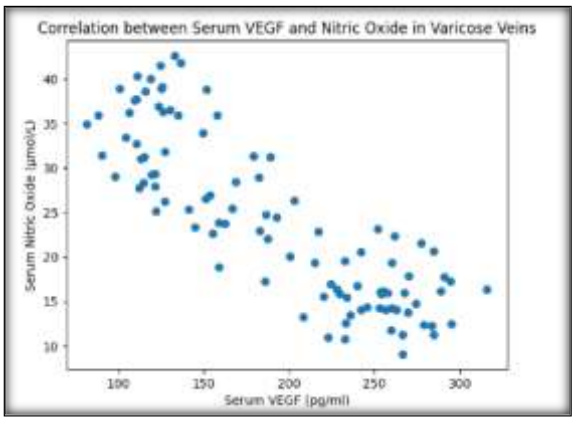

Figure 6

A strong negative correlation ($r = -0.847$, $p < 0.001$) between serum VEGF and Nitric Oxide levels confirms that endothelial dysfunction progresses with increasing disease severity. This imbalance forms the basis for grading endothelial dysfunction in varicose veins.

Table 11: Comparison of endothelial markers between mild and severe disease

	Severity Group	N	Mean	Std. Deviation	F-Value	P-Value
Serum VEGF pg per ml	Mild	34	118.83	16.90	4.655	0.000
	Severe	43	257.33	23.83		0.000
Serum Nitric Oxide umol L	Mild	34	34.82	4.74	8.256	0.000

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	Severe	43	15.49	3.24		0.000

Patients with severe varicose veins had significantly higher serum VEGF levels (257.33 ± 23.83 pg/ml) compared to those with mild disease (118.83 ± 16.90 pg/ml). Conversely, serum nitric oxide levels were significantly lower in the severe group (15.49 ± 3.24 µmol/L) compared to the mild group (34.82 ± 4.74 µmol/L). The differences in both endothelial markers were highly statistically significant ($p < 0.001$). This clearly demonstrates progressive endothelial dysfunction with increasing disease severity.

Table 12: Grading of Endothelial Dysfunction Based on VEGF and Nitric Oxide Levels

Grade	VEGF (pg/ml)	NO (µmol/L)	Correlating CEAP
Grade I	<150	>30	C2
Grade II	150–220	20–30	C3
Grade III	>220	<20	C4–C5

The proposed grading system demonstrates a clear and progressive relationship between serum VEGF levels, nitric oxide levels, and CEAP classification in patients with varicose veins.

Patients categorized under Grade I (VEGF <150 pg/ml and NO >30 µmol/L) correspond predominantly to CEAP class C2, indicating mild endothelial dysfunction with relatively preserved nitric oxide bioavailability and lower angiogenic activity.

In Grade II (VEGF 150–220 pg/ml and NO 20–30 µmol/L), corresponding mainly to CEAP class C3, there is a moderate increase in VEGF levels along with a reduction in nitric oxide levels, reflecting intermediate endothelial impairment.

Patients falling under Grade III (VEGF >220 pg/ml and NO <20 µmol/L) correlate with advanced CEAP classes C4–C5, demonstrating markedly elevated angiogenic activity and significant reduction in nitric oxide levels. This indicates severe endothelial dysfunction associated with advanced chronic venous disease.

The inverse relationship between VEGF and nitric oxide across the grades highlights a progressive imbalance between angiogenesis and endothelial vasodilatory function. This biomarker-based grading system provides an objective method for assessing the severity of endothelial dysfunction in varicose veins and may aid in clinical stratification and prognostic evaluation.

DISCUSSION

Varicose veins are among the most common manifestations of chronic venous disease and are traditionally viewed as the result of valvular incompetence, venous reflux, and venous hypertension. However, increasing evidence suggests that endothelial dysfunction plays a central role in the initiation and progression of venous wall damage, inflammation, angiogenesis, and structural remodeling. In this context, VEGF serves as a marker of angiogenic and endothelial activation, whereas nitric oxide reflects endothelial vasodilatory and protective function. The aim of the present study was to evaluate the significance of endothelial dysfunction in patients with varicose veins by assessing two important endothelial biomarkers, vascular endothelial growth factor (VEGF) and nitric oxide (NO), and to determine their relationship with clinical severity and CEAP classification.

The present study demonstrated that varicose veins predominantly affected middle-aged and elderly individuals, with the highest proportion of patients in the 56–60 year's age group (17%), while females showed a slight predominance (54%) over males (46%). Most patients reported a prolonged symptom duration, particularly between 3 and 7 years, reflecting the chronic and progressive nature of the disease. These findings support the concept that sustained venous hypertension, valvular incompetence, and endothelial dysfunction gradually lead to venous wall remodeling and clinical progression over time. Pascarella et al,[7] demonstrated that venous hypertension induces early structural and enzymatic alterations in the venous wall, including increased pro-MMP-2 activity and active MMP-2/MMP-9 expression, resulting in progressive valve damage and venous reflux. Similarly, Boisseau,[8] reported that leukocyte activation, endothelial adhesion, and inflammatory tissue infiltration contribute to the gradual development of symptoms and disease progression. Kucukguven and Khalil,[9] further highlighted the contribution of genetic, hormonal, and environmental factors to venous wall weakness and valvular dysfunction, while Castro-Ferreira et al,[10] emphasized the central role of endothelial activation and inflammation induced by altered shear stress and chronic venous hypertension in the pathogenesis of chronic venous disease. Collectively, these observations support the present findings that varicose veins are a long-standing disorder characterized by progressive endothelial injury and inflammatory venous wall remodeling, which become more evident with advancing age and prolonged disease duration.

The present study evaluated endothelial dysfunction in varicose veins using serum vascular endothelial growth factor (VEGF) and nitric oxide (NO) levels and demonstrated a strong association between these biomarkers and disease severity. According to the CEAP classification, 34% of patients belonged to C2, 23% to C3, 25% to C4, and 18% to C5, indicating that a considerable proportion of patients presented with advanced chronic venous disease. The combined prevalence of C4 and C5 disease (43%) suggests

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substantial progression of venous pathology before clinical intervention. This observation is consistent with the pathophysiological model described by Castro-Ferreira et al,[10] who identified endothelial activation, inflammation, and sustained venous hypertension as key mechanisms driving progression of chronic venous disease. Abrashev et al,[11] emphasized the role of oxidative stress and endothelial dysfunction in promoting disease progression.

Severity grading revealed that severe disease constituted the largest subgroup (43%), followed by mild (34%) and moderate disease (23%). This predominance of advanced disease supports the concept that many patients seek treatment only after significant venous wall remodeling and endothelial injury have occurred. Kucukguven and Khalil,[9] proposed that prolonged venous hypertension induces matrix metalloproteinase activation, extracellular matrix degradation, and progressive venous dilation, while Pascarella et al,[7] demonstrated that venous hypertension rapidly induces MMP-2 and MMP-9 activation, initiating structural alterations in the venous wall and valves. These mechanisms provide a biological explanation for the high proportion of severe disease observed in the present study.

The descriptive analysis demonstrated a mean serum VEGF level of 191.02 ± 65.14 pg/ml, indicating substantial angiogenic activity among patients with varicose veins. VEGF is a major mediator of angiogenesis, endothelial activation, and vascular remodeling. Flórez et al,[12] reported significantly increased VEGF gene expression in varicose veins compared with healthy veins and identified the diseased vein wall as an important source of VEGF overexpression. Similarly, Horecka et al,[13] demonstrated elevated VEGF concentrations within the varicose vein wall, while Zalewski et al,[14] reported significantly increased circulating VEGF-A levels in patients with varicose veins. These findings support the elevated VEGF levels observed in the present study and reinforce the importance of angiogenic activity in chronic venous disease.

The mean serum nitric oxide level in the present study was 24.15 ± 9.31 µmol/L, reflecting impaired endothelial vasodilatory function. Nitric oxide is a critical regulator of vascular homeostasis, endothelial integrity, platelet inhibition, and anti-inflammatory signaling. Cyr et al,[15] defined endothelial dysfunction as a state of reduced nitric oxide bioavailability and impaired nitric oxide signaling. Abrashev et al,[11] further explained that oxidative stress decreases nitric oxide availability through interactions with reactive oxygen species, thereby promoting vasoconstriction, hypoxia, and endothelial injury. The reduced nitric oxide levels observed in the present study therefore provide biochemical evidence of endothelial dysfunction in patients with varicose veins.

A major finding of this study was the progressive increase in serum VEGF levels across severity groups, from 118.83 ± 16.90 pg/ml in mild disease to 173.73 ± 24.96 pg/ml in moderate disease and 257.33 ± 23.83 pg/ml in severe disease ($F = 384.90$, $p < 0.001$). These findings indicate that angiogenic activity intensifies with worsening clinical severity. Similar observations have been reported by Flórez et al,[12] who demonstrated VEGF overexpression in varicose vein tissue, and by Horecka et al,[13] who reported increased VEGF concentrations associated with venous wall remodeling. The significant rise in VEGF with increasing disease severity suggests that VEGF may serve as a useful biomarker of progressive endothelial activation and vascular remodeling.

Conversely, serum nitric oxide levels declined significantly with increasing disease severity, decreasing from 34.82 ± 4.74 µmol/L in mild disease to 24.55 ± 3.68 µmol/L in moderate disease and 15.49 ± 3.24 µmol/L in severe disease ($F = 231.916$, $p < 0.001$). This progressive decline is consistent with the concept that endothelial dysfunction worsens as varicose veins advance. Cyr et al,[15] emphasized that nitric oxide deficiency is a hallmark of endothelial dysfunction, while Abrashev et al,[11] demonstrated that oxidative stress contributes to nitric oxide depletion during chronic venous disease progression. The present findings strongly support the role of nitric oxide as a marker of endothelial impairment and disease severity.

Analysis according to CEAP classification demonstrated a significant increase in serum VEGF levels from C2 to C5 ($F = 253.99$, $p < 0.001$). Patients in advanced CEAP classes exhibited markedly elevated VEGF concentrations, indicating greater angiogenic activity and endothelial activation. Flórez et al,[12] demonstrated that VEGF overexpression is present even in early venous disease and increases with progression, while Horecka et al,[13] reported elevated VEGF levels within the varicose vein wall in patients with clinically significant disease. The present findings therefore suggest that VEGF correlates closely with clinical staging and may complement CEAP classification in assessing disease severity.

Similarly, serum nitric oxide levels showed a significant decline with advancing CEAP class ($F = 153.03$, $p < 0.001$). Patients with C4 and C5 disease demonstrated the lowest nitric oxide concentrations, reflecting severe endothelial dysfunction. Cyr et al,[15] reported that nitric oxide deficiency promotes inflammation, thrombosis, and vascular rigidity, while Castro-Ferreira et al,[10] highlighted endothelial activation as a key event linking venous hypertension to clinical progression. The present results indicate that nitric oxide levels mirror clinical severity and may provide an objective measure of endothelial deterioration in chronic venous disease.

One of the most important findings of the study was the strong negative correlation between serum VEGF and nitric oxide levels ($r = -0.847$, $p < 0.001$). This relationship indicates that increasing angiogenic activation is accompanied by progressive loss of

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endothelial vasodilatory function. Flórez et al,[12] and Horecka et al,[13] demonstrated increased VEGF expression in varicose veins, whereas Cyr et al,[15] emphasized the importance of nitric oxide in maintaining endothelial homeostasis. The strong inverse correlation observed in the present study suggests that combined assessment of VEGF and nitric oxide provides a comprehensive evaluation of endothelial dysfunction.

Direct comparison between mild and severe disease further reinforced these observations. Severe disease was characterized by markedly elevated VEGF levels and significantly reduced nitric oxide concentrations compared with mild disease ($p < 0.001$). These findings indicate that progression from mild to severe varicose veins is accompanied by a profound biochemical shift toward endothelial dysfunction, increased angiogenesis, and impaired vascular homeostasis. Similar mechanisms have been proposed by Flórez et al,[12] Cyr et al,[15] and Abrashev et al,[11] all of whom emphasized the central role of endothelial injury in chronic venous disease progression.

Based on these findings, a novel grading system for endothelial dysfunction was developed using combined VEGF and nitric oxide measurements. Grade I dysfunction was characterized by VEGF <150 pg/ml and NO >30 µmol/L, Grade II by VEGF 150–220 pg/ml and NO 20–30 µmol/L, and Grade III by VEGF >220 pg/ml and NO <20 µmol/L. These grades corresponded closely with CEAP progression from C2 to C5. The proposed grading model integrates a rising pro-angiogenic marker with a declining protective endothelial mediator and is supported by previous evidence demonstrating VEGF overexpression in varicose veins,[12,13] and nitric oxide depletion as a hallmark of endothelial dysfunction.[11,15] This grading system may provide a clinically meaningful framework for objective assessment of endothelial dysfunction and risk stratification in patients with varicose veins.

CONCLUSION

From the above results, it can be concluded that the present study established that endothelial dysfunction is a fundamental component of varicose vein disease. Elevated serum VEGF and reduced serum nitric oxide were strongly associated with clinical severity and CEAP progression, and the strong inverse relationship between these biomarkers confirmed progressive endothelial imbalance. These findings demonstrate that VEGF and nitric oxide are useful indicators of endothelial dysfunction and may serve as valuable objective markers for assessing disease severity, stratifying patients, and improving understanding of the pathophysiology of chronic venous disease.

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