STUDY THE ASSOCIATION OF VARICOSE VEIN WITH INFLAMMATORY BIOMARKERS

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Dedicated to my Wife for her'
endless support and patience.

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ABSTRACT

The increase in the prevalence of the varicose vein has opened the gates for further research and exploration on this particular topic. The association between varicose veins and increased levels of inflammatory biomarkers has been studied previously, but no study has been done yet to assess the correlation between the changes occurring in the concentration of the inflammatory biomarkers in the blood and the changes occurring in the diameter of the varicose veins. To find out this correlation, patients suffering from varicose veins were recruited who fulfilled the inclusion criteria set for this study. This was a case-control study and consisted of 76 participants (38 cases, 38 controls). Doppler ultrasound was used to measure the changes that have occurred in the walls of the abnormally dilated veins. Blood samples from the abnormally dilated superficial veins in lower limb of each patient were collected to analyze the concentration of inflammatory biomarkers i.e., fibrinogen. The results obtained were analyzed statistically to determine any significant correlation. It was observed that fibrinogen levels were significantly elevated in patients suffering from varicose veins and ulceration, and this increase was significantly correlated with the changes in the diameter of the wall of the varicose veins (p= .000). The occurrence of the varicose vein among the different ethnicities of the country was also found to be statistically significant (p= .005). It can be concluded that fibrinogen can be used as a biomarker to monitor the severity and prognosis of this disease and can also be used to determine the efficacy of the management given to the patients who suffer from varicose vein.

Key words: Chronic venous insufficiency, varicose veins, inflammatory biomarkers, Doppler ultrasound, fibrinogen, venous hypertension, great saphenous veins, perforator veins, venous leg ulcer

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LIST OF SYMBOLS/ABBREVIATIONS

VV's	-	Varicose Veins
CVD	-	Chronic Venous Disorder
CVI	-	Chronic Venous Insufficiency
VLU		Venous Leg Ulcer
BMI	-	Body Mass Index
CEAP	-	Clinical, Etiological, Anatomy, Pathophysiology
SFJ	-	Saphenofemoral Junction
CRP	-	C-reactive Protein
GSV		Great Saphenous Vein
SSV	-	Small Saphenous Vein
BUHSCK	-	Bahria University Health Sciences Campus Karachi

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CHAPTER 1

INTRODUCTION

1.1 BACKGROUND

Varicose veins (VVs) are defined as dilated, twisted, tortuous superficial veins (fig 1.1) occurring in lower extremities typically measuring 3mm or greater. (Jawien, Grzela, and Ochwat, 2003). These are considered as the most common presentation of the chronic venous insufficiency (CVI). These abnormally dilated veins are thought to be present in 25% of female population and 15% of male population. Occurrence is more common in developed western countries in comparison with the developing countries. European population is more affected then black or Asian population (Tafur & Rathbun, 2013). Other venous abnormalities include reticular veins (fig 1.2) measuring about 1-3mm in diameter and are less tortuous as compared to VVs and spider vein or telangiectasias (fig 1.3) measure 1mm or less in diameter are also very common. These various types of abnormal veins depict the clinical progression of the disease (fig 1.4). According to a cross-sectional study conducted in Scotland the reticular veins and spider veins were seen in 80% of male population and 85% of female population. Population based epidemiological studies of VVs showed the prevalence of the disease ranges from 2 to 73% worldwide and 16 to 20% of Pakistani population suffer from this condition (Aslam et al., 2022). Clinically these abnormally dilated veins may present just as a cosmetic problem or with severe symptoms like venous ulcers (fig 1.5). On the basis of the pattern of the disease VVs are classified into uncomplicated varices and complicated varices. Uncomplicated VVs are asymptomatic, these may require cosmetic intervention or just reassurance. Whereas those patients with complicated VVs may develop fatigue,

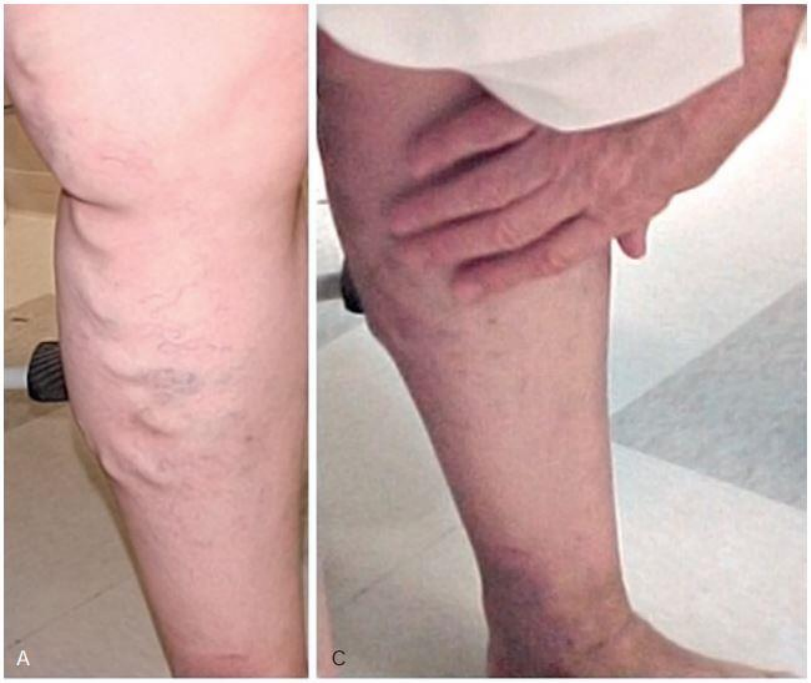


Fig 1.1 Varicose Veins (Tafur & Rathbun, 2013)

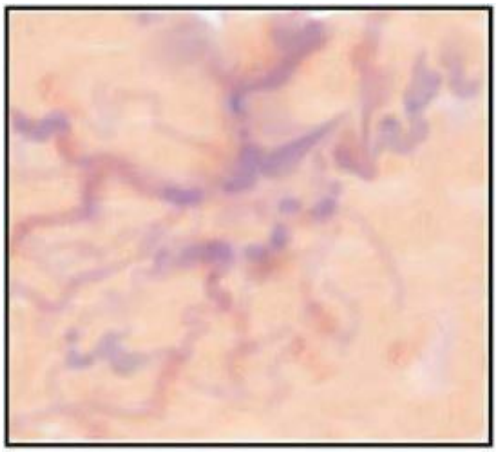


Fig 1.2 Reticular veins (Vitale-Lewis, 2008)

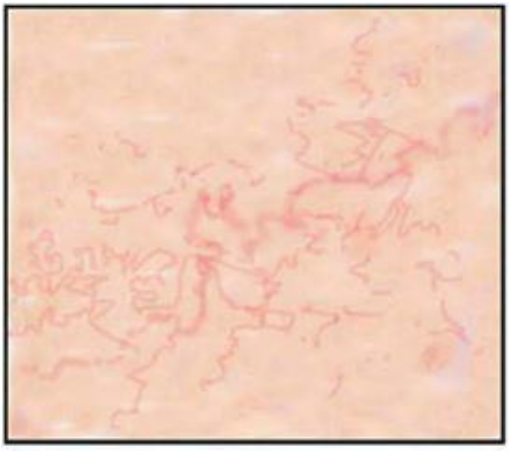


Fig 1.3 Spider veins (Vitale-Lewis, 2008)

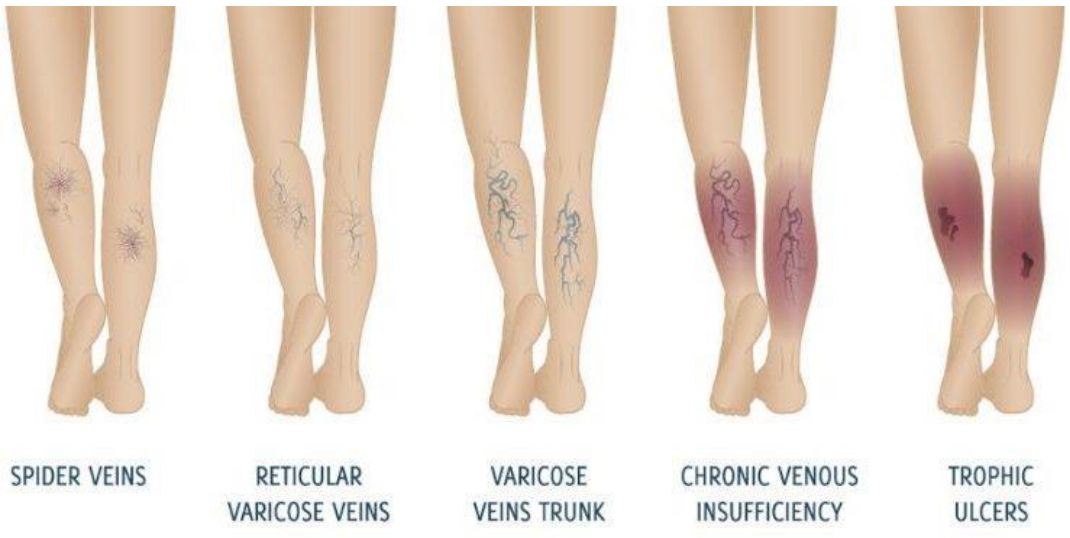


Fig 1.4 Stages of development of varicose veins (Orem, 2022)

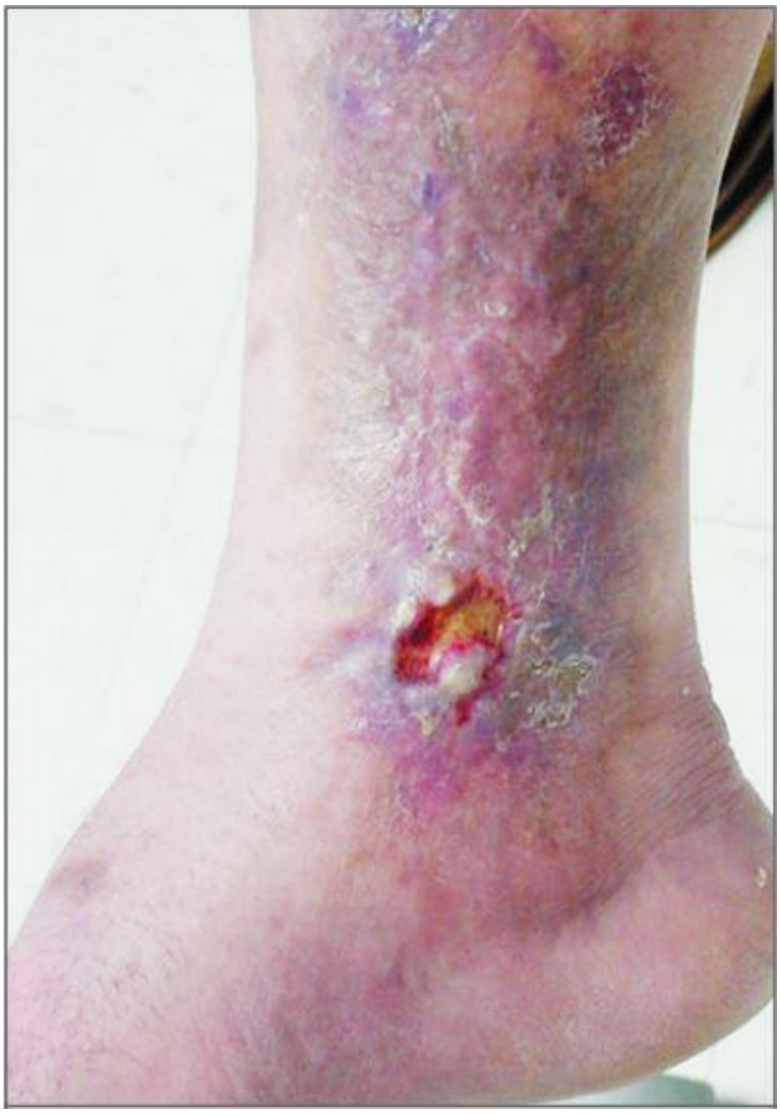


Fig 1.5 Venous leg ulcers (Raju & Neglen, 2009)

heavy legs, spontaneously bleeding ulcers and superficial thrombophlebitis (Tafur & Rathbun, 2013). Other symptoms that are reported to be associated with VVs are edema, pain and dermal changes e.g., stasis dermatitis. Complicated VVs have a great economical impact on the healthcare system of a country.

Venous leg ulcer (VLU) which are later complication of this disease generate a cost burden of 3 billion dollars on the healthcare system of united states of America only (Tafur & Rathbun, 2013). Venous abnormalities alone consume 2% of the national healthcare resources in United Kingdom (Clark, Harvey, and Fowkes, 2010). According to the updated clinical, etiological, anatomical and pathophysiological (CEAP) classification, a venous ulcer has been recently defined as "an open skin lesion of the leg or foot, that shows little or no tendency to spontaneous healing and occurs in the area affected by ambulatory venous hypertension and showing other signs of chronic venous insufficiency persisting for more than 30 days" (Ligi, Mosti, Croce, Raffetto, & Mannello, 2016). It also affects the quality of life of a person suffering from this condition. It is estimated that venous ulcer cause the loss of approximately 2 million working days every year (Tafur & Rathbun, 2013). Because of the high incidence of this disorder, the disease is thought to be a social disease. The social cost of CVI which include medical leaves, diagnostic investigations, hospitalization, and surgery is comparable to the diseases which are generally considered as more serious diseases e.g., cardiovascular diseases (Łastowiecka-Moras, 2021).

Many hypotheses have proposed the mechanism of occurrence of venous leg ulcers (VLU). According to the most widely accepted hypothesis, chronic venous hypertension in lower limbs aid in formation of venous stasis, endothelial cell activation, development of transcellular spaces or gaps between endothelial cells and lastly extravasation of red blood cells and leucocytes (Ligi et al., 2016). The major leucocytes are macrophages, T-lymphocytes and mast cells. These cells gather and activate in the interstitium of the dermis and augment the exposure of many adhesion molecules e.g., ICAM, VCAM, selectins from leucocytes and endothelial cells. This activation of leucocytes and endothelial cells causes liberation of many cytokines, chemokines, growth factors and proteases which alter the function of many resident cells like smooth muscle cells, fibroblasts and myofibroblasts leading to dermal changes and venous leg ulcers (VLU) formation (Ligi et al., 2016).

Ulcers arising due to varicose vein take more than 9 months to heal. According to (Tafur & Rathbun, 2013) about 66% of the ulcers took 5 years to heal. The position of the

ulcers gives a clue to determine the veins which are affected. Ulcers present close to medial malleolus involve GSV, posterior tibial veins and peroneal veins, ulcers that are located near the lateral malleolus usually involve the SSV and its tributaries and the peroneal veins. Ulcers on the anterior aspect affected GSV and anterior tibial veins (Labropoulos & Leon, 2005).

1.2 VARICOSE VEINS AND ASSOCIATED RISK FACTORS

Research depicts positive relationship between varicose veins with increasing age, family history, work routine which consists of long standing, corpulence (obesity), pregnancy, phlebitis and blood clot formation. However, heterogenous results have been observed when relating the varicose vein with cigarette smoking, constipation, oral contraceptive pills, hormone replacement therapy, raised blood pressure, injury, physical activity, diabetes and gender. During pregnancy many physiological changes occur in the body of the mother that can lead to venous dilatation and varicose veins. Increased levels of hormones (estrogen and progesterone) in the blood during pregnancy could also be a cause of VVs. Elevated blood volume, increased physiologic plasma volume, continuous fetal growth and weight gain, increase in intra-abdominal pressure and venous return are also considered as causes of VVs during pregnancy. In fatty women the plasma levels of estrogen are higher than the lean women, this could also be the reason for positive relationship of VVs among the obese women. Whereas in males BMI has shown no association with the occurrence of VVs (Raffeto & Khalil, 2021).

The global burden of venous diseases, their care and treatment are thus far enormous, consuming a good amount of healthcare resources, and is expected to continue to increase in upcoming decades as the population of older individual grows. Statistics for varicose vein procedures has been increased by 60% between 2013 and 2021 (Nicolaides & Labropoulos, 2019).

Professional negligence by many general practitioners who fail to neither assess the venous pathology nor the seriousness of the disease and even don’t refer the patient to the vascular surgeon for proper management, leads to the increase in the number of the patients suffering from chronic venous disease and reoccurrence of the disease. Ultimately it would finally end up in to a massive burden on the healthcare system of a country. This burden of disease can be avoided by proper counseling, early diagnosis and

management of the venous disease patients. It is necessary for a general practitioner to understand the early sign and symptoms of the disease i.e., pain, swelling and heaviness in lower limbs which is usually increased in intensity at the end of the day. Progression of the chronic venous disease and its signs and symptoms are mainly consequences of two mechanism which are venous hypertension and inflammation. Chronic hypertension causes the affected veins to dilate and endothelial disruption excites leukocyte activation and beginning of inflammatory process. Chronic inflammation will cause damage to the valves in the affected vessels which result in blood reflux and pooling hence, leading to varicose veins (Nicolaides & Labropoulos, 2019). 32% of the patients with early-stage venous disease will progress to higher CEAP class of the disease within next 6 years according to Bonn vein study conducted between November 2000 and march 2002. Whereas, 57.8% of the patients will progress to higher class of the disease in next 13 years according to Edinburgh vein study (Nicolaides & Labropoulos, 2019).

1.3 LOWER LIMBS VENOUS ANATOMY

Knowledge of anatomical structure of the veins present in lower limbs is essential to determine the pathophysiology and type of management necessary to provide to the patient suffering from this condition. Anatomically the venous system of lower limbs is divided into three parts, the superficial venous system, the deep venous system and perforator venous system (Youn & Lee, 2019).

1.3.1 THE SUPERFICIAL VENOUS SYSTEM

This network of veins lies within the subcutaneous tissue above the deep muscular fascia and drains the blood from skin and subcutaneous tissues. This venous system consists of great saphenous vein (GSV) and small saphenous vein (SSV) that are present between muscular fascia and saphenous sheath (fig 1.6).

Great saphenous vein arises from the medial side of the foot and ascend upward on the anterior aspect of the medial malleolus. Keep ascending upwards on the medial aspect of the calf and thigh to reach and drain into the common femoral vein. In the distal part of the lower limb around the tibial bone great saphenous vein receives two tributaries

called anterior accessory of the great saphenous vein (AASV) and posterior accessory of the great saphenous vein (PASV) also known as the vein of Leonardo. Saphenous vein ascends upward and drain into femoral vein in inguinal region. (Fig 1.7) showing the junction between GSV and common femoral vein (CFV) seen on doppler ultrasound. This junction is known as the saphenous arch or saphenofemoral junction (SFJ). On doppler ultrasound saphenous vein, common femoral artery and common femoral vein are identified by "Mickey mouse sign" forming the saphenofemoral junction (fig 1.8). Ultrasound appearance of GSV, fascia and sheath give an characteristic picture known as the Egyptian eye within saphenous compartment (fig 1.9), this feature is particular for identification of the saphenous vein on ultrasound (Youn & Lee, 2019). Anterior accessory part of great saphenous vein also drains into the SFJ and this AASV is a very common source of recurrent varicose veins so its diagnosis and treatment is important. Small saphenous vein arises from the dorsolateral aspect of the foot and passes from posterior aspect of the lateral malleolus, ascend through calf region to drain into popliteal vein and their junction is called the saphenopopliteal junction (SPJ) (Youn & Lee, 2019).

1.3.2 THE DEEP VENOUS SYSTEM

This system has greater volume but lesser pressure and accounts for nearly 90% of the venous blood flow in lower limbs. Veins of the deep venous system consist of thinner wall compared to superficial venous system but these are supported by the surrounding muscle and fascia. These muscle and fascia form a compartment which by contraction function to pump the blood toward right heart while walking. Deep venous system consists of anterior tibial vein, posterior tibial vein, peroneal vein, soleal vein and gastrocnemius vein lying infrapopliteal (Youn & Lee, 2019).

1.3.3 PERFORATING VEINS

These act as connecting channels between superficial and deep venous system. Perforators obliquely transverse the deep fascia and contain valves which prevent the back flow of blood from deep veins to superficial venous system. Perforators vary in their size, connection and arrangement but clinically four groups of perforators are identified

as follows: upper thigh group (hunterian), lower thigh group (dodd's), at knee level (boyd's) and calf region group (cockett's) (Youn & Lee, 2019). Incompetent perforator valves are always associated with chronic venous insufficiency.

Usually all veins contain valves except iliac and foot veins (Youn & Lee, 2019). Veins in above mentioned venous systems contain bicuspid valves and these valves make that sure unidirectional flow of blood occur from periphery of the body towards right heart against gravity. Abnormality of these valves will lead to retrograde blood flow as seen in varicose veins. The GSV consist of at least six valves, The SSV has seven to ten valves, and the tibial veins consist of valves at every 2cm (Youn & Lee, 2019).

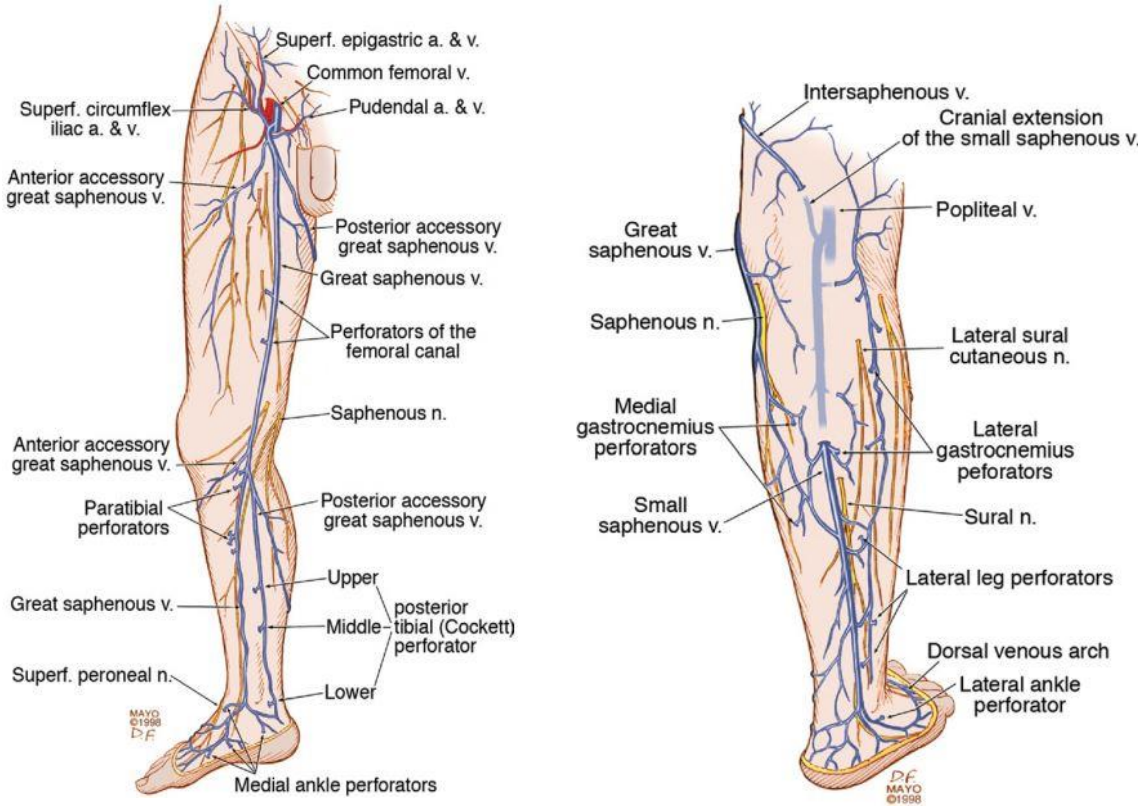


Fig 1.6 Venous anatomy of lower limbs (Gloviczki, Comerota, Dalsing, Eklof, Gillespie, et al., 2011)

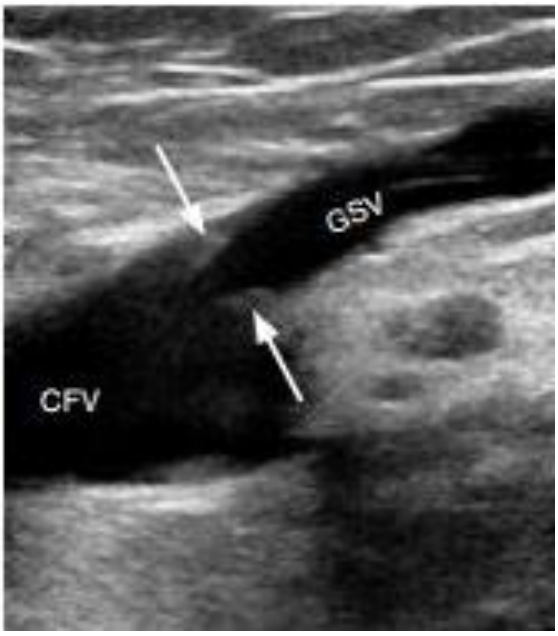


Fig 1.7 Saphenofemoral junction (Lee, Ahn, Kang, & Cho, 2017)

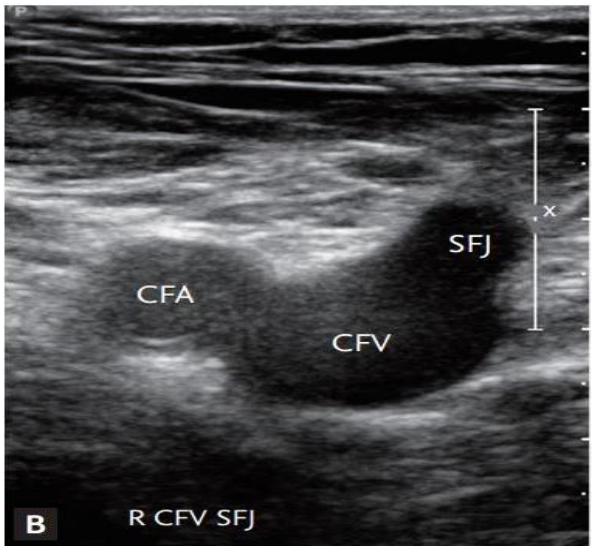


Fig 1.8 Mickey mouse view (Lee et al., 2017)

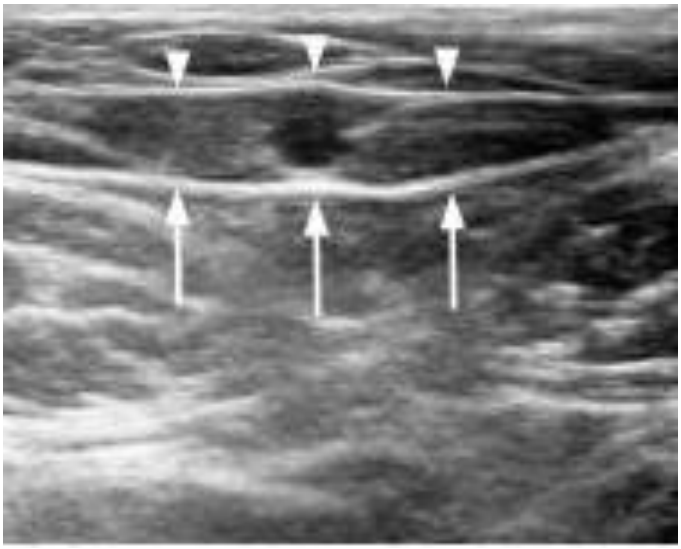


Fig 1.9 Saphenous compartment (Lee et al., 2017)

1.4 CLINICAL CLASSIFICATION OF CHRONIC VENOUS DISORDER

CVD is globally classified on the basis of CEAP (clinical manifestations, etiological factors, anatomic distribution, underlying pathophysiology) standardized classification system (Tab 1.1). Along with classification CEAP is also used to report the severity of the disease.

1.4.1 CLINICAL CLASSIFICATION

Clinical classification of CEAP consists of classes from Co to C6. In Co category there are no visible or palpable sign of venous disease, C1 consist of reticular veins or telangiectasia, C2 with varicose vein, C3 with edema, C4 skin and subcutaneous tissue changes, C5 is healed ulcers and C6 consist of active or recurrent venous ulcers (Lurie, Passman, Meisner, Dalsing, Masuda, et al., 2020).

1.4.2 ETIOLOGICAL CLASSIFICATION

Etiology consists of four categories. “Ep” is the primary etiological subclass and this occurs as a result of any degenerative process that involves the venous wall or venous valves causing vein wall or valve weakness and dilatation leading to pathological reflux of blood. “Es” is the secondary etiological subclass and this occurs as a result of any intravenous pathology or extravascular pathology. Intravenous causes that can damage the venous wall or valves include deep venous thrombosis (DVT), trauma induced arteriovenous fistulas, primary intravenous sarcoma. Extravenous causes of secondary etiological subclass include central venous hypertension due to obesity, congestive heart failure (CHF), pelvic and venous congestion, external compression by any mass e.g., extravenous tumor, retroperitoneal fibrosis, or muscle pump failure due to motor diseases e.g., paraplegia, arthritis, sedentary lifestyle, frozen ankle etc. Extravenous secondary causes do not involve the venous wall or valve but it affects venous hemodynamics either locally or systemically. “Ec” is the congenital subclass defined as the congenital abnormality that is present at the time of birth or can be recognized later in life. This

category involves conditions such as venous agenesis, venous malformation (Klippel-Trenaunay syndrome), arteriovenous malformations. “En” subclass of etiology include causes that are other than Ep, Es, Ec or when no cause is identified in presence of clinical sign and symptoms of that are typically associated with venous disease (Lurie, et al., 2020)(Lurie, Passman, Meisner, Dalsing, Masuda, et al., 2020).

1.4.3 ANATOMICAL CLASSIFICATION

Venous disease involves a particular region identified in this category. Classified as “As” that involves the superficial venous system, “Ad” that involve the deep venous system, “Ap” that involve the perforator venous system of the lower limbs. Single, double or all three systems can be involved in combination. The limb which is involved should also be identified as right[R] or left[L] (Lurie, et al., 2020).

1.4.4 PATHOPHYSIOLOGICAL CLASSIFICATION

Consists of Pr (reflux), Po (obstruction), Pr + o (reflux + obstruction), Pn (no pathophysiology identified).

General physical examination of a patient with varicose vein may show edematous lower limbs, prominent dilated veins, plethora, hyperpigmentation, lipodermatosclerosis, and active or healed ulcers (fig 1.10). Lipodermatosclerosis occurs as a result of localized chronic inflammation and fibrosis of the skin and subcutaneous tissues. Atrophie blanche is avascular and atrophied skin surrounded by hyperpigmentation and capillaries, all the above-mentioned conditions represent severe form of venous insufficiency. Another similar condition showing severe venous insufficiency is known as phlebectatic crown which is a small fan-shaped intradermal vein. Trendelenburg test are used to differentiate superficial and deep venous insufficiency (Lurie, et al., 2020).

In the year 2000, venous clinical severity score (VCSS) was introduced to complement the CEAP classification system (Tab 1.2). Although CEAP is sufficient for routine clinical use, VCSS gives better measuring standards to assess the outcome of the disease. In this system clinical features of CVD are graded from 0 to 3. CVD is graded from absent (0), mild (1), moderate (2) to severe (3) (Raju, Hollis, & Neglen, 2007).

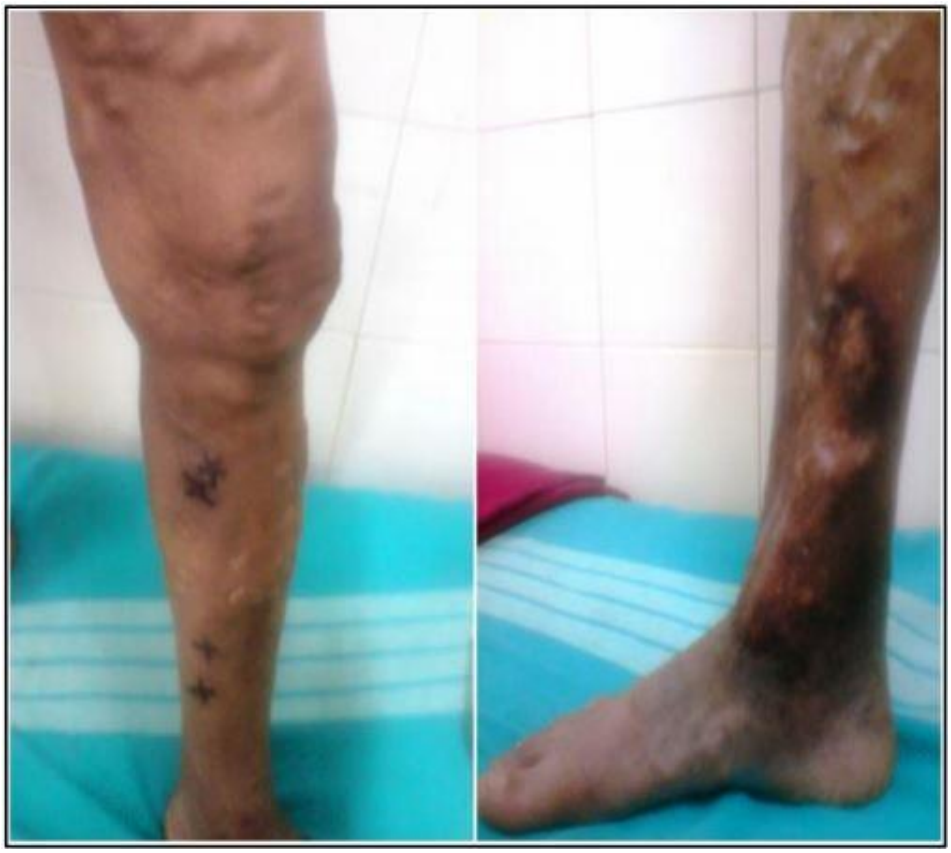


Fig 1.10 Hyperpigmentation
(Mulla & Pai, 2017)

Clinal Manifestation		Etiology		Anatomy		Pathophysiology	
C1	Telangiectasias Reticular vein	Ec	Congenital	As	Superficial Venous System	Po	obstruction
C2	Varicose vein Recurrent varicose vein **(C2r)**	Ep	Primary	AD	Deep venous System	Pr	Reflexes
C3	Edema	Es	Secondary	Ap	Perforators		
C4	Pigmentation **(C4a)** Lipodermatosclerosis **(C4b)** Corona Phlebectatica **(C4c)**						
C5	Healed Ulcer						
C6	Active venous Ulcer Recurrent Active venous Ulcer **(C6r)**						

Tab 1.1 CEAP Classification of Varicose Veins (Openmed, 2022)

Attribute	Absent = 0	Mild = 1	Moderate = 2	Severe = 3
Pain	None	Occasional, not restricting daily activity	Daily, interfering but not preventing daily activity	Daily, limits most daily activity
Varicose veins	None	Few, isolated branch varices, or clusters, includes ankle flare	Confined to calf or thigh	Involves calf and thigh
Venous edema	None	Limited to foot and ankle	Extends above the ankle but below knee	Extends to knee and above
Skin pigmentation	None or focal	Limited to perimalleolar	Diffuse, over lower third of calf	Wider distribution above lower third of calf
Inflammation	None	Mild cellulitis, ulcer margin limited to perimalleolar	Diffuse over lower third of calf	Wider distribution above lower third of calf
Induration	None	Limited to perimalleolar	Diffuse over lower third of calf	Wider distribution above lower third of calf
Ulcer number	0	1	2	≥ 3
Ulcer duration	NA	< 3 mon	> 3 mon but < 1 yr	Not healed > 1 yr
Ulcer size	NA	Diameter < 2 cm	Diameter 2–6 cm	Diameter > 6 cm
Compressive therapy	Not used	Intermittent	Most days	Full compliance

An aggregate score for the limb is calculated by adding the individual component scores. The range of the total score is 0 to 30.

Tab 1.2 Venous Clinical Severity Score (Youn & Lee, 2019)

To evaluate the cases of varicose veins doppler ultrasound is used worldwide. This test is performed while patient is standing, sitting or in supine position. However, ultrasound examination in supine position is not recommended due to significant chances of false positive or false negative reflux results. This procedure is quick, inexpensive and non-invasive. Doppler ultrasound is used to find out any acute or chronic thrombosis, reflux, post thrombotic changes, obstruction of the flow or incompetent deep veins and perforator veins. It is helpful to assess the natural history of CVD, in selection of patients for method of intervention and in follow-up of patients after treatment. It has increased the indications for minimally invasive procedures in CVD surgery. It is the test of choice to diagnose venous reflux and to assess reoccurrence (Labropoulos & Leon, 2005). While the patient is standing saphenofemoral junction (SFJ), common femoral vein (CFV) and the commencement of the both femoral veins are identified (fig 1.11). To check the blood reflux manual compression of the thigh and calf is performed. Valsalva maneuver can also be done for reflux assessment. After that, examination of the femoropopliteal junction and calf veins is performed in sitting posture. Distal compression of the calf region or foot is performed to assess the reflux in these areas. GSV within saphenous compartment and SSV within triangular fascia are also assessed with intermittent calf compressions. VVs on doppler ultrasound present as multiple, hypoechoic, round circles on the screen (fig 1.12). Other radiological investigation that are done in varicose vein patients are plethysmography to assess any venous obstruction or reflux in large veins proximal to knee joint. Venography is also done which aids in detection of pelvic veins obstruction and venous insufficiency.

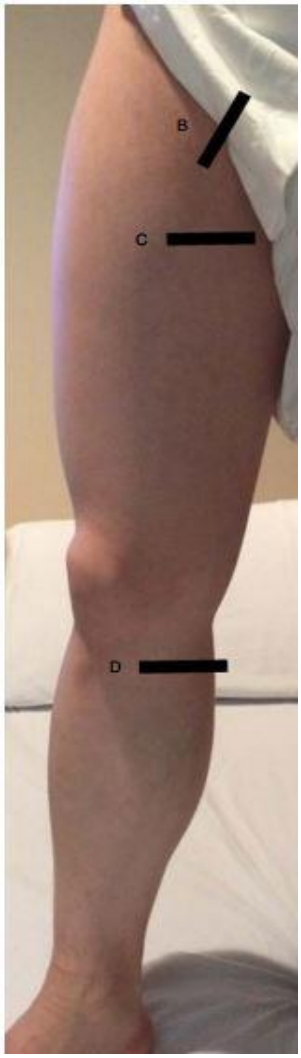


Fig 1.11 main sites for ultrasound in varicose veins (Lee et al., 2017)

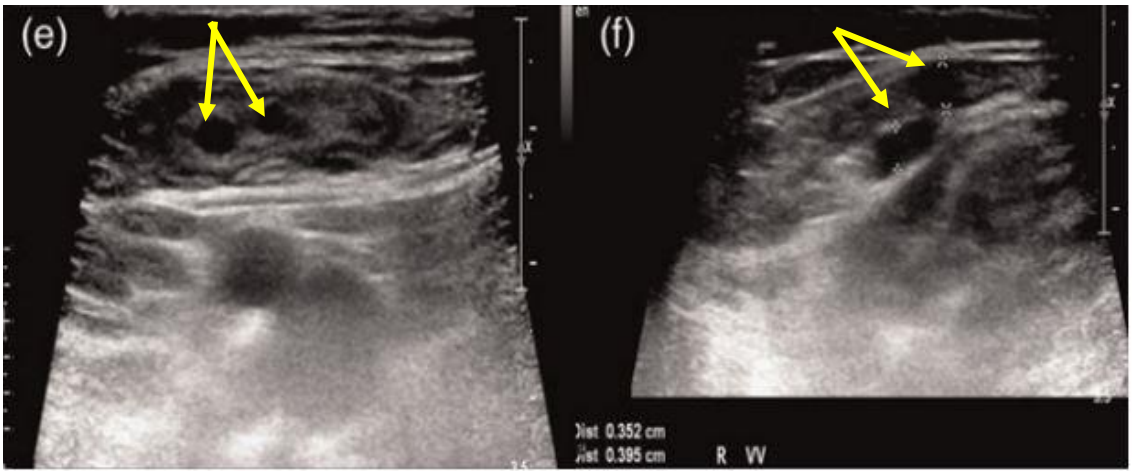


Fig 1.12 Varicose veins ultrasound (Malgor & Labropoulos, 2012)

Management of the varicose veins is divided into two categories. First one is the conservative treatment of the varicose veins which includes lifestyle modifications e.g., avoid prolonged standing, exercise to improve calf muscle function, lose weight, compression therapy with stockings or bandages aid in reducing pain and swelling and also inhibit the progression of varicose veins to ulcer formation, and pharmacotherapy. Secondly invasive procedures are used depending on the size of the abnormally dilated veins and the presence of complications. In surgical intervention most commonly done procedure is ablation of incompetent or great saphenous veins varicose to decompress more distal varicosities. Other surgical procedure includes endovenous laser therapy (EVLT), radiofrequency ablation (RFA), saphenofemoral ligation or stripping and phlebectomy. Patients with GSV varicosities 3-12mm in diameter are more suitable candidates for RFA. Whereas, EVLT is opted for varicosities larger than 3mm in diameter. Foam sclerotherapy another less invasive surgical procedure is performed in varicose veins of GSV smaller than 1cm in diameter (Tafur & Rathbun, 2013).

1.5 ROLE OF INFLAMMATION IN THE ETIOLOGY OF VARICOSE VEINS

Many studies revealed that chronic venous insufficiency is associated with venous hypertension which results due to valve failure and venous reflux. Venous reflux is defined as the "retrograde flow of blood which occur physiologically just before valve closure and pathologically occur due to valve absence or incompetence caused by dilatation, recanalization or remodeling" (Labropoulos & Leon, 2005).

Venous hypertension involves two processes, first process involves the weight of the blood column from right atrium to peripheral femoral vein and SFJ at the level of upper thigh. Second process involves the contraction of skeletal muscles in lower limbs. Valve failure leads to raised pressure in veins due to retrograde flow of the blood. This abnormal hemodynamic flow pattern causes morphological changes in superficial veins and venules which in comparison to deep veins have less mechanical support (Takase et al., 2004). Angioscopic examination during varicose vein surgery shows significant changes in incompetent saphenous veins e.g., perforated valvular cusp, torn cusp, elongated cusp and absent cusp. Tissue specimen from such limbs also revealed increased leukocyte levels and membrane adhesion molecules on the surface of venous walls and valves. It is thought that sudden raise in venous pressure causes a shift in venous hemodynamics and change in wall shear stress. Unstable shear stress and abnormally distended venous walls causes neutrophil, monocyte/macrophage, and endothelial cells activation. This causes leucocyte adherence to the endothelium of the vein wall and valve cusps. This cause apoptosis or necrosis in the endothelium, smooth muscle cells, fibroblasts, and parenchymal cells in the venous wall. This activation of inflammatory cells also causes degeneration of extracellular matrix components e.g., collagen, elastin, laminin and fibronectin by release of oxygen free radicals and matrix metalloproteinases (MMP). This entire process weakens and destroys the venous walls and valve cusps thereby producing gross clinically evident changes (Takase et al., 2004).

Microscopic examination of a normal saphenous vein is characterized by an endothelial layer overlying the media, mainly composed of elastic fibers, smooth muscle bundles and connective tissue. Intimal thickening is evident and adventitia is composed of collagen, fibroblasts, smooth muscle cells and micro-vessels (Woodside, Burke, Murakami, Pounds, Killewich, et al., 2003) (fig 1.13) . Histopathological examination of the section taken from varicose veins during therapeutic surgical procedures done for CVI

shows that in venous disease significant inflammation in tissues take place. Histopathological examination of VVs shows collagen intimal plaques beneath the endothelial lining, disorganization of medial smooth muscles bundles, adventitia presented with increased fibroblasts, smooth muscle cells and collagen (Woodside et al., 2003) (fig 1.14). Inflammatory indicators include endothelial attachment of leucocytes, monocytes, lymphocytes, mast cells infiltration into connective tissues and development of fibrotic tissue infiltrates (Pascarella et al., 2005).

Elevated venous hydrostatic pressure in lower limbs may result in injury to the vein endothelium, increase in the endothelial cells permeability, glycocalyx degeneration, leucocyte infiltration, and inflammation of the wall of the veins. Unsteady shear stress damages the glycocalyx structure and this is associated with increased inflammation in varicose vein patients. Glycocalyx is a glycoprotein and it has very crucial functions including mediating mechanotransduction, detects any alteration in shear stress, selective permeability, act as electrostatic barrier to cells and proteins, anti-adhesive, anti-inflammatory, and prevents endothelial damage induced by hemodynamic changes (Raffeto & Khalil, 2021). Many animal studies have shown that alteration in shear stress has a significant effect on the activation of leucocytes, inflammatory molecules, adhesion molecules and MMPs expression. Rat models with elevated venous pressure in hind limbs induced by femoral arterio-venous fistulas have shown increased pressure in saphenous veins, leucocyte infiltration, and inflammation of the wall of the veins (Raffeto & Khalil, 2021). Infiltration of leucocytes augments the production of MMPs which acts to destroy many components of the extracellular matrix (ECM) causing weakening of the wall of the veins, venous dilatation, valvular incompetency, thereby further elevating the venous pressure in lower limbs and progression of chronic venous disease (CVD) continuous. To confirm the role of inflammation in varicose veins specimen were obtained in many research studies which show elevated plasma levels of inflammatory markers e.g., ICAM-1, VCAM-1, angiotensin converting enzyme, L-selectin, MMPs, proinflammatory cytokines like IL-6, IL-8, alpha-TNF, and vascular endothelial growth factor (VEGF). These findings support a relationship between inflammation, cytokines secretion, MMPs activation in different stages of chronic venous insufficiency (CVI) (Raffeto & Khalil, 2021).

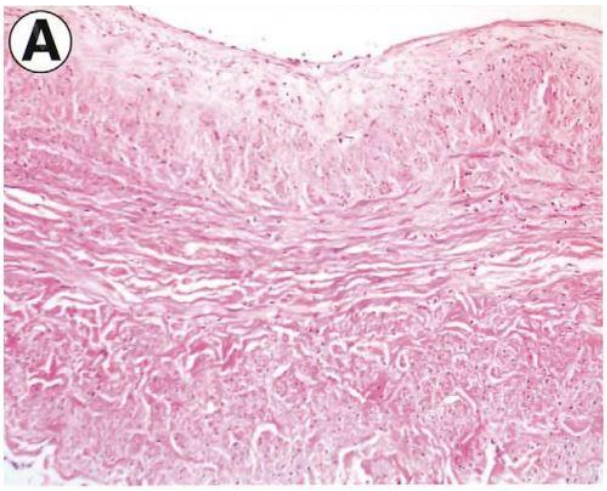


Fig 1.13 H & E staining of normal vein (Woodside et al., 2003)

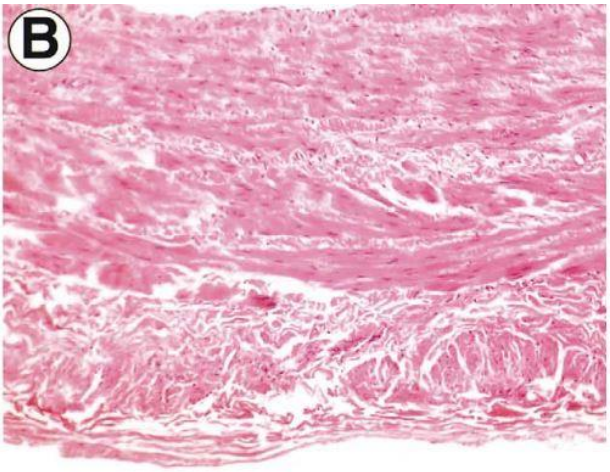


Fig 1.14 H & E staining of varicose veins (Woodside et al., 2003)

1.6 FIBRINOGEN AS AN INFLAMMATORY MARKER

In many other vascular diseases like atherosclerosis and abdominal aortic aneurysm (AAA) inflammation of the wall of the vessel is clearly observed. Numerous detectable inflammatory markers e.g., fibrinogen and C-reactive protein (CRP) are secreted in bloodstream. These inflammatory markers are also detected in the atheromatous plaques. Their elevated plasma levels are used to detect inflammation (Gomez, Benyahia, Dall, Payré, Louedec, et al., 2013). Raised plasma concentration of fibrinogen reflect the inflammatory state of the vascular wall. Similarly, pathogenesis of VVs has a significant inflammatory component and requires further exploration. Peripheral venous disease (PVD) may present widely from minor venous pathology and skin color changes to leg swelling, pain, and skin ulceration. PVD occurs commonly due to deep venous thrombosis (DVT) and post-thrombotic syndrome (PTS) or in general population as a consequence of CVI. Established risk factors for PVD e.g., obesity and older age are associated with elevated plasma levels of inflammatory markers. In animal studies where thrombus formation was induced by the researcher, inflammatory markers such as IL6, IL8, fibrinogen, MCP-1, VEGF, have been shown to act as early proinflammatory markers in response to thrombus formation (Cushman et al., 2014). Inflammation occurs in such conditions as a primary response to heal and resolve the formed thrombus and during this process damage to the vein wall or valves may result in venous insufficiency (Cushman et al., 2014).

The association of coagulation factors with the perivascular environment influences the occurrence of many pathological abnormalities in ways that extend beyond their function in acute hemostatic cascade. Key molecular components of the coagulation cascade e.g., tissue factor, thrombin and fibrinogen are epidemiologically and mechanistically linked with diseases with an inflammatory component (Davalos & Akassoglou, 2012). Recognition of important molecular mechanisms that link coagulation and inflammation has helped to identify many factors of coagulation cascade as new targets of therapeutic intervention in wide range of inflammatory diseases that occur in human beings. Fibrinogen has been identified to have a proinflammatory function in many vascular wall diseases e.g., hypertension & atherosclerosis, stroke, spinal cord injury, brain trauma, multiple sclerosis (MS), Alzheimer’s disease, bacterial

infections, rheumatoid arthritis, lung and renal fibrosis, and several types of cancer (Davalos & Akassoglou, 2012). Many research studies have revealed the important role of fibrinogen in determining the extent of local or systemic inflammation.

Fibrinogen is a soluble glycoprotein synthesized by liver hepatocytes with a molecular weight of 340-kDa. Fibrinogen consists of three different polypeptide chains known as alpha, beta and gamma chains. These three chains form an elongated molecule which via disulfide bond is attached to another identical molecule and form a homodimeric fibrinogen molecule that circulates in blood. Structure of this protein on molecular level was identified by using x-ray crystallography on human and chicken fibrinogen and fibrin. A single fibrinogen molecule consists of several binding sites on its different regions. These binding sites are needed for the binding of fibrinogen molecule to unique receptors or adhesion molecules present on the surface of cells present in hematopoietic, immune and nervous system of the body. In normal physiological conditions, the plasma levels of fibrinogen ranges from **2 – 4 g/L**. However in pathological conditions, like trauma, injury, disease associated with vascular disruption, infection, or inflammation, the plasma levels of fibrinogen protein rise several fold (Davalos & Akassoglou, 2012). This glycoprotein consists of a wide range of functions in the body such as it acts as a marker of vascular rupture, balance between hemostasis and thrombosis, coagulation and fibrosis, provide immunity against infection and prevent extensive inflammation (Davalos & Akassoglou, 2012). Several factors may cause variations in plasma concentration of this protein e.g., age, physical formation, smoking, excessive fat consumption, certain drugs and metabolic conditions like diabetes mellitus, lipoid proteinosis etc. (Pola, Tondi, Martini, & Gerardino, 1994).

Fibrinogen has a pivotal role in the coagulation cascade. The main purpose of the coagulation is to stop bleeding, and prevent excessive loss of body fluids usually from a damaged vessel by forming a blood clot. The major factors that begin, amplify, propagate, and end the coagulation cascade are free circulating factors in blood e.g., zymogens, cell bound platelets, or endothelial cells of vascular wall (Davalos & Akassoglou, 2012). Coagulation cascade is activated by one of three mechanism which include intrinsic pathway, extrinsic pathway and common pathway. Tissue factor (TF) is a glycoprotein present in perivascular tissues which is thought to be the primary initiator of the coagulation cascade after a vascular damage when blood enters the perivascular tissues. TF make a complex with factor VII and this complex activates factor X by extrinsic pathway (directly) or intrinsic pathway (factor IX is activated). These two pathways

intersect with each other with the activation of factor Xa. This factor Xa breaks down prothrombin into thrombin which than cleaves fibrinogen into fibrin monomer which upon polymerization form a fibrin clot. This clot acts to stop blood loss from an injured vessel. This whole cascade is maintained by anticoagulant proteins and natural inhibitors with multiple regulatory check zones that act in a typical negative or positive feedback mechanism. At the same time fibrinolytic system of the body is also acting simultaneously to control fibrin degradation to make sure no extensive fibrin is formed that can lead to any severe form of thrombotic disaster (Davalos & Akassoglou, 2012). The development of a typical blood clot needs the involvement of fibrinogen and platelets as substrate for the formation of platelet plug. Fibrinogen also aids in the formation of bridges between platelets thereby facilitating their aggregation. Plasmin is an important component of the fibrinolytic system which is responsible for the destruction of fibrin clot or inhibition of extensive fibrin clot formation as it can lead to thrombotic complications. This degradation of fibrin clot results in the formation of diffusible and soluble fibrin fragments e.g., D-dimer, fragment D & E. Under physiological conditions fibrin synthesis and destruction are perfectly balanced in the body. Abnormality in either process will result in severe form of clinical consequences ranging from thrombosis, hemorrhagic consequences to tissue fibrosis (Davalos & Akassoglou, 2012). Clotting factors have an identified crucial role not only in maintaining hemostasis but also in tissue repair, reproduction, inflammatory response to related infections or diseases. Some of the most prominent ones are tissue factor, thrombin and fibrinogen have been significantly implicated in inflammation (Davalos & Akassoglou, 2012). Many studies have shown that fibrinogen is a molecular mediator of the disease pathogenesis not only in bloodstream but also within tissues. Fibrinogen and its degradation products e.g., D-dimer have a significant role in regulation of the inflammatory response in several target tissues. Even before the leakage of fibrinogen in perivascular space, elevated fibrinogen levels in plasma is considered as an indicator of proinflammatory state and a high risk marker for developing vascular inflammatory diseases (Davalos & Akassoglou, 2012).

Inflammation includes the interaction between inflammatory cells, vascular cells, pro-inflammatory mediators and anti-inflammatory mediators. Inflammation take place in both intravascular fluid compartment and extravascular fluid compartment where blood cells, vessel hemodynamics, blood coagulation and fibrinolysis occur. The formed blood clot takes part in hemostasis and prevents the invasion of any foreign particle and inflammation occurs as a protective response by the immune system of the body to

counter the foreign invading agent and to repair the damage occurring in tissues. Sometime the repair mechanism fails to occur due to which the acute inflammation can transform into chronic inflammation, or systemic inflammation that may lead to multiple organ failure or death may occur as in cases of sepsis. Fibrinogen is also present at high plasma levels in inflammatory diseases thereby considered as an acute phase protein and considered as an pro-inflammatory factor responsible for vascular dysfunction (Saldanha & Silva, 2018). In CVI patients the antigravitational processes of the lower limbs have failed and this result in a sustained elevation in the venous pressure. Sign and symptoms that occur in CVI e.g., pain, edema, skin changes like hyperpigmentation, fibrosis or venous ulcer indicate the presence of inflammatory process happening that is facilitating tissue destruction (Lattimer, Kalodiki, Geroulakos, Hoppensteadt, & Fareed, 2016).

The molecular pathways that are responsible for cellular damage need more exploration but many factors that have been identified to play a vital role in this process include hypoxia, iron deposition, fibrinogen/fibrinolysis, and endothelial or glycocalyx malfunctioning. Inflammation act as the central process of damage occurring. Studies have been conducted in which concentration of inflammatory markers were compared between samples that were taken from the varicosed veins with the systemic blood that was collected from the veins of the upper limb or the normal non-varicosed veins of the same individual. The result revealed elevated levels of inflammatory markers e.g., IL-6, IL-8, D-dimer and von Willebrand factor (vWf) in VV blood than the corresponding systemic sample taken from normal veins of the same person (Poredos, Spirkoska, Rucigaj, Fareed, & Jezovnik, 2015). D-dimer protein is a degradation product of fibrin clot and its levels are assessed in many clotting disorders e.g., deep venous thrombosis (DVT), pulmonary embolism (PE), disseminated intravascular coagulation (DIC), stroke. IL-6 has high sensitivity for inflammation in CVI and IL-8 and MCP-1 are more specific for inflammation in CVI. Higher levels of inflammatory biomarkers in VVs in comparison to normal veins of same individual further strengthen the evidence to support the role of inflammation in the pathophysiology of the venous wall damage, valvular incompetency and etiology of the VVs (Lattimer et al., 2016).

1.7 RATIONALE

The aim of this research is to measure the diameter of varicose veins and compare this measurement with the plasma concentration of inflammatory biomarkers especially fibrinogen. This will be a useful prognostic tool in the management of patients with varicose veins; it will help assess the prognosis and severity of the illness, as well as the efficiency of the management and treatment of such patients, particularly in regions of the country with inadequate access to healthcare facilities.

1.7.1 THEORETICAL GAP

Previous studies on plasma levels of inflammatory biomarkers such as fibrinogen in patients with varicose veins have yielded conflicting results. Many studies have shown increased plasma fibrinogen levels in patients with varicose veins, but some studies found no evidence of an increase. This study will validate the results of previous studies and will also find an association between changes in varicose vein diameter and increased inflammatory biomarkers. The present study will also reveal any association that may exists between varicose vein and the patient's ethnicity and blood group which has not been checked earlier.

1.7.2 CONTEXTUAL GAP

To date, no studies have shown the relationship between Doppler ultrasonography results and blood inflammatory biomarker concentrations in patients with varicose veins. These biomarkers may play a role in the pathogenesis and progression of this disease. Assessing such correlations can help determine the course, severity, and effectiveness of treatments (such as stockings and surgery) given to patients, especially in areas of the country where medical facilities are scarce.

1.7.3 METHODOLOGY GAP

Literature on the respective topic was scarce so it was difficult to establish any known relation between the elevated levels of inflammatory biomarkers in the blood with the occurrence of varicose vein. It has been shown in several studies that inflammatory biomarkers raised significantly in patients suffering from abnormally dilated veins, but to assess the prognosis, severity and management efficacy after treatment i.e., stockings or surgery attempts are needed to explore less invasive, more accessible and inexpensive tools especially in areas where radiological investigations are not easily accessible.

1.8 PROBLEM STATEMENT

The occurrence of varicose veins is common among adults today. There are no methods or techniques available to help assess prognosis, severity, or efficacy of treatment in patients with varicose veins. This can lead to management failure and disease relapse. To prevent such accidents, such studies would be of great help to clinicians and physicians around the world, especially those working in remote and rural areas without medical facilities. Because varicose veins are usually asymptomatic, patients do not bother to visit a health facility or doctor. These small asymptomatic dilated veins then increase in size and severity and become symptomatic. These patients then present to the doctor with more complicated varicose veins e.g., Venous leg ulcers. This increases the financial burden on the healthcare system and increases the likelihood of recurrence. These complex varicose veins interfere with a patient's daily life and affect their ability to continue working and earn a living. All of these affect the patient's social, financial, and emotional well-being. Early treatment of varicose veins can relieve all such stresses for patients and reduce the financial burden on the country's health care system. The study aims to raise awareness among the public suffering from this disease, whether symptomatic or asymptomatic, to contact their health care providers and physicians to avoid future complications of varicose veins. The study will also encourage physicians to refer patients with varicose veins to the appropriate vascular surgery department or vascular surgeon who can treat them appropriately and prevent future burden.

1.9 RESEARCH QUESTIONS

1. Is the concentration of plasma fibrinogen as an inflammatory biomarker increased in the patients with varicose vein?
2. Is an increased concentration of fibrinogen as an inflammatory biomarker in the patients' blood, associated with changes in varicose vein wall diameter?
3. Is the frequency of varicose vein statistically related to patient age, sex, BMI, comorbidities, blood type and ethnicity?

1.10 OBJECTIVES

- To measure the diameter of varicose vein using doppler ultrasound
- To measure the concentration of fibrinogen as an inflammatory biomarker in blood
- To correlate between varicose vein diameter and levels of fibrinogen, an inflammatory biomarker in blood
- To analyze statistical correlation between varicose vein occurrence and patient age, gender, BMI, comorbidities, blood group and ethnicity

1.11 SIGNIFICANCE OF THE STUDY

To the best of our knowledge, the present study would be the very first to investigate the association between varicose veins diameter with the inflammatory biomarker's concentration in blood. A good amount of work has been done internationally to identify inflammatory biomarkers associated with varicose veins but no such work has been observed in Pakistan. Present study will not only identify the association of plasma fibrinogen levels as an inflammatory biomarker with diameters of varicose veins but also check the association of varicose veins with the ethnicity and blood group of the patient which has not been done before. We hope this present study will help clinicians and caregivers in the management and treatment of varicose veins in

light of the information yielded by analyzing the association between morphological changes that occur in varicose veins and changes that occur in the plasma levels of fibrinogen an inflammatory biomarker in the blood.

This study would be a useful prognostic tool and would also contribute to assess the severity and efficacy of the treatment given to varicose vein patients. This study highlights the complications that can occur when varicose veins are not treated timely, and convinces patients to seek treatment as soon as possible to avoid any future complications.

This research will help to reduce the financial burden on the nation's health care system by raising awareness among physicians and patients, improving treatment and reducing recurrence of varicose veins.

CHAPTER 2

LITERATURE REVIEW

In chronic venous insufficiency (CVI) patients usually have raised levels of leucocytes esterase (LE) in their blood due to leucocytes activation. In patients with deep venous thrombosis (DVT) the LE degrades fibrin from thrombus causing increased concentration of cross-linked fibrin degradation products (E-XDP) in the blood. Sinabulya and colleagues (2021) conducted a study to assess the relationship between plasma E-XDP levels and severity of CVD. Concentration of E-XDP were assessed by enzyme linked immunosorbent assay (ELISA). Plasma samples were collected from 142 diagnosed CVI patients (81 female, 61 male) with a mean age of 64 years. On the basis of severity, the patients were subdivided into three groups according to CEAP classification. C0-C1 was classified as reference group, C2-C3 as the second group and C4-C6 as the third group (most critically affected patients). Result revealed significantly elevated levels of E-XDP in third (C4-C6) group than the other two (C0-C1 and C2-C3) groups (Sinabulya, Silveira, Blomgren, & Roy, 2021). Significant association was seen between E-XDP concentration in plasma of the patients and increasing CVD severity across groups.

Association of inflammatory markers i.e., albumin, C-reactive protein (CRP), and CRP/albumin ratio with the diameters of great saphenous vein (the most commonly affected vein in CVI) was analyzed by Unal and colleagues (2021). This was a case control study. In this study about 150 subjects were selected, 114 were the patients suffering from varicose vein and were labeled as group I and group II comprising 36 normal control subjects. Doppler ultrasound was performed on each subject to assess venous insufficiency and increase in the diameter of the greater saphenous vein. Blood sample from each subject was collected to evaluate the biomarkers. The authors have used

an ultrasound scanner with transducer to measure the diameter of vessels. The diameter of the saphenous vein as measured was 3cm beneath the sapheno-femoral junction in the upright position of patient. Results showed mean diameter of vessels measured was 5.70mm in case group and 3.21 mm in control group. Whereas, the CRP and albumin levels measured were 6.18 mg/dl and 4.45 g/dl respectively in case group and 4.25mg/dl and 6.18g/dl respectively were measured in control group. The authors have observed positive correlation between the diameter of the great saphenous vein and the levels of CRP. Correlation between ratios of CRP/albumin was also found to be moderately positive. Similar analysis was performed for small saphenous, communicating, and deeper veins and results found were same as mentioned above (Unal et al., 2021).

Lattimer et al., (2016) have conducted a similar study as mentioned above but they looked for a group of interleukins (IL-1 to IL-10), vascular endothelial growth factor (VEGF), gamma interferon, alpha tumor necrosis factor, gamma interferon, monocyte chemotactic protein I (MCP-I), and epidermal growth factor were measured between citrated blood samples taken from the varicose veins and antecubital veins of the same patient. This research involved the study of several cytokines so that the most important ones can be identified that are elevated particularly in varicose vein. Their study consisted of 24 patients in cases and 24 controls who did not have varicose vein. Result depicts significantly elevated levels of interleukin-6, interleukin-8 and MCP-I biomarkers in the blood samples of patients with the varicose vein compared with the blood samples from the antecubital vein of the same patient. No rise in inflammatory biomarkers was observed in control group. Authors concluded that some inflammatory processes are activated in varicose veins leading to elevated concentrations of IL-6, IL-8 and MCP-1 biomarkers in patients suffering from varicose vein (Lattimer et al., 2016).

Pregnancy is an identified risk factor associated with CVD. It is estimated that about 1/3rd of the women with pregnancy develop CVD. Ortega et al., (2022) in their study focused on the spectrum of cytokines in the serum of mother with CVD (during pregnancy) and in the serum of newborn. No previous data was available in the literature, that could show alterations in the serum levels of cytokines during pregnancy. Two groups were formed. Group I consisted of 62 pregnant women with pregnancy induced CVD and group II consisted of 52 pregnant women as healthy controls and their newborn of both groups. Results showed significant changes in the levels of inflammatory cytokines e.g., IL-2, IL-4, IL-5, IL-6, IL-7, IL-10, IL-12, IL-13, IL-21, IL-23, TNF-α, GM-CSF, chemokines in pregnant women with pregnancy induced CVD as compared to

the control pregnant women. This maternal immunoinflammatory disturbance was also observed in the serum of newborns of pregnant women who presented with CVD. It was concluded that pregnancy induced CVD is correlated with a proinflammatory environment and this may lead to an alarming complication of this condition for the health of both mother and fetus.

Deep venous thrombosis (DVT) can lead to a chronic complication known as the post-thrombotic syndrome (PTS). This clinical syndrome is characterized by pain, itching, swelling or heaviness, felt in the limb effected by the DVT. The symptoms of this syndrome get worse when standing or walking and is relieved by rest. Nearly 20-50% of the patient suffering from DVT develop PTS. Bouman and colleagues (2012) conducted research to find out any association exists between elevated concentrations of factor VIII, CRP, D-dimer and occurrence of PTS in individual suffering from acute DVT. The authors used Villalta scale to analyze the PTS status of the patients. For this purpose, about 228 patients were recruited who were suffering from acute DVT. Blood from each participant was collected. After the 12 months of occurrence of DVT, concentration of D-dimer and CRP both were found elevated in patients with PTS as compared to those without PTS. Whereas, levels of factor VIII were observed insignificant. Multivariate regression analysis revealed a significant association between PTS and varicosities, elevated CRP levels, and existing ipsilateral DVT (Bouman et al., 2012).

Arase and colleagues (2019) did a research study to assess the association between systemic inflammatory biomarkers and efficacy of the surgical intervention of primary varicose veins of the lower limbs. Two study groups were established. First group consisted of 12 individuals who underwent stripping or endovenous laser ablation (EVLA) of primary VVs. Second group consist of six healthy individuals. Molecular and morphological alterations were examined by immunohistochemical staining. Concentrations of interleukin-6 (IL-6) and monocyte chemotactic protein-1 were measured in antecubital systemic blood before the treatment and 3 months after the surgical intervention. Results of immunohistochemical staining showed significant expression of MCP-1 positive endothelial cells in the walls of varices. Patient serum levels of IL-6 and MCP-1 in first group were significantly raised in comparison to the healthy controls. Results were correlated with the severity of CVI. 12 weeks after the surgical intervention the levels of (IL-6 and MCP-1) proteins were checked again, significantly reduced levels of (IL-6 and MCP-1) proteins were observed as compared to

the preoperative concentrations. Results obtained from stripping and EVLA were found similar. On the basis of these findings the authors have concluded that varicose veins with CVI increased the concentration of inflammatory biomarkers in local tissue as well as in systemic blood whereas, after appropriate management of the patients with varicose vein levels of these inflammatory biomarkers fall in the body (Arase et al., 2019).

Endovenous foam sclerotherapy (EFS) is a commonly performed procedure in United States for the management of VVs. In a prospective study Rathbun et al, (2016) collected data of clinical and coagulation biomarkers in order to perform EFS in symptomatic VVs patients. For this purpose, the authors have recruited those one hundred patients who had already undergone through EFS procedure for VVs earlier. Biomarker analysis and also the clinical characteristics of these patients was performed at baseline, and 1 week, 12 weeks and 26 weeks' follow-ups were carried out. 44% of the patients followed up after one week interval required second EFS injection. Whereas, after 24 weeks' follow-up 100% of patients showed nearly 80% of obliteration in their involved veins. However, 96% of the patients revealed improvement in the symptoms of venous stasis. Few minor side effects and 4 patients who underwent EFS presented with DVT as post-procedure complication. A significant surge in the levels of D-dimer was observed in patients after one week of EFS procedure but these levels gradually returned to their baseline values after 12 weeks of the procedure. An elevation in the levels of the fibrin monomer and platelet microparticles was also observed in patients after 1 week of EFS procedure. Results of this particular study concluded that EFS is an effective treatment for symptomatic VVs and elevated concentration of D-dimer are significantly related with obliteration of venous segments that hint an correlation among vein obliteration and activation of coagulation (Rathbun et al., 2016).

Function of endothelial cells in occurrence of VVs has been identified via many studies. Mikuła-Pietrasik and colleagues (2016) designed a study to determine the effect of VVs on the senescence of the endothelial cells and local or systemic reaction against this effect. They exposed commercially prepared human umbilical vein endothelial cells (HUVEC) with the sera of VVs patient and healthy controls. They observed increased expression of senescence markers, proliferation, and increased production of reactive oxygen species (ROS) in comparison to the serum from healthy controls. During this process young and senescence endothelial cells were plated in cultural discs and then exposed to the serum of VVs patient and healthy volunteers. HUVEC exposed to pathological sera (serum from VV's) produced greater amounts of intercellular adhesion

molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and P-selectin. This research recommended that presence of VVs causes a senescence related dysfunction of vascular endothelium that may lead to the occurrence of local and systemic proinflammatory environment (Mikuła-Pietrasik et al., 2016).

Tiwary and colleagues (2020) conducted a study to compare the levels of different inflammatory markers between the blood samples collected from the varicose vein of lower extremities and antecubital vein of the same individual. They assessed the levels of different inflammatory biomarkers including interleukin-6, hemoglobin, and fibrinogen. For this study the authors recruited 40 patients. On the basis of clinical examination and Doppler ultrasonography these patients were diagnosed as primary cases of varicose veins, and on the basis of CEAP classification these patients were categorized in C2 and C3 class. After all aseptic measures patients were laid in supine position to elicit any hydrostatic pressure on the vessels and blood was collected from patient's antecubital vein (considered as control) and leg with dilated superficial veins (considered as case group). Blood samples were centrifuged and plasma was collected and freezed at -20 °C for further use. The plasma levels of fibrinogen and interleukin-6 were assessed by utilizing Elisa technique. SPSS was used for statistical analysis. For qualitative variables chi-square test was used. Student t-test was used to measure difference between mean of cases and controls. Hemoglobin levels in the blood showed significant difference between varicose vein and antecubital vein. In the control group mean value of fibrinogen was 4.4666 and in cases it was 5.4439 (significantly higher). Interleukin levels were also found to be significantly different between case and control groups. This study confirms that in patients suffering from varicose veins some inflammatory processes are initiated which lead to an increase in levels of interleukin-6 and fibrinogen inflammatory biomarker (Tiwary et al., 2020).

In the year 2015, Poredos and colleagues assessed alterations in the blood samples collected from varicose vein and compared the changes in the systemic markers of inflammation and endothelial cell damage in the blood circulation of the same person. They selected 40 patients of primary VVs, majority of the patients were in C2 group of CEAP classification. Blood samples were collected from dilated, tortuous, superficial tributaries of the GSV (lower limbs) and also from the normal cubital vein (upper limbs). Basic hematologic blood tests were performed and compared the varicose venous blood with that of systemic circulation. Results showed significantly higher levels of the C-reactive protein (CRP), Von willebrand factor (vWF), IL-6 and D-dimer as compared to

the cubital veins. On the basis of these findings, it was concluded that few markers of inflammation and endothelial cell damage indicators are elevated in VVs blood. This was an outcome of abnormal flow of blood in dilated, twisted, superficial veins of lower limbs and increased venous pressure. Injury to the venous walls leads to a chronic inflammatory response along with procoagulant properties of local blood, may further enhance progression of the disease and thrombotic complications (Poredos et al., 2015).

Karahan and colleagues (2016) studied the inflammatory biomarkers present in blood to evaluate the clinical stage and intensity of the disease in patients suffering from chronic venous insufficiency as CVI is usually associated with VV. For this study 80 patients of chronic venous insufficiency were selected, who were at different clinical stages of the disease. On the basis of venous clinical severity scores (VCSS) the patients were grouped into three cohorts of mild (<9 VCSS), moderate (>9 and <18 VCSS) and severe (>18 VCSS), The authors also classified the patients according to CEAP classification of CVI. SPSS was used for the analysis of collected data. Their results revealed significant difference in the levels of white blood cells, albumin, fibrinogen and fibrinogen/albumin ratio with increasing intensity of the disease. They also observed that on the basis of CEAP (clinical, etiological, anatomical, and pathophysiological) there is significant difference in the levels of albumin, fibrinogen and fibrinogen/albumin ratio except for WBC's as in VCSS classification. The authors have also estimated the sensitivity and specificity of these parameters for predicting clinical stage and severity of the disease. Fibrinogen was found to 65% sensitive and 7% specific for detection of clinical class of CVI. Fibrinogen/albumin ratio 75% sensitive and 87.5% specific for clinical class identification. Whereas for detection of severity in the clinical disease fibrinogen was 80% sensitive and 70% specific and fibrinogen/albumin ratio were 90% sensitive and 88.3% specific. The researcher has concluded that serum fibrinogen and albumin levels can be used to judge / evaluate the clinical stage of chronic venous insufficiency. They also emphasized that Fibrinogen/albumin ratio is highly specific and sensitive for the detection of severity of the chronic venous insufficiency (Karahan et al., 2016).

In past, studies were carried out to determine the relationship between increase in the diameter of great saphenous vein and CEAP clinical classification of VVs. Gibson and colleagues (2012) aimed to correlate patient quality of life (QOL) measures with the diameter of GSV in VVs. Patients suffering from GSV reflux. For this purpose, they recruited 91 patients (19 male, 72 female) who were enrolled for VVs treatment with a

mean age of 45 years. These patients had symptomatic VVs with GSV reflux. Duplex ultrasound was performed to measure the maximum diameter of the GSV with patients in standing posture within 5cm of saphenofemoral junction (SFJ). To assess the quality of life (QOL) of these patients' questionnaires were filled before surgical intervention. These questionnaires include chronic venous insufficiency questionnaire 2 (CIVIQ-2), venous insufficiency epidemiological and economic study (VEINES-QOL) QOL, and venous clinical severity score (VCSS). Demographic data, weight, height, BMI of the participants were noted. Results obtained from this study showed mean diameter of GSV, that was 6.7 mm and the mean VCSS was 7.8. According to these results, weak correlation between increasing GSV diameter and VCSS was observed. The authors thereby, concluded that diameter of GSV is a poor indicator to determine the effect of VVs on QOL of the patients. Furthermore, use of GSV diameter alone for determining medical requirement for the treatment of GSV reflux is not appropriate (Gibson, Meissner, & Wright, 2012).

Several Studies have proven the correlation of increased plasma fibrinogen levels (hyperfibrinogenemia) with risk of cardiac diseases and vascular thrombus formation. But increased levels of fibrinogen act as a biomarker for the proinflammatory condition or has any role in its cause and etiology still needs to be found out (Machlus, Cardenas, Church, & Wolberg, 2011). Machlus and colleagues in the year 2011, conducted experimental research on mice. In these animals plasma, concentration of fibrinogen were increased by intravenous infusions and thrombus formation was induced by applying ferric chloride to the saphenous vein or carotid artery. The results obtained revealed elevated levels of fibrinogen significantly reduced the time of obliteration of these vessels. Hyperfibrinogenemia raised the fibrin content of thrombus, augmented rapid fibrin formation, and also increased the density, strength and stability of the fibrin network. Process of thrombolysis was also found to be decreased showing thrombus resistance. It was concluded in this study that elevated concentrations of fibrinogen directly assist in thrombus formation, which in turn contributes to thrombolysis resistance by improving fibrin formation and its stability. Hyperfibrinogenemia also has a significant role in the development of the acute thrombosis and its implication in thrombolysis therapy. These elevated levels of fibrinogen may be used to identify patients who are at increased risk of thrombus illness (Machlus et al., 2011).

Pascarella and colleagues (2005) designed an animal model to assess the mechanism of inflammation associated with venous hypertension. The study comprised of a total of 65 rats. Among them 60 rats were selected as case group and 5 rats as control. All the rats in case group underwent a surgical procedure in which an arteriovenous fistula was created in the femoral vein to induce venous hypertension in case group. Venous Pressure below the fistula was measured to assess valve functions. 7 days after surgery, rats with fistula presented with hind limbs swelling and raised venous pressures, venous reflux was also increased. Venous pressure was highest at 6th week after the surgical intervention. Abnormalities in the valves were also evident. All the animals were euthanized and biopsy samples from the affected veins were collected for histopathological study. Results revealed a great increase in the concentration of Immune markers i.e., MMP-2 and MMP-9. The authors concluded that increased venous hypertension causes inflammation which is detected by increased levels of inflammatory markers and this inflammation could be a cause of valve damage leading to occurrence of varicose vein (Pascarella et al., 2005).

Another study on rat model was carried out by Takase and colleagues (2004). In this study, the authors had added a treatment option with an anti-inflammatory medicine (micronized purified flavonoid fraction). The rats were divided into two groups comprising cases and controls. In the rats of case group, a fistula was made in femoral vein to produce venous hypertension, pressure and reflux in femoral vein, valve architecture, and inflammatory markers were evaluated using immunohistochemistry. At the end of the study effect of the above-mentioned drug was assessed on the inflammation. 3 weeks after the formation of femoral fistula, an increase in the pressure within the femoral vein was observed. Reflux was also detected in femoral vein, consequently the inflammatory markers were also assessed. The end result of the research was that the anti-inflammatory drug that was given to the animals was found effective against venous reflux that was caused by the venous hypertension but this drug was ineffective for the valves those became incompetent due to venous dilatation and shortening of the valvular leaflets at the same time. An increase was reported in the levels of inflammatory markers known as p-selectin and intercellular adhesion molecule 1 (ICAM-1) in the rat models (Takase et al., 2004).

Previous data about relationship between excessive body weight, CVD and its CEAP classes are not consistent. Vlajinac and colleagues (2013) analyzed correlation of

overweight (BMI 25-30), and obesity (BMI >30) with the CEAP classification of CVD. They performed a cross-sectional study in Serbian male and female patients age >18 years, who were enrolled to vascular specialist due to any venous issue in their lower limbs. The Demographic, anthropometric and clinical data of all patients was documented. Study cohort consisted of 1116 subjects, including 384 males, and 732 females with primary CVD. Body mass index of all the participants was measured, 464 subjects were having normal weight (BMI <25 kg m^2), 476 patients were found to be overweight (BMI 25-29.9 kg m^2) and 176 patients were obese (BMI > 30 kg m^2). C class on the basis of CEAP classification was significantly more advanced in overweight and obese patients of CVD and more evident in obese patients. In univariate logistic regression analysis, venous obliteration was associated with overweight and obese patients. The authors have concluded that C class of CEAP was significantly associated to overweight and obese subjects. This association was independent of age, gender and other postulated risk factors which can be confounding variables. However, it seems difficult to assess the association of BMI with CVD classification but it looks reasonable to suggest weight reduction to overweight and obese patients suffering from CVD (Vlajinac et al., 2013).

Van rij and colleagues (2008) conducted a somewhat similar study comparing venous physiology of the lower limbs (by air plethysmography) with body weight. The authors also assessed the effect of posture (standing, sitting, lying) on these measures. This association is assessed in a large group of obese and normal weight patients. For this study the authors selected 934 patients who visited hospital due to any venous related disorder. The patients were divided into obese and non-obese groups. Physiological venous functions of these patients were examined by using duplex ultrasound and air plethysmography. A smaller group which consisted of 20 VVs patient with a range of body weight > 90 kg (n=6), < 90 kg (n=14) were selected randomly. Foot vein pressure and superficial femoral vein diameter of patients were measured in standing posture, sitting and lying postures. Results revealed that clinically venous disease was more severe in obese patients, venous reflux was also worse in the same group, correlation of the weight of the patient with the diameter of the femoral vein (in smaller cohort) was found to be significant and foot vein pressure was significantly raised in all obese patients. Their result findings concluded that C class of CEAP venous disease classification is more advance and severe in obese patients in comparison to non-obese patients of CVD (van Rij et al., 2008).

Seidel and colleagues (2017) in a retrospective cross-sectional study (over a period of two years) have correlated the BMI with clinical classes of CVD according to CEAP classification. They also compared the severity of CVD in obese and non-obese patients. Study cohort consisted of 482 random sample patients who visited vascular center with complaints related to CVD. Patients' hospital record was used to obtain information about patients' age, sex, weight and height (BMI) and clinical class of CVD according to CEAP classification. Association between BMI and clinical class of CVD was found to be significantly positive for females and no significant correlation was seen between BMI and clinical class of CVD in male participants. Presence of obesity was more significant in CVD clinical class 3 and 4 of CEAP classification. On the basis of these findings, it was concluded that there is significant association between BMI and clinical class of CVD in women but not in men (Seidel et al., 2017).

Seidel and colleagues (2017) in another study analyzed the association between symptoms of VVs and GSV reflux observed on doppler ultrasound. This was a prospective study, consisted of 1384 patients (2669 limbs), age range was between 17-85 years. About 1227 patients were females. The most frequent symptoms presented by the patients according to the questionnaire were Pain, tiredness, limb heaviness, burning or tingling sensation, and cramps. Doppler ultrasound of all the participants was performed. The participants were later divided into three clinical groups (according to doppler ultrasound findings). In group I symptoms were present but VVs were absent, in group II symptoms were absent but VVs were present, in group III symptoms and VVs both were present. Statistical analysis was done and it was observed that chances of GSV reflux was 11.2 times and morbid obesity was 9.1 times greater in group III. Age group between 30-50 years was 43.1 times more prone to occurrence of GSV insufficiency. The authors have concluded that GSV insufficiency and obesity is more frequent in patients who have symptoms and evident VVs both simultaneously and patients were in the older group (Seidel et al., 2017).

Flore and colleagues (2015) in their study examined the serum inflammatory biomarkers in CVD patients with primary axial reflux of unilateral or bilateral lower limbs GSV. This was a case-control study in which case cohort consisted of 96 patients, presented with uncomplicated VVs and control consisted of 65, age and gender matched healthy individuals without any venous reflux. Among the case cohort 54 patients had unilateral CVD (U-CVD) and 42 patients had bilateral CVD (B-CVD). Echoduplex

scanning was performed to measure the venous reflux and continuous wave doppler ultrasound was performed to measure the mean venous pressure of both lower limbs at distal great saphenous vein (mGSVP). After radiological intervention blood samples of the participants were collected from the antecubital vein of the upper limb to analyze the blood inflammatory biomarkers and other parameters. Following markers and parameters were analyzed: reactive oxygen species (ROS), white blood cells (WBCs), hematocrit (HCT), neutrophils, platelets (PLTs), tissue plasminogen activator (t-PA) and its inhibitor 1 (PAI-1), blood viscosity and fibrinogen (FIB) levels. Results showed that patients with bilateral CVD had significantly elevated concentrations of fibrinogen and had higher mean venous pressure in comparison to those with unilateral CVD. It was concluded in this study that elevated concentrations of plasma fibrinogen (hyperfibrinogenemia) in patients with VVs may be an early warning sign, as it could be associated with long term progression of the CVD (Flore et al., 2015).

Pola and colleagues in the year 1994 analyzed the effect of posture on plasma fibrinogen levels in patients of VVs disease. This case-control study consisted of two cohorts. Case cohort comprised of 20 patients presented with primary VVs of the lower limbs and control cohort consisted of 10 normal healthy subjects who did not have any venous pathology. Plasma fibrinogen levels were analyzed in both groups at base-line conditions and after 1 hour of standing. Results showed that patients with primary VVs had elevated concentrations of fibrinogen in both upper and lower limbs in comparison with the control group. In varicose group (patients) after 1 hour of orthostatism plasma fibrinogen levels were significantly higher in both varicose veins and veins of the upper limbs in comparison with the normal values. Interestingly, after 1 hour of standing, plasma fibrinogen levels in upper and lower limb veins of the control group were increased but not at a significant level as it was observed in the case cohort. This increase/surge in the fibrinogen levels in control group might be due to the presence of vascular risk factors e.g., smoking, hypertension, diabetes, obesity etc. These results confirmed the effect of posture on the plasma fibrinogen levels and this study also confirmed that fibrinogen levels are also influenced in limbs that are not affected by the stasis (Pola et al., 1994).

All over the world doppler ultrasound is supposed to be a principal procedure for diagnosing and evaluating CVD but baseline laboratory investigations can also be used as an easier, cheaper and more accessible method to evaluate the progression of CVD.

Matei and coworkers in the year 2022 designed a unique retrospective study to analyze the routine laboratory investigations of the diagnosed cases of CVD. On the basis of CEAP classification of venous disease and depending on the clinical stage of the CVD, the patients were categorized into three groups according to the severity of the disease. Group I consisted of C2, C3 with mild CVD, group II consisted of C4 with moderate to severe CVD and group III consisted of C5, C6 with severe CVD. The standard lab protocols were analyzed in these patients including red blood cells (RBCs) count, white blood cells count (WBCs), platelet count (PLT), neutrophil and lymphocytes percentage, neutrophil to lymphocyte ratio (NLR), erythrocyte sedimentation rate (ESR), plasma fibrinogen concentration, C-reactive protein (CRP), prothrombin time (PT), activated partial thromboplastin time (aPTT), internal normalized ratio (INR), creatinine kinase (CK), alanine transaminase (ALT), aspartate transaminase (AST), total bilirubin and urea levels. The results of the current study revealed increased levels of NLR, ESR, CRP and fibrinogen with the progression of the CVD. The outcome of the study endorsed implication of inflammatory markers in the pathophysiology of the CVD including venous wall thickening and valvular modification. It was therefore, concluded that routine baseline laboratory investigations can be considered as useful markers in evaluating the progression of the CVD and may provide healthcare providers additional insight for the management of these patients (Matei et al., 2022).

Joh & Park (2013) designed a study to explore a correlation between the diameter of the saphenous vein and saphenous vein reflux. Increase in the diameter of the saphenous vein occurs as a result of saphenous vein reflux. But there are no prior studies done to verify this correlation. During a period of 2009 to 2012, around 777 patients were examined for venous reflux in a vascular laboratory. B-mode imaging was used to measure the diameter of the saphenous vein and reflux was determined based on valve closure time (0.5 to <1 second) using doppler spectral tracing technique. To decide the best saphenous vein diameter cutoff value for predicting reflux receiver operating characteristic curve (ROC statistical graph) analysis was applied. Results revealed that the mean diameter of normal GSV was 5.0 ± 2.4 mm and for reflux GSV it was 6.4 ± 2.0 mm. Similarly, the mean diameter for normal SSV was 3.1 ± 1.3 mm and for reflux SSV 5.2 ± 2.7 mm. The diameter distinction between normal and reflux GSV and SSV were 1.4 and 2.1 mm, respectively. These results were found to be statistically significant. The outcome of the study suggested that the diameter of GSV ≥ 5.05 mm has the best positive

predictive value for venous reflux and for SSV, whereas, the best cutoff diameter for pathologic reflux was 3.55 mm (Joh & Park, 2013).

2.1 OPERATIONAL DEFINITIONS

1. **CHRONIC VENOUS DISEASE:** A common vascular condition that affects the lower limbs. It covers a wide range of conditions that can be broadly categorized as varicose veins, chronic venous insufficiency and venous ulcers (Ulcers, Simpson, & Roderick, 2004).
2. **CHRONIC VENOUS INSUFFICIENCY:** A common disorder whose manifestations include varicose vein, skin changes such as venous dermatitis, hyperpigmentation, lipodermatosclerosis, and chronic leg ulcers. Venous hypertension leading to valvular incompetence is the main cause of CVI (Pascarella & Mekenas, 2006).
3. **VARICOSE VEINS:** These are defined as permanently dilated subcutaneous veins ≥ 3mm in diameter in the upright position (Tekin et al., 2016).
4. **VENOUS HYPERTENSION:** In VVs due to gravity exerting downward pressure on the vein, the vein valves begin to weaken and break over time increasing the pressure on valves present inferiorly. Eventually with most or all of the valve breaking inside the veins, the pressure will increase to very high levels (Perlmutter, 2022).
5. **VENOUS REFLUX:** The presence of reversed blood flow greater than 0.5 seconds following the release of a firm manual calf compression (Robertson et al., 2009).
6. **DOPPLER ULTRASOUND:** This is a highly accurate, non-invasive technique to diagnose vascular disease and organ perfusion. The Frequency of sound waves reflected by circulating blood is different from that of stagnant blood (Lewis et al., 1989).
7. **INFLAMMATION:** The succession of changes which occurs in a living tissue when it is injured provided that the injury is not of such a degree as to at once destroy its structure and vitality (Punchard et al., 2004).
8. **INFLAMMATORY BIOMARKERS:** In response to inflammatory processes in the body, many acute phase reactant proteins are released which indicate

excessive endothelial damage and increased procoagulative activities in the body. These biomarkers include cytokines and many other proteins (Tiwary et al., 2020).

9. **CHRONIC VENOUS ULCERS:** An area of discontinuity in the epidermis, persisting for 4 weeks or more and occurring as a result of venous hypertension (increased pressure) and calf muscle pump insufficiency (Simpson & Roderick, n.d.).
10. **KLIPPEL-TRĚNAUNAY SYNDROME:** A complex congenital anomaly characterized by varicosities and venous malformations (VMs) of one or more limbs, port-wine stains, and soft tissue and bone hypertrophy (Noel et al., 2000).
11. **CEAP CLASSIFICATION:** A internationally accepted standard for describing patients with chronic venous disorders and it has been used for reporting clinical research findings in scientific journals (Lurie et al., 2020).

CHAPTER 3

RESEARCH METHODOLOGY

3.1 STUDY DESIGN

This was a case-control study. Duration of this study was from January to June 2023. Sample collection was performed at the Department of Vascular Surgery and Radiology, Shaheed Mohtarma Benazir Bhutto Institute of Trauma Karachi (SMBBITK). Blood sample analysis was performed at “the Laboratory” lab in Karachi. Ethical approval was granted by the Ethics Review Committee (ERC) of Bahria University of Health Sciences Karachi Campus (BUHSCK) and the Ethics Review Board of SMBBITK.

Patients who visited the OPD of the department of vascular surgery with complaints of dilated superficial veins in their lower limbs were selected as the case group, and patients who visited the hospital with complaints other than vascular diseases were recruited as the control group. An ethics committee-approved consent form and questionnaire were distributed to all participants who consented to participate in this study. Each subject was asked to complete a provided questionnaire and personal information form. After meeting study requirements, subjects were analyzed clinically and radiologically.

3.2 SUBJECTS

The patients visit OPD, Vascular Surgery department, Shaheed Mohtarma Benazir Bhutto Institute of Trauma, Karachi (SMBBITK).

3.3 SETTING

Vascular Surgery Department, Radiology Department of Shaheed Mohtarma Benazir Bhutto Institute of Trauma Karachi (SMBBITK), and “The Laboratory" lab in Karachi

3.4 INCLUSION CRITERIA

- Patients with dilated superficial veins in the lower limbs aged between 20 and 70 years
- Patients (both genders) who visited the OPD, Department of Vascular Surgery, SMBBITK
- Patients with visibly dilated veins in one of both lower limbs
- Controls were the subjects who were not suffering from any chronic disorder, e.g., Diabetes mellitus, tuberculosis, arthritis, etc.

3.5 EXCLUSION CRITERIA

- Patients suffering from any bleeding disorder
- Patients with any venous disease other than varicose veins, i.e., hemorrhoids, varicocele, esophageal varices

3.6 DURATION OF STUDY

- Individual study period: 2-3 hours per patient
- Total period of study: 6 months

3.7 SAMPLE SIZE CALCULATION

The sample size for the current study, titled "Study the association of varicose veins with inflammatory biomarkers" was 76 (38 cases, 38 controls). This sample size was calculated using the population sample size frequency (www.openepi.com), open-source calculator version 3-SSproper. The following equation was used:

Sample size n = [DEFF*Np(1-p]/[(d^2/$Z^2$1-α/2*(N-1)+p*(1-p)]

3.8 SAMPLING TECHNIQUE

The sampling technique used in this study was purposive. After subject selection, samples were collected for a specific purpose with a predetermined assumption of options, based on inclusion and exclusion criteria.

3.9 HUMAN SUBJECTS AND CONSENT

An informed consent form (in English and Urdu), approved by ethical review, was completed with all relevant information regarding this study. Written informed consent was obtained from all participants.

3.10 MATERIALS USED (DRUGS/ CHEMICAL/ PROFORMA/ QUESTIONNAIRE AND ANY OTHER)

The materials used to conduct this study included a questionnaire, subject evaluation form, and a consent form in English and Urdu. Lab consumables, including Disposable gloves, Alcohol swabs, Bandages and Gauze, Digital weight machine, Measuring tape, “Blue topped” Blood Collection Tubes (3ml) containing sodium citrate as anti-coagulant (fig. 3.1), Centrifuge machine, 3cc Syringes (fig 3.2), Needle cutter for

syringes, Ice bag (fig. 3.3), Ultrasound gel (fig. 3.4), Ultrasound machine (GE LOGIQ model P7) (fig. 3.5), Fully automatic fibrinogen analyzer (Sysmex model CA-660 series 600) (fig. 3.6), Anti-sera for blood group (fig 3.7)

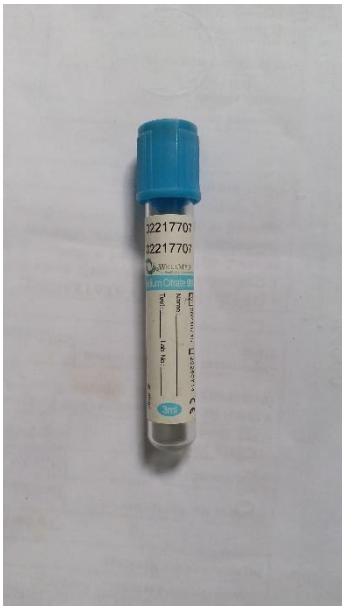


Figure 3.1: Blood collection tubes

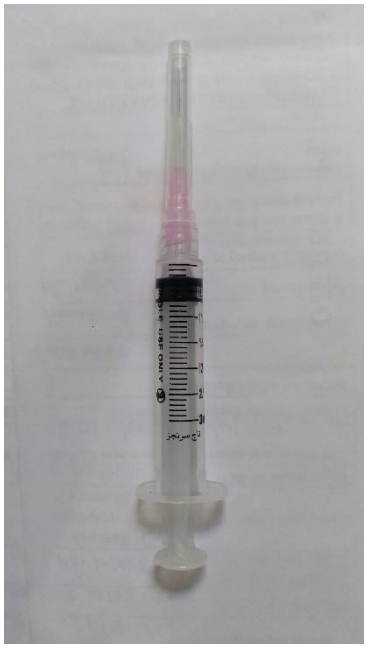


Figure 3.2: Syringe 3cc

Figure 3.3: Cold transportation bag with ice pack

Figure 3.4: Sky Gel Ultrasound Gel which was applied on the legs of the subjects prior to placing the transducer

Figure 3.5: Diagnostic ultrasound system (GE LOGIQ model P7)

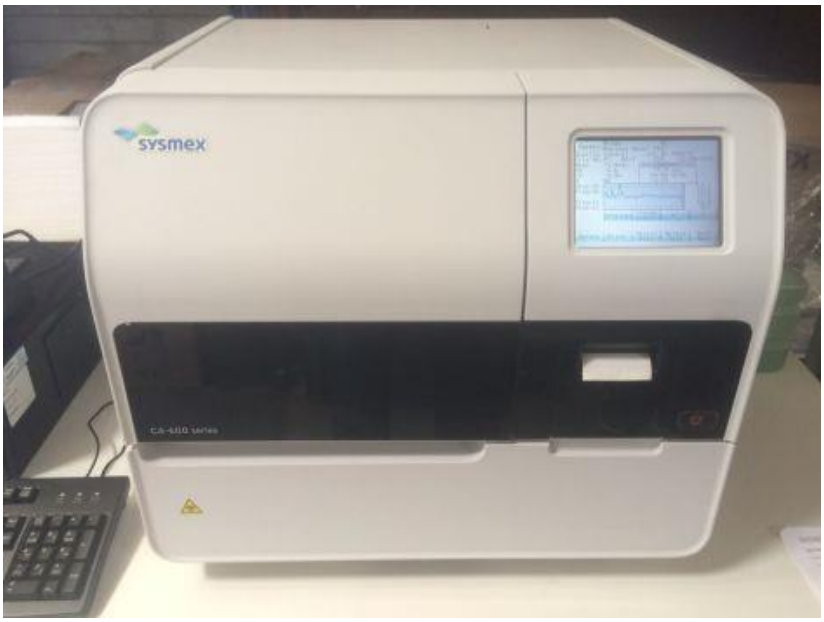


Figure 3.6: Sysmex coagulation analyzer model 660 series 600

Figure 3.7: Anti-sera used for blood grouping

3.11 PARAMETERS OF THE STUDY

- Age
- Weight
- Height
- BMI
- Gender
- Ethnicity
- Comorbidities
- ABO blood grouping
- Varicose vein diameter measurement
- Analysis of the levels of inflammatory biomarkers in blood (fibrinogen)

3.12 PROTOCOL OF STUDY

Data collection for the current study was performed using questionnaires and subject evaluation forms. Participant demographic details and medical history were collected from hospital records, questionnaires, and subject evaluation forms. To obtain informed consent from each participant, a written informed consent form (in English and Urdu) was distributed to all subjects to read and sign. Participants who were illiterate were notified verbally and their thumbprint was considered consent to participate in this study. After obtaining informed consent from each subject, a radiological examination was performed to assess the superficial venous structure of the participant's lower extremities. 3 mL blood sample was collected from each participant for analysis of fibrinogen as an inflammatory biomarker.

3.12.1 RADIOGRAPHIC EVALUATION

3.12.1.1 ULTRASOUND

After clinical examination, the patient was transferred to SMBBITK's radiology department. An ultrasound machine (GE LOGIQ P7) equipped with a linear array probe was used for ultrasound examination (fig 3.5). Ultrasonography was performed in both standing and supine positions. Patients were asked to expose their lower extremities for visibility and apply gel to the exposed leg. The transducer (probe) of the ultrasound device was applied to the skin. Fig 3.8 showing the procedure of ultrasound being performed in standing position.

Veins were distinguished from arteries on ultrasound, because of being non pulsatile, run superficial to the arteries, and have valves. During compression maneuvers, veins were susceptible to compression and had large, irregular lumen. At first, the saphenous femoral junction was identified by its characteristic appearance known as the "Mickey Mouse view". This is the site where the common femoral artery, common femoral vein, and great saphenous vein can be visualized with ultrasound. In varicose veins, the saphenofemoral junction is usually dilated. The great saphenous vein exists in the saphenous compartment (Fig. 3.9). This compartment is formed by the saphenous fascia and the muscular fascia.

The reflux of the blood in the patients was assessed with a manual compression technique, and it was noted to be greater than 0.5 seconds. Later on, the probe was moved further distally, and several dilated veins were identified as multiple hypoechoic round circles on the doppler machine screen (fig. 3.10). In order to measure the diameter of these dilated veins, the image was fixed on the screen, and two points, A and B, were marked on the outer walls of the dilated veins. The lumen diameters of these dilated veins were automatically recorded and displayed on the screen (Fig. 3.11). After the procedure, the gel was removed (with gauze) from the patient’s leg.

Figure 3.8: Ultrasound of the lower limb for varicose vein being performed in standing position

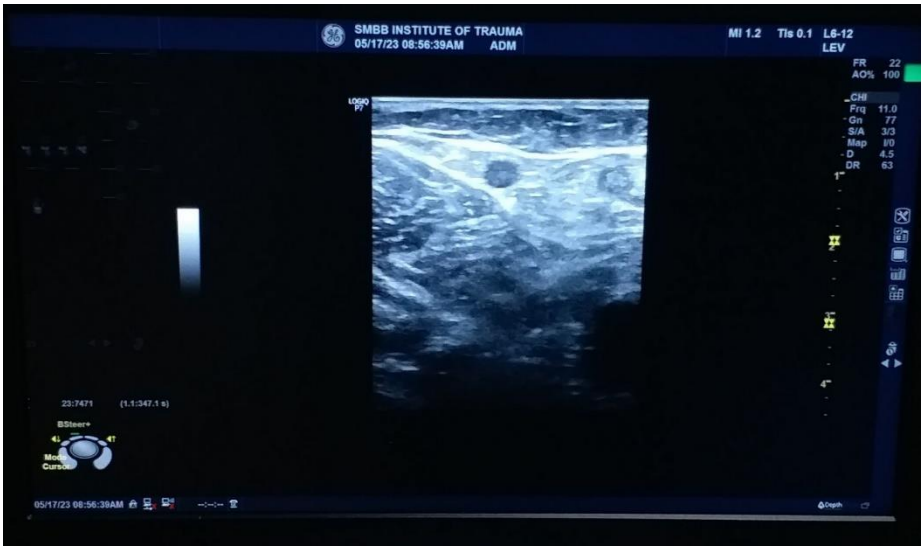


Figure 3.9: Ultrasound image of the saphenous compartment (Egyptian eye sign)

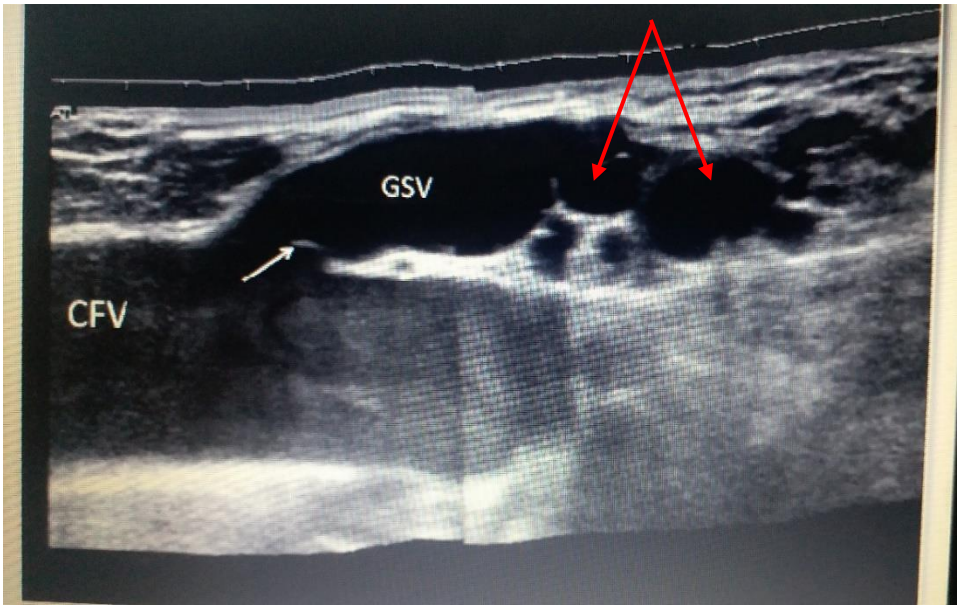


Figure 3.10: Ultrasound image showing a valve (white arrow), dilated great saphenous vein and several varicosities originating from GSV (red arrow)

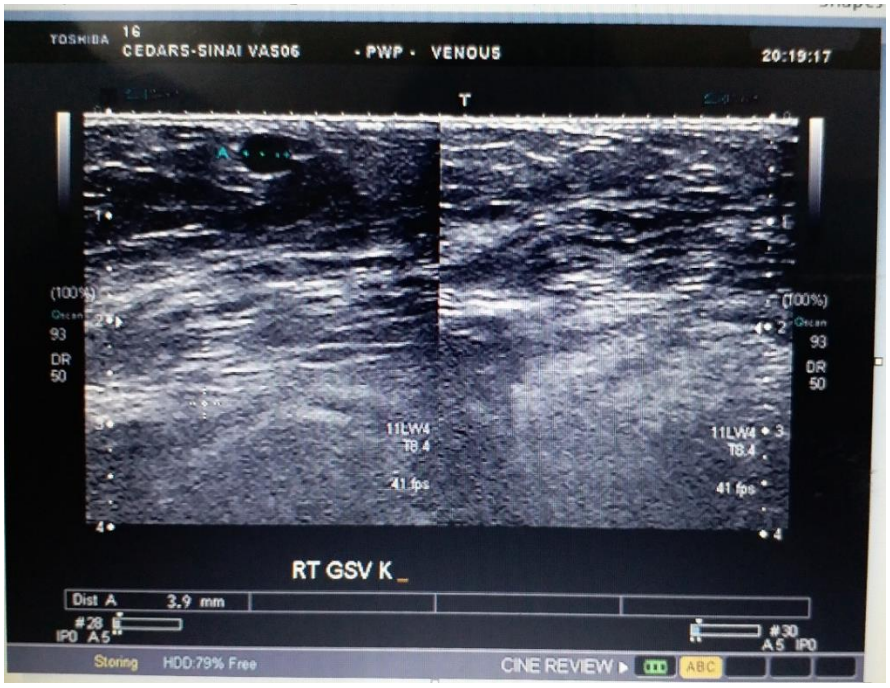


Figure 3.11: Ultrasound image of a dilated vein showing its diameter in the left bottom of the display

3.12.2 INFLAMMATORY BIOMARKER ANALYSIS

3.12.2.1 PHLEBOTOMY FOR THE ANALYSIS OF PLASMA INFLAMMATORY BIOMARKERS

After completing the ultrasound, phlebotomy was performed to analyze the inflammatory biomarkers in the plasma of the patients. The entire procedure of phlebotomy (blood sampling) was carried out in a very sterilized environment. All the consumables used were thoroughly sterilized. The patients were asked to lie in a supine position and were asked to expose both the lower limbs till knee height, but if the dilated veins were not seen in the supine posture, then the patients were asked to stand up with much of the leg above the knee remaining uncovered. The area of the most dilated veins in the legs were cleaned with an alcohol swab and let dry. To draw the blood, a 3cc sterile syringe was inserted into the vein and 3 ml of blood was collected and transferred to the blue-topped glass tubes containing sodium citrate. The anti-coagulant tubes were tilted up and down three to four times to ensure the mixing of chemicals with blood.

After collecting the blood sample, some pressure was applied with the help of sterile gauze to the site of the punctured vein (at least 2 minutes) and the area of the leg was tightly wrapped with sterile crape bandages to stop chances of further bleeding.

For sample identification, each tube was marked with the patient's name and phlebotomy date. The blood samples were transported to the laboratory situated in Sadder, Karachi in a portable ice pack within 1-2 hours of phlebotomy.

Blood samples from the participants of control group were collected in a similar technique. The only difference was that the samples were collected from the veins of the upper limbs.

3.12.2.2 SEPARATION OF PLASMA FROM BLOOD

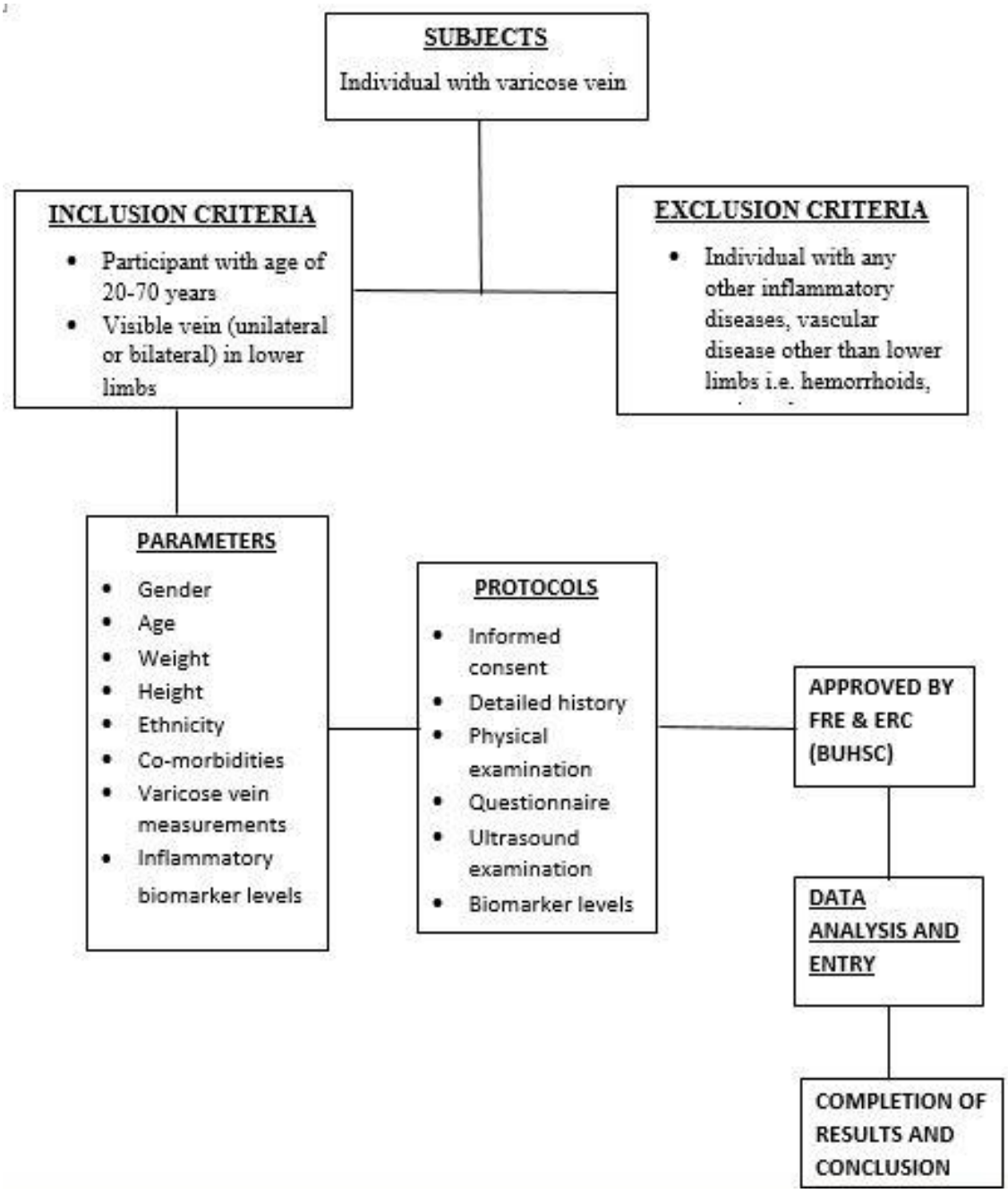
In the lab, blood samples were placed in a cool place and were checked first for any clot or foreign material before plasma separation. Blood samples were centrifuged for 10 minutes at 40000 rounds per minute (RPM) in a centrifuge machine. The tube was removed from the centrifuge and plasma was checked to see if the plasma was clear

without any signs of hemolysis. It was then transferred into a cuvette (a container with a specific tube number) with the help of a 10 ml pippete and the plasma samples were placed in a tube rack which contained another cuvette containing a fibrinogen determination reagent. The tube rack was placed in the Sysmex CA-660 chemistry analyzer. On this rack, multiple samples can be run simultaneously. Patient biodata with specific tube number was entered into the analyzer and the process of fibrinogen measurement was initiated. Within 10-15 minutes, the results of all the samples were displayed on the monitor of the analyzer. All used tubes and reagents were disposed off at the end of the procedure. For accuracy, reliability and authenticity of the results, it was mandatory to run the commercially prepared standard sample in the reagent box to calibrate the chemistry analyzer before running the sample.

3.13 STATISTICS

Statistical analysis was done using the IBMM SPSS 23.0. Correlation between the ultrasound measurements and the levels of inflammatory biomarkers was analyzed by the Pearson correlation test. The Chi-square test & Fisher's exact test was applied on categorical variables. The *p* value was set to ≤ 0.05.

3.14 ALGORITHM OF STUDY



CHAPTER 4

RESULTS

The present study was a case-control study that consisted of 76 individuals (38 cases, 38 controls). Fibrinogen levels were analyzed and compared in both of these groups, whereas Doppler ultrasound was performed only in the case group as the hospital resources were limited and it was against the policy of the hospital to perform Doppler ultrasound on patients without any evident indication. The patients visited the hospital with the complaints of pain, swelling, heaviness, discomfort, and itching sensation in the affected areas of the involved lower limb. Whereas, venous leg ulcers were the most common sign (60.5%) presented by these patients. Other signs included were skin color changes, hyperpigmentation and eczema. The majority of the participants in both cases and controls were married which account for 60 (75.95%) of the participants. Hypertension was the most common comorbidity to be found in the cases group. It was present in 3 (7.89%) of the cases. 1 (2.6%) patient had previous history of myocardial infarction.

4.1 VARICOSE VEIN DIAMETER IN CASE GROUP

To evaluate the severity of varicose veins in the case group, the diameter of the dilated veins was measured by Doppler ultrasound. On the basis of the ranges that were assessed in the diameter of varicose veins, we divided our patients according to the severity of varicose vein disease into three groups: A (mild varicose), B (moderate varicose), and C (severe varicose).

In the group A (mild varicose), the diameter of the varicose vein ranged between 3mm and 3.9 mm. In three patients out of 38 (7.9%), the diameter of varicose veins measured was less than 3mm. Eleven patients out of 38 (28.9%) had vein diameters ranging between

3.0 and 3.4 mm, and they were considered the largest group of cases (Table 4.1). In the other five patients (13.2%), the range of diameter was between 3.5 and 3.9 mm.

In the group B (moderate varicose), the diameter of the varicose vein ranged between 4mm and 5.9 mm: Four patients (10.5%) had the diameter of distended veins ranging between 4.0 and 4.4 mm; six patients (15.8%) had the diameter of dilated veins ranging between 4.5 and 4.9 mm; and in three patients (7.9%), the diameter of dilated veins ranged between 5.0 and 5.9 mm. (Table 4.1). These were the patients who had skin discoloration, hyper pigmentation, pain and swelling.

In the group C (severe varicose), the diameter of the varicose veins ranged between 6mm and 8mm. Three patients (7.9%) had a diameter of distended veins measured ranging between 6.0 and 6.9 mm, one patient (2.6%) had a diameter of dilated veins between 7.0 and 7.9 mm, and in two patients (5.3%), the diameter of dilated veins was greater than 8 mm. (Table 4.1). These patients had necrosis and ulcers with scar formation, pus discharge and bleeding from ulcers.

4.2 PLASMA FIBRINOGEN LEVELS IN CASES AND CONTROLS

Plasma fibrinogen levels were assessed in the case and control groups. The levels of fibrinogen in the blood were described as above or high, below or low, and within the normal or reference range. Normal plasma fibrinogen levels range between 1.5 g/l and 3.5 g/l. (Arbez and Casini, 2018).

In the present study 38 (100%) subjects in the control group had a normal fibrinogen level between 3.5 g/l and 3.999 g/l (Table 4.2). Whereas, among case group 2 varicose vein patients out of 38 (5.3%) fell within the normal range of 3.5g/l and 3.999 g/l. The rest of the 36 patients (94.7%) presented plasma fibrinogen levels above the normal range (Table 4.3), with a mean plasma fibrinogen level of 2.7105±2 (Table 4.4). According to the ranges of fibrinogen levels achieved in the present study, we divided patients according to fibrinogen levels into Group A (high), Group B (higher), and group C (highest).

Group A: High fibrinogen levels (4.0 g/l and 4.449 g/l), including 14 varicose vein patients (36.8%), were presented with plasma fibrinogen levels ranging between 4.0 and 4.399 g/l, and 2 patients (5.3%) had plasma fibrinogen levels between 4.400 and 4.449 g/l.

Group B: Higher fibrinogen levels (4.450g/l and 4.699g/l) were found 9 patients (23.7%) with a raised plasma fibrinogen level ranging between 4.450 and 4.599 g/l, and 5 patients (13.2%) had fibrinogen levels between 4.600 and 4.699 g/l.

Group C: The highest fibrinogen levels in our study ranged from 4.700 g/l to > 5.0 g/l, including 2 patients (5.3%) with plasma fibrinogen levels ranging from 4.700 to 4.749 g/l, followed by another 2 patients (5.3%) with plasma fibrinogen levels between 4.800 and 4.999 g/l, 1 patient (2.6%) with plasma fibrinogen levels between 4.750 and 4.799 g/l, and 1 patient (2.6%) with plasma fibrinogen levels greater than 5.0 g/l. A Pearson Chi-square test was run to assess the association of raised plasma fibrinogen levels with the varicose veins. It was found to be statistically significant (p =.000) (Table 4.5).

4.3 PLASMA FIBRINOGEN LEVELS CORRELATION WITH VARICOSE VEINS DIAMETER

In the present study, we tried to correlate the changes occurring in the plasma concentration of fibrinogen with the changes occurring in the diameter of varicose veins, as has been observed on Doppler ultrasound. It was observed in this study, group A (mild) increase in varicose veins diameter was the largest group comprising 11 (28.95%) patients, had a vein diameter of 3.0 to 3.4 mm on Doppler ultrasound. Similarly, group A with high fibrinogen levels had the largest number of patients, 14 (36.84%), with elevated fibrinogen levels ranging between 4.0 and 4.399 g/l. To adjust a correlation between Group A (mild) increase in varicose vein diameter on Doppler ultrasound and group A with a high plasma fibrinogen level, a Pearson correlation test was run, and it was found to be statistically significant (p =.000) (Table 4.7), and the strength of this correlation was.764**.

4.4 CEAP CLASSIFICATION OF VARICOSE VEINS

The clinical category of the CEAP classification was also checked in patients of varicose veins. 20 (52.63%) of the patients coming to the hospital presented with C6 (active ulcers) category, this was found to be the most common presenting sign and symptom of varicose veins. It was followed by the C3 (edema) category which was

present in 6 (15.8%) of the cases, the C2 (VVs) and C4 (skin involvement) category had the equal number of patients, accounting for 4 (10.5%) patients in each group, 3 (7.9%) patients presented with C5 (healed ulcers) category and only 1 (2.6%) patient presented with C1(spider veins) category of the CEAP classification (Table 4.8).

4.5 AGE ASSOCIATION WITH VARICOSE VEINS

In the present study, a total of 76 participants had an age range of 20 to 80 years. Twelve participants out of 76 (15.7%) were young adults aged less than 30 years. Six participants (7.89%) were the oldest patients having age greater than 60 years and fifty-seven participants out of seventy-six (75%) were aged between 30-60 years. In the case group as well as in the control group, the majority of the participants were adults age ranging between 30-60 years. Which account for 24 participants out of 38 (63.16%) in the cases and 33 participants out of 38 (86.8%) in controls (Table 4.9).

In the present study it was observed that the occurrence of varicose veins was among the adults with a minimum age of 20 years and a maximum age of 65 years. Table 4.10 represent the association of the age of the patients with changes occurring in the diameter of the varicose veins observed on Doppler ultrasound. Statistical analysis showed insignificant association between the age of the patients and the occurrence of varicose veins (p= .777) (Table 4.11).

4.6 GENDER ASSOCIATION WITH VARICOSE VEINS

Of the thirty-eight patients in the case group, twenty-eight (73.68%) patients were male and only ten (26.32%) patients were female. Similarly, in the control group, thirty three out of thirty-eight (86.8%) controls were male and only five (13.2%) were female (Table 4.12). In the current study, the majority of participants in both groups were male. Table 4.13 represent the association of the gender of the respondents with the changes occurring in the diameter of the varicose veins observed on Doppler ultrasound. Statistically, there was no significant correlation between patient gender and varicose vein incidence (p = .315) (Table 4.14).

4.7 BODY MASS INDEX ASSICIATION WITH VARICOSE VEINS

The height and weight of patients and controls were measured and BMI was calculated using the imperial formula (BMI = weight [lbs] / height [inch]^2). In the control group, fourteen (36.8%) participants had normal BMI (18-20 kg/m^2) and five (13.2%) participants were obese (BMI >30 kg/m^2). In the case group, twelve patients (31.6%) had a normal BMI (18-25 kg/m^2) and eleven patients (28.9%) were obese (BMI >30 kg/m^2). In both the case and control groups, the majority of the participants were overweight (BMI 25-30 kg/m^2). Fifteen (39.47%) participants were overweight in the case group and nineteen (50%) participants in the control group (BMI 25–30 kg/m^2) (Table 4.15). Table 4.16 represent the association of the BMI of the patients with the changes occurring in the diameter of the varicose veins observed on Doppler ultrasound. No statistically significant relationship was found between the BMI and the incidence of varicose veins (p = .537) (Table 4.17).

4.8 ETHNICITY ASSOCIATION WITH VARICOSE VEINS

Participant ethnicity was assessed to determine the incidence of varicose veins in specific ethnicities. Twenty-two (27.85%) participants in this study were Pathans. Fourteen (17.7%) of the participants were Mohajirs (a group of people who migrated from India to Pakistan in the year 1947)**,** and eleven (13.9%) of the participants were of Baloch ethnicity. Two (2.5%) participants were Punjabi and one (1.3%) participant was non-Muslim. In both case and control groups, most of the participants were local Sindhis, accounting for 14 (36.84%) and 12 (31.58%) in the control group and cases, respectively (Table 4.18). Table 4.19 represent the association of the ethnicity of the patients with the changes occurring in the diameter of the varicose veins observed on Doppler ultrasound. A statistically insignificant association was found between ethnicity and o**c**currence of varicose veins (p= .137) (Table 4.20).

4.9 ABO BLOOD GROUP ASSOCIATION WITH VARICOSE VEIN

In this study ABO blood typing was performed for both case and control groups. The most common blood group in this study was A+ve, present in fifteen (19.7%) of the participants. The second commonest group was B+ve occurred in thirteen (17.1%) participants, eleven participants had AB+ve (14.5%). Ten had A-ve (13.2%). AB-ve group was present in eight (10.5%) participants, group O+ve was found in seven (9.2%) participants, and group B-ve and group O-ve was found in six (7.9%) of the participants respectively.

A+ve was found to be the most common blood group in the controls as nine individuals (23.7%) had this particular group and B+ve was found to be the most common blood group in cases which account for 9 (23.7%) of the cases (Table 4.21). Table 4.22 represent the association of the blood group of the patients with the changes occurring in the diameter of the varicose veins observed on Doppler ultrasound. Statistically significant association was present between the ABO blood group of the patients with the occurrence of varicose veins (p= .030) (Table 4.23).

4.10 OCCUPATION ASSOCIATION WITH VARICOSE VEIN

The participants in this study belonged to different occupational groups. Fifteen (19%) of the participants were government employees, eleven (13.9%) of the participants were students, twelve (15.2%) of the participants were house wives. The majority of the participants in both controls as well as patients, were labors, fourteen (36.84%) and twenty-four (63.16%) among the controls and cases respectively (Table 4.24). Table 4.25 represent the association of the occupation of the respondents with the changes occurring in the diameter of the varicose veins observed on Doppler ultrasound. Statistically no significant association was seen between the occupation of the participants with the occurrence of the varicose veins (p= .175) (Table 4.26).

4.11 SMOKING ASSOCIATION WITH VARICOSE VEINS

In the current study, most participants in both groups were nonsmokers, accounting for 22 (57.89%) and 28 (73.68%) controls and cases, respectively. Table 4.27 represent the association of smoking with the changes occurring in the diameter of the varicose veins observed on Doppler ultrasound. There was no statistically significant

association between participants' smoking and the incidence of varicose veins (p= 0.853) (Table 4.28).

Table 4.1: The frequency of vein diameter among cases measured on Doppler ultrasound

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	<3 mm	3	7.9	7.9	7.9
	3.0 to 3.4 mm	11	28.9	28.9	36.8
	3.5 to 3.9 mm	5	13.2	13.2	50.0
	4.0 to 4.4 mm	4	10.5	10.5	60.5
	4.5 to 4.9 mm	6	15.8	15.8	76.3
	5.0 to 5.9 mm	3	7.9	7.9	84.2
	6.0 to 6.9 mm	3	7.9	7.9	92.1
	7.0 to 7.9 mm	1	2.6	2.6	94.7
	> 8 mm	2	5.3	5.3	100.0
	Total	38	100.0	100.0	

Table 4.2: The percentage of fibrinogen levels in case and control groups

Fibrinogen levels (g/l)	**Controls (percentage)**	**Cases (percentage)**	**Total (percentage)**
3.5 to 3.999	38 (100%)	2 (5.3%)	40 (52.6%)
4.0 to 4.3999	0 0.0%	14 (36.8%)	14 (18.4%)
4.400 to 4.449	0 0.0%	2 (5.3%)	2 (2.6%)
4.450 to 4.599	0 0.0%	9 (23.7%)	9 (11.8%)
4.600 to 4.699	0 0.0%	5 (13.2%)	5 (6.6%)
4.700 to 4.749	0 0.0%	2 (5.3%)	2 (2.6%)
4.750 to 4.799	0 0.0%	1 (2.6%)	1 (1.3%)
4.800 to 4.999	0 0.0%	2 (5.3%)	2 (2.6%)
>5.0	0 0.0%	1 (2.6%)	1 (1.3%)
Total	38 (100%)	38 (100%)	76 (100%)

Table 4.3: The frequency and percentage of fibrinogen levels in the case group

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	3.5 to 3.999 g/l	2	5.3	5.3	5.3
	4.0 to 4.399 g/l	14	36.8	36.8	42.1
	4.400 to 4.449 g/l	2	5.3	5.3	47.4
	4.450 to 4.599 g/l	9	23.7	23.7	71.1
	4.600 to 4.699 g/l	5	13.2	13.2	84.2
	4.700 to 4.749 g/l	2	5.3	5.3	89.5
	4.750 to 4.799 g/l	1	2.6	2.6	92.1
	4.800 to 4.999 g/l	2	5.3	5.3	97.4
	> 5.0 g/l	1	2.6	2.6	100.0
	Total	38	100.0	100.0	

Table 4.4: Mean fibrinogen levels in blood of case group

N	Valid	38
	Missing	0
Mean		2.7105
Std. Deviation		2.02562
Variance		4.103
Range		8.00
Minimum		.00
Maximum		8.00

Table 4.5: Association between fibrinogen level with varicose veins

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	68.400[a]	8	.000
Likelihood Ratio	89.477	8	.000
Linear-by-Linear Association	35.927	1	.000
N of Valid Cases	76		

***p*-value < 0.05 = statistically significant (*), <0.01 = highly statistically significant (**)**

Test applied = Pearson Chi-square

Table 4.6: Mean diameter of varicose veins on Doppler ultrasound and mean fibrinogen levels in blood

	Mean	Std. Deviation	N
Diameter of the varicose veins on ultrasound	2.9737	2.24796	38
Fibrinogen levels in blood	2.7105	2.02562	38

Table 4.7: Correlation between the changes in diameter of varicose vein and levels of fibrinogen

		Diameter of the varicose veins on ultrasound	Fibrinogen levels in blood
Diameter of the varicose veins on ultrasound	Pearson Correlation	1	.764**
	Sig. (2-tailed)		.000
	N	38	38
Fibrinogen levels in blood	Pearson Correlation	.764**	1
	Sig. (2-tailed)	.000	
	N	38	38

***p*-value < 0.05 = statistically significant (*), <0.01 = highly statistically significant (**)**

Test applied = Pearson Correlation

Table 4.8: The frequency of clinical category of CEAP classification in cases

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	C1 (Spider or reticular veins)	1	2.6	2.6	2.6
	C2 (VVs)	4	10.5	10.5	13.2
	C3 (Edema)	6	15.8	15.8	28.9
	C4 (Discoloration)	4	10.5	10.5	39.5
	C5 (Healed ulcers)	3	7.9	7.9	47.4
	C6 (Active ulcers)	20	52.6	52.6	100.0
	Total	38	100.0	100.0	

Table 4.9: Bar graph representing comparison of age percentage among the groups

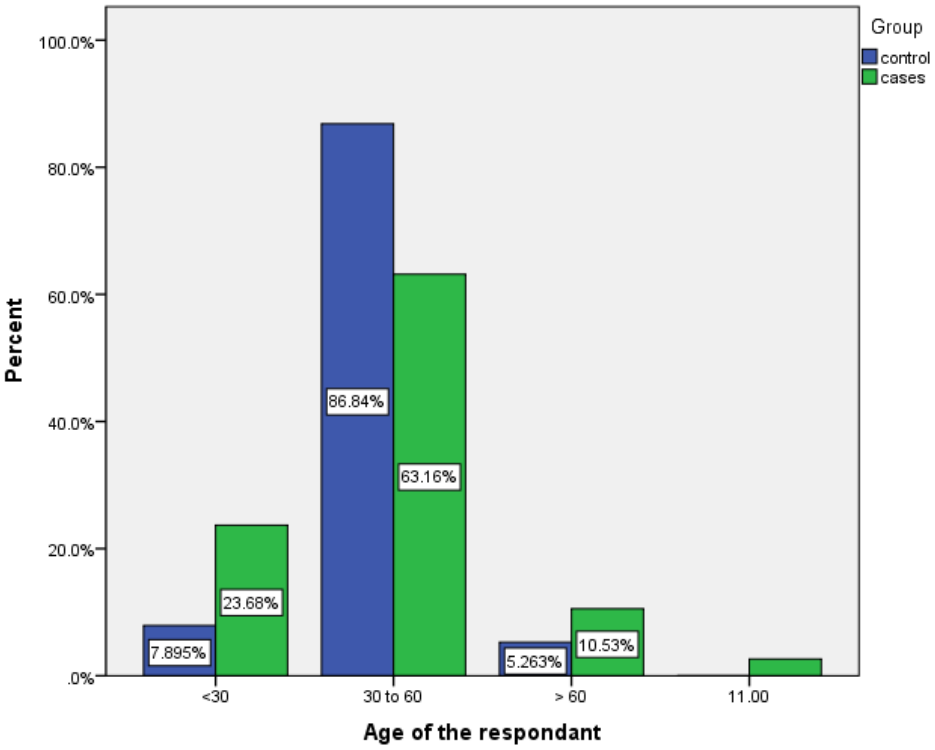


Table 4.10: Age of the respondent *Diameter of varicose vein on Doppler ultrasound

Age		Diameter of the varicose veins on doppler ultrasound									Total
		<3mm	3.0 to 3.4 mm	3.5 to 3.9 mm	4.0 to 4.4 mm	4.5 to 4.9 mm	5.0 to 5.9 mm	6.0 to 6.9 mm	7.0 to 7.9 mm	> 8 mm	
<30	Count	1	2	2	1	1	0	1	1	0	9
	Expected Count	7	2.6	1.2	.9	1.4	.7	.7	.2	.5	9.0
	% Within Diameter of the Varicose Veins on Ultrasound	33.3%	18.2%	40.0 %	25.0%	16.7%	0.0%	33.3%	100%	0.0%	23.7%
30 to 60	Count	2	7	3	3	4	3	1	0	1	24
	Expected Count	1.9	6.9	3.2	2.5	3.8	1.9	1.9	.6	1.3	24.0
	% Within Diameter of the Varicose Veins on Ultrasound	66.7%	63.6%	60.0 %	75.0%	66.7%	100%	33.3%	0.0%	50.0%	63.2%
>60	Count	0	2	0	0	1	0	0	0	1	4
	Expected Count	3	1.2	.5	.4	.6	.3	.3	.1	.2	4.0
	% Within Diameter of the Varicose Veins on Ultrasound	0.0%	18.2%	0.0%	0.0%	16.7%	0.0%	0.0%	0.0%	50.0%	10.5%
11.00	Count	0	0	0	0	0	0	1	0	0	1
	Expected Count	.1	.3	.1	.1	.2	.1	.1	.0	.1	1.0
	% Within Diameter of the Varicose Veins on Ultrasound	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	33.3%	0.0%	0.0%	2.6%
Total	Count	3	11	5	4	6	3	3	1	2	38
	Expected Count	3.0	11.0	5.0	4.0	6.0	3.0	3.0	1.0	2.0	38.0
	% Within Diameter of the Varicose Veins on Ultrasound	100.0%	100.0 %	100.0 %	100.0 %	100.0 %	100.0 %	100.0 %	100.0 %	100.0 %	100.0 %

Table 4.11: Association of age with varicose vein

	Value	Df	Asymp. Sig (2-Sided)	Exact Sig. (2-Sided)	Exact Sig. (1-Sided)	Point Probability
Pearson Chi-Square	24.032[a]	24	.460	.472		
Likelihood Ratio	18.481	24	.779	.788		
Fisher's Exact test	23.469			.777		
Linear-By-Linear Association	1.934[b]	1	.164	.152	.112	.006
N of Valid Cases	38					

***p*-value < 0.05 = statistically significant (*), <0.01 = highly statistically significant (**)**

Test applied = Chi-square & Fisher's exact test

Table 4.12: The gender percentage of the participants presented as a Bar graph

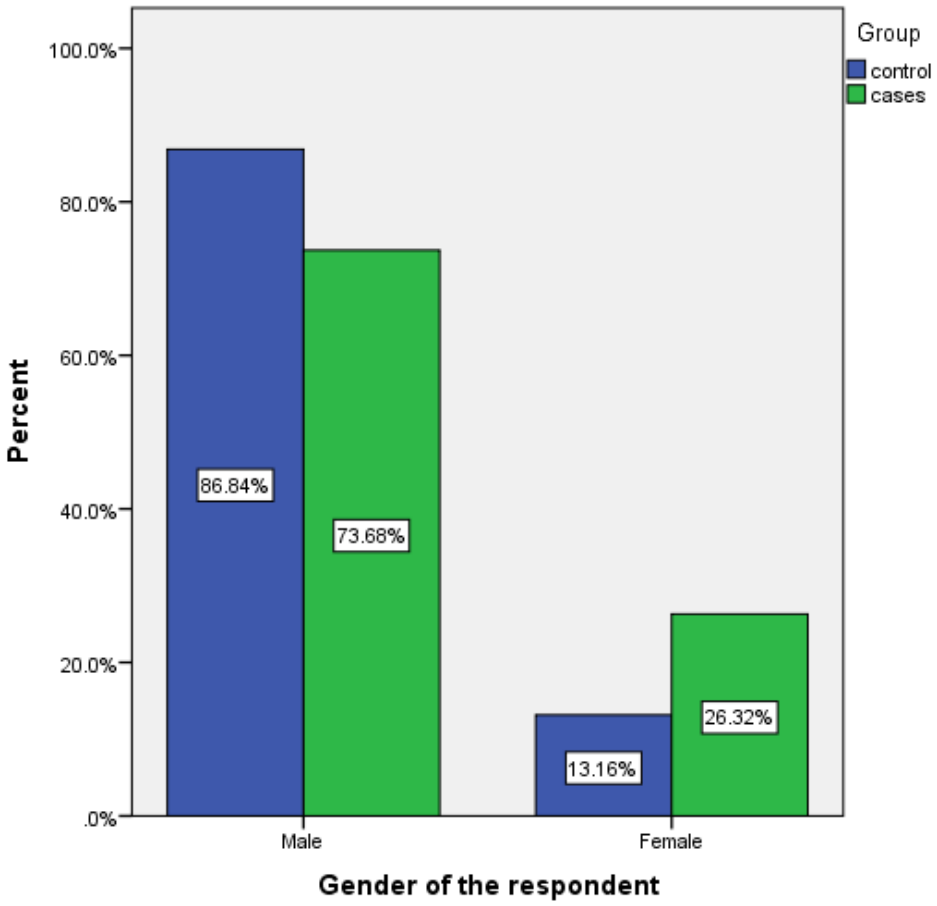


Table 4.13: Gender of the respondent * Diameter of varicose vein on Doppler ultrasound

Gender		Diameter of the varicose veins on doppler ultrasound									Total
		<3mm	3.0 to 3.4 mm	3.5 to 3.9 mm	4.0 to 4.4 mm	4.5 to 4.9 mm	5.0 to 5.9 mm	6.0 to 6.9 mm	7.0 to 7.9 mm	> 8 mm	
Male	Count	1	7	5	3	5	3	2	0	2	28
	Expected Count	2.2	8.1	3.7	2.9	4.4	2.2	2.2	.7	1.5	28.0
	% Within Diameter of the Varicose Veins on Ultrasound	33.3%	63.3%	100.0 %	75.0%	83.3%	100.0 %	100.0 %	0.0%	100.0 %	73.7%
Female	Count	2	4	0	1	1	0	1	1	0	10
	Expected Count	.8	2.9	1.3	1.1	1.6	.8	.8	.3	.5	10.0
	% Within Diameter of the Varicose Veins on Ultrasound	66.7%	36.4%	0.0%	25.0%	16.7%	0.0%	33.3%	100.0 %	0.0%	26.3%
Total	Count	3	11	5	4	6	3	3	1	2	38
	Expected Count	3.0	11.0	5.0	4.0	6.0	3.0	3.0	1.0	2.0	38.0
	% Within Diameter of the Varicose Veins on Ultrasound	100.0%	100.0 %	100.0 %	100.0 %	100.0 %	100.0 %	100.0 %	100.0 %	100.0 %	100.0 %

Table 4.14: Association of gender with varicose veins

	Value	Df	Asymp. Sig (2-Sided)	Exact Sig. (2-Sided)
Pearson Chi-Square	9.831[a]	8	.277	.272
Likelihood Ratio	11.837	8	.159	.275
Fisher's Exact test	8.483			.315
N of Valid Cases	38			

***p*-value < 0.05 = statistically significant (*), <0.01 = highly statistically significant (**)**

Test applied = Chi-square & Fisher's exact test

Table 4.15: Bar graph representing BMI percentage among the groups

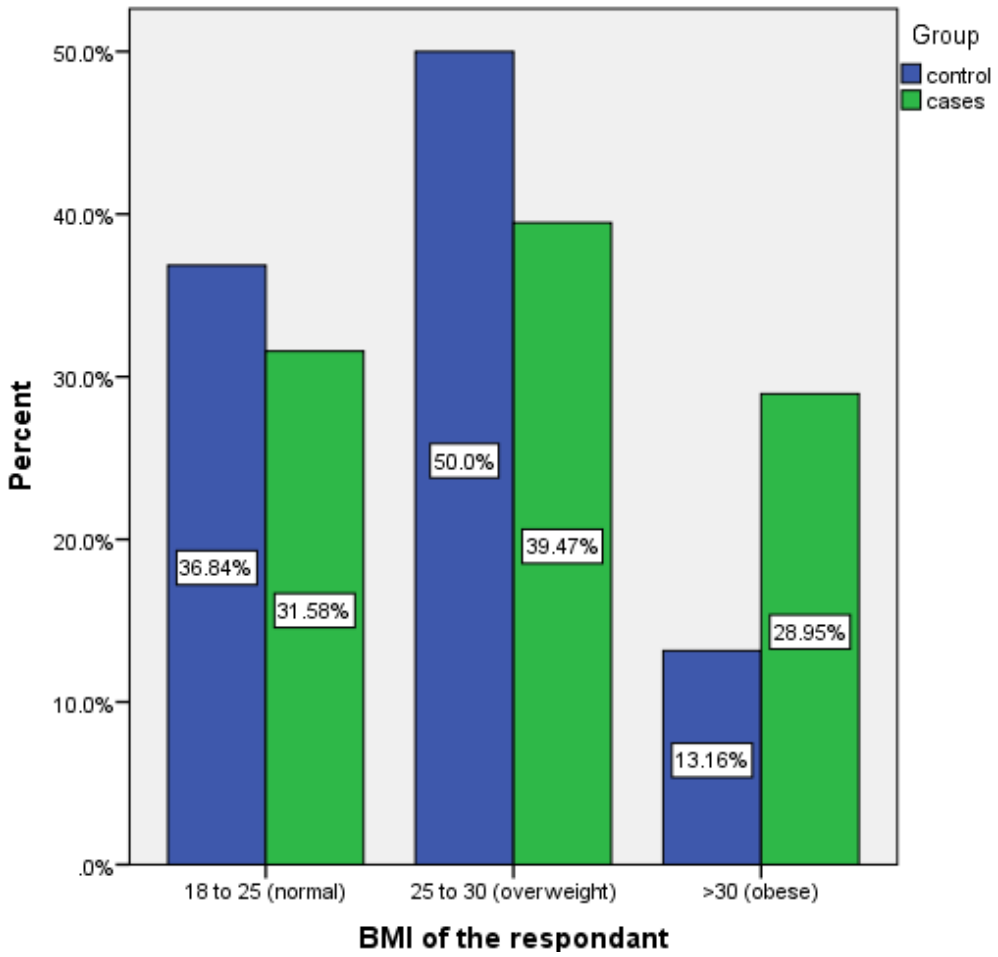


Table 4.16: BMI of the respondent *Diameter of varicose vein on Doppler ultrasound

BMI (kg/m²)		Diameter of the varicose veins on doppler ultrasound									Total
		<3mm	3.0 to 3.4 mm	3.5 to 3.9 mm	4.0 to 4.4 mm	4.5 to 4.9 mm	5.0 to 5.9 mm	6.0 to 6.9 mm	7.0 to 7.9 mm	> 8 mm	
18-25 (Normal)	Count	1	4	1	2	1	1	0	1	1	12
	Expected Count	.9	3.5	1.6	1.3	1.9	.9	.9	.3	.6	12.0
	% Within Diameter of the Varicose Veins on Ultrasound	33.3%	36.4%	20.0%	50.0%	16.7%	33.3%	0.0%	100.0 %	50.0%	31.6%
25 to 30 (Overweigh t)	Count	1	6	2	2	2	1	0	0	1	15
	Expected Count	1.2	4.3	2.0	1.6	2.4	1.2	1.2	.4	.8	15.0
	% Within Diameter of the Varicose Veins on Ultrasound	33.3%	54.5%	40.0%	50.0%	33.3%	33.3%	0.0%	0.0%	50.0%	39.5%
>30 (Obese)	Count	1	1	2	0	3	1	3	0	0	11
	Expected Count	.9	3.2	1.4	1.2	1.7	.9	.9	.3	.6	11.0
	% Within Diameter of the Varicose Veins on Ultrasound	33.3%	9.1%	40.0%	0.0%	50.0%	33.3%	100.0 %	0.0%	0.0%	28.9%
Total	Count	3	11	5	4	6	3	3	1	2	38
	Expected Count	3.0	11.0	5.0	4.0	6.0	3.0	3.0	1.0	2.0	38.0
	% Within Diameter of the Varicose Veins on Ultrasound	100.0 %	100.0 %	100.0 %	100.0 %	100.0 %	100.0 %	100.0 %	100.0 %	100.0 %	100.0 %

Table 4.17: Association of BMI with varicose veins

	Value	Df	Asymp. Sig (2-Sided)	Exact Sig. (2-Sided)	Exact Sig. (1-Sided)	Point Probability
Pearson Chi-Square	16.216[a]	16	.438	.482		
Likelihood Ratio	18.474	16	.297	.622		
Fisher's Exact test	16.637			.537		
Linear-By-Linear Association	.419[b]	1	.517	.553	.277	.030
N of Valid Cases	38					

***p*-value < 0.05 = statistically significant (*), <0.01 = highly statistically significant (**)**

Test applied = Chi-square & Fisher's exact test

Table 4.18: The ethnicity percentage among the groups presented as a Bar graph

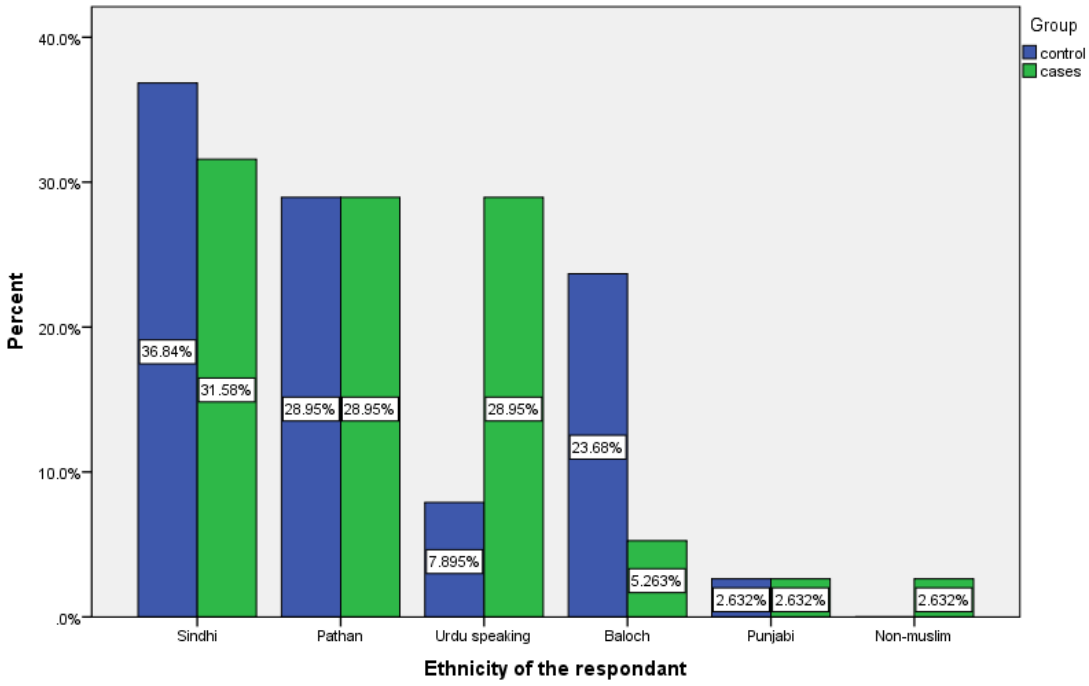


Table 4.19: Ethnicity of the respondent * Diameter of varicose vein on Doppler ultrasound

Ethnicity		Diameter of the varicose veins on doppler ultrasound									Total
		<3mm	3.0 to 3.4 mm	3.5 to 3.9 mm	4.0 to 4.4 mm	4.5 to 4.9 mm	5.0 to 5.9 mm	6.0 to 6.9 mm	7.0 to 7.9 mm	> 8 mm	
Sindhi	Count	0	5	1	1	1	1	2	0	1	12
	Expected Count	.9	3.5	1.6	1.3	1.9	.9	.9	.3	.6	12.0
	% Within Diameter of the Varicose Veins on Ultrasound	0.0%	45.5%	20.0 %	25.0%	16.7%	33.3%	66.7%	0.0%	50.0%	31.6%
Pathan	Count	1	1	4	2	2	0	1	0	0	11
	Expected Count	.9	3.2	1.4	1.2	1.7	.9	.9	.3	.6	11.0
	% Within Diameter of the Varicose Veins on Ultrasound	33.3%	9.1%	80.0 %	50.0%	33.3%	0.0%	33.3%	0.0%	0.0%	28.9%
Urdu Speaking	Count	2	4	0	1	3	1	0	0	1	11
	Expected Count	.9	3.2	1.4	1.2	1.7	.9	.9	.3	.6	11.0
	% Within Diameter of the Varicose Veins on Ultrasound	66.7%	36.4%	0.0%	25.0%	50.0%	33.3%	0.0%	0.0%	0.0%	28.9%
Baloch	Count	0	1	0	0	0	0	0	0	0	2
	Expected Count	.2	.6	.3	.2	.3	.2	.2	.1	.1	2.0
	% Within Diameter of the Varicose Veins on Ultrasound	0.0%	9.1%	0.0%	0.0%	0.0%	0.0%	0.0%	100.0 %	0.0%	5.3%
Punjabi	Count	0	0	0	0	0	0	0	0	1	1
	Expected Count	.1	.3	.1	.1	.2	.1	.1	.0	.1	1.0
	% Within Diameter of the Varicose Veins on Ultrasound	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	50.0%	2.6%
Non-Muslim	Count	0	0	0	0	0	1	0	0	0	1
	Expected Count	.1	.3	.1	.1	.2	.1	.1	.0	.1	1.0
	% Within Diameter of the Varicose Veins on Ultrasound	0.0%	0.0%	0.0%	0.0%	0.0%	33.3%	0.0%	0.0%	0.0%	2.6%
Total	Count	3	11	5	4	6	3	3	1	2	38
	Expected Count	3.0	11.0	5.0	4.0	6.0	3.0	3.0	1.0	2.0	38.0
	% Within Diameter of the Varicose Veins on Ultrasound	100.0%	100.0 %	100.0 %	100.0 %	100.0 %	100.0 %	100.0 %	100.0 %	100.0 %	100.0 %

Table 4.20: Association of ethnicity with varicose veins

	Value	Df	Asymp. Sig (2-Sided)	Exact Sig. (2-Sided)
Pearson Chi-Square	66.662[a]	40	.005	.[b]
Likelihood Ratio	40.509	40	.448	.153
Fisher's Exact test	49.366			.137
N of Valid Cases	38			

***p*-value < 0.05 = statistically significant (*), <0.01 = highly statistically significant (**)**

Test applied = Chi-square & Fisher's exact test

Table 4.21: Bar graph representing ABO blood group percentage among the groups

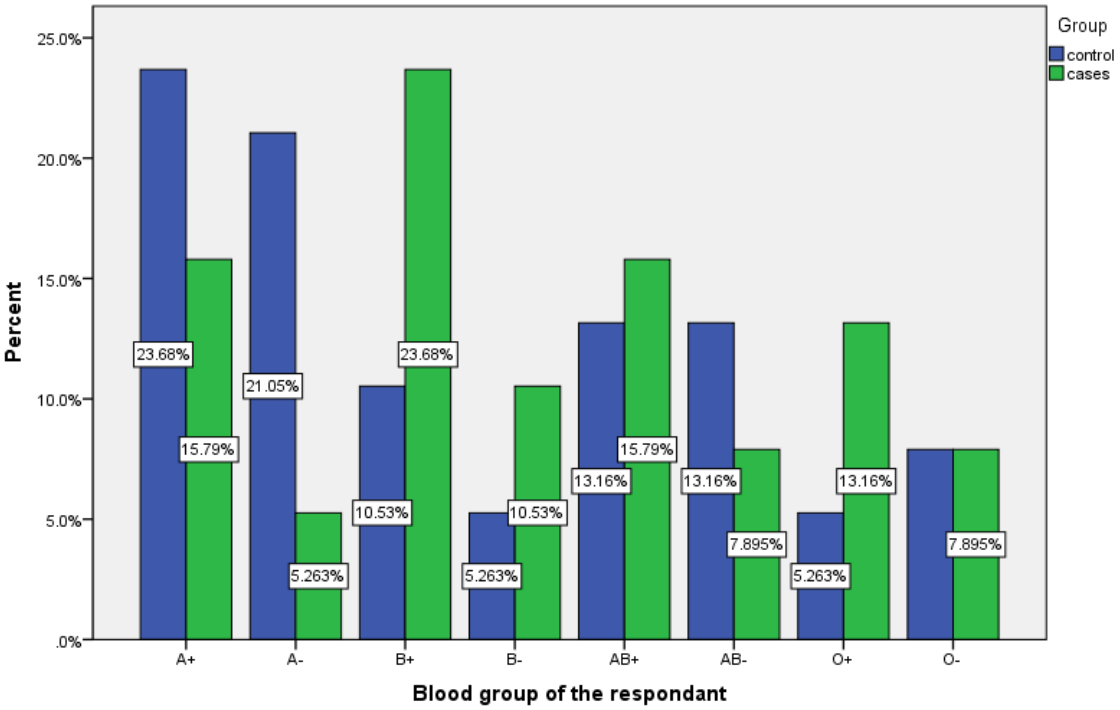


Table 4.22: Association of blood group with the diameter of varicose veins on Doppler ultrasound

Blood Group		Diameter of the varicose veins on doppler ultrasound									Total
		<3mm	3.0 to 3.4 mm	3.5 to 3.9 mm	4.0 to 4.4 mm	4.5 to 4.9 mm	5.0 to 5.9 mm	6.0 to 6.9 mm	7.0 to 7.9 mm	> 8 mm	
A+	Count	0	1	0	2	0	1	2	0	0	6
	Expected Count	.5	1.7	.8	.6	.9	.5	.5	.2	.3	6.0
	% Within Diameter of the Varicose Veins on Ultrasound	0.0%	9.1%	0.0%	50.0%	0.0%	33.3%	66.7%	0.0%	0.0%	15.8%
A-	Count	0	1	0	0	1	0	0	0	0	2
	Expected Count	.2	.6	.3	.2	.3	.2	.2	.1	.1	2.0
	% Within Diameter of the Varicose Veins on Ultrasound	0.0%	9.1%	0.0%	0.0%	16.7%	0.0%	0.0%	0.0%	0.0%	5.3%
B+	Count	2	0	1	0	4	1	0	0	1	9
	Expected Count	.7	2.6	1.2	.9	1.4	.7	.7	.2	.5	9.0
	% Within Diameter of the Varicose Veins on Ultrasound	66.7%	0.0%	20.0 %	0.0%	66.7%	33.3%	0.0%	0.0%	50.0%	23.7%
B-	Count	1	3	0	0	0	0	0	0	0	4
	Expected Count	.3	1.2	.5	.4	.6	.3	.3	.1	.2	4.0
	% Within Diameter of the Varicose Veins on Ultrasound	33.3%	27.3%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	10.5%
AB+	Count	0	2	0	2	1	1	0	0	0	6
	Expected Count	.5	1.7	.8	.6	.9	.5	.5	.2	.3	6.0
	% Within Diameter of the Varicose Veins on Ultrasound	0.0%	18.2%	0.0%	50.0%	16.7%	33.3%	0.0%	0.0%	0.0%	15.8%
AB-	Count	0	0	2	0	0	0	0	0	1	3
	Expected Count	.2	.9	40.0 %	.3	.5	.2	.2	.1	.2	3.0
	% Within Diameter of the Varicose Veins on Ultrasound	0.0%	0.0%		0.0%	0.0%	0.0%	0.0%	0.0%	50.0%	7.9%
O+	Count	0	3	1	0	0	0	1	0	0	5
	Expected Count	.4	1.4	.7	.5	.8	.4	.4	.1	.3	5.0
	% Within Diameter of the Varicose Veins on Ultrasound	0.0%	27.3%	20.0 %	0.0%	0.0%	0.0%	33.3%	0.0%	0.0%	13.2%
O-	Count	0	1	1	0	0	0	0	1	0	3
	Expected Count	.2	.9	.4	.3	.5	.2	.2	.1	.2	3.0
	% Within Diameter of the Varicose Veins on Ultrasound	0.0%	9.1%	20.0 %	0.0%	0.0%	0.0%	0.0%	100.0 %	0.0%	7.9%
Total	Count	3	11	5	4	6	3	3	1	2	38
	Expected Count	3.0	11.0	5.0	4.0	6.0	3.0	3.0	1.0	2.0	38.0
	% Within Diameter of the Varicose Veins on Ultrasound	100.0%	100.0 %	100.0 %	100.0 %	100.0 %	100.0 %	100.0 %	100.0 %	100.0 %	100.0 %

Table 4.23: Association of ABO blood group with varicose veins

	Value	Df	Asymp. Sig (2-Sided)	Exact Sig. (2-Sided)
Pearson Chi-Square	71.443[a]	56	.080	.[b]
Likelihood Ratio	67.686	56	.136	.027
Fisher's Exact test	58.933			.030
N of Valid Cases	38			

***p*-value < 0.05 = statistically significant (*), <0.01 = highly statistically significant (**)**

Test applied = Chi-square & Fisher's exact test

Table 4.24: Bar graph representing occupation percentage among the groups

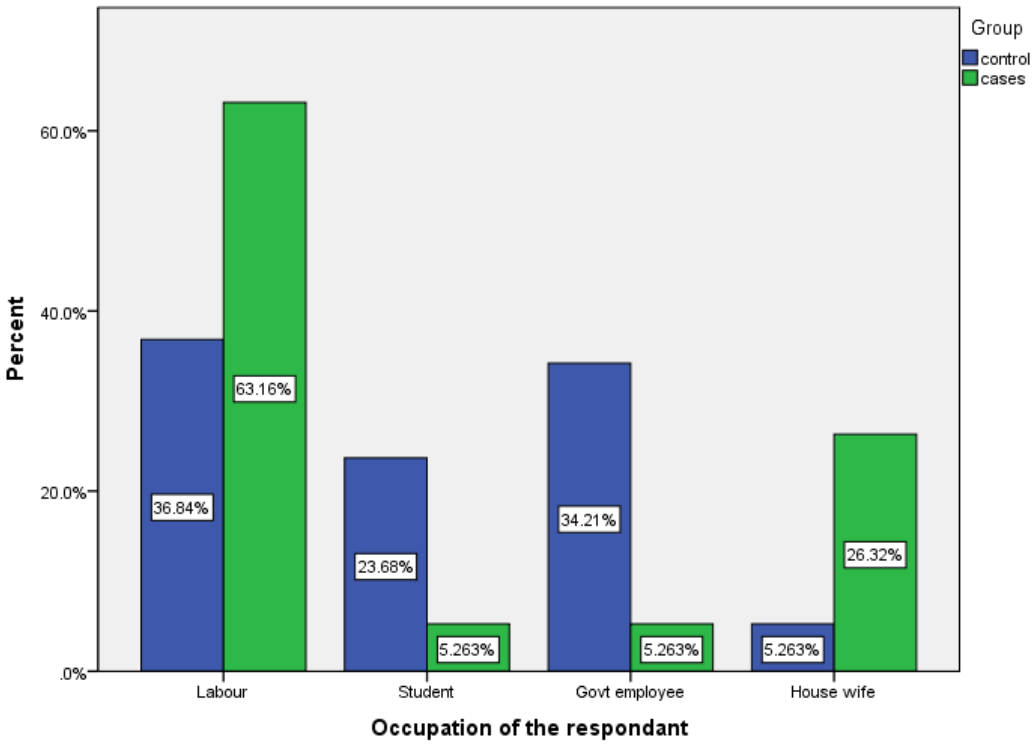


Table 4.25: Association of occupation with the diameter of varicose vein on Doppler ultrasound

Occupation		Diameter of the varicose veins on doppler ultrasound									Total
		<3mm	3.0 to 3.4 mm	3.5 to 3.9 mm	4.0 to 4.4 mm	4.5 to 4.9 mm	5.0 to 5.9 mm	6.0 to 6.9 mm	7.0 to 7.9 mm	> 8 mm	
Laborer	Count	0	5	1	1	1	1	2	0	1	12
	Expected Count	.9	3.5	1.6	1.3	1.9	.9	.9	.3	.6	12.0
	% Within Diameter of the Varicose Veins on Ultrasound	0.0%	45.5%	20.0%	25.0%	16.7%	33.3%	66.7%	0.0%	50.0%	31.6%
Student	Count	1	1	4	2	2	0	1	0	0	11
	Expected Count	.9	3.2	1.4	1.2	1.7	.9	.9	.3	.6	11.0
	% Within Diameter of the Varicose Veins on Ultrasound	33.3%	9.1%	80.0%	50.0%	33.3%	0.0%	33.3%	0.0%	0.0%	28.9%
Government Employee	Count	2	4	0	1	3	1	0	0	1	11
	Expected Count	.9	3.2	1.4	1.2	1.7	.9	.9	.3	.6	11.0
	% Within Diameter of the Varicose Veins on Ultrasound	66.7%	36.4%	0.0%	25.0%	50.0%	33.3%	0.0%	0.0%	0.0%	28.9%
Housewife	Count	0	1	0	0	0	0	0	0	0	2
	Expected Count	.2	.6	.3	.2	.3	.2	.2	.1	.1	2.0
	% Within Diameter of the Varicose Veins on Ultrasound	0.0%	9.1%	0.0%	0.0%	0.0%	0.0%	0.0%	100.0 %	0.0%	5.3%
Total	Count	3	11	5	4	6	3	3	1	2	38
	Expected Count	3.0	11.0	5.0	4.0	6.0	3.0	3.0	1.0	2.0	38.0
	% Within Diameter of the Varicose Veins on Ultrasound	100.0 %	100.0 %	100.0 %	100.0 %	100.0 %	100.0 %	100.0 %	100.0 %	100.0 %	100.0 %

Table 4.26: Association of occupation with varicose veins

	Value	Df	Asymp. Sig (2-Sided)	Exact Sig. (2-Sided)
Pearson Chi-Square	56.662[a]	36	.007	.[b]
Likelihood Ratio	30.509	36	.446	.153
Fisher's Exact test	49.366			.175
N of Valid Cases	38			

***p*-value < 0.05 = statistically significant (*), <0.01 = highly statistically significant (**)**

Test applied = Chi-Square & Fisher's exact test

Table 4.27: Smoking of the respondent * Diameter of varicose vein on Doppler ultrasound

Smoking History		Diameter of the varicose veins on doppler ultrasound									Total
		<3mm	3.0 to 3.4 mm	3.5 to 3.9 mm	4.0 to 4.4 mm	4.5 to 4.9 mm	5.0 to 5.9 mm	6.0 to 6.9 mm	7.0 to 7.9 mm	> 8 mm	
Smoker	Count	0	4	1	1	3	0	1	0	0	10
	Expected Count	.8	2.9	1.3	1.1	1.6	.8	.8	.3	.5	10.0
	% Within Diameter of the Varicose Veins on Ultrasound	0.0%	36.4%	20.0 %	25.0%	50.0%	0.0%	33.3%	0.0%	0.0%	26.3%
Non-Smoker	Count	3	7	4	3	3	3	2	1	2	28
	Expected Count	2.2	8.1	3.7	2.9	4.4	2.2	2.2	.7	1.5	28.0
	% Within Diameter of the Varicose Veins on Ultrasound	100.0%	63.6%	80.0 %	75.0%	50.0%	100.0 %	66.7%	100.0 %	100.0 %	73.7%
Total	Count	3	11	5	4	6	3	3	1	2	38
	Expected Count	3.0	11.0	5.0	4.0	6.0	3.0	3.0	1.0	2.0	38.0
	% Within Diameter of the Varicose Veins on Ultrasound	100.0%	100.0 %	100.0 %	100.0 %	100.0 %	100.0 %	100.0 %	100.0 %	100.0 %	100.0 %

Table 4.28: Association of smoking with varicose veins

	Value	Df	Asymp. Sig (2-Sided)	Exact Sig. (2-Sided)	Exact Sig. (1-Sided)	Point Probability
Pearson Chi-Square	5.705[a]	8	.680	.759		
Likelihood Ratio	7.741	8	.459	.697		
Fisher's Exact test	5.053			.853		
Linear-By-Linear Association	.201[b]	1	.654	.689	.366	.060
N of Valid Cases	38					

***p*-value < 0.05 = statistically significant (*), <0.01 = highly statistically significant (**)**

Test applied = Chi-square & Fisher's exact test

CHAPTER 5

DISCUSSION

5.1 SEQUENCE OF DISCUSSION, ACCORDING TO EXPERIMENT OR HYPOTHESIS

The present study was conducted to investigate the association of varicose vein with levels of inflammatory biomarker. In this study, we analyzed the prevalence and severity of varicose veins according to the CEAP classification, which indicates categories C1 to C6. We have also analyzed chronic venous insufficiency, and the relationship between the diameter of dilated veins and plasma levels of fibrinogen, an inflammatory biomarker, as well as risk factors like age, gender, BMI, smoking, ethnicity, ABO blood group and obesity.

This research was approved by the faculty research committee of the Bahria University of Health Science (FRC-BUHS-50/2022-515), and after receiving approval from the ethical review board, the letter approval number (ERC 62/2022) was issued.

All the Participants were recruited from the Shaheed Mohtarma Benazir Bhutto Institute of Trauma (SMBBIT) Karachi (after seeking permission from the SMBBIT authorities). SMBBIT was inaugurated on December 23rd, 2015. It is the number one trauma center in Sindh. It provides free of charge treatment to traumatically injured patients through specially trained medical personnel with expertise in emergency medicine, and the Centre is at the forefront of providing emergency care with the resources and degree of preparedness to provide total care to patients from prevention to treatment.

The participants belonged to different ethnicities residing in Karachi. Karachi is the largest city in Pakistan and the capital of the province of Sindh, Pakistan. Situated in the southern region of the country, spread along the Arabian Sea coast. It is a heavily populated city of over 20 million and is Pakistan's major industrial and financial center. People from all over the country throng to Karachi to earn their livelihood; therefore, it is linguistically, ethnically, and religiously diverse.

5.1.1 STUDY PARTICIPANTS, AGE AND GENDER

The total number of study participants was 76, including 38 patients and 38 controls. Only those participants were selected who fulfilled our study's inclusion and exclusion criteria and those who had consented to participate in the present study and had signed and filled out the ER-approved consent form and questionnaire.

All the participants belonged to both genders, but men were predominant (73.62%) as compared to women (26.32%). The men-to-women ratio was 2.8:1. The same was found in (kudchadkar et al., 2019) in India, who mentioned a total of 50 patients, including 30 men and 20 women, and men-to-women ratio was 1.5:1. Widmer (1978), in Switzerland, mentioned a Men:Women ratio of 1:1, which means 50% of the participants were men and 50% were women. Leipnitz et al., (1989) in Germany described a ratio of men to women of 1:2. In their study, women participated more as compared to men. Carpentier et al., (2004) collected data from random population of four areas of France. They selected 2000 individuals from each of the four areas and showed women were predominant as 50.5% of the participants were women and 30.1% of the participants were men.

The lower number of women patients in our study was either because women usually avoid seeing clinicians for treatment of this disease due to negligence or due to social constraints. The other reason may be that women do not do as strenuous muscular work as men do. In our study, male participants were mostly laborers doing more strenuous work.

The age of the participants in this study ranged between 20 to 70 years, and majority of the patients (75%) aged between 30 to 60 years; 15.79% of the participants aged less than 30 years; and 7.89% of the patients were senior patients aged more than 60 years. Venous insufficiency is a known progressive illness. VVs can develop at any age, whether young or old. Many studies have mentioned venous reflux even in school-going children of age ranging from 11 to 18 years (Thaler et al., 2001), and the incidence of

disease increases as they grow older. However, older people are at greater risk of developing VVs than younger individuals (Abramson, Hopp, and Epstien, 1981). In a study from India, 50 patients had an age range of 21-80 years (Kudchadkar et al., 2020); in England, 1338 patients had an age range of 20-75 years (Abramson et al., 1981). These studies confirm the present study.

5.1.2 PREVALANCE OF VARICOSE VEINS

Varicose veins are dilated, tortuous, superficial veins with a diameter of three millimeters or more. These are thought to be the most typical manifestation of chronic venous insufficiency (CVI), occurring in the lower limbs (Jawien et al., 2003).

The disease affects all populations and ethnicities worldwide. Varicose veins are reported to affect between 2% to 73% of people worldwide (Aslam et al., 2022). Due to the small sample size, and hospital-based study we were unable to calculate the prevalence in the present study. However, according to (Aslam et al., 2022), 16%–20% of Pakistani population suffer from varicose veins. It affects the 5% of the Indian population (Mishra, Ali, and Singh, 2016).

5.1.3 LIMBS INVOLVEMENT

Chronic venous insufficiency causing dilated veins affects one or both the lower limbs. In the present study, 28 out of 38 patients (72%) presented with unilateral varicosities, and the right lower limb was the most commonly involved. The rest of the ten participants (28%) in the case group presented with bilateral varicosities i.e., both of the lower limbs were involved. The majority of these patients were old subjects. (Kudchadkar et al., 2019) mentioned that the majority of their patients (58%) had unilateral cases involving mostly the left leg. Our findings were contrary to (Kudchadkar et al., 2019). The etiology of one or both legs is not confirmed but it could be due to trauma to the veins in one or both the legs i.e., injury damages the valves within veins in a certain area, and may lead to the formation of varicose veins in the affected leg (Kudchadkar et al., 2019).

5.1.4 SIGN AND SYMPTOMS

All the study subjects in the present study visited the clinic with complaints of pain, swelling, heaviness and itching in the affected areas of the lower limbs. Pain was usually associated with active hours of the day, swelling and feeling of heaviness in lower limb was more evident at the end of the day. Other studies have also described similar complaints. Seidel et al, (2017) observed pain, swelling, tiredness, a sense of heaviness, burning, cramps, and tingling sensation in Brazilian patients. Similar symptoms were mentioned in the Edinburgh vein study, a cross-sectional survey of men and women aged 18 to 64 years resident in Edinburgh, Scotland (Bradbury et al., 1999). Radhakrishnan et al., (2018), Kudchadkar et al., (2019) and Ghosh, Mamun, & Majumder, (2023) also mentioned that the most common symptoms of varicose veins are distended visible veins, pain, aching, swelling, itching, skin changes, ulceration, thrombophlebitis and bleeding in Indian patients.

5.1.5 COMPLICATIONS

It was observed in the present study that the majority of the patients who presented with venous leg ulcers also presented with accompanying skin color changes or hyperpigmentation of the skin around the malleoli and with eczema. Similar complications have been mentioned by Robertson and colleagues from Edinburgh, United Kingdom (2009), who found that in patients who had VVs with skin changes, e.g., lipodermatosclerosis, corona phlebectatica, and eczema, such patients were more likely to progress to a severe form of CVI. Skin trophic changes such as pigmentation or pigmented dermatitis, dermatitis or venous eczema, lipodermatosclerosis, white atrophy, ulcer scars, and active leg ulcers were carefully evaluated, and the presence of pitting edema in the skin over the tibia or malleolar areas was reported in French patients presented with varicose venous disease (Carpentier et al., 2004). Radhakrishnan et al., (2018) from Kerala, India, correlated the above-mentioned skin complications with the severity and diameter of varicose veins. These findings were in accordance with the present study.

5.1.6 SEVERITY OF VARICOSE VEINS ACCORDING TO CEAP CLASSIFICATION

The American Venous Forum developed the first CEAP classification in 1994 to provide a comprehensive classification pertaining to venous diseases. It is now internationally accepted, and almost all clinical research on venous diseases uses the CEAP classification to mention the degree of severity of varicosity. It is in two parts: (i) classification (ii) severity scoring of lower extremity vein disease. It ranges from C0 (no venous disease) to C6 (an open and active ulcer) (Eklof et al., 2004).

In the present study, a total of 38 patients were graded according to CEAP classification on the basis of the severity of their VVs. 60.5% of patients (23 out of 38) presented with venous leg ulcers; these patients were graded the most severe CEAP grading group "C5 and C6". Among the patients in C5 grade, ulcers were present but healing types. In patients in C6 grade, ulcers were of the worst variety and also bled. 2.6% of patients were classified in the C1 grade because they had only telangiectasia (aka thread veins, spider veins, or broken veins) on their affected legs. 10.5% of patients presented with leg edema and were classified as C2 grade. These patients were advised to wear compression stockings to improve their symptoms of the disease, and 15.8% of them were classified as C3 grade. They had somewhat dilated varicose veins evident in their legs, and they complained of swelling in their lower limbs that went worse and worse after standing for long periods. 10.5% of patients are classified as C4-grade because they had varicose veins associated with changes in skin color, hyperpigmentation around the ankles, or eczema in the affected areas of their legs. 7.9% of patients presented with varicose veins associated with healing types of ulcers in their affected regions of the legs and were identified as the C5 category of the CEAP classification. Our findings support those of Poulose and colleagues (2021), who evaluated the severity of CVI according to CEAP grades in a cohort of 57 Indian patients with a male-to-female ratio of 6.1:1 and a mean age of 51.68 years. They discovered 49.12% (C4a), 21.05% (C6), 15.7% (C4b), 7.01% (C3), and 3.50% (C2 and C5). Mishra et al., (2016) reported more or less similar findings in a total of 60 Indian patients, 20 patients (C2), 15 (C3), 15 patients (C4) and 10 patients (C5).

5.1.7 ASSOCIATION OF CEAP CLASSIFICATION WITH DILATED VEINS DIAMETER

In our study, we found that patients with more dilated veins presented with more complicated varicose veins. Stuart and colleagues (2000) assessed the morphology of the veins and its relationship with the severity of CVI. They found that C0 patients had a vein diameter ranging between 1-3 mm, while C2 and C3 patients had vein diameters ranging between 2-4 mm. C4, C5, C6 patients had a vein diameter of 3-5 mm. Results in both studies were in accordance, as an increase in the diameter of the vein was associated with a clinically more advanced stage of varicose veins.

5.1.8 VENOUS HYPERTENSION AND ULCERATION

Sustained ambulatory pressure is termed "venous hypertension", which is also referred to as "chronic venous insufficiency" or "incompetency." It causes obstruction of nutrients and oxygen to the affected region, which results in tissue necrosis and ulcer formation. Venous ulcers are also known as nonhealing wounds or venous stasis ulcers. These are open wounds around the ankle (around the malleolar region) or lower leg, extending into the subcutaneous regions of the leg or foot. Many hypotheses have been postulated for the occurrence of venous ulcers, including the fibrin cuff theory. (Brown Browse and Burnand,1982 cited by Valencia, Falabella, Kirsner, & Eaglstein, 2001) due to sustained venous hypertension, the capillary endothelial gap junctions enlarge, causing leakage of macromolecules, including fibrinogen, from capillaries into surrounding subcutaneous tissue in the leg. Fibrinogen polymerizes into fibrin, which accumulates around the capillaries as a fibrin cuff and acts as a barrier for oxygenation and nutrition of the skin, which leads to necrosis of tissue and ulcers are formed. This hypothesis was supported by Harris, Eaglstein, & Falanga, (1993) in an immunofluorescence study of lipodermatosclerotic nonulcerative skin samples taken from the edges of the venous ulcers and from the ulcer bed, which showed the presence of pericapillary fibrin cuffs. In an in vitro study, Burnand et al. (1982) revealed that the presence of fibrin sheets causes a hindrance in the diffusion of oxygen. Decreased cutaneous oxygenation in patients with chronic venous insufficiency has been reported in the literature. Another hypothesis for venous ulcer formation was white blood cell trapping (Coleridge Smith et al., 1988, cited by Valencia et al., 2001). Due to venous hypertension, the WBCs adhere (trap) to the

capillaries' endothelial lining and initiate the release of proteolytic enzymes, including collagenase and elastase, cytokines, and superoxide metabolites, which would promote vascular permeability and release of macromolecules as fibrinogen into the tissue around the capillaries hence leads to venous ulceration (Valencia et al., 2001). The majority of the patients in the present study (60.5%, 23 patients out of 38) presented with venous leg ulcers; these patients were categorized into the most severe CEAP grading group of C5 and C6, which was characterized by the presence of venous ulcers in the legs.

5.1.9 ANALYSIS OF PLASMA FIBRINOGEN CONCENTRATIONS

Fibrinogen is a soluble plasma glycoprotein that plays an essential role in blood clotting or coagulation. It is polymerized into fibrin by thrombin through the coagulation pathway to form the main bulk of the clot (Salomon et al., 2019).

Fibrinogen is a 340-kDa dimeric glycoprotein synthesized in the liver. Each dimer consists of three different polypeptide chains known as α-, β-, and γ- chains, held together by 29 disulfide bonds. These α, β-, and γ polypeptide chains are encoded by 3 different genes, FGA, FGB, and FGG, all of which are grouped on chromosome 4 in region q^{32} (Vu and Neerman, 2007).

In healthy individuals, fibrinogen circulates in plasma at high concentrations (2–4 mg/mL). However, fibrinogen is an acute phase protein, and during acute inflammation, plasma fibrinogen levels can exceed 7 mg/mL, which is essential for a variety of processes, including blood clot formation, wound healing, inflammation, and blood vessel growth (Kattula, Byrnes, & Wolberg, 2017). Studies have shown elevated plasma fibrinogen levels in patients with venous disease and ulceration (Nicolaides., 2000).

Increased plasma fibrinogen levels, pericapillary fibrin deposits, and increased levels of fibrinogen degradation products have been observed in patients with venous ulcers, which is indicative of a rapid turnover of the fibrinogen macromolecule, and decreased fibrinolysis leads to a condition of hyperfibrinogenemia mentioned in patients with severe and non-healing types of ulcers (Nicolaides., 2000; Van de Scheur & Falanga, 1997).

The implications of fibrinogen in causing severity of varicosity and ulcer formation were confirmed in the present study and the studies mentioned hereto.

In the present study, plasma fibrinogen levels were analyzed in both cases and controls. In controls, plasma fibrinogen levels were found to be within normal ranges (< 4 g/l), whereas in the case group, fibrinogen levels were found to be significantly raised, ranging from 3.5 g/l to 5 g/l. These levels were higher in the more advanced stages of the varicose veins. Mean fibrinogen levels in plasma were 2.7105 ± 2.02562 g/l (Table 4.4). Higher levels of fibrinogen in more advanced stages of disease in our study support the hypothesis that perivascular fibrin cuffs and delayed fibrinolysis is a main cause in skin color changes and ulcer formation.

Our results are supported by Matei and colleagues (2022), who analyzed several parameters in the blood of participants, including fibrinogen concentrations. Levels of fibrinogen were highest (412.69 ± 111.29 mg/dl; p = 0.0095) in patients with severe disease groups C5 and C6 of the CEAP classification. The mean fibrinogen level was 317.18 ± 80.32 mg/dl (p = 0.0004), in patients in C2-C3 grades. The mean fibrinogen level was 368.48 ± 104.90 mg/dl (p = 0.0001) in patients with a C4 grade. These results coincide with our results (p =.000), which were significant (Table 4.5).

Further affirmation to our study results was made by Tiwary and colleagues (2020) who evaluated the interleukin-6 (IL-6), hemoglobin (Hb), and fibrinogen levels between the blood of varicose veins of the lower limb and the normal peripheral blood of antecubital veins of the same individuals and found that concentrations of hemoglobin (p = 0.012), IL-6 (p =0.001), and fibrinogen (p =0.001) were significantly higher in the blood of varicose veins from the lower limb as compared to the blood from antecubital veins of the same individuals. They concluded that the mean plasma fibrinogen level of 5.4439 ± 1.317 in the blood is indicative of the severity of VVs. These elevated plasma fibrinogen levels in VVs patients are in accord with the present study.

Increased levels of fibrinogen degradation products i.e., D-dimer have been observed in the blood of varicose veins as compared to systemic circulation in the same individual and were confirmed to be indicative of a rapid turnover of the fibrinogen macromolecule in the pathogenicity of the varicosity and ulceration. Although, fibrinogen levels were also higher in blood collected from VVs in comparison with systemic peripheral blood of the same patient, however, no statistically significant difference was present in fibrinogen concentration in VVs blood and systemic blood (p = 0.93) (Poredos et al., 2015). In the current study D-dimer levels were not analyzed in VVs patients.

Flore and colleagues (2015) compared the concentrations of biomarkers including fibrinogen in the blood of patients who presented with unilateral CVD (U-CVD) and

bilateral CVD (B-CVD). Mean fibrinogen levels in U-CVD were 260.31 ± 53.67 mg/dl, and in B-CVD were 278.12 ± 48.26 mg/dl. Significantly higher fibrinogen levels were observed in B-CVD (p = 0.005) as compared to U-CVD. This elevated concentration of fibrinogen in B-CVD is similar to the significantly elevated fibrinogen levels mentioned in the present study.

Karahan and colleagues (2016) evaluated the changes in inflammatory marker levels (WBCs, platelets, albumin, D-dimer, fibrinogen, and the fibrinogen to albumin ratio), which can help in the prediction of the progression and severity of VVs disease. They divided 80 patients into mild, moderate, and severe VVs. Mean fibrinogen levels were 210.00 ± 63.08, 277.31 ± 64.38, and 312.70 ± 90.85 µg/ml in mild, moderate, and severe clinical classes of VVs, respectively. Results were statistically significant (p = 0.001). Fibrinogen was also found to be statistically significant as a predictor marker for the progression and severity of the VVs in moderate to severe cases (p = 0.006).

Gomez and colleagues in 2013 assessed CRP and fibrinogen levels in the blood of patients suffering from VVs and healthy individuals. CRP was found to be significantly lower in VVs patients, and there was no difference observed in fibrinogen levels in either group. These results were contradictory to the findings mentioned here.

Pola and colleagues in 1994 analyzed the plasma fibrinogen levels in 20 VVs patients and 10 healthy individuals. A sample was collected twice from the participants. After the initial blood sample collection, participants were asked to stand for one hour, and then a second blood sample was collected. It was observed that plasma fibrinogen levels were significantly higher in patients with VVs when compared with healthy participants. These levels were found to increase further after one hour of standing. Compared with the baseline values, fibrinogen was found to be higher in healthy individuals after one hour of standing but less significant than in the VVs group. There was a statistically significant difference in the fibrinogen concentration at baseline and after one hour of standing (p = 0.01). These elevated fibrinogen levels in VVs patients are similar to those in the present study.

5.1.10 BODY MASS INDEX AND OBESITY

Obesity is considered a risk factor for the occurrence of VVs by many authors. But the results are heterogeneous, as in many studies, BMI has shown no association with

the occurrence of varicose veins. In the present study, a larger proportion of the cases were found to be overweight or obese. In this larger group of patients with abnormally high BMI, 39.47% of the cases were overweight (BMI 25–30 kg/m²), and 28.95% of the patients were obese (BMI > 30 kg/m²). A large number of these patients presented with the C5 and C6 clinical classes of chronic venous disease (CVD), and the majority of these cases were male (73.68%). It was observed in the present study that the clinical category of the CEAP classification was higher in overweight and obese individuals. This is why the role of a higher BMI in the severity of varicose veins cannot be ignored. In the current study, statistically, no significant association was seen between BMI and the occurrence of VVs (p =.438). Research with a larger number of participants may provide more appropriate results about the association of BMI with the occurrence of varicose veins.

Shrestha and Karmacharya (2020) compared the severity of the CVD in obese and non-obese patients in Nepal; a significant positive correlation between BMI and the clinical class of the CVD was observed in female patients but not in male patients with CVD. Obesity was significantly more frequent in the C3 and C4 clinical classes of CVD. They also observed a significant association between age and the clinical staging of VVs (p = 0.05). In the current study, we did not find any significant association of age with the occurrence of VVs (p =.460).

Vlajinac and colleagues (2013) observed the association of BMI with the clinical class of CVD. 65.6% of the participants were female patients, the majority of the patients (42.7%) were overweight and advanced category of the CEAP classification was found to be significantly present in both overweight and obese participants independent of age and gender (p = 0.001). More advanced clinical stages of CVD in overweight and obese individuals are considered to occur as a consequence of raised intra-abdominal pressure leading to greater reflux, increased vein diameter, and venous pressure in these individuals (van Rij et al., 2008).

Robertson and colleagues (2009), in their study found a statistically significant association between BMI (p =.006) and smoking (p =.009) and the clinical severity of the VVs. These results were contradictory to what we observed in the current study. Smoking was not found to be significant in our study.

The majority of the patients in our study, accounting for 63.16% of the cases, were laborers and had a history of long standing during working hours. Selçuk, Uzun, Fen, & Kapisiz, (2014) observed the association between BMI, age, number of pregnancies, standing for more than 8 hours without a break, comorbidities, and family history in the

etiology of VVs. They found a positively significant association between standing for a long period of time and the occurrence of VVs (p = 0.001). These results are similar to those in the present study.

Hypertension was found to be the most common comorbidity (28.6%) in the study of Selçuk Kapisiz and collegues (2014) which was also found to be the most common comorbidity in present study (7.89%).

5.1.11 POSTURE AND OCCUPATION

The majority of the participants in the current study were daily working laborers like shopkeepers, barbers, and tailors. They had a history of several hours of standing while performing their jobs. Łastowiecka-Moras (2021) found a statistically significant positive correlation (p<0.05) between the occurrence of VVs and the posture of participants while performing their duties e.g., sitting more than 4 hours/day, working in standing position, picking up and carrying heavy objects, fast paced work and computer work and a negative correlation was seen between presence of VVs and use of footrest while working on a computer. Łastowiecka-Moras (2021) also found a statistically significant positive correlation (p<0.05) between occurrence of VVs and age, smoking, number of pregnancies and fast food consumption and these results were condradictory to the present study. In our study no statistically significant correlation was seen between posture during occupation (p =.520).

5.1.12 ABO BLOOD GROUP ASSOCIATION WITH VARICOSE VEINS

The most prevailing blood group system in humans is the ABO blood group system. The best-known ABO phenotypes among the population are A, B, AB, and O. Several associations between particular ABO phenotypes and increased susceptibility to disease have been identified (Mitra, Mishra, & Rath, 2014). A link between the ABO phenotype and stomach ulcers has been identified more commonly in individuals in the O group; and gastric cancer was associated with group A individuals (Mitra et al., 2014). The association between ABO blood groups and venous thromboembolism (VTE) was first reported in 1969 by Jick et al (Yu et al., 2017). Many studies have shown the relationship between ABO blood groups and deep vein thrombosis (DVT). Some studies

have shown that non-O blood groups, particularly those with the A-allele, are associated with a higher incidence of DVT than blood group O (Gallerani, Reverberi, & Manfredini, 2014). Yu et al., (2017) in the Chinese population, found the same association: individuals having A, B, and AB blood types had a higher risk of DVT than individuals having O blood types (Yu et al., 2017).

In the present study, we performed ABO blood typing in VVs patients and controls. A+ve was found to be the most common blood group in the controls, as nine individuals (23.7%) had this particular group, and B+ve was found to be the most common blood group in cases, which account for nine (23.7%) of the cases (Table 4.15). Statistically, no significant association was present between the ABO blood group of the patients and the occurrence of varicose veins (p =.080) (Table 4.29). The association between varicose vein disease and the ABO group is not sufficiently mentioned in the literature. However, our results are in accord with the findings of Yu et al. (2017) and Gallerani et al. (2014) that non-O blood groups had a higher risk of DVT or a venous disease than O blood types.

5.2 IMPLICATIONS OF THE STUDY

5.2.1 THEORETICAL IMPLICATIONS

We evaluated the occurrence of CVI among local ethnicities in Karachi, Pakistan. This is the very first study in Pakistan in which the frequency of the most commonly affected blood groups in varicose vein disease was analyzed for the first time. Measurement of the inflammatory markers i.e., fibrinogen, and its correlation with the severity of varicose vein disease in patients in Pakistan has not been done before. This is the very first study in Pakistan that successfully confirmed the association of hyperfibrinogenemia with the increase in the diameter of the varicose vein and the CEAP classification. This study endorses the theory that inflammation plays a role in the etiology of varicose veins and ulcer formation.

5.2.2 PRACTICAL IMPLICATIONS

To the best of our knowledge, this study has provided substantial material in the existing literature in the context of the association of fibrinogen levels with varicose veins. The results of the present study showed that fibrinogen concentrations are raised in patients suffering from varicose veins, and this increase in the plasma levels of inflammatory biomarkers is associated with the variation in the diameter of the varicose veins and ulceration. The caretakers and doctors could include the assay of inflammatory biomarkers, i.e., fibrinogen, in the assessment and prognosis of varicose vein and its severity, and to ensure the efficacy of the treatment given to varicose vein patients.

5.2.3 POLICY IMPLICATIONS

Radiological facilities are inadequate in most of the hospitals present in the rural and suburban areas of Pakistan. Based on the results of this study, policies can be made to use inflammatory biomarkers to analyze the severity, prognosis, and efficacy of management for patients suffering from varicose veins. These biomarkers, such as fibrinogen, are comparatively cheaper, easier to obtain, and more accessible than Doppler ultrasound.

5.3 LIMITATIONS

- This was a single center study
- The sample size was small because we recruited only those who consented to participate in our study and fulfilled our research criteria
- The results of several parameters were insignificant because of small sample size
- Vascular surgery departments were not available in most of the public sector hospitals so it was difficult to approach varicose vein patients and SMBBIT is the only public sector hospital catering the entire population from Sindh and other provinces of Pakistan
- Due to cultural constraints, female patients avoid visiting hospitals, making it impossible to have an equal number of genders in each group

5.4 STRENGTHS

- To the best of our knowledge such research work has been done for the first time in Pakistan
- This is a pioneer study as the association of Doppler ultrasound findings with inflammatory biomarkers has never been done before

5.5 RECOMMENDATIONS

- Further studies should be done in future with a larger sample size
- Studies should be done at molecular levels to identify the genes associated with varicose vein disease
- Other inflammatory biomarkers should be analyzed that are involved in venous diseases
- Multicentric studies should be done

5.6 CONCLUSION

The pathophysiology of varicose veins is associated with levels of fibrinogen. An increase in the levels of fibrinogen causes an increase in the diameter of abnormally dilated superficial veins and changes in the color of the skin of the affected area (lipodermatosclerosis) due to a lack of oxygen and nourishment, which results in ulcer or scar formation. Fibrinogen, as an inflammatory biomarker, can be used to assess the severity of CVI, the prognosis, and the efficacy of the surgical management provided to patients with varicose veins. Hyperfibrinogenemia in the present study confirmed the role of inflammatory biomarkers (fibrinogen) in the etiology of varicose veins.

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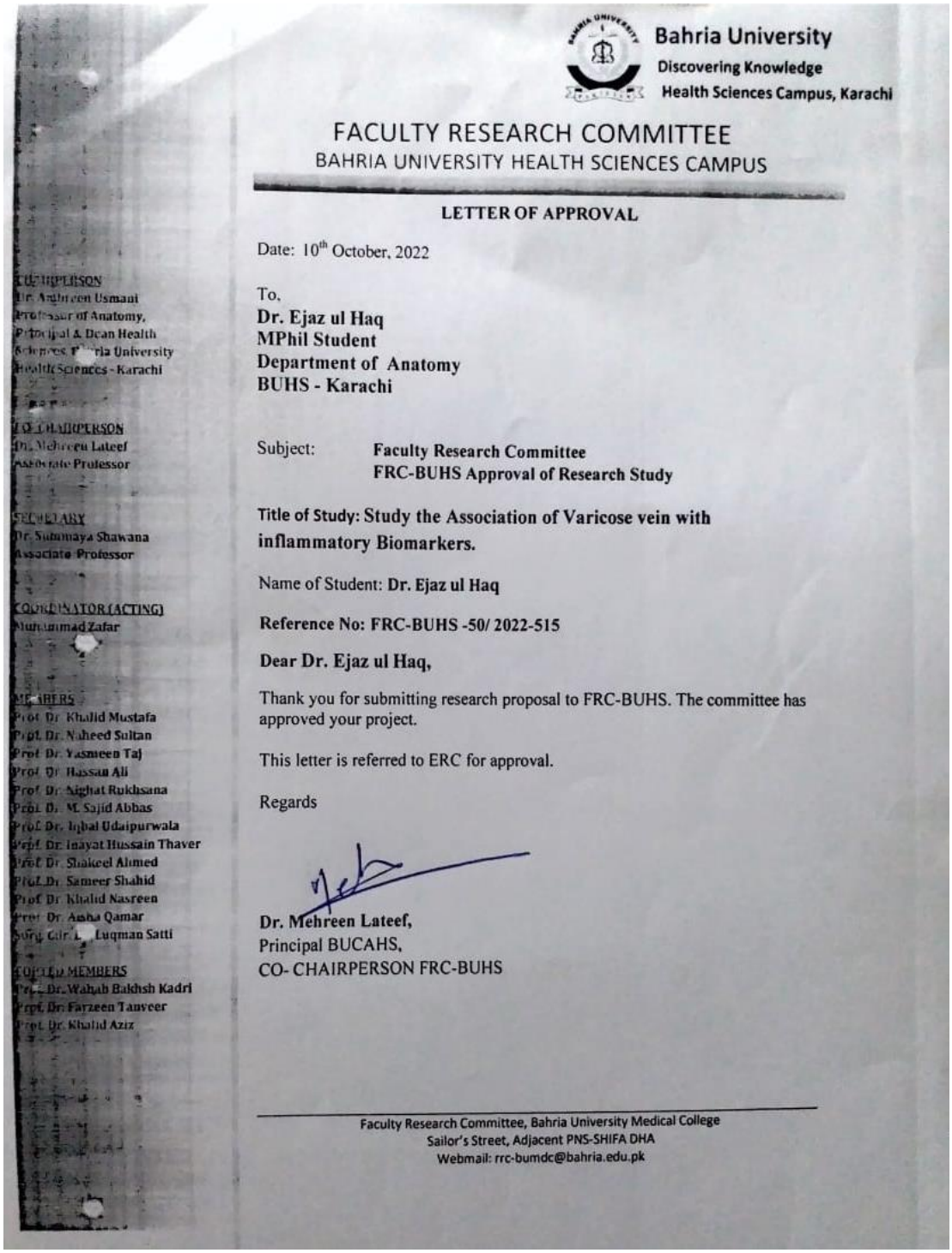
APPENDIX A: FRC



Bahria University
Discovering Knowledge
Health Sciences Campus, Karachi

FACULTY RESEARCH COMMITTEE
BAHRIA UNIVERSITY HEALTH SCIENCES CAMPUS

LETTER OF APPROVAL

Date: 10th October, 2022

To,
Dr. Ejaz ul Haq
MPhil Student
Department of Anatomy
BUHS - Karachi

Subject: **Faculty Research Committee**
FRC-BUHS Approval of Research Study

Title of Study: Study the Association of Varicose vein with inflammatory Biomarkers.

Name of Student: **Dr. Ejaz ul Haq**

Reference No: FRC-BUHS -50/ 2022-515

Dear Dr. Ejaz ul Haq,

Thank you for submitting research proposal to FRC-BUHS. The committee has approved your project.

This letter is referred to ERC for approval.

Regards

Dr. Mehreen Lateef,
Principal BUCAHS,
CO- CHAIRPERSON FRC-BUHS

CO-CHAIRPERSON
Dr. Mehreen Usmani
Professor of Anatomy,
Principal & Dean Health
Sciences Bahria University
Health Sciences - Karachi

CO-CHAIRPERSON
Dr. Mehreen Lateef
Associate Professor

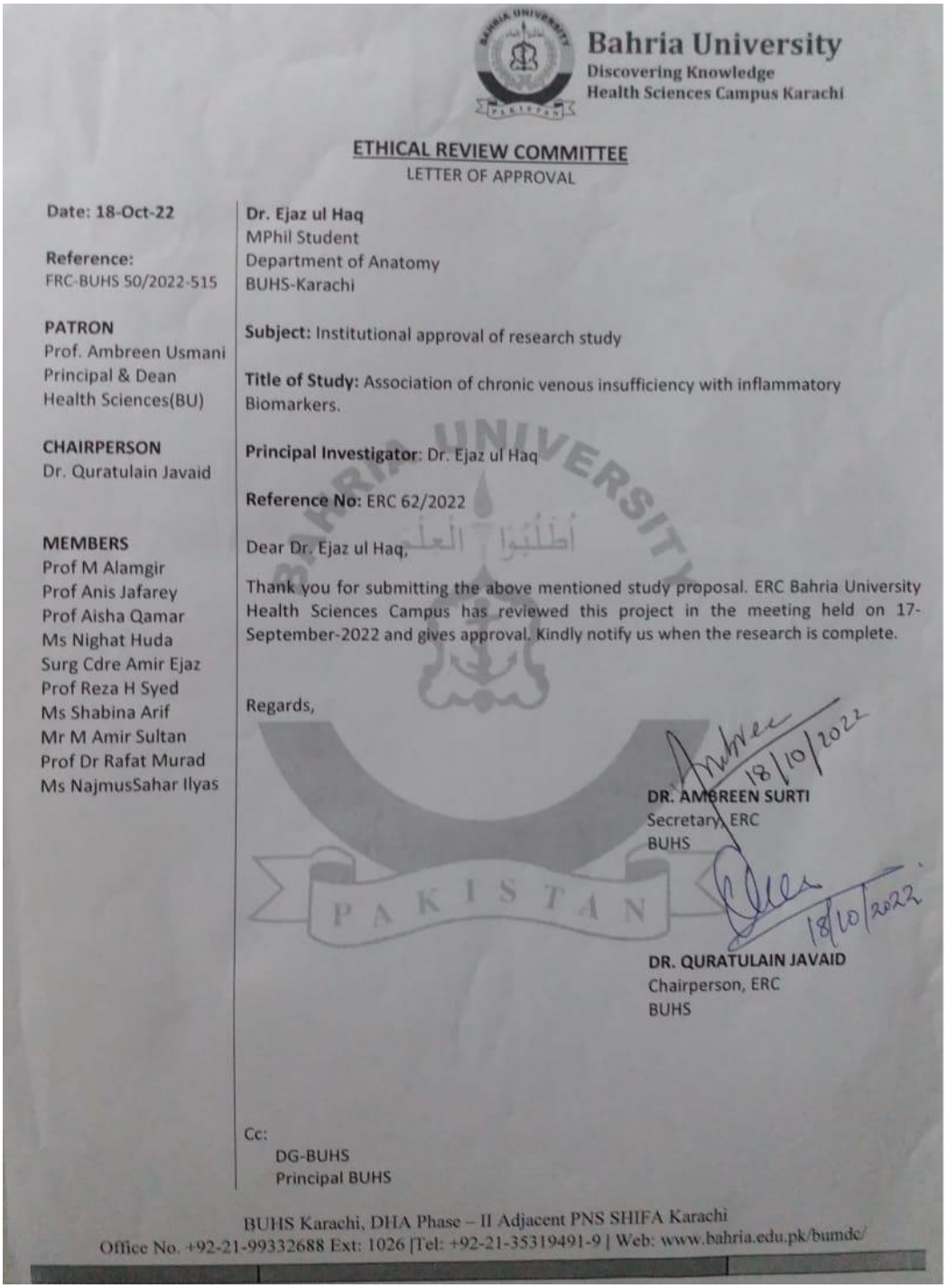
SECRETARY
Dr. Subhaya Shawana
Associate Professor

COORDINATOR (ACTING)
Muhammad Zafar

MEMBERS
Prof. Dr. Khalid Mustafa
Prof. Dr. Naheed Sultan
Prof. Dr. Yasmeen Taj
Prof. Dr. Hassan Ali
Prof. Dr. Aighat Rukhsana
Prof. Dr. M. Sajid Abbas
Prof. Dr. Iqbal Udaipurwala
Prof. Dr. Inayat Hussain Thaver
Prof. Dr. Shakeel Ahmed
Prof. Dr. Sameer Shahid
Prof. Dr. Khalid Nasreen
Prof. Dr. Amna Qamar
Surg. Cdr. (R) Luqman Satti

CO-OPTED MEMBERS
Prof. Dr. Wahab Baloch Kadri
Prof. Dr. Farzeen Tanveer
Prof. Dr. Khalid Aziz

APPENDIX B: ERC



Bahria University
Discovering Knowledge
Health Sciences Campus Karachi

ETHICAL REVIEW COMMITTEE
LETTER OF APPROVAL

Date: 18-Oct-22

Reference:
FRC-BUHS 50/2022-515

PATRON
Prof. Ambreen Usmani
Principal & Dean
Health Sciences(BU)

CHAIRPERSON
Dr. Quratulain Javaid

MEMBERS
Prof M Alamgir
Prof Anis Jafarey
Prof Aisha Qamar
Ms Nighat Huda
Surg Cdre Amir Ejaz
Prof Reza H Syed
Ms Shabina Arif
Mr M Amir Sultan
Prof Dr Rafat Murad
Ms NajmusSahar Ilyas

Dr. Ejaz ul Haq
MPhil Student
Department of Anatomy
BUHS-Karachi

Subject: Institutional approval of research study

Title of Study: Association of chronic venous insufficiency with inflammatory Biomarkers.

Principal Investigator: Dr. Ejaz ul Haq

Reference No: ERC 62/2022

Dear Dr. Ejaz ul Haq,

Thank you for submitting the above mentioned study proposal. ERC Bahria University Health Sciences Campus has reviewed this project in the meeting held on 17-September-2022 and gives approval. Kindly notify us when the research is complete.

Regards,

18/10/2022

DR. AMBREEN SURTI
Secretary, ERC
BUHS

18/10/2022

DR. QURATULAIN JAVAID
Chairperson, ERC
BUHS

Cc:
DG-BUHS
Principal BUHS

BUHS Karachi, DHA Phase – II Adjacent PNS SHIFA Karachi
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APPENDIX C: SUBJECT CONSENT FORM - ENGLISH

WRITTEN INFORMED CONSENT FORM OF PATIENT

You are being asked to participate in a research study designed to assess the relationship between abnormally dilated blood vessel in your legs with an inflammatory biomarker called fibrinogen in your blood.

You will be asked to undergo Doppler ultrasound of dilated veins to measure the changes that have occurred in your veins at the same time minute quantity of your blood is drawn from upper limb to assess fibrinogen levels in your body. This will take 1-2 hours only.

You have been explained that ultrasound and blood sample is collected to examine and evaluate your health status. Ultrasound is a safe, noninvasive and radiation less procedure and blood will be collected in a minute concentration that has no effect on your health.

You have been explained that this study will help to notify you about prognosis, treatment efficacy and will also help to determine the severity of your condition.

You have been explained that there is no financial compensation for your participation in this research.

You have been told that your identity in this study will be treated as confidential, the results of the study maybe published for scientific purpose but will not give your name or identifiable reference to you. However any record or data may be inspected by sponsor or SMMBIT ERC members.

You are clearly explained that you are free to choose whether or not to participate in this study. There will be no penalty if you choose not to participate. You will be provided with any new significant finding during course of this study that may relate to you or influence your wellbeing to continue participation. In addition your participation in study may be terminated by the investigator or sponsor without your consent.

You are advised to contact **Dr Ejaz ul haq** on mobile number **03136116011** in case of any query.

I have read and understood this consent form and I volunteer to participate in this research study. I understand I will receive a copy of this form.

Participant Name: _____ Date _____

Signature of Participant: _____ Date _____

Name of Researcher: _____ Date _____

Signature of Researcher: _____ Date _____

APPENDIX D: SUBJECT CONSENT FORM - URDU

مریض کے لئے اجازت فارم

آپ سے ایک تحقیقی مطالعہ میں حصہ لینے کے لیے کہا جا رہا ہے جو آپ کے خون میں فائبرنوجن نامی سوزش والے بائیو مارکر کے ساتھ آپ کی ٹانگوں میں غیر معمولی طور پر پھیلی ہوئی خون کی نالیوں کے درمیان تعلق کا جائزہ لینے کے لیے ڈیزائن کیا گیا ہے۔

آپ کو آپ کی رگوں میں ہونے والی تبدیلیوں کی پیمائش کرنے کے لیے خستہ حال رگوں کا ٹوپلر الٹراساؤنڈ کرانے کے لیے کہا جائے گا، اسی وقت آپ کے جسم میں فائبرنوجن کی سطح کا اندازہ لگانے کے لیے آپ کے خون کی مقدار اوپری اعضاء سے نکالی جاتی ہے۔ اس میں صرف 1-2 گھنٹے لگیں گے۔

آپ کو بتایا گیا ہے کہ الٹراساؤنڈ اور خون کا نمونہ آپ کی صحت کی حالت کو جانچنے اور جانچنے کے لیے جمع کیا جاتا ہے۔ الٹراساؤنڈ ایک محفوظ، غیر حملہ آور اور تابکاری سے کم طریقہ کار ہے اور خون ایک منٹ کے ارتکاز میں جمع کیا جائے گا جس کا آپ کی صحت پر کوئی اثر نہیں پڑے گا۔

آپ کو بتایا گیا ہے کہ یہ مطالعہ آپ کو تشخیص، علاج کی افادیت کے بارے میں مطلع کرنے میں مدد کرے گا اور آپ کی حالت کی شدت کا تعین کرنے میں بھی مدد کرے گا۔

آپ کو وضاحت کی گئی ہے کہ اس تحقیق میں آپ کی شرکت کے لیے کوئی مالی معاوضہ نہیں ہے۔

آپ کو بتایا گیا ہے کہ اس مطالعہ میں آپ کی شناخت کو خفیہ رکھا جائے گا، مطالعہ کے نتائج سائنسی مقصد کے لیے شائع کیے جا سکتے ہیں لیکن آپ کا نام یا آپ کو قابل شناخت حوالہ نہیں دیا جائے گا۔ تاہم کسی بھی ریگولیٹری باڈی کا ممبران کر سکتے ہیں۔ SMMBIT ERC معائنہ اسپانسر یا

آپ کو واضح طور پر وضاحت کی گئی ہے کہ آپ اس مطالعہ میں حصہ لینے یا نہ کرنے کا انتخاب کرنے کے لیے آزاد ہیں۔ اگر آپ شرکت نہ کرنے کا انتخاب کرتے ہیں تو کوئی جرمانہ نہیں ہوگا۔ اس مطالعہ کے دوران آپ کو کوئی بھی نئی اہم تلاش فراہم کی جائے گی جو آپ سے متعلق ہو سکتی ہے یا شرکت جاری رکھنے کے لیے آپ کی صحت کو متاثر کر سکتی ہے۔ اس کے علاوہ تحقیق کار یا کفیل آپ کی رضامندی کے بغیر مطالعہ میں آپ کی شرکت کو ختم کر سکتا ہے۔

آپ کو مشورہ دیا جاتا ہے کہ کسی بھی سوال کی صورت میں ڈاکٹر اعجاز الحق سے موبائل نمبر 03136116011 پر رابطہ کریں۔

میں نے رضامندی کے اس فارم کو پڑھ اور سمجھ لیا ہے اور میں اس تحقیقی مطالعہ میں حصہ لینے کے لیے رضاکارانہ طور پر تیار ہوں۔ میں سمجھتا ہوں کہ مجھے اس فارم کی ایک کاپی مل جائے گی۔

شریک کنندہ کا نام:_____تاریخ _____

شرکت کنندہ کے دستخط:_____ تاریخ _____

محقق کا نام:_____تاریخ _____

محقق کے دستخط:_____تاریخ _____

APPENDIX E: QUESTIONNAIRE

QUESTIONNAIRE

Name _____ Father/ Husband Name _____

Age _____Years Gender: Male / Female

Address _____

Contact _____

Weight _____ kg Height _____ m BMI _____

Hypertension Yes / No Diabetes Miletus Yes / No

Stroke Yes / No Myocardial Infarction Yes / No

1. Do you have dilated veins in lower limbs? Yes / No
2. Do you feel pain in effected leg? Yes / No
3. Do you stand for hours at your job? Yes / No
4. Do you feel any swelling in lower limbs? Yes / No
5. Do you feel heaviness in lower limbs? Yes / No
6. Does it affect your daily routine activities? Yes / No
7. Do you have a history of coagulation disorder i.e. DVT? Yes / No
8. Currently are you taking any medicines? Yes / No
9. Do you feel any skin color changes over distended veins? Yes / No
10. Have you ever had lower limb ulcer formation? Yes / No
11. Did you had any vascular disease before? Yes / No
12. Do you feel heaviness in your legs at the end of the day? Yes / No
13. Any previous history of injury which involved lower limbs? Yes / No
14. Did you ever had any bone fracture involving legs? Yes / No
15. Did you ever had itching sensation over dilated veins? Yes / No
16. Do you feel any hardness over dilated veins? Yes / No

APPENDIX F: HOSPITAL CONSENT FORM

SHAHEED MOHTARMA BENAZIR BHUTTO INSTITUTE OF TRAUMA, KARACHI

Ethics Review Committee(ERC)

Ref #: ERC-000052/SMBBIT/Approval/2022 **Dated:** Aug 04, 2022

Revised Date: Apr 18, 2023

Dr. Ejaz Ul Haq
M Phil Scholar
Departmen of Anatomy
Bahria University Health Sciences Karachi Campus

Subject: **Ethics Review Committee's approval for a research proposal**

Revised Title: **Study the Association of Varicosvein with inflammatory Biomarkers.**

Dear Dr. Ejaz-Ul-Haq

Thank you for submitting the application regarding changing the research study title, "Study the Association of Chronic venous insufficiency with inflammatory biomarkers and blood cell parameters".This proposal was reviewed on August 4th, 2022 by the ERC-SMBB Institute of Trauma, and was approved to conduct this study.

Your revised study title is mentioned above. Any change in the protocol or extension in the study period must be notified to the Board for approval. Interim reports on the progress of the study should be submitted to ERC in six months.

Dr. Waryam
Consultant Vascular Surgery Department
Chair of Ethics Review Committee
SMBB Institute of Trauma,
Karachi.

Dr. Muhammad Sabir Memon
Executive Director
SMBB Institute of Trauma,
Karachi.

Chand Bibi Road, Karachi-74200, Pakistan. Tel: 99215740-45, Fax: 99216490
E-mail: info@smbbtc.gos.pk Website: http://www.smbbtc.gos.pk

APPENDIX G: SUBJECT EVALUATION FORM

S.NO: _____

Name _____ Father/ Husband Name _____

Age _____Years Gender: Male / Female

Ethnicity _____ Marital status Married / Unmarried

Weight _____ kg Height _____ m BMI _____

Co-Morbid

Hypertension Yes / No Diabetes Yes / No

Occupation include hours of upright posture Yes / No

Year varicose veins was diagnosed _____

Most Common Symptoms _____

Any visible ulcer formation _____

Any skin color changes evident _____

Associated Symptoms _____

Any psychological issues _____

Any family member having same illness _____

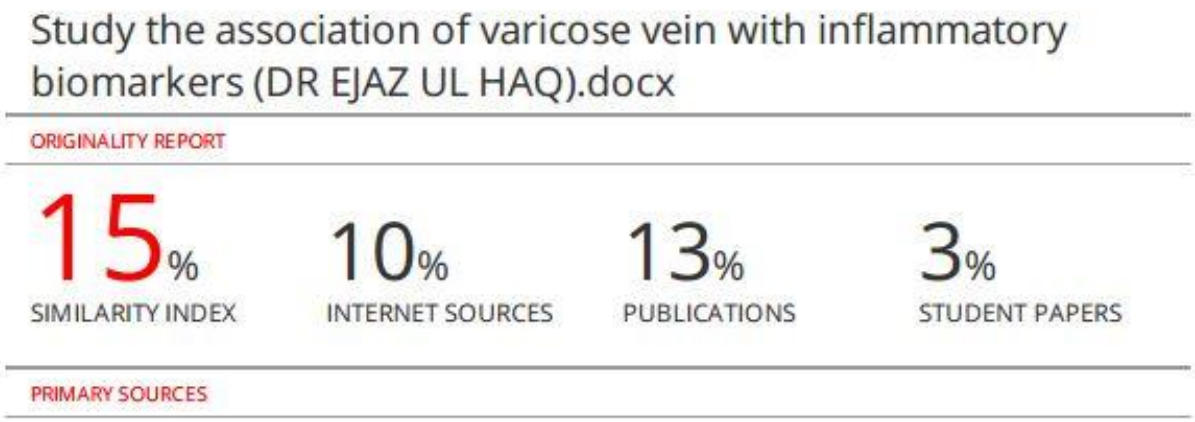
Currently taking any medicine _____

Other co-existing medical conditions _____

MEDICATION HISTORY: Please list any medications you are currently taking

MEDICATION	**DOSE**	**REASON**

APPENDIX H: TURNITIN PLAGIARISM CHECK REPORT



Study the association of varicose vein with inflammatory biomarkers (DR EJAZ UL HAQ).docx

ORIGINALITY REPORT

15%	10%	13%	3%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES