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Evaluation of the pathophysiology of Varicose veins in pregnancy

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Abstract

Introduction: Varicose veins (VVs) are characterised by tortuous and dilated superficial veins located in the lower extremities. The manifestation of VVs symptoms is contingent upon the severity and progression of the disease. The prevalence of VVs is greater among women, particularly those with a history of pregnancy, in comparison to men as well as women who have not given birth. VVs complications impact patients' quality of life (QOL) and healthcare costs. Understanding VVs causes may aid patients' well-being and cut healthcare expenses.

Hypothesis: The development of post-partum VVs is predictable and caused by both mechanical and hormonal factors.

Methodology: This longitudinal study recruited 70 pregnant women (140 limbs) randomly to assess VV development during pregnancy. Participants were monitored across trimesters and postpartum with six assessments. These included demographic data collection, Duplex Ultrasonography (DUS), Vector Flow Imaging (VFI), Photoplethysmography (PPG), Truncated cone limb volume measurement, hormonal blood tests, and Chronic Venous Insufficiency Quality of Life Questionnaire (CIVIQ-20) assessments.

Results: The present study has inked several demographic factors to VVs: older age, multiparity, family history, higher body mass index (BMI), white ethnicity, and jobs involving prolonged standing. Patients with VVs showed larger veins, particularly in the legs, with increased iliac vein compression, progressing to May-Thurner syndrome. DUS highlighted higher reflux time and peak velocity in those with VVs. VFI detected flow changes better than DUS. PPG matched DUS in reflux assessment but not location. Patients with VVs had increased limb volume, higher progesterone and Estroil levels, and lower QoL per CIVIQ-20 scores.

Conclusion: The study affirmed that VVs in pregnancy stem from mechanical and hormonal factors, contributing to existing knowledge. It emphasized VVs' detrimental impact on pregnant women's well-being, stressing the need for early detection, timely treatment, and preventive measures to safeguard pregnant women's QoL.

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Statement of Originality:

I confirm that the work presented here is solely my creation, and all external sources utilised have been appropriately acknowledged and referenced.

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Abbreviations:

APG	Air Plethysmpgraphy
AVF	American Venous Forum
AVVQ	Aberdeen Varicose Veins Questionnaire
AAGSV	Anterior accessory great saphenous vein
ACV	Anterior circumflex vein
AASV	Anterior accessory saphenous vein
AVP	Ambulatory venous pressure
ADH	Antidiuretic Hormone
ACTH	Adrenocorticotropic hormone
AC	Abdominal circumference
BMI	Body mass index
bFGF	Basic fibroblast growth factors
BP	Blood pressure
CIVIQ-20	QoL assessment using the Chronic Venous Insufficiency Quality of Life Questionnaire
CVI	Chronic Venous Insufficiency
CEAP	Clinical, Etiology, Anatomy distribution and Pathophysiology classifications
CIVs	Common iliac veins
CFV	Common femoral vein
CXVUQ	The Charing Cross Venous Ulceration Questionnaire
CCG	The Clinical commissioning groups
CO	Cardiac output
CVD	Chronic venous disease
CBG	Corticosteroid-binding globulin
COCL2	Cobalt chloride
CTV	Computed Tomography venography
CHIVA	The ambulatory conservative hemodynamic correction of venous insufficiency
CLOCK	Circadian locomotor output cycles kaput
COL1A2	Type I collagen gene
DUS	Duplex Ultrasonography
DVT	Deep vein thrombosis
DBP	Diastolic blood pressure
DNA	Deoxyribonucleic acid
3-D	Three dimensional
E3	Estroil
EIV	External iliac vein
EF	Ejection fraction

E1	Estrone
E2	Estradiol
ERs	Estrogen receptors
EDV	End diastolic volume
ESV	End-systolic volume
E2/fT	Serum estradiol/free testosterone
ECM	Extracellular matrix
EVLT	Endovenous laser therapy
EVLA	Endovenous laser ablation
EHIT	Endovenous heat induced thrombosis
FV	Femoral vein
FOXC2	Forkhead Box C2
FL	Femur length
GP	General practitioner
GSV	Great saphenous vein
GFR	Glomerular filtration rates
GWAS	Genome-wide association studies
GH	Serum growth hormone
hCG	Human chorionic gonadotropin
HDL	High-density lipoprotein levels
HL/S	high ligation and stripping
HRT	Hormone replacement therapy
HR	Heart rate
HC	Head circumference
IPG	Impedance Plethysmography
IVC	Inferior vena cava
IIV	Internal iliac vein
IVUS	Intravascular Ultrasound
ICC	intraclass correlation coefficients
IAP	increases abdominal pressure
IUP	International Union of Phlebology
ICT	Intermittent compression therapy
IQ	Intelligence quotient
lncRNAs	Long non coding RNAs
KTS	Klippel-Trenaunay syndrome
LDL	Low-density lipoprotein
LVEDP	Left ventricular end-diastolic pressure
MTS	May-Thurner syndrome
MMP	Matrix metalloproteinases

MV	Minute ventilation
MVO	Maximum venous outflow
MRV	Magnetic Resonance venography
MTHFR	Methylenetetrahydrofolate reductase
MPFF	Micronized purified flavonoid fraction
MGP	Matrix Gla Protein
MMPs	Matrix metalloproteinases
NICE	National Institute for Health and Care Excellence
NHS	The UK National Health Service
NJP	Non-junctional perforators
NO	Nitric oxide
OPN	Osteopontin
PRV	Peak reflux velocity
PELV	Pelvic veins
PPG	Photoplethysmography
PE	Pulmonary embolism
PCV	Posterior circumflex vein
PAGSV	Posterior accessory great saphenous vein
PVs	Perforator veins
PDGF	Platelet-growth factors
POPV	Popliteal vein
PRF	Pulse repetition frequency
PI3K/Akt	Phosphoinositide-3-kinase-protein kinase B/Akt
QoL	Quality of life
RT	Reflux time
RAP	Right atrial pressure
RAAS	Renin-angiotensin-aldosterone system
RBC	Red blood cell
RPF	Renal plasma flow
RAA	Renin-angiotensin-aldosterone
RVF	Residual volume fraction
RFA	Endovenous radiofrequency ablation
SF-36	Short Form 36 item
SQOR-V	Specific Quality of Life and Outcome ResponseVenous questionnaire
SVT	Superficial venous thrombosis
SMCs	Smooth muscle cells
SSV	Small saphenous veins
SFJ	Sapheno-femoral junction
SPJ	Sapheno-popliteal junction

SV	Stroke volume
SBP	Systolic blood pressure
SVR	Systemic vascular resistance
STP	Superficial thrombophlebitis
SD	Standard deviation
SFV	Superficial femoral vein
SGP	Strain-Gauge Plethysmography
TE	The thigh extension
TSH	Thyroid-stimulating hormone
TBG	Thyroxine-binding globulin
T_4	Thyroxine
T_3	Triiodothyronine
TIMPs	Tissue inhibitors of Matrix metalloproteinases
TO	Transverse oscillation
t-PA	Tissue plasminogen activator
TIPP	Transilluminated powered phlebectomy
TOF	Time of flight
UK	United Kingdom
UGFS	Ultrasound-guided foam sclerotherapy
UA	Uric acid
VVs	Varicose veins
VFI	Vector Flow Imaging
VRT	Venous refilling times
VDS	Venous Disability Score
VCSS	Venous Clinical Severity Score
VEINES	Venous Insufficiency Epidemiological and Economic Study instrument
VLUs	Venous leg ulcers
VG	Vein of Giacomini
VAD	Veno-active drugs
VEGF	Vascular endothelial growth factor
vWF	Von Willebrand factor

1 Chapter 1 Introduction

1.1 Background

Varicose veins (VVs) are an early indicator of Chronic Venous Insufficiency (CVI), which is characterised by abnormal tortuosity and dilation of the superficial veins of the lower extremities (Naoum *et al.*, 2007) (Fig1.1). VVs have a diameter of 3 mm or more and can occur in either the superficial saphenous veins and tributaries or non-saphenous superficial veins in the lower extremities (Berti-Hearn and Elliott, 2019). CVI refers to the inadequate venous blood return and pooling of blood in the veins, leading to venous hypertension (Germon, 1997). The symptoms and signs of CVI vary depending on the stage and severity of the disease (Adhikari *et al.*, 2000). In the early stages, patients may experience symptoms such as VVs, pain, leg heaviness, and mild swelling. However, in later stages, skin changes and ulceration may develop (Shadrina *et al.*, 2018). Complications of CVI not only negatively impact the quality of life (QoL) of patients (Kaplan *et al.*, 2003), but also impose a significant economic burden on society (Guest *et al.*, 2015). Therefore, a better understanding of the etiology of VVs can improve patients' QoL and provide valuable insights for reducing healthcare costs.



Fig 1.1: The presence of VVs showing abnormal tortuosity and dilation of the superficial veins of the lower extremities in a 39-year-old woman (De Maeseneer *et al.*, 2011).

1.2 Classification of CVI diseases

Reporting CVI diseases previously experienced lack of accuracy which caused inconsistent management and treatment (Eklöf *et al.*, 2004). This is because clinical manifestation of CVI disease differ between patients with similar pathology, which makes it difficult to address the clinical manifestation of CVI disease (Lurie *et al.*, 2020). Therefore, obtaining a standard system for describing patients with CVI diseases is significant to achieve a standard reporting and therefore uniform management and treatment for CVI patients (Meissner *et al.*, 2007). In 1994, CEAP classification system was developed by the international ad hoc committee of the American Venous Forum (AVF) with the support of the Society for Vascular Surgery (Porter, Moneta and An International Consensus Committee on Chronic Venous Disease, 1995). In 1995, CEAP system was added into the ‘’Reporting Standards in Venous Disease” (Eklöf *et al.*, 2004). This classification system has been used worldwide and it is currently considered the gold standard system for classifying CVI diseases (Santler and Goerge, 2017; Lurie *et al.*, 2020). Although CEAP classification system is significant in reporting CVI diseases, however, it has some limitations such as its inability to report recurrent VVs after surgery and its inability to differ between initial and recurrent ulcer (Cornu-Thénard et al., 2004). Therefore, CEAP classification needed continuous revision to ensure constant universal use. It was first revised in 2004. This revision involved improving the definitions of CVI diseases and C classes as well as adding ‘’n’’ which means no CVI diseases recognised (Table 1.1) (Eklöf *et al.*, 2004). Later in 2017, another revision took a place as the (AVF) committee developed the CEAP Task Force were they focused on four principles including; the practicality for clinical use, compatibility with previous versions, evidence based and the continuous reproducibility of CEAP (Lurie *et al.*, 2020). Last revision was made in 2020, the changes involved adding C4c subclass which presents corona phlebectatica as well as introducing ‘r’ for recurrent VVs and venous ulcers and substituting numeric descriptions of the venous segments by their common abbreviations (Lurie *et al.*, 2020) (Table 1.2).

CEAP classification	Description
Clinical classification (C):	C0: no visible or palpable signs of venous disease. C1: telangiectasies or reticular veins. C2: varicose veins C3: edema C4a: pigmentation or eczema C4b: lipodermatosclerosis or atrophic blanche C5: healed venous ulcer C6: active venous ulcer
Etiologic classification (E):	Ec: congenital Ep: primary Es: secondary (post-thrombotic) En: no venous cause identified
Anatomic classification (A)	As: superficial veins: 1: Telangiectasia 1: Reticular veins 2: Great saphenous vein above the knee 3: Great saphenous vein below the knee 4: Small saphenous vein 5: Nonsaphenous vein Ad: deep veins: 6: Inferior Vena Cava 7: Common iliac vein 8: Internal iliac vein 9: External iliac vein 10: Pelvic veins 11: Common femoral vein 12: Deep femoral vein 13: Femoral vein 14: Popliteal vein 15: Crural (tibial) vein 15: Peroneal vein 15: Anterior tibial vein 15: Posterior tibial vein 16: Muscular veins 16: Gastrocnemius vein 16: Soleal vein Ap: perforator veins: 17: Thigh perforator vein 18: Calf perforator vein An: no venous location identified
Pathophysiologic (P)	Pr: reflux Po: obstruction Pr,o: reflux and obstruction Pn: no venous pathophysiology identifiable
(S) (A)	(S) Symptomatic: including ache, pain, tightness, skin irritation, heaviness, muscle cramps and other complaints. (A) Asymptomatic.

Table 1.1: The 2004 revision of the CEAP classification of chronic venous disease adapted from (Eklöf *et al.*, 2004).

CEAP classification	Description
Clinical classification (C):	C0: no visible or palpable signs of venous disease. C1: telangiectasies or reticular veins. C2: varicose veins C3: edema C4a: pigmentation or eczema C4b: lipodermatosclerosis or atrophic blanche C4c: Corona phlebectatica C5: healed venous ulcer C6: active venous ulcer C6r: recurrent active venous ulcer
Etiologic classification (E):	Ec: congenital Ep: primary Es: secondary (post-thrombotic) Esi: Secondary-intravenous Ese: Secondary-extravenous En: no venous cause identified
Anatomic classification (A)	As: superficial veins: Tel: Telangiectasia Ret: Reticular veins GSVa: Great saphenous vein above the knee GSVb: Great saphenous vein below the knee SSV: small saphenous vein AASV: Anterior accessory saphenous vein NSV: nonsaphenous vein Ad: deep veins: IVC: Inferior vena cava CIV: Common iliac vein IIV: Internal iliac vein EIV: External iliac vein PELV: Pelvic veins CFV: Common femoral vein DFV: Deep femoral vein FV: Femoral vein POPV: Popliteal vein TIBV: Crural (tibial) vein PRV: Peroneal vein ATV: Anterior tibial vein PTV: Posterior tibial vein MUSV: Muscular veins GAV: Gastrocnemius vein SOV: Soleal vein Ap: perforator veins: TPV: Thigh perforator vein CPV: Calf perforator vein An: no venous location identified
Pathophysiologic (P)	Pr: reflux Po: obstruction Pr,o: reflux and obstruction Pn: no venous pathophysiology identifiable
(S) (A)	(S) Symptomatic: including ache, pain, tightness, skin irritation, heaviness, muscle cramps and other complaints. (A) Asymptomatic.

Table 1.2: The 2020 revision of the CEAP classification of chronic venous disease adapted from (Lurie *et al.*, 2020).

The CEAP classification system is comprised of four main components: clinical manifestation, etiology, anatomical distribution, and pathophysiology (Beebe *et al.*, 1996). The clinical manifestation component is further divided into seven clinical classes (C): (C0) indicating no visible signs of venous disease, (C1) referring to limbs with telangiectasias, (C2) referring to varicose veins (VV), (C3) indicating edema, (C4a) indicating skin changes with ulceration, (C4b) indicating skin changes without ulceration, (C5) indicating healed ulcers, and (C6) indicating active ulcers (Meissner *et al.*, 2007). Conversely, the etiology of venous disease can be classified into congenital, primary, or secondary categories (Cornu-Thénard, Uhl and Carpentier, 2004). Primary venous diseases are not caused by any venous abnormality event, while secondary venous diseases are caused by an event of venous dysfunction such as an episode of acute deep vein thrombosis (DVT) (Lurie *et al.*, 2020). With respect to the underlying pathophysiology, either reflux or obstruction can be precisely located in the lower limb venous segments (Eklöf *et al.*, 2004).

1.3 Clinical signs and symptoms

Typical indications and symptoms of CVI are characterised by leg heaviness, itching, edema, pain, or leg cramps (Eberhardt and Raffetto, 2014). The signs and symptoms of CVI may vary depending on the stage and severity of the condition (Adhikari *et al.*, 2000) (Fig 1.2). For instance, in the early stages of CVI (CEAP class C1-C2), no specific symptoms are typically associated (Santler and Goerge, 2017). Instead, the signs in these stages include telangiectases or "spider veins" and paraplantar corona phlebectatica or "reticular veins" often found around the ankles (Musil et al., 2016). Telangiectases are described as intradermal veins that are enlarged (diameter less than 1 mm), while paraplantar corona phlebectatica are described as subcutaneously enlarged veins (diameter between 1 to 3 mm) (Partsch, 2009) Although these initial signs of CVI are not considered as definitive evidence of the condition, they serve as warning signs and are typically a cosmetic concern for patients only (Rabe *et al.*, 2003).

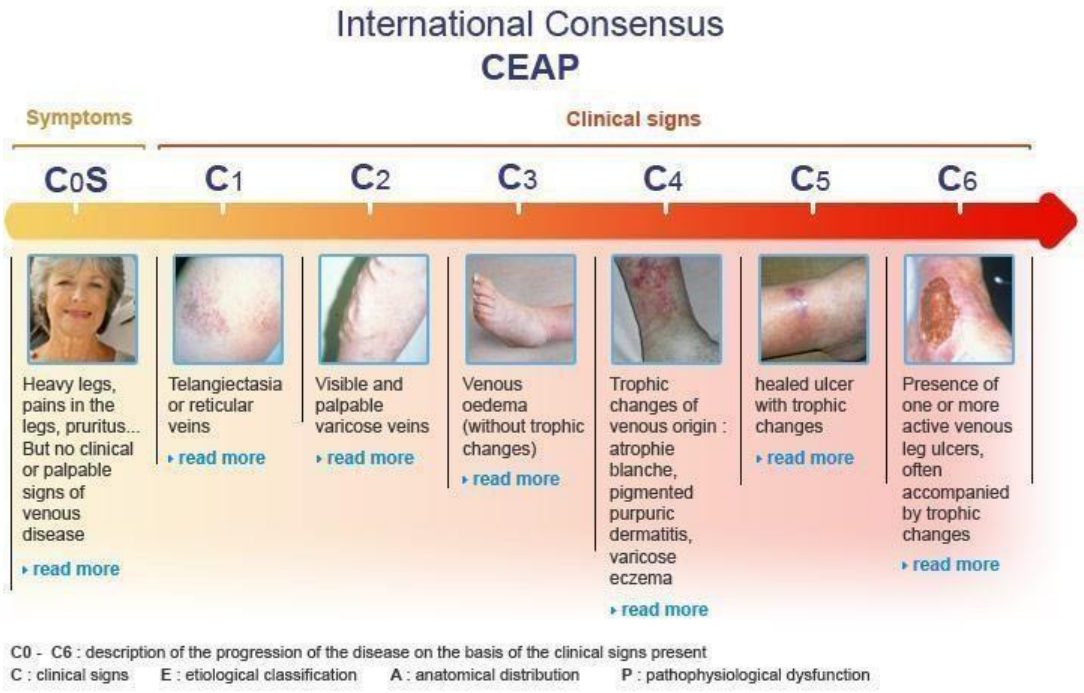


Fig 1.2: Signs and symptoms of CVI (Wolinsky and Waldorf, 2009).

The CEAP classification system identifies various stages of CVI disease. The second stage is CEAP class C2, which indicates the appearance of VVs. VVs are characterized as subcutaneous veins with a diameter greater than 3 mm and incompetence (Piazza, 2014). Without treatment, the vein diameter will continue to expand until it is visible to the naked eye (Youn and Lee, 2019). The subsequent stage is CEAP class C3, which is characterized by the presence of leg edema combined with VVs (Perrin and Ramelet, 2011). In this stage, edema initially occurs overnight but can persist throughout the day if left untreated (Keast *et al.*, 2015). CEAP class C4 indicates the progression of CVI and includes skin changes associated with the severity of venous disease (Allegra *et al.*, 2003). This stage is further subdivided into two subclasses to differentiate between the severity of these skin changes (Keast *et al.*, 2015). For example, (class C4a) presents pigmentation and eczema resulting from chronic inflammation in the dermis and subcutis (Santler and Goerge, 2017). Although these two signs typically occur, they do not frequently lead to ulcers. In contrast, (class C4b) presents lipodermatosclerosis and atrophie blanche, which usually indicate the progression towards leg ulcers (Choonhakarn et al., 2016). Class C5 represents

healed leg ulcers, while class C6 is the most severe clinical class of CEAP classification, including active venous ulcers caused by CVI that do not heal (Marston *et al.*, 2017).

1.4 CVI severity assessment tools

In the last decade, there was an increase in the prevalence of chronic diseases among patients which ultimately has a negative impact on patients' QoL (Megari, 2013). Therefore, recently, increased scientific attention to assess patients' QoL has been sighted (Landry, 2022). This is because the negative impact of a disease on patients' QoL is underestimated by physicians, as physicians focus only on the physiological and biochemical factors only (Carter *et al.*, 2016). Evaluating patients' QoL is essential to understand patients' subjective perspectives regarding the emotional, physical, and social impacts of a disease and therefore clinicians can provide new insights into patient management and treatment (McGee, 2001). QoL is an important outcome measure in the treatment of chronic diseases including chronic venous disease (Kuet *et al.*, 2013)

Several quality assessment tools are used to measure the functional impact of a disease on patients' QoL as perceived by the patient (Van Korlaar *et al.*, 2003). Both generic and disease-specific measures tools are used to estimate patients' quality of life status (Fayers P. M. and Machin D., 2013). Generic tools contain general items that are applicable to populations with or without chronic disease and these tools do not focus on precise symptoms of a specific disease (Kuet *et al.*, 2013). Therefore, generic tools are not suitable in testing the effectiveness of new medications and are less sensitive to measuring the effect of a disease on a patient's daily functioning (Dunderdale *et al.*, 2005). An example of a generic tool is the Medical Outcomes Study Short-Form 36 (SF-36) Health Survey, which is used commonly in several chronic diseases and can also be applied to a specific disease such as CVD (Chan *et al.*, 2014). On the other hand, disease-specific QoL assessment tools are most commonly used in clinical trials (Thomas *et al.*, 1995) as it focuses on measuring specific items of a specific disease. Therefore, disease-specific QoL tools are considered suitable to measure the effect of treatment-related variations compared to generic tools (Wiebe

et al., 2003). They are progressively used in the studies related to VVs are they are considered more sensitive for evaluating the outcomes of venous diseases (Kuet *et al.*, 2014). The most used disease-specific QoL assessment tools are the Chronic Venous Insufficiency quality of life Questionnaire5 (CIVIQ), the Aberdeen Varicose Vein Questionnaire4 (AVVQ) and the Venous Insufficiency Epidemiological and Economic Study instrument (VEINES) (Launois, 2015). Other examples of disease-specific QoL questionnaires include the Specific Quality of Life and Outcome ResponseVenous questionnaire (SQOR-V) (Guex *et al.*, 2007) and the Charing Cross Venous Ulceration Questionnaire (CXVUQ) (Tafernaberry *et al.*, 2016).

1.5 Complications of CVI

Untreated CVI can result in significant disability and severe complications, both acute and chronic (Bryant and Nix, 2016). Acute complications include superficial venous thrombosis (SVT), DVT and pulmonary embolism (PE), while chronic complications include venous leg ulcers (VLUs) that result from venous hypertension caused by venous reflux or obstruction (Vasudevan, 2014; Lal, 2015; Musil, Kaletova and Herman, 2016). VLUs are open skin lesions mainly located between the knee and ankle but typically affect the medial malleolus area of the leg (Santler and Goerge, 2017) (Fig 1.3). They are prevalent in approximately 60% of cases of chronic wounds caused by VLUs (Frykberg and Banks, 2015). Risk factors associated with the development of VLUs include older age, obesity, history and family history of CVI diseases such as VVs, ulcers and severe lipodermatosclerosis, increased number of pregnancies, reduced physical activity, and history of superficial or deep venous thrombosis and PE (Millan et al., 2019). In the UK, the approximate prevalence of venous leg ulcers varies between 0.1% to 0.3% and increases with older age (National Institute for Health and Care Excellence (NICE), 2021). Studies have shown that the period from the diagnosis of CVI to the incidence of ulceration is typically five years (Heit *et al.*, 2001), with the first ulceration occurring on average three years after diagnosis (Darwin *et al.*, 2021). In this study, the incidence of ulceration was reported to be 4.49% in a five-year follow-up period.



Fig 1.3: Venous Ulcers on the medial malleolus, accompanied by eczema and lipodermatosclerotic skin (Eberhardt and Raffetto, 2014a).

Chronic wounds resulting from VLUs often require extended periods of treatment (Dissemond *et al.*, 2021). Reports indicate that healing rates for such wounds are around 60% within three months, with 75% of these wounds recurring within three weeks after healing (Fernandes Abbade and Lastória, 2005). The most common management approach for VLUs is compression therapy and various wound dressings (Tatsioni *et al.*, 2007) , while other treatments such as exercise, pentoxifylline, and early surgical interventions, including venous ablation for treating superficial venous reflux, are also available (Millan et al., 2019). Despite surgical intervention being recommended for treating VLUs, several randomized controlled trials have failed to demonstrate that it can help improve healing rates, although it may aid in reducing recurrence rates (Pierik *et al.*, 1997; Barwell *et al.*, 2004). Thus, VLUs in CVI patients remain a significant concern for vascular surgeons and physicians.

1.5.1 The impact of CVI complications

1.5.1.1 Social impact (QoL)

CVI, particularly in severe stages, has a considerable negative impact on the QOL of affected individuals (Branisteanu *et al.*, 2019). As the severity of CVI increases, QOL tends to decrease (Kaplan *et al.*, 2003; John J Bergan *et al.*, 2006). For instance, the Venous Insufficiency Epidemiological and Economical Study (VEINES) revealed that

65% of patients with VVs and other venous diseases experienced low physical and mental QOL scores as the disease progressed (Laing, 1992). The QOL of patients classified under C5 and C6 in the CEAP classification was comparable to that of patients with heart failure (Andreozzi *et al.*, 2005). Furthermore, in late stages of CVI, patients experienced lower QOL compared to those with back pain, chronic lung disease, and arthritis (Laing, 1992). Patients with VLUs reported severe pain in these advanced stages, which impeded their physical activity and contributed to symptoms of depression and social isolation (Lindholm *et al.*, 1993). Among the problems that patients with VLUs commonly encounter, social isolation is the most significant, as malodour and wound leakage from ulcers can lead to embarrassment and reluctance to participate in social activities (Ebbeskog and Ekman, 2001). Consequently, patients tend to avoid social contact to prevent further harm to their legs and the recurrence of ulcers. Additionally, as patients are often unable to work, they become dependent on their family members, leading to a further economic impact (Hopkins, 2004; Douglas, 2014).

1.5.1.2 Economic impact

In addition to the negative impact of severe stages of CVI on patient QoL, the management of chronic wounds caused by VLUs imposes a considerable economic burden on society (Kurz *et al.*, 2001). Chronic wounds are defined as wounds that do not heal within the expected time frame of a reconstruction process, which is typically three months, to achieve anatomical and functional outcomes (Werdin *et al.*, 2009). The costs associated with managing chronic wounds can be categorized as direct or indirect (Carter, 2018). Direct costs include expenses related to medications, dressings, bandages, and hospital care facilities, such as general practitioner (GP) visits and nurse appointments, while indirect costs are associated with reduced patient QoL and productivity, the value of which is challenging to quantify (Simka and Majewski, 2003). The direct cost of managing chronic wounds is rising due to an increase in the number of resources and goods required for diagnosis and treatment (Posnett and Franks, 2007). Compared to managing healed wounds, managing uncured wounds necessitates a greater number of resources, such as a 40% increase in drug prescriptions, 13% more general practitioner appointments, 18% more hospital outpatient appointments, 20% more practice nurse appointments, and 104% more

community nurse appointments (Han and Ceilley, 2017; Guest, Fuller and Vowden, 2020) The duration of wound healing also plays a significant role in increasing the cost, along with nursing time, product cost, and the number of dressings required (Tennvall and Hjelmgren, 2005).

The management of chronic wounds incurs substantial costs to patients and healthcare systems worldwide system (Evans *et al.*, 1994; Cesarone *et al.*, 2002; Barnsbee *et al.*, 2019). For example, a study in the United States estimated that the cost of managing chronic wounds in outpatient clinics over a 10-week period ranged from $1444 to $271 (Marston *et al.*, 1999), while another study estimated the annual burden of chronic wounds at $14.9 billion (Rice *et al.*, 2014). In Australia, the yearly healthcare cost for treating chronic wounds caused by VLUs was over $3 billion Australian dollars (Weller and Evans, 2012). In many western countries, the cost of managing chronic wounds accounts for approximately 1% of the total public healthcare budget (O'Donnell *et al.*, 2014). In the United Kingdom, chronic wounds pose a significant burden to patients and the healthcare system, with the cost of managing them ranging from 2.3 to 3.1 billion pounds sterling, equivalent to 3% of the total healthcare budget, during 2005 and 2006 (Posnett and Franks, 2007).

Later in the UK, chronic wound management represents a substantial economic burden to the healthcare system. In 2012-2013, the NHS incurred a cost of approximately 5.3 billion-pound sterling, equivalent to approximately 4% of the total UK healthcare budget, for managing 2.2 million chronic wounds (Guest *et al.*, 2015). The annual cost of treating these wounds, post-modifying for two or more chronic diseases, was estimated to be between 4.5-5.1 billion pounds sterling (Guest *et al.*, 2015). Notably, the annual cost of managing specific wounds varied, with an estimated cost of 1.94 billion pounds sterling for treating 731,000 leg ulcers and 89.6 million pounds sterling for managing 87,000 burns (Guest *et al.*, 2017). The study further revealed that of the 2.2 million wounds, 61% were cured, while 39% remained uncured. The annual cost of managing cured wounds was approximately 2.1 billion-pound sterling, while uncured wounds cost 3.2 billion-pound sterling. Moreover, during the study period, the mean cost of patient care was 135% higher in uncured wounds, ranging from 1719 to 5976 pound-sterling, compared to cured wounds, ranging from 698 to 3998 pound-sterling (Guest *et al.*, 2017).

Moreover, the NHS clinical-led groups in England, Wales, and Scotland highlighted the substantial costs associated with managing chronic wounds in 2012-2013. These groups, which oversee healthcare service delivery at the community and local levels to optimize health outcomes, evaluated an average of 11,200 wounds during this period (Guest, Vowden and Vowden, 2017). The wounds were categorized as acute (40%), chronic (48%), or undiagnosed (12%). The study showed an annual prevalence growth rate of 9% for acute wounds, 12% for chronic wounds, and 13% for undiagnosed wounds. The authors recommended a minimum 1% increase in the rate of wound healing for all wound types to curb the prevalence growth. Failure to do so could lead to a predicted cost of £50 million to manage 23,200 wounds and associated chronic conditions by Clinical commissioning groups (CCG) and health boards in 2019/2020.

In recent years, there has been an increasing number of studies investigating the costs associated with managing chronic wounds in the UK. For instance, (Phillips *et al.*, 2020) reported on the direct cost of managing chronic wounds caused by VLUs in the Welsh NHS for an 11-year period from 2007-2017. Their findings revealed that the cost of managing patients with chronic wounds was £7706 per patient per annum, which extrapolates to over £2 billion when generalised to the UK population. Similarly, (Guest, Fuller and Vowden, 2020) reported on the prevalence and cost of chronic wounds in the UK. Their study found that the prevalence of chronic wounds had increased by 71% over the 5 years to 3.8 million wounds. Of these wounds, 70% healed, while the remaining 30% were considered unhealed. The NHS cost of managing these wounds had increased by 48% to account for £8.3 billion (£2.7 billion for healed wounds and £5.6 billion for unhealed wounds). District and community nurse visits accounted for the primary cost of 29% of the total cost, while GP office visits accounted for a secondary cost of 18%. Health care workers' visits, practice nurses' visits, and wound care budgets accounted for 17%, 7%, and 6% of the costs, respectively. These costs consumed 81% of the total annual NHS healthcare budget. Given the substantial burden of acute and chronic wounds and the increasing costs on the healthcare system, a better understanding of the pathophysiology of VLUs and the associated risk factors may inform strategies to deliver better treatment and

prevent or reduce the risk of progression, with positive impacts on socio-economic and QoL outcomes.

1.6 Aetiology of Varicose veins in the general population and pregnancy

The etiology of VVs remains uncertain. Historically, venous hypertension has been regarded as the primary factor contributing to venous dilation and subsequent valve incompetence and reflux (Bradbury, 2011a; Tabatabaeifar *et al.*, 2015). However, this theory fails to explain the changes that occur prior to the onset of venous hypertension and venous wall dilatation (London and Nash, 2000). Recent evidence challenges this hypothesis and emphasises the histopathological changes in the extracellular matrix of the vein walls, which may precede valvular dysfunction (Lim and Davies, 2009; Segiet *et al.*, 2015). Moreover, large number of cross-sectional studies have focused on the identification of risk factors associated with VVs development (Bergan *et al.*, 2006). Incidence studies are useful for investigating the etiology of a disease and assessing causal relationships (Ahti *et al.*, 2009). These studies have identified various risk factors, including family history, age, obesity, greater height, gender, occupational factors, and pregnancy (B Komsuoglu *et al.*, 1994).

The pathophysiology of VVs particularly in pregnant women, is not well understood despite its common occurrence during pregnancy (Cornu-Thenard and Boivin, 2014a). This may be attributed to the dearth of longitudinal studies investigating the risk factors involved in the development of the disease and tracking the changes in lower limb veins during and after pregnancy. Prior research proposed three hypotheses regarding pregnancy and its association with VVs formation (Mashiah, 1999; Ismail *et al.*, 2016; García-Honduvilla *et al.*, 2018). The first two hypotheses were related to mechanical factors, with one suggesting that increased gravid uterus in pregnancy raises iliac vein pressure, thereby causing blood flow disturbance, and the other positing that increased blood volume in pregnancy results in high blood pressure in lower limb extremities. The third hypothesis was linked to chemical factors, as it proposed that increased levels of progesterone and estrogen hormones in pregnancy contribute to

the development of VVs. Despite these existing hypotheses, information is insufficient to establish the causes of VVs in the general population and specifically in pregnant women.

1.7 Epidemiology

The prevalence of VVs is widely variable across different studies (Table 1.3). This variability can be attributed to various factors, such as variations in study design, disease definitions, imaging techniques used, demographic characteristics of the population, including age, gender, and ethnicity, and geographical location (Beebe-Dimmer *et al.*, 2005). For instance, in the United States, the Framingham study reported a higher prevalence of VVs in women (1-73%) than in men (2-56%), with a yearly incidence of 2.6% in women and 1.9% in men (Brand *et al.*, 1988). Similarly, the Edinburgh vein study in the UK found a higher age-adjusted prevalence of VVs in men (39.7%) than in women (32.2%) (Evans *et al.*, 1999). However, after 13 years of follow-up, (Robertson, *et al.*, 2013) reported a higher incidence of VVs among women (17.4%) than men (15.2%), which was attributed to family history, age, and obesity. In Germany, the Bonn vein study reported a prevalence rate of 22.7-25.1% in both sexes (Rabe *et al.*, 2010).

Year of publication	Reference	Country	Sample size	women	men	Both genders
1998	(S Canonico *et al.*, 1998)	Italy	1319	35.2% (40.5% in pregnant women, 38.2% non-pregnant)	17%	27.4%
1988	(Brand *et al.*, 1988)	USA	3822	1-73%	2.56%	_____
1999	(Evans *et al.*, 1999)	UK	1566	32.2%	39.7%	_____
2010	(Rabe *et al.*, 2010)	Germany	1978	_____	_____	22.7-25.1%
2013	(Robertson, *et al.*, 2013)	UK	880	17.4%	15.2%	18.2%

Table 1.3: Gender related estimates of prevalence of VVs.

1.8 Anatomy

1.8.1 The cardio vascular system:

The cardiovascular system is a complex network of blood vessels and the heart, which together facilitate the circulation of blood throughout the body. Comprised of three main subsystems - the arterial, venous, and capillary systems - this intricate network serves to transport oxygen and nutrients to body tissues and organs, while removing waste products and carbon dioxide (Formaggia, et al., 2010). Specifically, the arterial system consists of arteries and arterioles, which transport blood away from the heart at high pressure. The venous system, on the other hand, includes veins and venules, which return blood to the heart at low pressure. Finally, the capillary system is comprised of small blood vessels that facilitate the exchange of oxygen and nutrients with tissues and organs (Attinger, 1979; Bit, 2020). Blood flows through this system in a coordinated manner, with high-pressure blood being delivered to smaller arteries, arterioles, and eventually to capillaries. Here, the exchange of oxygen and nutrients with tissues and organs occurs. The low-pressure blood is then collected from capillaries by venules, which ultimately flow into veins and return to the heart (Formaggia, Quarteroni and Veneziani, 2009). Overall, the cardiovascular system plays a vital role in maintaining bodily homeostasis and ensuring the proper functioning of organs and tissues.

Veins and arteries perform distinct functions and exhibit marked differences in their wall structure. Arteries, as per (Bit, 2020), transport oxygenated blood from the heart to tissues and organs at high pressure, necessitating thicker and more muscular walls. Conversely, veins collect deoxygenated blood from organs and tissues at low pressure, requiring thinner and less muscular walls. Despite the differences in wall thickness, arteries and veins share similar structural components, as noted by (Meissner, 2005). Specifically, both veins and arteries exhibit three histological layers, namely, the tunica intima, tunica media, and tunica adventitia (Szasz *et al.*, 2007) (Fig 1.4). The thickness of the outer layer (tunica adventitia) and the medial layer (tunica media) differentiates veins from arteries, with veins having a thicker outer layer and a thinner medial layer compared to arteries, as reported by (Chantler, 2009).

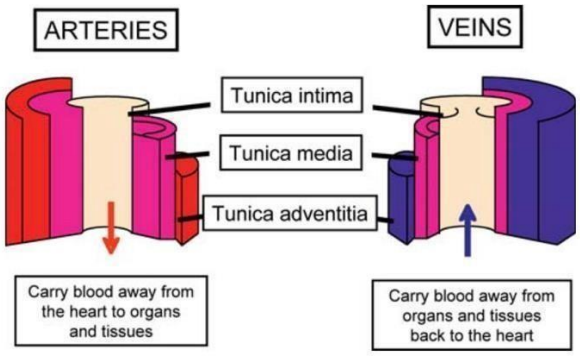


Fig 1.4: The three layers of the blood vessel wall in arteries and veins (Szasz *et al.*, 2007)

1.8.2 Histology of blood vessels:

The tunica intima, which constitutes the innermost layer of blood vessels, comprises a single layer of epithelial cells known as endothelium, as reported by (Chantler, 2009; Formaggia et al., 2009). The endothelium lies in direct contact with the blood flow, providing slight resistance to its passage, as per (Marieb and Hoehn, 2015). Moreover, it generates the chemical nitric oxide, which relaxes smooth muscle cells (SMCs) in the vessel walls and prevents clotting, according to (Chen et al., 2008). When the endothelium becomes damaged, as explained by (Bit, 2020), blood may be exposed to the collagenous fibers beneath, potentially leading to blood clots. Various risk factors, such as injury, smoking, immune disease, hypertension, diabetes, atherosclerosis, infection, and environmental factors, may contribute to endothelial damage, as noted by (Raffetto, 2018). The sub-endothelium, a connective tissue bed underlying the endothelium, is attached to the basal lamina, a membrane that provides both flexibility and strength and is permeable to substances, as reported by (Bit, 2020). In addition, some veins in the lower extremities feature thick endothelium and connective tissue layers extending into this lamina, as reported by (Mazurek *et al.*, 2017). The basal lamina separates the tunica intima from the second layer, the tunica media.

The tunica media represents the second layer of a blood vessel, and it is thicker in arteries compared to veins. It is composed of layers of SMCs which are supported by

connective tissue (MacColl and Khalil, 2015a). The SMCs within the tunica media are responsible for the production of extracellular matrix, which includes collagen and elastin (Mazurek *et al.*, 2017). The tunica media layer is characterised by layers of SMCs, collagenous fibers, and elastic fibers that are arranged around the vessel, with collagenous fibers aiding in the attachment of the tunica media to another layer (Bit, 2020). The activity of the SMCs in the tunica media is regulated by the activation or deactivation of the sympathetic nervous system, specifically the vascular nerves that are present at the blood vessel walls (Tonar *et al.*, 2012). When these vascular nerves are activated, they cause contraction of the SMCs, which results in vasoconstriction, i.e. narrowing of the blood vessel diameter (Chapados *et al.*, 2006). In contrast, when the vascular nerves are deactivated, the SMCs relax, resulting in vasodilation or widening of the blood vessel diameter (Schiffrin, 2002). As such, the physiological functions of blood vessels, including vasoconstriction and vasodilation, are maintained mainly by the SMCs, which are influenced by neurotransmitters and their receptors, as well as other chemicals and hormones (Bit, 2020).

The normal distribution of SMCs and extracellular matrix components, such as collagen and elastin, is crucial for maintaining normal wall distensibility in veins (Travers *et al.*, 1996). Deviations in the distribution of these components may lead to various vascular diseases, including hypertension, atherosclerosis, and VVs (Mazurek *et al.*, 2017). Multiple studies have reported significant alterations in these components in VVs, even in the presence of a seemingly normal saphenofemoral valve (Rose and Ahmed, 1986b; Sansilvestri-Morel *et al.*, 2001, 2005). Specifically, decreased levels of collagen and elastin (Psaila and Melhuish, 1989), increased levels of collagen without changes in elastin (Travers *et al.*, 1996), and increased collagen levels with reduced elastin in VVs segments compared to normal segments have been observed (Gandhi *et al.*, 1993).

The outermost layer of a blood vessel is known as the adventitia layer, which primarily consists of connective tissue fibers, including fibroblasts, collagen, and elastin, providing structural support and connecting the blood vessel to the surrounding peripheral tissues (Bit, 2020). Compared to arteries, veins exhibit a thicker adventitia layer, as it serves to protect the vein from damage and collapse, especially since veins can be superficially located (Meissner, 2005; Vilches and Macedo Dias, 2015). Vasa

vasorum, small blood vessels that deliver nutrients to the vascular wall itself, are also present and merge with the adventitia of larger vessels (Szasz *et al.*, 2007; Taylor and Bordoni, 2020). These three layers together form the vein wall and play a significant role in determining its behavior. Alterations to the venous wall structure occur in various conditions such as VVs and intraluminal thrombus, which can result in the addition of a new internal layer adjacent to the intima, thereby inducing mechanical reactions (Laredo and Lee, 2018).

1.8.3 Lower Extremity Venous Anatomy

Veins are essential components of the circulatory system that facilitate the return of deoxygenated blood from the lower extremities to the heart (Oğuzkurt, 2012). Unlike arteries, veins operate under low pressure and rely on muscular contractions to assist with blood flow (Bit, 2020). The anatomy of the venous system in the lower extremities is variable in some segments and constant in others (Cavezzi et al., 2006). In this regard, the lower limb venous system can be divided into three systems: superficial, deep, and perforator veins, which play a pivotal role in counteracting hydrostatic venous pressure (MacColl and Khalil, 2015). The classification of the lower limb venous system is based on its connection to the muscular fascia that surrounds the calf and leg muscles and, hence, its location in either the superficial or deep part (Meissner, 2005).

1.8.3.1 Superficial veins and junctions

The venous system of the lower extremities is composed of the superficial veins, which are located between the dermis and the muscle fascia and are responsible for draining cutaneous microcirculation into the deep veins system (Oğuzkurt, 2012). The major superficial veins include the great saphenous vein (GSV) and the small saphenous veins (SSV) (Santler and Goerge, 2017). The terminology for these veins has evolved over time, with previous terms such as "the long saphenous vein" for the GSV and "lesser saphenous vein" for the SSV. To standardize the terminology and avoid ambiguity, these terms should no longer be used (Cavezzi *et al.*, 2006; D. K. Lee *et al.*, 2017). In addition, the superficial venous system contains the saphenous junctions,

which are the sapheno-femoral junction (SFJ) and the sapheno-popliteal junction (SPJ). This study will provide a detailed discussion of each component of the superficial veins and junctions.

1.8.3.1.1 Great saphenous vein (GSV)

The great saphenous vein (GSV) is the longest vein in the human body, extending from the foot to the saphenofemoral junction (Oğuzkurt, 2012). The GSV originates from the dorsal venous arch of the foot, passing anteriorly to the medial malleolus, and then posterior-medially towards the knee. At the knee level, the GSV courses medially through the thigh, penetrating the muscular fascia to join the common femoral vein at the saphenofemoral junction located below the inguinal ligament (MacColl and Khalil, 2015a; D. K. Lee *et al.*, 2017). The GSV typically contains 8-12 valves, with more valves found below the knee compared to above the knee level (Hoballah and Bechara, 2021). Accessory branches and tributaries of the GSV may also play a role in the pathophysiology of CVI (Meissner, 2005).

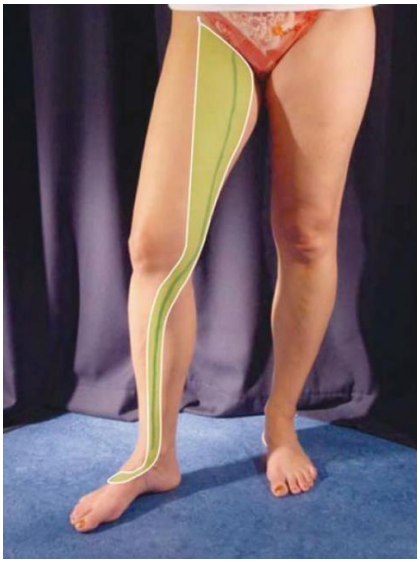


Fig 1.5: The great saphenous vein (Mendoza, Lattimer and Morrison, 2014).

1.8.3.1.2 Tributaries of the GSV

There is variability in the branching (tributaries) of the GSV among individuals, and standardization of their terminology has been attempted. One approach proposed by (Caggiati *et al.*, 2002) is to name these branches based on their location relative to the GSV (Fig 1.6). However, due to the use of different names and the difficulty in naming some of the branches, standardization of GSV branch terminology remains a challenge (Lee *et al.*, 2017; Santler and Goerge, 2017).

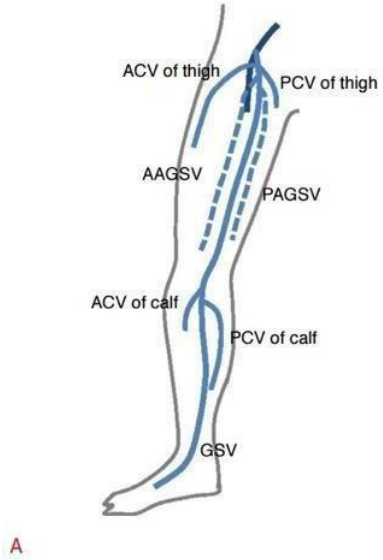


Fig 1.6: The GSV and its branches (D. K. Lee *et al.*, 2017).

1.8.3.1.2.1 the anterior accessory GSV (AAGSV)

The anterior accessory GSV tributary (AAGSV) is a vein located anteriorly and parallel to the GSV in the thigh, which commonly drains into the anterior circumflex vein (ACV) of the thigh (Oğuzkurt, 2012). AAGSV is of clinical significance in patients with primary VVs, as it may be incompetent while the GSV remains competent (Ricci, 2017). Studies have reported that incompetent AAGSV is present in approximately 14% of patients with VVs (Cavezzi *et al.*, 2006), although in some cases, reflux may occur in both the AAGSV and GSV (Ricci and Georgiev, 2002), Moreover, the AAGSV may be the origin of recurrent VVs, making it significant for diagnosis and treatment (Theivacumar, et al.,2009).

1.8.3.1.2.2 posterior accessory GSV (PAGSV).

The posterior accessory GSV (PAGSV) is a vein that runs parallel to the GSV in the posterior aspect of the thigh, but it is not consistently present and may not always connect with the GSV (Cavezzi *et al.*, 2006; Caggiati *et al.*, 2014). Its clinical importance lies in its association with venous ulceration, as the posterior tibial perforators are connected with the PAGSV and the posterior tibial veins, but not the distal part of the GSV (Youn and Lee, 2019).

1.8.3.1.2.3 posterior circumflex vein (PCV) of the thigh

The posterior circumflex vein (PCV) of the thigh tributary is located posteriorly and obliquely to the GSV in the thigh (Cavezzi *et al.*, 2006) (Fig 1.7).

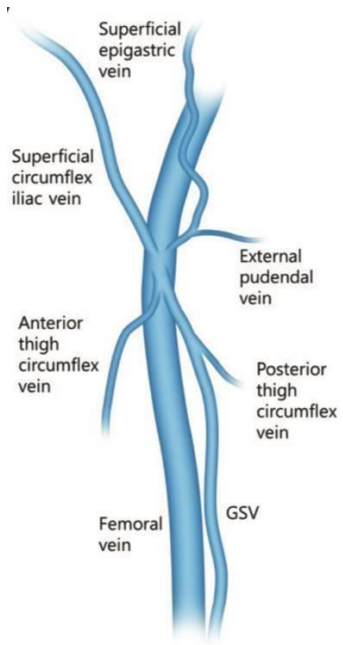


Fig 1.7: Anatomy of the anterior thigh circumflex vein and the posterior thigh circumflex vein. These veins drain venous flow from the anterolateral and posteromedial thigh, respectively (Kim *et al.*, 2017).

1.8.3.1.2.4 anterior circumflex vein (ACV) of the thigh

The anterior circumflex vein (PCV) of the thigh tributary is located anteriorly and obliquely to the GSV in the thigh (Cavezzi *et al.*, 2006).

1.8.3.1.2.5 posterior circumflex vein (PCV) of the calf

The posterior circumflex vein (PCV) of the calf tributary is located posteriorly and obliquely to the GSV in the calf (Cavezzi *et al.*, 2006).

1.8.3.1.2.6 the anterior circumflex vein (ACV) of the calf

The anterior circumflex vein (PCV) of the calf tributary is located anteriorly and obliquely to the GSV in the calf (Cavezzi *et al.*, 2006).

1.8.3.1.3 Saphenofemoral junction (SFJ)

The saphenofemoral junction (SFJ) represents the point at which the GSV terminates and is located at the level of the inguinal ligament (groin crease) (Cavezzi *et al.*, 2006). However, the anatomy of SFJ is not uniform among individuals, and there can be significant variations (Souroullas *et al.*, 2017). It is crucial to understand these anatomical differences between individuals for effective surgical treatment and to minimize the risk of recurrence. The SFJ plays a crucial role in treatment methods, flow patterns, and recurrent varicosities, making it an important area of study (Youn and Lee, 2019). The SFJ can be visualized through Duplex ultrasound as a "Mickey mouse" sign, which depicts the common femoral artery, common femoral vein, and the GSV (Fig 1.8) (Igari *et al.*, 2013).

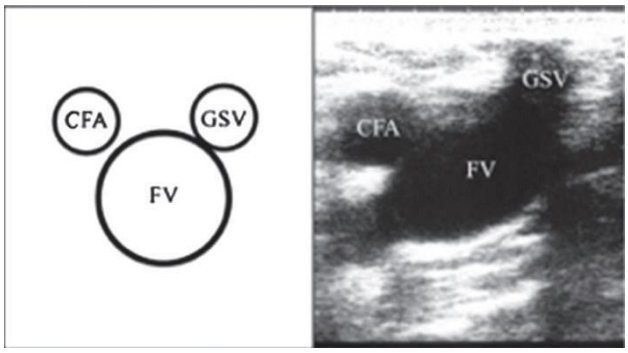


Fig 1.8: A cross-sectional image of the SFJ, also referred to as the "Mickey Mouse" sign, demonstrates the femoral vein, common femoral artery, and the GSV (Igari *et al.*, 2013).

1.8.3.1.4 Small saphenous vein (SSV)

The small saphenous vein (SSV) is positioned in the posterior aspect of the leg, extending from the ankle level along the mid-calf region until it reaches the sapheno-popliteal junction at the level of the popliteal vein where it concludes its course, as illustrated in (Figure 1.9) (MacColl and Khalil, 2015). It is noteworthy that, while the termination of the small saphenous vein typically occurs at the popliteal fossa level, variations in termination location can occur, with some cases exhibiting termination below or above this level (Oğuzkurt, 2012).

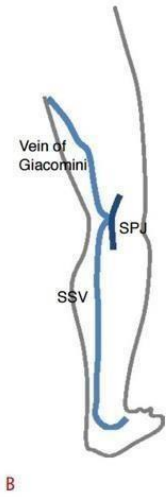


Fig 1.9: The small saphenous vein (SSV) (D. K. Lee *et al.*, 2017).

1.8.3.1.5 The thigh extension (TE) of the SSV:

The literature suggests that the termination location of the SSV exhibits variability. Specifically, the SSV commonly terminates at the popliteal fossa through a direct connection with the popliteal vein or indirectly through the gastrocnemius vein, observed in 50% to 80% of extremities according to previous studies (Dodd, 1965; Vasdekis *et al.*, 1989). Conversely, in 0% to 10% of extremities, the SSV terminates at a lower point by joining either the gastrocnemius or the GSV in the posterior proximal calf (Dodd, 1955; Engel *et al.*, 1994). Furthermore, in some instances, the

SSV may terminate above the popliteal fossa in 14% to 47% of extremities (Moosman and Hartwell, 1964; Thomas and Chan, 1988).This variability in termination location is of utmost clinical significance, as it impacts the optimal surgical treatment for patients with SSV incompetence who require SSV ablation to ensure the best clinical outcomes.

The termination location of the small saphenous vein (SSV) above the popliteal fossa displays significant variation (Min et al., 2003). Specifically, the thigh extension of the SSV may either continue connecting with the femoral vein (known as the femoro-popliteal vein) or continue connecting with the intersaphenous vein (formerly referred to as the vein of Giacomini (VG)) (Oğuzkurt, 2012b; Kim *et al.*, 2017) (Fig 1.10). This thigh extension terminates in the femoro-popliteal vein by connecting through one or more superficial or perforating veins of the thigh or gluteal region, but not in the GSV (Natsis *et al.*, 2015). The femoro-popliteal vein runs through the groove between the biceps femoris and semimembranosus muscles and is present in 72% to 95% of extremities (Georgiev, Myers and Belcaro, 2003). Conversely, the SSV may terminate in the VG when it branches upwards in the popliteal fossa and connects with the GSV through the posterior communicating vein of the thigh (Georgiev et al., 2003). For example, in 14% of the population, the SSV connects to the VG, then into the GSV. The vein of Giacomini also makes a connection branch between the GSV and the SSV, which is often overlooked in classic textbooks (Bush and Hammond, 2007). This vein is significant, as venous insufficiency may not only occur in the GSV, SSV, and accessory saphenous branches, but also in the VG combined with reflux in the upper or lower part of the thigh (Theivacumar *et al.*, 2007). The prevalence of venous insufficiency in the vein of Giacomini varies between 2.5% to 86% (Zierau, Küllmer and Künkel, 1996; Georgiev et al., 2003).

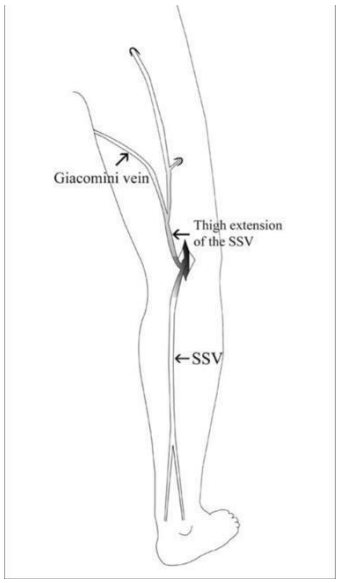


Figure 1.10: The termination of the SSV may continue to the femoral vein by one or more superficial or perforating veins of the thigh or gluteal region or rise up medially to connects with the GSV through the posterior circumflex vein (PCV) of the thigh to form the Giacomini vein (Oğuzkurt, 2012).

1.8.3.1.6 Saphenopopliteal junction (SPJ):

The Saphenopopliteal junction (SPJ) is where the SSV ends with the popliteal vein. Usually SPJ is located 2-4 cm above the popliteal crease, however, the exact location varies between individuals (Cavezzi *et al.*, 2006).

1.8.3.2 Deep veins

The lower extremity venous system comprises deep veins that are situated beneath the muscle fascia and are responsible for draining the blood from the muscles of the lower extremities back to the heart (Cina *et al.*, 2005). Deep veins typically run parallel to the corresponding arteries and transport about 85% of the total blood volume (Santler and Goerge, 2017). The deep venous system can be divided into the veins of the calf, the veins of the thigh, and the popliteal vein that is located behind the knee joint (Lee *et al.*, 2017). According to (Reček, 2006) the veins of the calf consist of pairs of anterior tibial, posterior tibial, and peroneal veins, which are situated on either side of the artery. The deep veins of the thigh include the common femoral vein, the femoral

vein, and the deep femoral vein (Fig 1.11), which will be discussed in detail in this study.

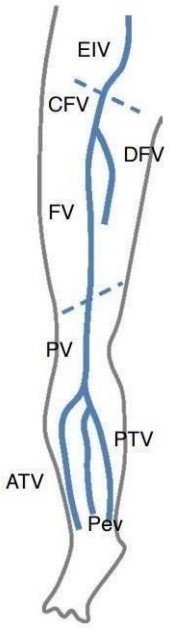


Fig 1.11: The deep veins of the lower extremity (D. K. Lee *et al.*, 2017).

1.8.3.2.1 The deep veins of the calf

1.8.3.2.1.1 Anterior tibial veins

The anterior tibial vein is a continuation of the dorsal pedal vein and courses along the anterior aspect of the leg, running between the tibia and fibula over the interosseous membrane. Subsequently, it anastomoses with the posterior tibial vein, contributing to the formation of the tibioperoneal trunk and popliteal vein (Lee *et al.*, 2017). Its main physiological role is to drain venous blood from the knee, ankle, tibiofibular joints and the anterior compartment of the lower leg (Binstead et al., 2017).

1.8.3.2.1.2 Posterior tibial veins

The posterior tibial vein is situated at the posterior aspect of the tibia and terminates by joining the popliteal vein at the posterior aspect of the knee. Its drainage area includes the medial and lateral plantar veins, the posterior part of the leg, and the surface of the foot (Lee *et al.*, 2017). The main function of the posterior tibial vein is to receive venous blood from the posterior muscles of the lower leg, the lateral and

medial plantar veins, and the plantar surface of the foot, and to transport it towards the larger veins of the lower limb (Binstead et al., 2017).

1.8.3.2.1.3 Peroneal veins

The peroneal vein is extends alongside the posteromedial part of the fibula and connects the posterior tibial vein (Lee *et al.*, 2017). The peroneal veins main function is to carry the blood from the lateral part of the leg and then withdraw the blood into the posterior tibial vein (Binstead et al., 2017).

1.8.3.2.2 The deep veins of the thigh

1.8.3.2.2.1 Common femoral veins

The common femoral vein (CFV) is located medially to the common femoral artery and separated from the external iliac vein by the groin (inguinal) ligament (Lee *et al.*, 2017). The common femoral vein function is to drain the lower limb extremities (Bit, 2020).

1.8.3.2.2.2 Femoral veins

Femoral vein (FM) is a new term replaced by an old term superficial femoral vein (Caggiati *et al.*, 2002). The old term is not recommended to use because this vein is a deep femoral vein not a superficial vein (Bundens *et al.*, 1995). Femoral vein links the popliteal vein to the common femoral vein (Gloviczki *et al.*, 2011). According to (Hoballah and Bechara, 2021), this vein is an extension of the popliteal vein at the level of the adductor magnus tendon which runs upwards anteromedially to the femoral artery at the thigh to collect blood from numerous muscular branches. The femoral vein then ascends to collect the blood from the deep femoral vein (profundal femoral vein) and the GSV where they finally meet at the CFV.

1.8.3.2.2.3 Deep femoral veins

The deep femoral veins (in another term the profundal femoral vein), is located medially to the common femoral vein and runs alongside the deep femoral artery connecting with the femoral vein to form the common femoral vein (Lee *et al.*, 2017). This vein drains the blood from the muscles in the posterior and lateral thigh and the deep femoral communicating veins (Caggiati *et al.*, 2002).

1.8.3.2.2.4 Popliteal veins

The popliteal vein is located medially to the popliteal artery and runs along the lower posterior knee to the distal part of the anteromedial thigh. This vein is formed when the anterior and posterior tibial veins are connected together (Lee *et al.*, 2017). Popliteal veins function is to drain the blood from the lower leg (Sawyer et al., 2020).

1.8.3.3 Perforator veins (PVs)

The lower extremities' venous system comprises the perforating veins (PVs), which traverse the muscle fascia and link the superficial veins with the deep veins (Meissner, 2005). These PVs play a crucial role in draining blood from the superficial system to the deep system (Mozes and Gloviczki, 2007). Although there are numerous perforators in the lower extremities, only a few are clinically significant, and their size and location vary. Among the clinically significant perforators, those situated in the mid-calf region are the most noteworthy (Oğuzkurt, 2012) (Fig 1.12). Typically, perforators are named based on their location in the leg (Ghali et al., 2005). According to (Lee *et al.*, 2017) perforator names are classified into groups and subgroups. Groups indicate the linguodental locations, which include the thigh, knee, leg, and ankle perforators, while subgroups denote the side locations, which include anterior, posterior, medial, and lateral perforators. Therefore, the name of the perforator is primarily a combination of its linguodental location and side location, such as the medial thigh perforator (Fig 1.13) or the anterior ankle perforator. Furthermore, (Caggiati *et al.*, 2002) stated that more comprehensive subgroups exist for the medial thigh and medial leg perforators. The medial thigh perforators are further subdivided

into the perforating vein of the femoral canal and the inguinal perforating vein, while the medial leg perforators are subdivided into the paratibial and posterior tibial perforating veins.

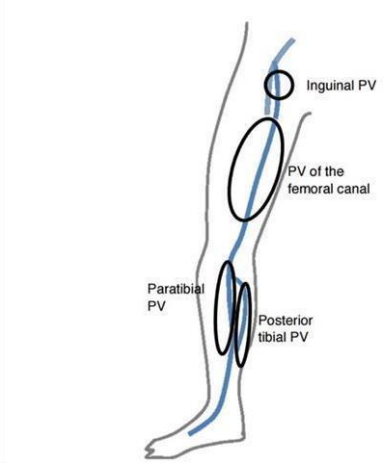


Fig 1.12: The perforating veins alongside the medial aspect of the lower extremity (D. K. Lee *et al.*, 2017).

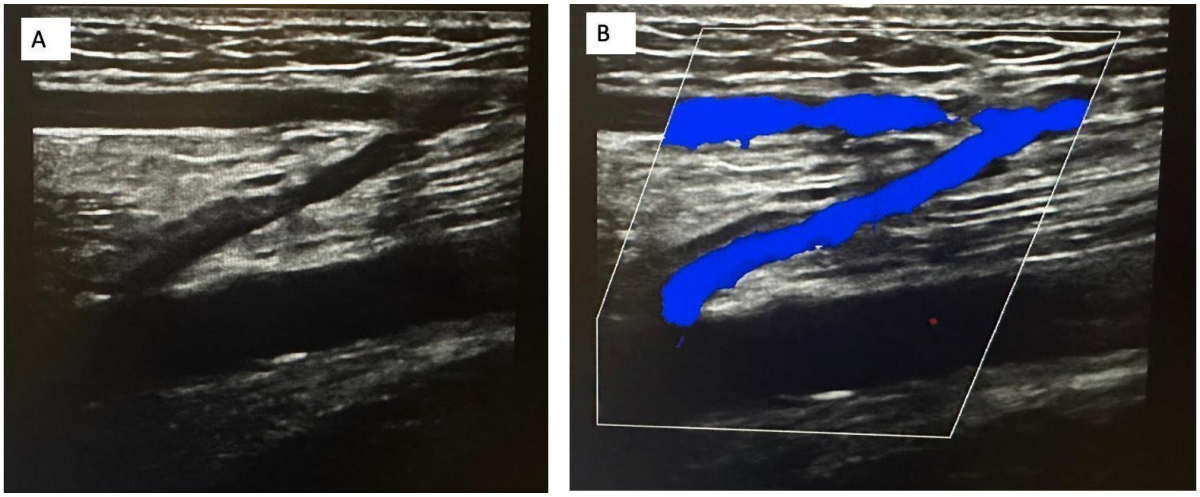


Fig 1.13: A competent perforator seen in the mid-thigh using B-mode ultrasound in image A and colour doppler ultrasound in image B.

1.8.3.4 Venous valves

The venous system of the lower limb contains valves which are present in the superficial, deep, and perforating veins (Meissner, 2005). Valves are typically located in the firmer extended segments of the vein and can take on two forms: a thick and strong form or a thin and transparent form, and are often bicuspid (Necas, 2010). The

leaflets of the valves move towards the center of the vein when closing, acting as automatic gates that allow blood flow to pass centrally while inhibiting its passage peripherally (Van Bemmelen *et al.*, 1990b; Lurie, Kistner, *et al.*, 2003) Valves are composed of connective tissue, elastic fibers, and a small number of SMCs that are covered with endothelium (Formaggia, et al., 2010). Venous valves play an important role in venous hemodynamics.

The normal function of venous valves is essential for proper blood flow and venous hemodynamics. Firstly, competent venous valves can resist increased hydrostatic pressure in the lower limb's peripheral regions (Meissner, 2005). Secondly, these valves play a crucial role in preventing blood flow from reversing from the deep system to the superficial system and preventing reversed blood flow from the proximal to the distal parts (PIERI *et al.*, 1997; Hach, 2006; Gloviczki and Dalsing, 2009). The valves open to allow blood flow to pass and close to prevent blood flow from reversing (Schweighofer, Mühlberger and Brenner, 2010) (Fig 1.14). In addition, as noted by (Van Bemmelen *et al.*, 1990), these valves remain open when lying down in the supine position and close when standing. Blood flows normally in an upright direction called the "antegrade direction" when the valves are open. However, while standing, blood flow initially moves in the opposite direction, known as the "retrograde direction," for a brief period of time (less than 0.5 sec) before returning to the normal upright (antegrade) position (Lurie, 2009). This reflux of blood flow is considered normal. Pathological reflux (incompetent venous valves) is defined when the retrograde time exceeds 0.5 sec.

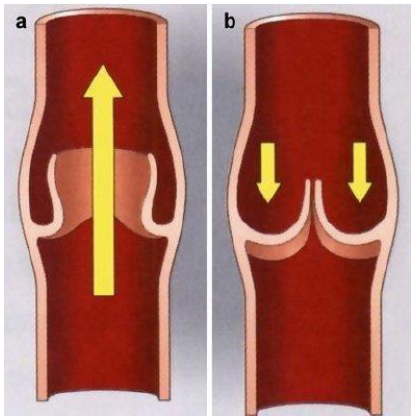


Fig 1.14: The venous valves in the lower limb. (a) Valves open when ascending flow (b) valve closes when there is a return flow (Mendoza et al., 2014).

Incompetent venous valves may arise from various risk factors, such as endothelial dysfunction, hormonal imbalances, genetic predisposition, increased venous pressure, and other lifestyle factors (Sadick, 1992). Once the valve is damaged, venous reflux can occur (Raju *et al.*, 2000). According to (Meissner, 2005), in primary cases of venous reflux in the superficial veins, a phenomenon known as "the domino effect" can take place. The domino effect refers to the occurrence of venous reflux starting from the proximal to distal parts, beginning with the failed terminal and subterminal valves of the GSV located at the top of GSV. Subsequently, the upper part of GSV becomes distended, causing increased pressure on the following valve distally, which in turn fails. This failed venous valve process continues distally, causing failure of the weaker valves along the way. Consequently, the dilated vein's normal straight texture will transform into tortuous VVs.

The venous system contains a varying number of valves, which differ among the superficial, deep, and perforator veins. Typically, as the veins move distally away from the central circulation, the number of valves increases and decreases when moving upward (Meissner, 2005; Lee *et al.*, 2016). The IVC and iliac veins do not contain any valves, while terminal and subterminal valves exist at the top of GSV in the superficial venous system (Portugal *et al.*, 2014). In the thigh and leg parts of the GSV and SSV, respectively, there are 1-2 and 10-12 valves (Moore et al., 2011). The number of valves in the PVs ranges from one to three, located beneath the fascia (Laredo and Lee, 2018). In the deep venous system, one valve is located at the level of the inguinal ligament in the CVF, while 1-6 valves are located in the FV within 3 cm of the deep femoral vein tributary (Mühlberger et al., 2008). The popliteal vein contains 0-4 valves close to the adductor hiatus and 12 valves in the deep calf veins (Kwakye, 1971).Although the deep venous system valves are typically located in the same location in the deep veins of the leg, their location may vary among individuals. Thus, new imaging modalities may be useful in providing accurate diagnoses and understanding the association of these valves with the development of venous disease, which may provide novel insights into the treatment of deep venous disease (Moore et al., 2011).

1.8.4 Ultrasound markers of the venous anatomy

1.8.4.1 The Egyptian eye

DUS is a valuable tool for identifying the saphenous compartment and its surrounding fascia, as noted by Caggiati, 1997). The superficial compartment, which contains the saphenous veins and their tributaries, is located within the subcutaneous tissue and is separated from the muscular compartment by the saphenous fascia (Cavezzi *et al.*, 2006). This fascia is thinner than the muscular fascia, and both appear hyperechogenic on duplex ultrasound. The junction of the saphenous and muscular fascia forms the saphenous compartment, which houses the GSV and the ultrasonographic "eye sign," also known as the Egyptian sign (Ricci and Georgiev, 2002). This sign is so named because in a transverse ultrasound scan, the saphenous veins passing through the saphenous compartment resemble an eye, with the saphenous lumen as the iris, the saphenous fascia as the upper eyelid, and the muscular fascia as the lower eyelid (Oğuzkurt, 2012). The Egyptian sign allows for easy identification of the saphenous veins and differentiation from parallel subcutaneous tributaries, and is present in both the GSV and SSV (Oğuzkurt, 2012) (see Figure 1.15).

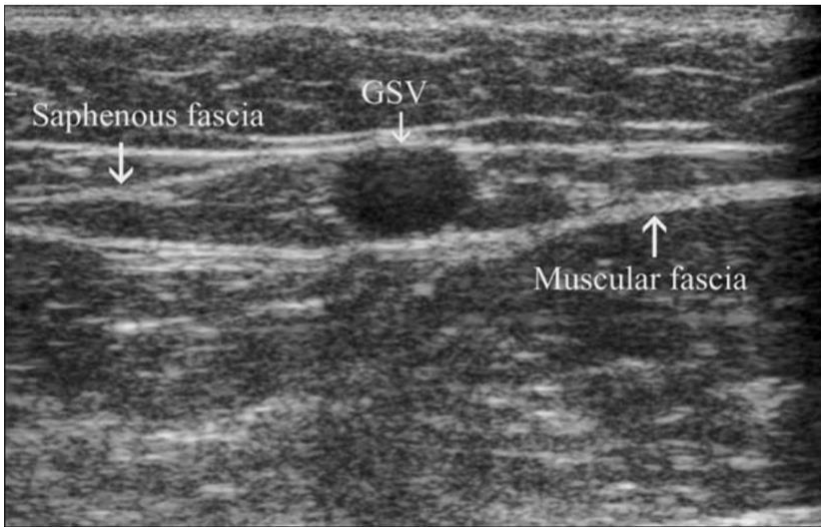


Figure 1.15: "The Egyptian sign" showed in a transverse section of an ultrasound image (Oğuzkurt, 2012).

1.8.4.2 *Alignment sign*

In a transverse ultrasound image of the upper third of the thigh, the alignment sign is observed, indicating the presence of two "saphenous eyes" corresponding to the GSV and the Anterior Accessory Saphenous Vein (AASV), respectively (Oğuzkurt, 2012) (Figure 1.16). To differentiate between the GSV and the AASV, the latter is located anterior lateral to the GSV and is aligned with the femoral vessels, including the deep femoral artery, superficial femoral artery, and femoral vein (Cavezzi *et al.*, 2006). In some cases where the GSV is absent or hypoplastic, only one "saphenous eye" corresponding to the AASV is observed while the GSV is not visible (Ricci and Caggiati, 1999).

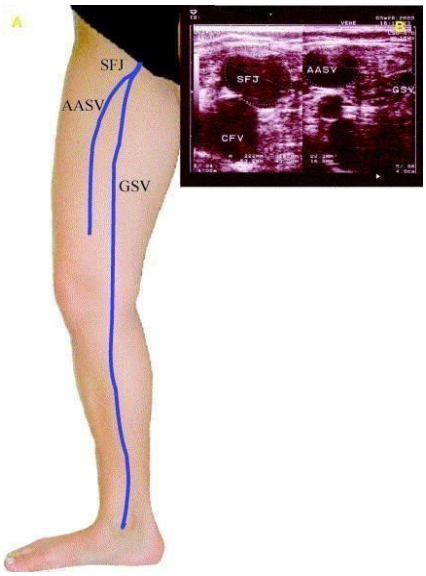


Fig 1.16: The alignment sign. (A) The AASV is located anterior lateral to the GSV where these two veins end in the SFJ. (B) The left image presents the SFJ while the right image presents the AASV located anterior lateral to the GSV. The AASV aligns with the femoral vessels (Cavezzi *et al.*, 2006).

1.8.4.3 *Tibio–gastrocnemius angle sign*

In a transverse ultrasound image of the GSV below the knee level, a triangular sign known as the tibio-gastrocnemius angle sign can be observed (Spinedi *et al.*, 2016). This sign is formed by the medial gastrocnemius muscle, the tibia, and the facial sheet,

and can be used to identify and distinguish the GSV from adjacent GSV tributaries (Chen and Prasad, 2009) (Fig 1.17). Moreover, the presence of an empty saphenous space in this region may indicate the absence or hypoplasia of the GSV (Cavezzi *et al.*, 2006).

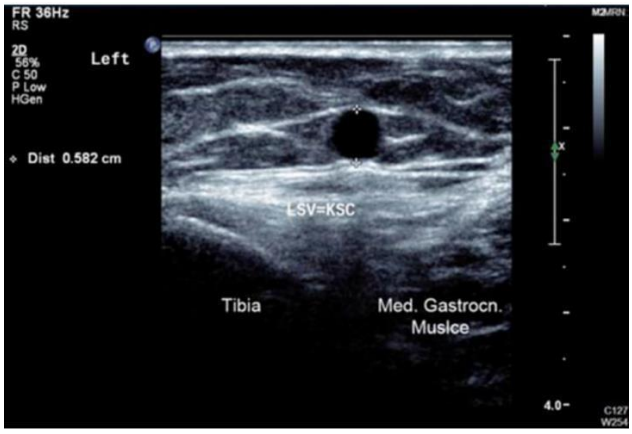


Fig 1.17: The tibiogastrocnemius angle sign where the GSV is located between tibia and medial gastrocnemius muscle below the knee (Chen and Prasad, 2009).

1.8.4.4 Tributaries

Tributaries are situated external to the saphenous compartment and travel in a parallel direction to the saphenous vein (Cavezzi *et al.*, 2006). Ultrasound imaging confirms that these veins are located beyond the saphenous eye, as illustrated in Figure (1.18), as noted by (Ricci *et al.*, 1999).

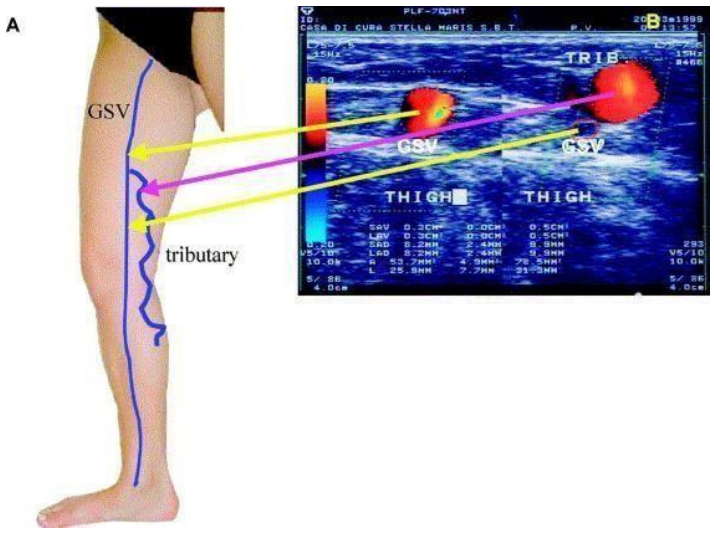


Fig 1.18: An ultrasound image of the mid-thigh area showing the relationship between the GSV and a tributary (Cavezzi *et al.*, 2006).

1.9 Physiology

1.9.1 The venous system

The venous system is a network of vessels that facilitate the transport of deoxygenated blood from various organs and tissues back to the heart, counteracting the force of gravity in the process (Bradbury, 2011). As per (Laredo and Lee, 2018), this blood is first directed to the right atrium before it is transferred to the right ventricle, which pumps it through the pulmonary circulation for oxygenation. Subsequently, the oxygen-rich blood travels from the lungs to the left atrium and left ventricle, which then circulates it throughout the body's organs and tissues via the arterial system.

The neuromuscular system regulates venous blood flow, while contractions of the muscle pump system, which consists of the foot, calf, and thigh muscles, are responsible for facilitating the return of venous blood (Recek, 2013). The velocity of venous blood flow is highly variable, ranging from high flow rates to no flow (Lee et al., 2016). The venous system is more complex than the arterial system, as it relies on valves and the muscle pump to return venous blood against the force of gravity (Meissner *et al.*, 2007).

The venous system of the lower extremities plays a crucial role in the body, serving as both conduits for facilitating blood flow against gravity and capacitance vessels capable of accommodating a significant proportion of the total blood volume (Pang, 2001; Laredo and Lee, 2018). In fact, veins in the lower extremities can hold up to two-thirds of the total blood volume, making them a vital blood reservoir (Hall JE., 2016). This remarkable capacity is attributed to the structural composition of veins, which comprise collagen, elastin, and SMCs (Buijtenhuijs *et al.*, 2004; Tansey *et al.*, 2019). Specifically, the SMCs in the superficial veins of the lower extremities function to counterbalance changes in blood volume resulting from alterations in hydrostatic pressure (Shepherd and Vanhoutte, 1975). Compared to arterial structure, veins possess less muscle and elastic tissue, thinner walls, and a larger diameter (William, Yingyot, Arora and Kunal, 2021). The regulation of venous tone is essential for maintaining the blood volume in the venous system, which, in turn, works in tandem with arterial tone to regulate venous return and cardiac output (CO) (Mark H. Meissner *et al.*, 2007). Consequently, any alterations in venous capacitance can have a

significant impact on CO due to the essential role of the venous system in serving as the primary capacitance in the body (Pang, 2000).

Moreover, the venous system possesses a high degree of vascular compliance, referring to the degree of distensibility in a blood vessel in response to changes in pressure (Magder, 2016; Tansey *et al.*, 2019). For instance, when exposed to high pressure, the vein will distend, increasing its capacity to accommodate blood volume, whereas it will contract and decrease in volume when subjected to low pressure (Mark H. Meissner *et al.*, 2007). Notably, the compliance ability of veins is 40 times higher than that of arteries (Deschamps and Magder, 1994), making them a suitable choice as arterial bypass grafts (Tansey *et al.*, 2019). Blood volume compliance can be calculated by the following equation (Aggarwal *et al.*, 2015):

$$\text{Blood volume compliance (C)} = \frac{\textbf{Volume of blood (V) in (mL)}}{\textbf{The amount of pressure (P) in (mmHg)} \text{ that the blood is experiencing}}$$

A thorough understanding of the relationship between blood volume and blood pressure is essential to comprehend normal and abnormal venous function (Katz et al., 1969). Changes in venous compliance can significantly affect the volume of blood entering the heart, ultimately impacting the CO (Tansey *et al.*, 2019). In healthy individuals, venous return equals CO, indicating that the heart cannot pump more blood than it receives (Patterson and Starling, 1914; Mark H. Meissner *et al.*, 2007). Hence, a comprehensive understanding of the venous system should acknowledge that alterations in venous pressure can lead to changes in CO, which in turn can affect arterial blood pressure (Young, 2010).

1.9.2 The effect of the Hydrostatic pressure

Hydrostatic venous pressure, also known as right atrial pressure (RAP), is a physical force that arises from the weight of the column of blood from the level of the right atrium to a specific point (Tansey *et al.*, 2019). As such, hydrostatic venous pressure is influenced by the height of the blood column and therefore varies with an individual's height (Laredo and Lee, 2018). The excessive pressure may lead to dilation of the veins (Recek, 2013). In patients with CVI, the hydrostatic pressure in the lower extremities may not decrease when elevating the feet due to venous flow obstruction or incompetent valves (Youn and Lee, 2019).

The hydrostatic venous pressure is affected by changes in body posture due to the position of the vein relative to the heart (Tansey *et al.*, 2019). The pressure begins to increase below the right atrium, and the larger the distance from the right atrium, the greater the column of blood and the higher the pressure, which is especially pronounced in the feet (Meissner *et al.*, 2007) (Figure 1.19). For example, when healthy individuals lie supine and elevate their legs, the hydrostatic pressure at the ankle decreases to a negative value, whereas when their legs lie flat, the pressure at the ankle slightly increases to approximately 10 mmHg (Laredo and Lee, 2018). In a standing position with relaxed calf muscles, venous valves initially remain in a neutral position (closed), but gravitational forces cause hydrostatic venous pressure in the foot to increase due to the excessive compliance of veins, which can redistribute up to approximately 500 ml of blood to the peripheral veins (Levick, 2013). The hydrostatic pressure at the level of the right atrium is zero and increases by 0.8 mm Hg/cm until it reaches 90 mmHg at the ankle level, depending on the individual's height (Meissner *et al.*, 2007; Recek, 2013). According to (Bradbury, 2011), this pressure is increased due to an undivided blood column from the right atrium to the foot, and when the venous pressure in one segment exceeds the pressure in the upper segment, the valves open.

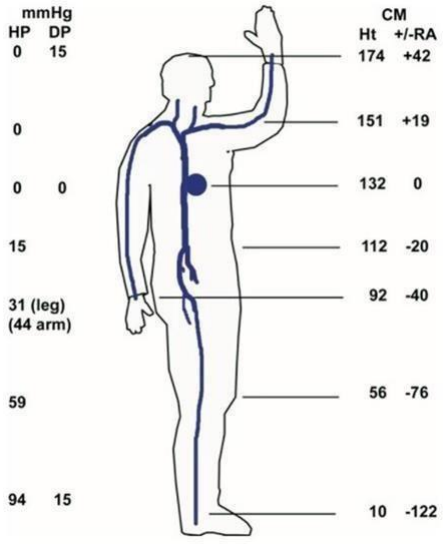


Fig 1.19: Hydrostatic venous pressure (Hp)at various heights (Ht) (Meissner *et al.*, 2007).

In addition, (Bradbury, 2011) has elucidated that during physical activity such as walking and running, the compression of deep veins and sinuses increases the pressure, pushing the blood upwards. Valves that function efficiently prevent the reversal of blood flow within 0.5-1.0 seconds. These competent valves also play a crucial role in dividing the single high-pressure venous blood column into smaller low-pressure columns during muscular contraction (systole) and relaxation (diastole) (Franceschi C., 2003). Consequently, the hydrostatic pressure drops from 90 to 30 mmHg to reach the normal ambulatory venous pressure (AVP) (Lee et al,. 2016). Reduced venous pressure is instrumental in minimizing oedema build-up in the feet and reducing venous pooling (Suzuki *et al.*, 2009). Venous pooling is defined as the extended transfer time of blood within the venous circulation (Tansey *et al.*, 2019). Therefore, gravity and hydrostatic pressure exert a physiological impact that counteracts venous return. However, the venous valves and pump system effectively overcome this issue (Alimi et al., 1994).

1.9.3 The venous muscle pump systems

The venous muscle pump system comprises the foot, calf, and thigh muscles, which are responsible for facilitating 90% of the venous return from the lower limbs to the heart through the deep veins (Goldman and Fronek, 1989; Bradbury, 2011). Moderate movement of the feet and legs in a seated position can promote venous blood return and decrease lower venous pressure (Stranden, 2000; Lee et al., 2016). In contrast, during walking, the pump system undergoes three phases of contraction (Laredo and Lee, 2018). Firstly, raising the foot during dorsiflexion expands the Achilles tendon, resulting in the drainage of venous blood from the peroneal and posterior tibial veins (Lawrence L. Tretbar, 1999). Dorsiflexion also stimulates the calf muscle pump (Lee et al., 2016). The gastrocnemius and soleus muscles, which contain numerous venous sinuses, comprise the calf muscle pump. Contraction of these muscles compresses the venous sinuses, which leads to the transfer of blood to the deep system and upward to the thigh (Meissner, 2005; Bradbury, 2011). During calf muscle contraction, calf muscle pressure can reach 250 mm Hg, while calf blood volume is reduced by 80% (Kuster et al., 1968; Eberhardt and Raffetto, 2005).

In addition, the reversal of blood from the deep venous system to the superficial system is prevented during muscle contraction not by valve closure, but rather by the closure of the junctions at the saphenofemoral and saphenopopliteal regions, as well as the closure of non-junctional perforators (NJP) (Bradbury, 2011). Furthermore, the facia and muscle that pass through the perforators apply external pressure, which contributes to the prevention of reversed blood flow (Baliyan *et al.*, 2016). This external pressure is essential in protecting the skin, subcutaneous tissue, and superficial veins from the high pressure of the deep venous system (up to 250 mmHg) generated during systole by the calf muscle pump (Mani *et al.*, 2019).

The second phase of the pump system involves overcoming hydrostatic pressure through the planter venous plexus within the calf deep system (Lee, NicoLaides and Myers, 2016). This is achieved through a combination of body weight and contraction of the planter venous plexus, which in turn causes contraction of the lateral planter veins, leading to distension of the middle part of the lateral planter veins acting as a reservoir (Uhl and Gillot, 2012). During each step, a small volume of blood, approximately 20-30 ml, is extracted (Scurr and Smith, 1993). The third phase of the pump system starts when the foot strikes the ground and the toes touch the floor during Planter flexion, which stimulates the foot pump due to the weight of the body and contraction of the planter muscles of the foot (Corley *et al.*, 2010). Additionally, contraction of both gastrocnemius and soleus muscles occurs to drain the calf muscle sinuses (Laredo and Lee, 2018).

1.9.4 The calf pumps

The calf muscle is known to be the most efficient pump muscle in the lower extremity when compared to the foot and thigh muscles (Burnand K G. In: Gloviczki P, Yao JST, 2001). This is due to its high ejection fraction (EF) which is the percentage of blood pumped outside the calf with each contraction, as well as its ability to produce high pressure and hold a large capacitance compared to other muscles (Ludbrook, 1966; Meissner, 2005). During walking, the systole phase (calf muscle contraction)

generates a pressure gradient between the deep veins in the calf and the popliteal veins, allowing for rapid transfer of blood from the calf to the upper extremities (Laredo and Lee, 2018). The posterior fascial compartment of the calf can experience venous pressure of up to 250 mm Hg during calf contraction (Eberhardt and Raffetto, 2005), and this pressure increase is distributed among the deep and superficial posterior compartments of the calf, the anterior tibial compartment, the popliteal vein, and the great saphenous vein (Alimi, et al., 1994). Competent valve closure is responsible for preventing backward blood flow when venous pressure exceeds intramuscular pressure in the calf compartment, and this valve function is supported by the deep fascia of the leg which presses the muscles during contraction to allow high pressure to be released within the muscular compartment (Meissner, 2005; J. F. Uhl and Gillot, 2012).

During diastole, when the calf muscles are relaxed, the veins are drained of blood, and the venous pressure decreases to a range of 15 to 30 mm Hg (Eberhardt and Raffetto, 2005). In addition, the pressure in the deep venous system decreases until it falls below the pressure in the superficial venous system (Laredo and Lee, 2018). Although the pressure decreases during calf muscle relaxation, the pressure in the popliteal vein remains constant, as reported in this study. The superficial veins collect blood from the superficial tissues and transfer it to the deep venous system through perforator veins that fill the sinuses (Meissner, 2005). Finally, during diastole, valves closure prevents backward flow of blood (Uhl and Gillot, 2012).

1.9.5 Effect of exercise on venous function

Regular exercise has been widely acknowledged to confer health benefits by reducing the risk of developing cardiovascular diseases and associated mortality (Paffenbarger *et al.*, 1986). Those who engage in regular physical activity exhibit lower blood pressure, better plasma lipoprotein profile, and increased insulin sensitivity (Nystoriak and Bhatnagar, 2018). Moreover, consistent exercise has been demonstrated to improve arterial vascular function and structure, enhancing arterial endothelial function (Birk *et al.*, 2012) and reducing arterial stiffness (Kakiyama *et al.*, 2005). In addition, regular exercise has been shown to improve venous compliance, with studies

indicating that trained individuals possess greater calf venous compliance compared to sedentary individuals (Hernandez and Franke, 2005). Additionally, regular exercise plays a significant role in promoting venous drainage, leading to a substantial reduction in hydrostatic pressure (Laredo and Lee, 2018).

In the context of changing body position from horizontal to standing, individuals with and without venous insufficiency experience a similar increase in hydrostatic pressure by 80-90 mmHg in both arteries and veins of the foot (Laredo and Lee, 2018). The magnitude of this pressure elevation is dependent on the distance between the right atrium and the foot (Tansey *et al.*, 2019), and it is equivalent to the pressure at the ankle (Pollack *et al.*, 1949). In normal individuals, walking reduces hydrostatic pressure by around 60 mmHg after 3-12 steps, ultimately reaching a steady-state pressure of 22 mmHg at a speed of 1.7 miles per hour, due to the muscles and competent valves preventing retrograde blood flow during relaxation periods (Pollack and Wood, 1949). However, in patients with primary VVs, venous valves are unable to prevent retrograde blood flow, resulting in a faster return of the venous hydrostatic pressure to the hydrostatic level than in normal individuals, leading to higher mean pressure during walking and faster return to hydrostatic control level after completing the walk (Pollack *et al.*, 1949).

1.10 Aetiology

The etiology of CVI has been the subject of ongoing investigation. Although some aspects remain incompletely understood, recent research has revealed that CVI arises from elevated venous blood pressure resulting from a complex interplay of functional, biochemical, and structural changes (Meissner, 2005). These changes have been classified in the literature as either primary or secondary causes of CVI (Ortega *et al.*, 2021), with primary causes accounting for the majority (70%) of cases, and secondary causes for the remaining 30% (Patel and Surowiec, 2021). While secondary CVI primarily arises from obstruction, primary CVI may be caused by various mechanisms.

1.10.1 Primary CVI:

In the medical literature, primary CVI is characterised as a progressive process that occurs without an inciting event in the venous wall or valves, resulting in venous remodeling (Ortega *et al.*, 2021). These primary changes lead to venous weakening and dilation, ultimately resulting in the development of CVI (Meissner, 2005). These mechanisms include:

1.10.1.1 Venous hypertension and venous stasis:

CVI is primarily caused by venous hypertension, which is characterised by the expansion of the diameter of superficial veins in the lower limb, often in combination with other factors (Bradbury, 2011). The underlying mechanism of venous hypertension is frequently attributed to risk factors such as obesity, pregnancy, age, occupational or environmental factors, and family history, which increase the likelihood of developing CVI (Jawien, 2003). Venous hypertension typically occurs below the knee due to either incompetent venous valves or venous obstruction, or a combination of both, and is unable to be alleviated in the upright position (Eberhardt and Raffetto, 2014; Santler and Goerge, 2017). Moreover, calf muscle pump failure exacerbates venous hypertension (Goldman and Fronek, 1989). In healthy individuals with competent venous valves and relaxed muscle, the hydrostatic venous pressure in the standing position is typically high, ranging from 80 to 90 mmHg (Youn and Lee, 2019). However, during walking, the hydrostatic venous pressure is significantly reduced to approximately 30 mmHg due to the peripheral heart effect system, which engages the calf muscle contractions and the coordinated actions of the deep venous system and venous valves to facilitate the return of deoxygenated blood from the lower extremities back to the heart against gravity (Boisseau, 2003; J. J. Bergan *et al.*, 2006).

In contrast, patients with CVI exhibit a different pattern of hydrostatic pressure while walking, known as ambulatory venous hypertension (AVH). AVH occurs when calf muscle contraction generates high pressure in the deep venous system, which

subsequently transfers the blood to the superficial system due to incompetent perforator veins and then to the skin microcirculation (Mark H. Meissner *et al.*, 2007). Notably, AVH persists even during walking, leading to slow venous blood circulation in the lower limbs, also referred to as venous stasis (Laverick *et al.*, 2016).AVH is associated with various clinical manifestations, such as pain, heavy legs, swelling, skin pigmentation, and subcutaneous fibrosis. In cases of ulceration, AVH occurs at pressures higher than 90 mm Hg (Plate *et al.*, 1986; Jawien, 2003). It is worth noting that while several studies have shown that sustained AVH can damage venous valves and walls, leading to the progression of CVI, some experimental studies have suggested that more complex structural mechanisms may underlie the development of venous hypertension (Ortega *et al.*, 2021).

1.10.1.2 Functional abnormalities:

1.10.1.2.1 Valvular incompetence:

The primary functions of venous valves include preventing pathological venous reflux and segmenting the hydrostatic pressure column of blood to prevent full pressure column of blood from applying force on the distal vein (Golgman and Fronek, 1989; Lee *et al.*, 2016). Pathological venous reflux is characterized by retrograde flow that occurs when the venous valves are either congenitally absent or malfunctioned, also known as incompetent valves (Natsis *et al.*, 2015). These incompetent valves can result from primary venous disease, such as degenerative processes like inflammatory infiltrate, or secondary venous disease, such as thrombus (Back *et al.*, 1995; Ono *et al.*, 1998). In the absence of functioning valves, retrograde blood flow during calf muscle relaxation halts the normal decrease in pressure and the fast-venous refilling that occurred from the retrograde flow of blood and the capillary inflow (Hallett, 2009). As a result, high venous pressure can be transferred from the deep venous system through the incompetent perforators to the superficial venous system, causing reflux (Youn and Lee, 2019). The incidence of venous reflux in the lower extremities varies at different sites, with 68% of reflux occurring in the saphenous below the knee, 55% above the knee, and 30% at the saphenofemoral junction (Boisseau, 2003). In patients with ulceration, venous reflux occurs severely in more than one segment (Eberhardt and Raffetto, 2014).

Previous research proposed that venous reflux was the main hemodynamic malfunction leading to the development of CVI (Cotton, 1961). However, recent evidence suggests that valve failure may not be the primary cause of CVI, as there may be underlying primary valve abnormalities (Meissner, 2005). Several studies have indicated the presence of venous valve abnormalities prior to the onset of valvular incompetence (Rose and Ahmed, 1986a; Gandhi *et al.*, 1993). Therefore, researchers have shifted their attention to exploring structural and biochemical abnormalities of the venous wall, including the "weak wall theory," which attributes the development of CVI to changes in connective tissue and venous tone (Vanhoutte et al., 1997).

1.10.1.2.2 Calf muscle dysfunction:

In addition to valvular abnormalities, functional abnormalities such as muscle pump failure may also contribute to the development of chronic venous disease (CVD) (Back *et al.*, 1995). Patients with stiff joints or immobilized individuals are particularly at risk for muscle pump failure (Santler and Goerge, 2017). Damage to the musculoskeletal components of the calf muscle pump, resulting in venous incompetence, can progress to CVD (Williams *et al.*, 2014). Muscle pump failure occurs when there is severe reflux or obstruction, causing unsuccessful venous blood draining from the distal parts of lower limbs and resulting in increased hydrostatic pressure similar to that experienced after extended standing (Araki *et al.*, 1994a; Mark H. Meissner *et al.*, 2007). Primary conditions, such as muscle-wasting syndromes and neuromuscular disorders, rarely cause muscle pump dysfunction (Christopoulos *et al.*, 1989). This mechanism of muscle pump dysfunction can significantly contribute to the progression of venous ulcers (Araki *et al.*, 1994). In limbs with reflux but no ulceration, the calf muscle ejection fraction percentage is highest (53%), but it decreases in limbs with healed ulceration (49%) and is the lowest in limbs with active ulceration (35%) (Meissner, 2005). This decrease may be associated with the severity of the disease and the continuous decrease in ankle range of motion (Back *et al.*, 1995).

1.10.1.3 Venous wall hypoxia

Vein wall hypoxia is characterised by insufficient oxygen supply to the lumen or vasa vasorum or both (Lim *et al.*, 2011). This condition occurs periodically and after prolonged standing (Boisseau, 2002). Two types of hypoxic states affect the venous wall: Media hypoxia and Stasis-induced hypoxia (Pak *et al.*, 2007). The former results from the compression of the vasa vasorum by distension of the vein due to blood stasis and elevated hydrostatic pressure (Malone and Agutter, 2009). In contrast, endoluminal hypoxia occurs when venous blood flow stasis reduces oxygen refill compared to normal venous flow (Michiels et al., 2000). In this case, the endothelial and inner layers of the vein wall are the first to be affected (Nicolaides, 2003). The weight of the blood column, which acts on the small diameter of the capillary-like vessels, and flow damage to the vasa vasorum during prolonged standing cause these two types of hypoxia (Boisseau, 2003).

In advanced stages of CVI, the progression of stasis dermatitis and cellulitis is attributed to a combination of tissue hypoxia and venous blood flow stasis (Mathes, 2011). Tissue hypoxia refers to an insufficient supply of oxygen and nutrients to the cells, which can lead to reduced metabolic demands and poor tissue nutrition (Wheaton and Chandel, 2011). Additionally, in later stages of CVI, ulceration develops due to a combination of factors including hypoxia, venous hypertension, and venous flow stasis, which can trigger an inflammatory response and fibrosclerotic remodeling of the skin (Kabnick and Scovell, 2020). Ulceration can also result from injury and cellular death (Gefen *et al.*, 2008). Studies have shown that ulceration was observed in 74% of patients due to various factors such as edema, injury, cellulitis, dry skin, insect bites, and dermatitis (Johnson et al., 2015). This occurs due to reduced venous circulation and weakened biochemical passage necessary for immune and inflammatory reactions to the infected area (Berti-Hearn and Elliott, 2019). Therefore, patients with ulceration are at a higher risk of infection.

Studies on cell culture and ex vivo plants have shown that hypoxia stimulates leucocytes and endothelium to release mediators that regulate vein wall remodeling, as observed in varicosities (Lim *et al.*, 2011). Veno-active drugs (VAD) have been shown to enhance venous oxygenation and prevent the activation of leucocytes and

endothelium by hypoxia (Boisseau, 2002). However, the relationship between hypoxia and the development of varicosities remains unclear due to heterogeneity and poor study designs in published in vivo studies (Malone and Agutter, 2009; Lim *et al.*, 2011). Molecular studies have demonstrated the effect of hypoxia on causing inflammatory changes and remodeling of the vein wall, as observed in varicosities (Michiels et al., 2002). Further research is necessary to expand our understanding of the role of hypoxia and to develop effective treatments.

1.10.1.4 Histopathology changes:

Histopathological changes are a common occurrence in dilated saphenous veins due to valvular remodeling, which involves alterations in the collagen and elastin content throughout the vessel wall (Gandhi *et al.*, 1993; MacColl and Khalil, 2015). VVs segments have been found to exhibit increased collagen and reduced elastin and laminin levels when compared to normal non-varicose segments (Wali and Eid, 2002). Interestingly, reduced elastin levels have been observed in both healthy segments and VVs segments at high risk of developing venous insufficiency (Clarke *et al.*, 1992). Additionally, connective tissue irregularities have been observed in the upper limb veins of patients with VVs (Vanhoutte, et al., 1997). Collectively, these findings suggest that histopathological changes in venous structure contribute to the progression of valvular reflux and the development of CVI.

1.10.1.5 Inflammation:

Incompetent valves and vein wall remodeling in CVI may be caused by inflammation (Grudzińska and Czuba, 2014). It has been suggested that tissue damage resulting from an inflammatory response may initiate this (Schmid-Schönbeinet al., 2001). Understanding the mechanisms that trigger inflammation in CVI is crucial (Chen *et al.*, 2018). One such mechanism suggested by current evidence is alterations in blood flow shear stress, which can lead to the release of proinflammatory messengers from areas of low shear stress and hemodynamic changes under the influence of increased venous pressure (Berk *et al.*, 2001). Conversely, normal blood flow shear stress promotes anti-inflammatory effects (Sorescu *et al.*, 2004). In addition, several

inflammatory markers have been implicated in the development of primary venous valve dysfunction, including increased endothelial permeability leading to leukocyte attachment, mast cell infiltration into connective tissue, lymphocytes, fibrotic tissue infiltration, and various molecular markers such as membrane adhesion molecule generation and growth factor production (Schmid-Schönbein et al.,2001). These markers can be observed in the early stages of CVI, facilitating early diagnosis and timely management and treatment to prevent disease progression.

Several studies have established a link between the development of VVs and the activity of matrix metalloproteinases (MMPs), adhesion molecules, macrophage-released growth factors, and proinflammatory cytokines (Sayer and Smith, 2004; Sprague and Khalil, 2009). In particular, macrophage/monocyte white blood cells have been identified in different locations within incompetent veins, including the adventitia and vasa vasorum, but not in the muscular layer (Ono *et al.*, 1998; Knaapen *et al.*, 2005). The infiltration of macrophages near venous valves, along with the secretion of proteolytic enzymes, may contribute to the weakening and damage of the valve (Ono *et al.*, 1998). Additionally, a large number of monocyte-platelet complexes have been found in both lower and upper limbs of patients with venous ulcers, and these complexes persist even after surgical intervention, suggesting their involvement in the early pathophysiology of VVs (Peyton *et al.*, 1998; Rohrer *et al.*, 2002). These findings provide important insights into the inflammatory processes that contribute to the development of VVs and may inform the development of targeted interventions to prevent or manage this condition.

1.10.1.6 Genetics:

Primary CVI can also be caused by genetic factors. It was reported that 17% of CVI is caused from genetic factors (Ortega *et al.*, 2021). Patients with CVI carried specific polymorphisms and gene mutations and variants that play a significant role in developing CVI (Laredo and Lee, 2018). For instance, the association between the C282Y polymorphism in hemochromatosis (HFE gene) and venous ulceration has

been reported in the study by (Gemmati *et al.*, 2009). Likewise, epigenetic changes have also been discovered in patients with CVI.

1.10.1.7 Hormonal:

In recent years, there has been a growing body of literature exploring the relationship between hormonal factors and the development of CVI. Among the hormones implicated in the pathogenesis of CVI are progesterone and estrogen (Mashiah, 1999). Progesterone has been shown to cause smooth muscle cell relaxation, vasodilation, and valvular incompetence (Ropacka-Lesiak, Kasperczak and Breborowicz, 2012). Similarly, estrogen has been found to cause vasodilation, which can also contribute to valvular incompetence (Serra *et al.*, 2016). Pregnancy is a period of significant physiological changes that affect the venous system, including hormonal, hemodynamic, and mechanical factors that have been linked to the development of CVI (Ortega *et al.*, 2021).

1.10.2 Secondary CVI:

1.10.2.1 Venous obstruction:

Secondary CVI is a result of venous obstruction leading to venous hypertension (Neglén ., 2003). The obstruction, such as DVT, activates inflammation, valve scarring, adhesion, and luminal narrowing, resulting in damage to the vein wall and valves (Patel and Surowiec, 2021). Additionally, DVT may restrict blood outflow, causing elevated venous pressure during muscle contraction, leading to secondary muscle pump failure (Eberhardt and Raffetto, 2014). This study suggests that venous obstruction may also be caused by venous stenosis or extrinsic compression, in addition to DVT. Post-thrombotic syndrome, which occurs after DVT, results from residual venous flow obstruction and valve reflux caused by valve damage (Kahn *et al.*, 2014), leading to venous hypertension.

The association between venous obstruction, venous reflux and the development of venous ulceration has been extensively studied. (Labropoulos *et al.*, 2007) reported that both obstruction and venous reflux are commonly found in patients with venous ulceration. However, (Killewich *et al.*, 1985) suggested that symptoms of ulceration may be more closely related to a decrease in venous refilling time rather than continuous abnormalities of venous outflow, such as valve incompetence. To accurately diagnose severe post-thrombotic syndrome, it is crucial to identify the anatomy of venous reflux and obstruction (Labropoulos *et al.*, 1996). Asymptomatic and mildly symptomatic patients typically present with isolated and segmental reflux, whereas patients with ulceration and skin changes often exhibit deep, superficial and perforator vein reflux that is typically multi-segmental. Furthermore, nearly two thirds of patients with ulceration have a multisystem disease that affects multiple anatomical venous systems (Hanrahan *et al.*, 1991). Post-thrombotic skin changes are predominantly associated with superficial venous reflux and reflux in the popliteal and posterior tibial veins (van Bemmelen *et al.*, 1991). Lastly, (Meissner *et al.*, 1998) highlighted that constant obstruction in the popliteal vein is a significant indicator for confirming the severity of CVD signs.

1.11 Varicose veins in pregnancy:

Pregnancy is a well-established risk factor for the development of VVs in women, which can subsequently progress to CVI and leg edema (Stansby, 2000). The symptoms of VVs during pregnancy are similar to those in the general population, including pain, leg heaviness, swollen legs, night cramps, itchiness, and numbness (Smyth, Aflaifel and Bamigboye, 2015). These symptoms typically appear in the first trimester in 70-80% of pregnant women (Carr, 2006). A study by (Dindelli *et al.*, 1993) examined the risk factors for venous diseases before and during pregnancy in 611 women. This study found that women who had one previous pregnancy had a relative risk of 1.2 for developing venous disease, whereas women who had two or more pregnancies had a relative risk of 3.8. Moreover, 28% of nulliparous women who had not had any previous pregnancies developed venous disease during pregnancy. These findings were consistent with studies by (J. Beebe-Dimmer *et al.*, 2005; Ismail *et al.*, 2016), which indicated that multiparity women are at higher risk of developing

VVs and have a higher incidence of VVs compared to nulliparous women. Despite the abundance of research on the relationship between pregnancy and VVs, it remains unclear whether pregnancy is an independent risk factor or simply exacerbates pre-existing risk factors (Stansby, 2000).



Fig 1.20: Large size VVs runs parallel to the path of the GSV became visible during the fourth month of pregnancy (Cornu-Thenard and Boivin, 2014).

There are three main theories described in the literature for the development of VVs during pregnancy. These theories are mechanical, hereditary, and hormonal in nature. The mechanical theory encompasses three sub-theories. Firstly, the increased size of the gravid uterus which compresses the iliac and pelvic veins, thereby disturbing venous return from the lower limbs back to the heart (Mashiah, 1999). However, this theory has been abandoned since clinical evidence shows that venous dilatations begin their development in the first weeks of pregnancy when the gravid uterus size is still insignificant (de Barros *et al.*, 2010). Secondly, increased blood volume during pregnancy (Ismail *et al.*, 2016), which is mainly due to plasma activity, causes an increase in blood pressure that makes it difficult for veins to move blood from the lower body back to the heart, thus influencing the development of VVs (Bamigboye and Smyth, 2007). The third mechanical theory is due to increased uterine blood circulation (de Barros *et al.*, 2010), which results in increased pelvic pressure that causes iliac

vein obstruction and reduced draining capacity of the lower extremity veins (Boivin et al.,2000).

Hereditary theory is widely reported in a large number of studies, highlighting the importance of family history as a risk factor in the development of VVs (Fiebig *et al.*, 2010; Krysa, Jones and van Rij, 2012; Msh *et al.*, 2013; Fukaya *et al.*, 2018). Currently, the most widely accepted theory is the hormonal theory. Estrogen and progesterone hormones during pregnancy have been found to be strongly associated with the development of VVs (Ismail *et al.*, 2016). Progesterone hormone relaxes the muscular walls of the blood vessels, increasing venous distensibility, while estrogen hormone expands venous walls, causing venous distensibility (Dindelli *et al.*, 1993). Despite the existing theories for the development of VVs during pregnancy, there is still a lack of information to establish the cause of VVs in the general population and specifically in pregnant women.

The long-term consequences of VVs that develop during pregnancy have been the subject of limited quantitative analysis. (Asbeutah *et al.*, 2015) conducted a study using DUS to assess venous structure and function during pregnancy and in the postpartum period. Their findings revealed a significant increase in venous diameters and venous reflux, particularly during the third trimester. However, these changes reverted to normal values after delivery. Similarly, a small study conducted by (Sparey *et al.*, 1999) reported that pregnancy causes vein dilatation, but the valves remain competent. Nonetheless, larger studies with extended follow-up periods are necessary to provide a comprehensive understanding of the long-term impact of venous changes that occur during pregnancy.

1.12 Epidemiology of VV in pregnancy:

According to existing literature, the prevalence of VVs during pregnancy exhibits a wide range of variation, owing to the utilisation of varied concepts, classifications, and epidemiological analysis methods, as well as regional and racial disparities. Several studies pertaining to this subject provide only an approximation of the prevalence of varicose disease during pregnancy (as shown in Table 1.4). This approximation ranges from 25 to 82% of pregnant women, taking into account all types of VVs. In

light of the scarcity of studies on VVs during pregnancy, undertaking the proposed study would also offer an accurate and up-to-date estimate of the prevalence of VVs among pregnant women.

Study	Prevalence (%)
(Boivin and Hutinel, 1987)	30-40%
(Dindelli *et al.*, 1990)	57.9 %
(Sciannameo *et al.*, 1993)	48.17%
(Canonico *et al.*, 1998)	40.5%
(Boivin, et al.,2000)	64.39%
(Coughlin *et al.*, 2001)	25%
(de Barros *et al.*, 2010)	72.7%
(Ismail *et al.*, 2016)	82%

Table 1.4: Prevalence of VVs during pregnancy, according to literature.

1.13 Anatomical and physiological changes during pregnancy

Women undergo significant anatomical and physiological changes during pregnancy to support fetal growth and development (Soma-Pillay *et al.*, 2016). These changes commence at conception, peak in the third trimester, and return to normal in the postpartum period (Morton, 2021). While healthy females can adapt to these changes, those with preexisting medical conditions may experience exacerbations of pregnancy-related disorders (Bhatia and Chhabra, 2018). Recognising the normal anatomical and physiological alterations during pregnancy is essential for distinguishing them from pathological changes (Soma-Pillay *et al.*, 2016). This knowledge serves as a fundamental basis for clinicians in their clinical decision-making. The present study solely aims to elucidate the normal cardiovascular anatomical and physiological changes to provide a foundation for comprehending the pathophysiology of VVs during pregnancy. The ensuing changes are delineated below:

1.13.1 Anatomical changes

During pregnancy, women undergo a series of anatomical changes, which include an increase in the size of the gravid uterus. As a result, the heart is displaced in the cephalad direction and laterally, as reported by (Hall et al., 2011). Additionally, by the 20th week of gestation, the gravid uterus causes pressure on the inferior vena cava (IVC) and the descending aorta when the pregnant woman is in the supine position, as noted by (Krywko and King, 2021). This position can result in maternal hypotensive syndrome, leading to a decrease in central venous return, as observed by (Kim and Wang, 2014). Symptoms of hypotensive syndrome, such as temporary tachycardia followed by bradycardia, hypotension, dizziness, sweating, and nausea, have been reported by (Lanni, Tillinghast and Silver, 2002). To prevent the risk of unconsciousness or sudden maternal death, it is recommended that pregnant women be placed on their lateral decubitus position, as unresolved maternal hypotension can lead to such fatal outcomes (De-Giorgio *et al.*, 2012).

1.13.2 Physiological changes

1.13.2.1 Hormonal changes

1.13.2.1.1 Serum progesterone

Pregnancy requires an increase in progesterone hormone levels to cause structural and functional changes in the endometrium that prepare for embryo implantation and pregnancy establishment, as noted by (Kumar and Magon, 2012). The source of progesterone hormone secretion varies from early to late pregnancy, according to (Ku *et al.*, 2021; Cable and Grider, 2022) reported that serum progesterone is primarily produced from the corpus luteum before the 6th week of gestational age, whereas after the 7th week of pregnancy, the placenta becomes the main source of production, eventually accounting for almost all progesterone secretion after the 10th week of pregnancy. Most of the progesterone secreted from the placenta enters the maternal circulation, as observed by (Hinson et al., 2010). Maternal progesterone concentration levels increase from 6- to 8-fold from the luteal phase to term, as reported by (Reshef and Taylor, 2000). These elevated progesterone levels have been linked with

increased levels of low-density lipoprotein (LDL) cholesterol, as noted by (An-Na *et al.*, 1995). The placenta's limited ability to synthesize cholesterol de novo, coupled with cholesterol accessing the trophoblasts as LDL cholesterol during pregnancy, results in LDL cholesterol being the primary substrate for placental progesterone synthesis, as reported by (Yañez and Leiva, 2022). and confirmed by (Edison *et al.*, 2007; Pecks *et al.*, 2016).

Progesterone hormone concentrations vary throughout the menstrual cycle and in each trimester of pregnancy, with slight variation among different laboratories. According to (Reshef and Taylor, 2000), progesterone levels range between 11.2 to 90.0 nanograms per milliliter (ng/mL) in the first trimester of pregnancy, 25.6 to 89.4 ng/mL in the second trimester of pregnancy, and 48 to 150 to 300 or more ng/mL in the third trimester of pregnancy. In the postpartum period, progesterone measurements were either less than 1 nanogram per milliliter (ng/mL) during the preovulation period or 5 to 20 ng/mL during the mid-cycle period, as observed by (Carmina and Lobo, 2009). Progesterone hormone reaches its highest levels during the third trimester, indicating its potential impact on venous pathology in late pregnancy.

The impact of progesterone hormone on the vascular system is significant (Stachenfeld, 2008). Multiple studies have reported the association between elevated levels of progesterone hormone and the development of VVs in the superficial venous system of lower limbs, particularly in pregnant women (J. Beebe-Dimmer *et al.*, 2005), with a higher prevalence in women with multiple pregnancies (Ismail *et al.*, 2016). Serum progesterone concentrations were observed to be higher in pregnant women with VVs compared to those without VVs (Lenkovic *et al.*, 2009). Furthermore, a study by (Mashiah, 1999) reported a significant increase in progesterone receptors in the walls of VVs segments compared to normal venous segments, especially in women. Progesterone activates receptors in the nuclei of cells within the walls of veins, leading to the relaxation of smooth muscle and collagen fibers, resulting in vasodilation and the potential development of valve reflux and VVs (Mashiah, 1999; Ropacka-Lesiak, Kasperczak and Breborowicz, 2012). Moreover, progesterone increases plasma volume in pregnancy by reducing protein diffusion through capillary endothelium,

thereby avoiding the expected increase in blood pressure via (Stachenfeld and Taylor, 2005; Lenkovic *et al.*, 2009).

1.13.2.1.2 Estrogens

In addition to progesterone, elevated levels of estrogen hormones are also necessary during pregnancy. The role of estrogen in pregnancy is crucial as it facilitates the maintenance of uteroplacental blood flow by increasing plasma levels and uterine blood flow (Mandalà, 2020). During the early stages of pregnancy, the corpus luteum is responsible for the production of estrogen, while from around the 9th week of gestation, the placenta takes over this function (Berkane *et al.*, 2017). The process of estrogen synthesis during pregnancy involves three types of estrogens, namely Estrone (E1), Estradiol (E2), and Estriol (E3) (Cui, Shen and Li, 2013). Upon production by the placenta, E1 and E2 estrogens are converted into estriol (E3) and estetrol (E4) (Shimizu, 2010). Estriol is the predominant estrogen during pregnancy and reaches its peak during the third trimester before returning to normal levels post-delivery (Draca, 2006). According to Quest, estriol levels vary depending on the stage of pregnancy, ranging from ≤0.21 ng/mL during the normal menstrual cycle to ≤2.50 ng/mL, ≤9.60 ng/mL, and ≤14.60 ng/mL during the first, second, and third trimesters, respectively. However, these levels are subject to daily variation and may differ across laboratories based on the equipment and chemicals utilised.

Increased levels of estrogen hormones have been found to significantly affect the vascular system, specifically the superficial venous system of the lower limbs, and are associated with the development of VVs, particularly in women, including pregnant women (Serra *et al.*, 2016). Studies have reported a remarkable increase in estrogen receptors in patients with CVI compared to controls, which was also associated with the severity of venous disease (Serra *et al.*, 2016). Additionally, (Mashiah, 1999) found a significant increase in estrogen receptors in the walls of VVs segments compared to normal venous segments of the same vein, especially in females. Similar to progesterone, the effect of estrogen on the vascular system is mediated by estrogen receptors (ERs), which cause smooth muscle and collagen fiber relaxation resulting in vasodilation and potential valve reflux and VVs development (Mashiah, 1999; Ropacka-Lesiak, Kasperczak and Breborowicz, 2012).

Estrogen promotes vasodilation by activating the synthesis of prostacyclin and nitric oxide while reducing the creation of vasoconstrictor agents such as reactive oxygen species, cyclooxygenase-derived products, angiotensin II, and endothelin-1 (Miller and Duckles, 2008). Estrogen has also been found to damage collagen fiber connections and synthesis, which can lead to VVs without venous hypertension (Ropacka-Lesiak, Kasperczak and Breborowicz, 2012). Additionally, estrogen increases plasma volume in pregnancy by reducing protein diffusion through the capillary endothelium, which prevents the anticipated increase in blood pressure through vasodilation (Stachenfeld and Taylor, 2005; Lenkovic *et al.*, 2009). These findings suggest that estrogen plays a crucial role in the development of VVs during pregnancy and that modulation of estrogen levels may be a potential strategy for VVs prevention and treatment.

1.13.2.1.3 Relaxin

Increased levels of Relaxin hormones are essential during pregnancy as they play a crucial role in preparing the female body for pregnancy. Relaxin, a peptide hormone produced by the corpus luteum and placenta, is released in early pregnancy during the luteal phase of the ovulatory cycle (Stachenfeld and Taylor, 2005). This hormone is responsible for preventing impulsive uterine contractions, relaxing pelvic ligaments, repining the cervix, and activating the mammary gland, which are all crucial steps for pregnancy (Sherwood, 2004). Additionally, Relaxin stimulates vascular proliferation in the endometrium, which is combined with the rise of uterine blood flow during pregnancy, ultimately encouraging placenta and uterine growth (Sherwood, 2004). The hormone also plays a significant role in regulating maternal cardiovascular changes during pregnancy and mediating renal vasodilation adaptations that are essential for both maternal and fetal health (Conrad, 2011). Relaxin is involved in maternal kidney physiology and acts through the endothelial endothelin B receptor-nitric oxide (NO) pathway to mediate the vasodilation of renal arteries (Teichman *et al.*, 2010). This hormone also prepares the female body for implantation by activating endometrial decidualization and increasing the concentration of local immune cells

needed for implantation (Strauss and Williams, 2019). Overall, Relaxin plays a crucial role in preparing the female body for pregnancy, facilitating placental and uterine growth, regulating maternal cardiovascular changes, and mediating renal vasodilation adaptations that are essential for both maternal and fetal health.

Upon conception, Elastin hormone levels reach their peak at the end of the first trimester of pregnancy and subsequently decrease to an intermediary level throughout pregnancy (Soma-Pillay *et al.*, 2016). The maintenance of appropriate levels of Relaxin hormone is crucial for a healthy pregnancy. The work by (Weiss *et al.*, 1993) elucidated that excessive levels of Relaxin can cause premature labor by disrupting the cervical connective architecture. Conversely, inadequate levels of Relaxin during pregnancy may have an adverse effect on glucose metabolism (Goldsmith and Weiss, 2009).

Increased levels of Elastin hormone have a significant impact on the vascular system. Like progesterone and Estrogen hormones, Elastin acts as a vasodilator hormone, promoting venous dilation, which may ultimately result in valve dysfunction and the development of VVs (Kristiansson and Wang, 2001; Skøtt and Carter, 2002; Dschietzig *et al.*, 2003; Elamrawy *et al.*, 2021). Relaxin, in turn, enhances the synthesis of matrix metalloproteinase (MMP)-induced collagen and reduces the effects of the tissue inhibitors of MMPs (Adams *et al.*, 2012). This hormone mediates the relaxation of smooth muscle cells and weakens the collagen and connective tissue, both of which play an essential role in the pathogenesis of VVs (Conrad, 2011).

1.13.2.2 Hemodynamic changes

1.13.2.2.1 Cardiac output (CO)

Normal pregnancy is known to be associated with a substantial increase in cardiac output (CO) (Bader *et al.*, 1955; Vanoppen, Stigter and Bruinse, 1996). This increase in CO can be as high as 40-50% above the normal levels and typically peaks at mid-gestation (Robson *et al.*, 1989; Hunter and Robson, 1992a; Edouard *et al.*, 1998). CO is determined by two key factors: heart rate (HR) and stroke volume (SV) (Bruss and

Raja, 2021). HR is the number of heartbeats per minute, while SV is the volume of blood ejected with each stroke of the heart (Feher, 2012). In early pregnancy, the rise in SV moderates CO, whereas in late pregnancy, the increase in CO is primarily driven by an increase in HR (Sanghavi and Rutherford, 2014). The overall change in HR during pregnancy is estimated to be between 20% and 25% above baseline, with a progressive elevation from the first trimester and the highest rates in the third trimester, increasing by 10 to 20 bpm (Mahendru *et al.*, 2014). In contrast, SV increases from early pregnancy until 28-32 (Metcalfe et al., 1981). SV increases slowly until the end of the second trimester and then either stabilizes or decreases later in pregnancy (Sanghavi and Rutherford, 2014). The underlying mechanisms for the increase in SV during pregnancy remain unclear. Studies in pregnant rats and humans have suggested that the rise in cardiac contractility may be a contributing factor (Slangen *et al.*, 1996), while others have proposed that the increase in venous return may play a significant role (Edouard *et al.*, 1998).

CO is commonly assessed in pregnancy using non-invasive echocardiography techniques (Sanghavi and Rutherford, 2014). To minimise positional disparity, CO measurements using echocardiography are typically obtained with the mother in a left lateral position, as it has been shown that CO is reduced by 16.4% when the mother is in the supine position (Humphries *et al.*, 2019). Cardiac magnetic resonance imaging (MRI) is another method that can be used to examine CO in pregnancy (Prokšelj and Brida, 2021). In a study of 34 normal pregnant women in their third trimester and three months post-delivery, echocardiography and cardiac magnetic resonance were compared, revealing a rise in left ventricular diastolic volume, left ventricular diameter, and overall CO (Ducas *et al.*, 2014). Although these CO changes occur in singleton pregnancies, the changes in CO differ between singleton and twin pregnancies. In twin pregnancies, CO is 15% higher with a significant increase in the diameter of the left ventricle and the left ventricular diastolic volume (Hunter and Robson, 1992).

1.13.2.2.2 Blood pressure (BP)

In healthy pregnancies, there is a decrease in arterial pressure, which is composed of systolic blood pressure (SBP) and diastolic blood pressure (DBP) (Hall, George and

Granger, 2011). The reduction in arterial pressure during pregnancy is gradual and more pronounced in DBP and mean arterial pressure compared to SBP (Sanghavi and Rutherford, 2014). This reduction begins in the first trimester, around 6-8 weeks of gestation, and results in a 5-10 mm Hg decrease below pre-pregnancy levels values (Mahendru *et al.*, 2014). The decrease in arterial pressure is accompanied by a reduction in vascular resistance and an increase in CO (Chapman *et al.*, 1997). The lowest values of Blood pressure (BP) are reached at around 22-24 weeks, with a subsequent increase from the 28th week until reaching pre-pregnancy levels by 36 weeks of pregnancy and postpartum (Ochsenbein-Kölble *et al.*, 2004) (Fig. 1.21). During the second trimester, BP is reduced by approximately 10 mmHg, with mean values of 105/60 mmHg. This decrease may be attributed to changes in the renin-angiotensin-aldosterone system (RAAS) and hormonal changes during pregnancy (Cheung and Lafayette, 2013). It is important to compare BP values during pregnancy with pre-pregnancy levels instead of early pregnancy, when alterations have already begun (Sanghavi and Rutherford, 2014).

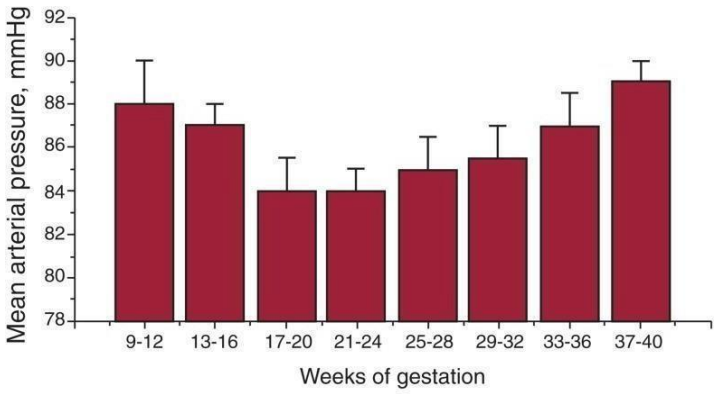


Fig 1.21: Mean arterial pressure during normal pregnancy (Moutquin *et al.*, 1985).

Several studies have investigated BP changes during pregnancy (Grindheim *et al.*, 2012; Mahendru *et al.*, 2014; Sanghavi and Rutherford, 2014). While some studies have reported a decrease in BP during pregnancy, others have drawn attention to its increase (JONES, 1979; Nama *et al.*, 2011). Preeclampsia, a hypertensive disorder characterised by proteinuria after 20 weeks of gestation, can result in fetal growth restriction and prematurity and is a leading cause of intensive care unit admissions related to (Xiao *et al.*, 2009; Porreco and Barkey, 2010; Hutcheon, Lisonkova and

Joseph, 2011). Risk factors associated with hypertension during gestation include advanced maternal age and higher BMI (Christianson, 1976). Females with a pre-pregnancy BMI >25 kg/m2 have been found to have higher mean arterial pressure, systolic and diastolic BP during and after pregnancy compared to those with a lower BMI (Gaillard *et al.*, 2011; Grindheim *et al.*, 2012).

Previous studies have reported an association between high pre-pregnancy BMI and hypertension during pregnancy. However, conflicting findings exist in the literature regarding the relationship between pre-pregnancy BMI, weight gain during pregnancy, and hypertension (Gaillard *et al.*, 2013; O'Dwyer *et al.*, 2013). One potential explanation for these discrepancies is the use of different BP measurement devices, such as finger arterial pressure and automated oscillometric devices, which can yield dissimilar data (Penáz, Voigt and Teichmann, 1976). Another factor to consider is ethnicity, as studies have shown that the effect of ethnicity on increasing BP during pregnancy may vary (Ochsenbein-Kölble *et al.*, 2004; Bouthoorn *et al.*, 2012). For instance, a study found that Caucasians had slightly higher BP compared to blacks and Asians, which may be due to lower BMI in Asians and blacks, and the fact that they are more likely to be multiparous compared to Caucasians. However, another study found no significant difference in the presentation of hypertension between multiparous and primiparous groups, suggesting that other pathophysiological factors may be at play (Gleicher *et al.*, 1986).

1.13.2.2.3 Contractility

During pregnancy, the cardiovascular system of the mother undergoes various adaptations to cope with the hemodynamic load. One of these adaptations is left ventricular contractility, which is the ability of the myocardial muscle to generate the force required for pump function (Pirani, Campbell and MacGillivray, 1973; Crystal, Assaad and Heerdt, 2019). The increase in contractility leads to an increase in CO, while impaired contractility results in a decrease in CO. However, the cardiac effort should not exceed a certain limit, or it may result in fatigue and even complete collapse, such as in cases of excessive inotropic support leading to high mortality rates (Vincent, 2008). Assessing contractility clinically is challenging, and hence, ejection

fraction and CO are the common clinical parameters used to evaluate cardiac function (David Sidebotham, 2007).

Apart from contractility, other cardiac mechanics, including preload and afterload, also play a crucial role in cardiac function. Preload is represented by the left ventricular end-diastolic pressure (LVEDP), which reflects the degree of ventricular stretch at the end of diastole (O'Keefe and Singh, 2021). According to (Vincent, 2008), the force of contraction of the myocardial cells depends on their stretch length. As the stretch increases (within specific limits), the force of contraction also increases, leading to elevated CO. Preload is related to the amount of ventricular filling and is considered one of the elements of CO, as venous return equals CO (Geva *et al.*, 1997). Therefore, understanding the relationship between these cardiac mechanics is vital in the assessment of maternal cardiovascular adaptation during pregnancy.

On the other hand, afterload is the force applied against the ventricles to pump blood away from the heart, depending on arterial blood pressure and vascular tone (Vincent, 2008). Reduced afterload can increase CO in cases of impaired contractility. Contractility, preload, and afterload are three factors that influence SV, which affects CO and overall cardiac function (O'Keefe and Singh, 2021). (David Sidebotham, 2007) explained that increased contraction force leads to contractility shifts, resulting in increased SV for a given end-diastolic volume (EDV) and smaller end-systolic volume (ESV). Conversely, reduced contraction force leads to decreased contractility shifts, resulting in reduced SV for a given diastolic volume and higher ESV. SV is related to ejection fraction, which represents the percentage of blood in the left ventricle ejected during systole (Feher, 2012). Understanding these cardiac factors is essential to comprehend overall cardiac physiology and deliver optimal treatments (Sheth *et al.*, 2015).

The literature presents conflicting findings regarding the impact of the hemodynamic load of pregnancy on left ventricle contractility. Some studies, such as those conducted by (Poppas *et al.*, 1997a; Ducas *et al.*, 2014), report no change in myocardial contractility or left and right ejection fractions during pregnancy. However, (Geva *et al.*, 1997) investigated the cardiac function of 34 healthy pregnant women at six intervals during pregnancy and postpartum and observed an association between

pregnancy and increased preload, as well as reduced afterload. Specifically, their results showed a 10.5% increase in left ventricular myocardial preload, which corresponded with an increase in stroke volume, and a 28.8% decrease in end-systolic stress, an index of myocardial afterload. This decrease was attributed to reduced end-systolic pressure and an elevated ratio of left ventricular thickness and diameter. Despite these changes, ejection phase indexes during and after pregnancy remained unchanged, which is consistent with the findings of (Savu *et al.*, 2010; Estensen *et al.*, 2013; Melchiorre *et al.*, 2016). These findings highlight the need for further research to clarify the impact of pregnancy on left ventricle contractility and other aspects of cardiac function.

1.13.2.2.4 Blood volume

Normal pregnancy is associated with a considerable increase in total blood volume comprising plasma volume, red blood cell (RBC), white blood cells, and platelets (Aguree and Gernand, 2019a; Ragav Sharma, 2022). The most significant expansion of total blood volume, between 40% to 50%, occurs in plasma volume from the 6th to the 24th week of pregnancy (Aguree and Gernand, 2019). This increase in plasma volume starts early in the first trimester and continues to rise gradually until term (Sanghavi and Rutherford, 2014). The production of estrogen hormone during pregnancy is responsible for the increase in plasma volume, leading to an increase in aldosterone production via the renin-angiotensin system, resulting in renal Na+ reabsorption and water retention and, consequently, plasma volume expansion (Longo, 1983). This increase in plasma volume is necessary to meet the circulatory demands of maternal organs and the placenta (Faupel-Badger *et al.*, 2007). Preterm birth, fetal growth restriction, and preeclampsia may occur in cases of inadequate increase in blood plasma volume during pregnancy (Duley, Williams and Henderson-Smart, 1999). Along with the significant increase in blood plasma volume, there is also a 20% to 35% increase in RBC production compared to prepregnant values (Head, Ferguson III and Ferguson II, 2009). However, the proportional increase in blood plasma volume surpasses the increase in RBC production, resulting in physiological anemia due to hemodilution (Vricella, 2017).

1.13.2.2.5 Arterial and venous change

In addition to the hemodynamic changes observed during pregnancy, which include increases in blood volume, CO, heart rate, SV, and cardiac contractility, there are also consequences of arterial and venous changes that contribute to adjustments in cardiac structure and function (Edouard *et al.*, 1998). These vascular changes occur in response to the systemic vasodilation mechanism (Sanghavi and Rutherford, 2014). With regards to venous changes during pregnancy, vascular compliance and venous wall distensibility increase from the first trimester, and return to normal values three months post-delivery (Poppas *et al.*, 1997; Gyselaers *et al.*, 2011). The increase in venous compliance and distensibility during pregnancy may be due to increased blood pressure, which can lead to venous overdistension, blood stasis, slower venous flow velocity, increased red cell aggregation, and increased blood flow echogenicity (Mares and Brun, 2000). It should be noted that blood flow echogenicity is a normal finding during pregnancy and should not be misdiagnosed as DVT (Rabhi *et al.*, 2000). The extent of the increase in venous capacitance varies between studies, depending on the location of measurement of different veins (Goodlin, 1986; Smith *et al.*, 1995). (Edouard *et al.*, 1998) highlighted the need for longitudinal studies that provide clear insights about venous changes over time, as well as accurate measurement methods, given the lack of studies that investigate venous changes in early pregnancy. Additionally, venous changes are often assessed alone in pregnancy, without taking into account changes in arterial function. The authors suggested that investigating changes in venous function from early pregnancy and correlating these changes with potential venous diseases developed during pregnancy is crucial.

In terms of arterial changes during pregnancy, an increase in aortic augmentation index, which reflects aortic stiffness, has been reported (Wilkinson *et al.*, 2000). Aortic stiffness decreases significantly during early pregnancy, with the lowest measurement occurring in the second trimester, and then gradually increases during the third trimester (Fujime *et al.*, 2012). Even four months after delivery, aortic stiffness measurement can still be higher than pre-pregnancy levels (Sanghavi and Rutherford, 2014). Additionally, higher aortic stiffness measurements have been observed in women with multiple pregnancies compared to nulliparous women (Singh *et al.*, 2020).

1.13.2.3 Changes in the renal system

During pregnancy, the renal system undergoes significant functional changes due to hormonal influences, outflow obstruction caused by the gravid uterus, and the metabolic needs of the developing fetus (Cheung and Lafayette, 2013). Renal vasodilation and systemic vasculature occur as a result of increased nitric oxide (NO) synthesis, which is a normal mechanism that increases blood flow to oxygen and nutrient-deficient tissues in the body (Gongora and Wenger, 2015; Ramanlal and Gupta, 2022). Vasodilation starts as early as 5 weeks of pregnancy to support full placentation and uteroplacental development (Hellsten *et al.*, 2012). As a consequence, there is a 50% increase in glomerular filtration rates (GFR) and an 80% increase in renal plasma flow (RPF), leading to decreased serum concentrations of creatinine, uric acid, and urea (Cheung and Lafayette, 2013c). Vasodilation also leads to a decrease in systemic vascular resistance (SVR), an increase in blood flow, and ultimately a decrease in blood pressure (Ramanlal and Gupta, 2022). The decrease in systemic vascular resistance has a significant impact on renal vasculature, accounting for approximately 40% (Mahendru *et al.*, 2014). This decrease is also associated with an elevated renal blood flow state, which is contrary to other conditions such as arteriovenous fistula, cirrhosis, and sepsis (Melo and Peters, 1999). Maternal hemodynamic and systemic vascular resistance return to normal levels postpartum (ROBSON *et al.*, 1987; Clapp and Capeless, 1997). It is essential to study these changes longitudinally to better understand the implications of these physiological adaptations during pregnancy.

Numerous studies have reported significant physiological changes in the renal system during pregnancy. One such change is the elevation of urinary protein excretion, which is considered normal during pregnancy but abnormal when it exceeds 300 mg daily (Higby *et al.*, 1994). In addition, regulation of water and sodium is altered during pregnancy (Tkachenko, Shchekochikhin and Schrier, 2014). Although all components of the renin-angiotensin system increase during pregnancy, opposition to the pressor effects, along with water and sodium retention, contributes to a greater rise in extracellular volume (Gold, 1986). This results in a decrease in normal plasma osmolality and plasma sodium concentration during pregnancy, and the release of

Antidiuretic Hormone (ADH) from the pituitary as a normal physiological response to these osmolality changes (Bhatia and Chhabra, 2018). Despite the increased filtration by the kidneys, the body maintains sodium by controlling reabsorption mechanisms in the distal tubules (Kazma *et al.*, 2020). It is worth noting that all renal functional changes during pregnancy return to normal in the postpartum period (Cheung and Lafayette, 2013).

In addition to the renal physiological changes during pregnancy, pregnant women also experience renal anatomical changes. The first anatomical change is the development of maternal hydronephrosis, which is the dilation of one or both kidneys due to accumulated urine inside the kidney pelvis or calyces during pregnancy (Pates and Dashe, 2006). Maternal hydronephrosis occurs in 43% to 100% of pregnant women and usually develops during the second trimester of pregnancy, disappearing within a few weeks post-delivery (Rasmussen and Nielsen, 1988; Ciciu *et al.*, 2022). The dilatation in the maternal kidney's collecting system can hold 200 to 300 ml of urine, causing urine stasis and a high risk of pyelonephritis with asymptomatic bacteriuria compared to non-pregnant females (Mandal, Saha and Pal, 2017). Maternal hydronephrosis can occur because of the progesterone hormone and the mechanical compression of the gravid uterus (Mevorach, Hulbert and Rabinowitz, 2007). While progesterone hormone can decrease contraction pressure, peristalsis, and ureteral tone, leading to dilating the ureters, the primary cause of hydronephrosis in pregnancy is the mechanical compression on the ureters (Cheung and Lafayette, 2013a). Anatomically, the right ureter passes over the iliac and ovarian vessels before entering the pelvis at a specific angle, while the left ureter passes parallel to the ovarian vein (Lescay, Jiang and Tuma, 2022). This explains the high prevalence (86%) of hydronephrosis in the right kidney compared to the left kidney, as this aspect has no relation to the hormonal effect (Schulman and Herlinger, 1975). Studies have found no association between the effect of progesterone and the development of hydronephrosis in non-pregnant females and no relation between estrogen and progesterone hormone concentrations and the severity of calyceal dilatation (Chapman *et al.*, 1997).

Secondly, kidney length size increases during pregnancy by 1 to 1.5 cm, and kidney volume increases by about 30% and this length and volume return to normal

measurements after six months post-delivery (Chauhan and Tadi, 2022). Initially, this increase in size was associated with hydronephrosis. However, the growth was also related to an increase in kidney vascular and interstitial volume without any alterations in the number of nephrons (Strevens *et al.*, 2003).

1.13.2.4 Changes in the respiratory system

Pregnancy is associated with significant changes in the respiratory system in order to satisfy the metabolic requirements of both the mother and the developing fetus (Hegewald and Crapo, 2011). Respiration, a metabolic process that involves the exchange of gases between the air and body tissues, plays a critical role in this regard (Molnar and Gair, 2015). Respiratory function is enhanced during pregnancy, characterized by increases in minute ventilation (MV) and resting oxygen consumption (Kolarzyk, Szot and Lyszczarz, 2005). MV shows a steady rise from the first trimester, followed by a rapid increase (20-40%) above baseline during the third trimester (Jensen *et al.*, 2008). Additionally, oxygen consumption increases gradually during the first trimester and then shows a 20-33% increase during the third trimester (Lee *et al.*, 2017). During pregnancy, the placenta, fetus, and uterine muscle utilize approximately two-thirds of the total oxygen consumption, while the remaining one-third is consumed by the maternal kidneys, myocardium, mammary gland, and respiratory muscles due to the increased oxygen demand (Seriki and Mfem, 2021). Multiple gestations have a higher metabolic demand compared to singleton gestations, resulting in increased oxygen consumption (Suzuki *et al.*, 2005). During labor, oxygen consumption is doubled or tripled during uterine contraction due to a decline in fetal oxygen saturation after each contraction (McNamara and Johnson, 1995).

The respiratory system undergoes marked anatomical changes during pregnancy that are necessary to meet the metabolic demands of the mother and fetus. One such change is the elevation of the resting position of the diaphragm towards the cephalad position, which occurs in response to the increased size of the gravid uterus in late pregnancy (LoMauro and Aliverti, 2015). This elevation of the diaphragm leads to a reduction in functional residual capacity and expiratory reserve volume, without inhibiting thoracic cage movement (Lee *et al.*, 2017). Consequently, the amount of

exhaled oxygen after maximum inspiration remains relatively unchanged or slightly increased during pregnancy (Contreras *et al.*, 2012). Furthermore, the upper respiratory tract and airway mucosa also undergo changes during pregnancy. These changes result from hormonal alterations, as increased estrogen during pregnancy leads to airway mucosal hyperemia, hypersecretion, edema, and friable mucosa (Koehler *et al.*, 2005). Despite these changes, the gas exchange process remains constant (Lee *et al.*, 2017). Moreover, increased levels of estrogen and progesterone hormones during pregnancy also cause hyperventilation, as progesterone elevates the sensitivity center to oxygen, while estrogen supports the number and sensitivity of progesterone receptors in the central neuronal respiratory locations, including the hypothalamus and medulla (Bayliss and Millhorn, 1992). Although physiological and anatomical changes occur during pregnancy, gas exchange in healthy pregnant women remains unchanged.

1.13.2.5 Changes in the digestive system

Adaptive changes occur in the digestive system during pregnancy, and one of the most common symptoms is nausea and vomiting (Soma-Pillay *et al.*, 2016). This symptom is experienced by 50-90% of all pregnant women during their first pregnancy (Boyd, 2003). While some pregnant women experience an improvement in symptoms by the 20th week of pregnancy, others may continue to experience nausea and vomiting until delivery (Lee and Saha, 2011). Management of electrolyte levels and dietary adjustments have been shown to reduce symptoms in some cases (Creanga *et al.*, 2014). However, approximately 0.3-2% of pregnant women may develop severe and persistent nausea and vomiting, known as hyperemesis gravidum, which can lead to dehydration, weight loss, and electrolyte imbalances (Dodds *et al.*, 2006). Treatment for hyperemesis gravidum typically involves the use of intravenous fluids and vitamin supplements (Miller, 2002).

The exact etiology of nausea and vomiting during pregnancy remains unknown, but it is believed to be multifactorial (Murphy Goodwin, 2002). One of the main factors is hormonal, with increased levels of estrogen, progesterone, and human chorionic gonadotropin (hCG) being associated with the development of these symptoms during

pregnancy (Creanga *et al.*, 2014). Studies have shown that higher levels of hCG in early pregnancy are associated with more significant nausea and vomiting symptoms, particularly in cases of multiple gestations where hCG levels are higher (Soma-Pillay *et al.*, 2016). Additionally, abnormal levels of thyroid-stimulating hormone (TSH) during pregnancy have been correlated with stronger symptoms of nausea and vomiting (Liu *et al.*, 2022). Other factors that may contribute to the development of nausea and vomiting during pregnancy include genetic, psychological, immunological, Helicobacter pylori infection, and nutritional insufficiency (Verberg *et al.*, 2005).

During pregnancy, the increased size of the gravid uterus leads to mechanical changes in the digestive system. The stomach is displaced to the cephalad direction, causing elevated intragastric pressure and reduced tone of the esophageal sphincter, which can result in symptoms of reflux, nausea, and vomiting (Koch, 2002; Soma-Pillay *et al.*, 2016). Additionally, the displacement of the bowel may result in appendicitis due to obstruction of the appendiceal lumen and bacterial accumulation in the appendix, leading to inflammation and abscess development (Schulman and Herlinger, 1975; Duque and Mohney, 2022). Moreover, increased levels of estrogen and progesterone during pregnancy can cause structural changes in the gastrointestinal tract, including alterations in the smooth muscle function and gastric neural activity, resulting in gastroparesis and gastric dysrhythmia (Soma-Pillay *et al.*, 2016; Chen *et al.*, 2019). These changes are more likely to occur in women with a history of gastrointestinal diseases such as inflammatory bowel disease, gastric bypass surgery, diabetic gastroparesis, and gastroesophageal reflux disease (Clark, Costantine and Hankins, 2012).

1.13.2.6 Changes in skeletal and bone density

Anatomical and hormonal changes during pregnancy result in various musculoskeletal adaptations (Kesikburun *et al.*, 2018). These adaptations include an increase in the inward curvature of the lumbar spine, known as lordosis (Yoo, Shin and Song, 2015), as well as lumbar spine laxity, which is associated with an increased risk of low back pain and injury (Katonis *et al.*, 2011). The sacroiliac joints and pubic symphysis also

experience increased mobility and widening (Stolarczyk *et al.*, 2021). Joint laxity during pregnancy is primarily due to increased levels of Relaxin hormone, which peaks during the third trimester and plays a significant role in collagen remodeling and the relaxation of pelvic joint ligaments to facilitate fetal delivery (Ireland et al., 2000; Sherwood, 2004). Additionally, progesterone and serum estradiol also contribute to joint laxity during pregnancy (Marnach *et al.*, 2003). Furthermore, during pregnancy, the neck flexes forward, and the shoulders move downward (Soma-Pillay *et al.*, 2016). Musculoskeletal changes generally return to normal within 4-12 weeks postpartum (Kesikburun *et al.*, 2018).

Pregnancy can have significant effects on maternal bone density (Marnach *et al.*, 2003), with loss of bone density reported in the literature (Naylor *et al.*, 2000). This is due to changes in calcium metabolism, which are necessary for the transfer of calcium from the mother to the developing fetus in order to ensure complete fetal bone growth (Mahadevan et al., 2012). Calcium metabolism changes are most pronounced in the third trimester of pregnancy due to increased fetal s keleton growth and calcification (Kovacs and Kronenberg, 1997). The exact mechanism of maternal bone density loss is not fully understood (Sanz-Salvador., 2015); however, it has been suggested that insufficient drainage of calcium from the mother to the fetus may play a role (Kovacs, 2016). Pregnancy may also be associated with the development of osteoporosis (Yun *et al.*, 2017), although there is controversy regarding the underlying reasons. Some suggest that pregnancy itself, with its intense changes in calcium metabolism, may contribute to osteoporosis, while others propose that pre-existing osteopenia may result in structural failure during pregnancy (Sanz-Salvador et al., 2015). Notably, pregnancy has not been identified as a risk factor for postmenopausal osteoporosis (Salari et al., 2014).

1.13.2.7 Endocrine changes

1.13.2.7.1 Thyroid gland

The thyroid gland undergoes various modifications during pregnancy, as documented in the scientific literature (Vaidya and Chan, 2017). Firstly, a substantial decline in iodine levels occurs in pregnancy, leading to iodine deficiency (Sheila, 2011). This phenomenon is attributed to increased renal iodide clearance resulting from the

elevated maternal GFR, and the transfer of iodine from the mother to the developing fetus and placenta (Pearce, 2008). To prevent an enlarged thyroid gland, iodine intake should be maintained during pregnancy (Soma-Pillay *et al.*, 2016). Secondly, the liver increases the production of Thyroxine-binding globulin (TBG) early in pregnancy, and the levels remain constant until post-delivery, when they return to normal (Sahay and Nagesh, 2012). This is due to elevated estrogen hormone levels and reduced hepatic elimination of TBG as a result of estrogen-induced sialylation (Ain, Mori and Refetoff, 1987). High levels of TBG during pregnancy lead to increased thyroxine (T4) and triiodothyronine (T3) hormones (Soldin, 2006), which are critical in determining whether the patient has a normal or abnormal thyroid function (Sheehan, 2016). In the second and third trimesters of pregnancy, T3 and T4 hormones are slightly reduced, but this has no clinical significance (Soldin, 2006).

Thirdly, during the first trimester of pregnancy, Thyroid-stimulating hormone (TSH) hormone levels slightly decrease, followed by a slight increase towards the end of the trimester trimester (Sahay and Nagesh, 2012).This change is due to the release of hCG hormone during pregnancy (Alemu *et al.*, 2016). Maternal hypothyroidism occurs when TSH hormone levels are elevated with reduced T4 levels, a condition that is prevalent among pregnant women (Casey *et al.*, 2005). Untreated maternal hypothyroidism leads to fetal respiratory distress, reduced fetal birth weight, and preterm birth (Sahay and Nagesh, 2012). Furthermore, children born to mothers with hypothyroidism may experience low Intelligence Quotient (IQ) scores and difficulties in learning abilities later in life (Pop *et al.*, 1999). In contrast, maternal hyperthyroidism occurs when TSH hormone levels decrease with increased T3, T4, or both during pregnancy (Stagnaro-Green and Pearce, 2012). Untreated maternal hyperthyroidism may result in early labor, pre-eclampsia, fetal tachycardia, decreased fetal weight, prematurity, stillbirths, and congenital deformities (Michalska *et al.*, 2008). Therefore, adequate thyroid hormone levels during pregnancy are essential for a healthy pregnancy, and all thyroid hormone changes typically return to normal post-partum (Cooper and Laurberg, 2013).

1.13.2.7.2 Adrenal gland

The maternal adrenal gland plays a crucial role in sustaining normal pregnancy by undergoing physiological adaptations (Ishimoto and Jaffe, 2011). During pregnancy, reduced vascular resistance and BP levels activate the renin-angiotensin-aldosterone (RAA) system, resulting in a three-fold increase in aldosterone steroid hormone levels secreted by the adrenal cortex in early pregnancy and a ten-fold increase in late pregnancy (Kurlak *et al.*, 2019). These elevated levels of aldosterone are responsible for the expansion of plasma volume (Scaife and Mohaupt, 2017). Additionally, there is a 3-4 fold increase in the levels of the renin hormone compared to the prepregnant levels (Sanghavi and Rutherford, 2014). The renin hormone is a kidney-secreted hormone that plays a vital role in maintaining the production of aldosterone hormone (Scott et al., 2022). Moreover, there is a 2-4 fold increase in angiotensin II levels, a peptide hormone of the RAA system responsible for regulating blood pressure by constricting veins (Morris et al., 2022). During pregnancy, a state of physiological hypercortisolism occurs due to increased levels of corticosteroid-binding globulin (CBG), adrenocorticotropic hormone (ACTH), cortisol, free cortisol, and deoxycorticosterone (Soma-Pillay *et al.*, 2016a). This increase in total cortisol levels causing hypercortisolism begins at the end of the first trimester and reaches a three-fold increase at term compared to prepregnant levels. Hypercortisolism at term is one of the reasons that stimulates the onset of labor (Pourali et al., 2017). Balanced levels of adrenocortical hormones released by the adrenal gland are required for a healthy pregnancy, as overactive or underactive adrenal hormones secretion can result in adrenal hormone disorders (Hadden, 1995).

1.13.2.7.3 Pituitary gland

During pregnancy, significant anatomical and physiological changes occur in the pituitary gland (Laway and Mir, 2013). These changes include an increase in the size of the gland due to proliferation of prolactin-producing cells in the anterior lobe, stimulated by increased levels of estradiol (Chourpiliadi and Paparodis, 2022).

Prolactin levels in the serum also increase in early pregnancy and become ten times higher in the third trimester (Ekinci *et al.*, 2017). In addition, growth hormone (GH) levels increase during pregnancy, which is essential for growth, postnatal cell production, and regeneration (Chourpiliadi and Paparodis, 2022). Humans have two GH genes, the pituitary version and the placental version, with the latter associated with fetal growth (Liao *et al.*, 2018). During mid to late pregnancy, the pituitary version is replaced by the placental version in the maternal circulation to adapt to maternal changes (Liao *et al.*, 2018). Oxytocin levels in the posterior part of the pituitary gland also increase, reaching their maximum levels in the third trimester (Prevost *et al.*, 2014). While antidiuretic hormone (ADH) levels remain constant, reduced sodium levels during pregnancy result in a decrease in ADH hormone osmolality (Cheung and Lafayette, 2013). These changes in the pituitary gland return to normal around six months post-delivery (Laway and Mir, 2013).

1.13.2.8 Metabolism changes

1.13.2.8.1 Glucose metabolism

During pregnancy, the maternal glucose metabolism undergoes significant adaptive physiological changes (Catalano *et al.*, 1998). In early pregnancy, there is a decrease in fasting blood glucose levels which is attributed to an increase in insulin sensitivity that allows for the accumulation of large quantities of glucose for fetal growth (Plows *et al.*, 2018). However, as pregnancy progresses, maternal insulin sensitivity reduces and there is a 50% increase in maternal insulin resistance which results in increased postprandial glucose levels and an extended glucose peak (Stern *et al.*, 2021). This rise in insulin resistance is associated with increased levels of diabetogenic hormones including cortisol, prolactin, growth hormone, progesterone, and human placental lactogen (Rassie *et al.*, 2022). The maternal insulin resistance leads to increased maternal blood glucose levels which are transported through the placenta to support fetal growth (Arshad et al., 2014). This transfer occurs through the gradient compartment between the mother and the fetus which is regulated by hyperplasia in insulin-secreting pancreatic beta-cells (Stern *et al.*, 2021). These cells exhibit comparative glucose insensitivity, resulting in lower fetal blood glucose levels compared to maternal glucose levels (Soma-Pillay *et al.*, 2016). Maternal insulin

resistance is expressed in peripheral tissues such as skeletal muscles and adipocytes, serving as a maternal fuel source to assist fetal growth, particularly at term (Sonagra *et al.*, 2014).

1.13.2.8.2 Lipid metabolism

Adaptive alterations in lipid metabolism are necessary for fetal growth and development (Steinhauser *et al.*, 2021). The hormonal changes that occur during pregnancy result in increased levels of various lipid parameters such as total serum cholesterol, LDL, and triglycerides, particularly in the second and third trimesters (Bartels and O'Donoghue, 2011). The rise in lipid parameters begins in the second trimester, peaks in the third trimester, and returns to normal levels approximately one month post-delivery (Pusukuru *et al.*, 2016). The increase in triglyceride levels is primarily due to a decrease in lipoprotein lipase activity and an increase in liver synthesis, leading to a reduction in adipose tissue catabolism (Wild and Feingold, 2023). This increase in triglyceride levels is crucial for meeting the maternal energy requirements, while glucose plays a crucial role in supporting fetal energy needs (Gootjes *et al.*, 2022). Additionally, maternal LDL cholesterol levels increase by approximately 50% during the third trimester, which is essential for placental steroidogenesis (Chatuphonprasert et l., 2018). High-density lipoprotein levels (HDL) increase during early pregnancy but decrease at term (Adank *et al.*, 2019), with levels being approximately 15% higher than pre-pregnancy levels (Salameh and Mastrogiannis, 1994). Abnormal increases in cholesterol levels during pregnancy can lead to hypertension, while inadequate cholesterol levels can result in premature labor and reduced fetal weight (Catov *et al.*, 2007). Therefore, moderate levels of cholesterol are critical for the health of both the mother and fetus.

1.13.2.8.3 Protein metabolism

During pregnancy, an increase in protein intake is necessary to support fetal growth and development, as amino acids are transported across the placenta (Avagliano, Garò and Marconi, 2012). A reduction in protein catabolism occurs during pregnancy, as fat stores are utilized to supply energy metabolism (Lourdais *et al.*, 2004). Thus,

adequate protein intake is crucial for ensuring that the maternal and fetal nutritional requirements are met during pregnancy.

1.13.2.8.4 Calcium metabolism

Maternal calcium metabolism undergoes significant physiological changes to maintain a healthy pregnancy (Soma-Pillay *et al.*, 2016). These changes are necessary to meet the increasing demand for calcium required for fetal growth and development. During pregnancy, there is a decrease in the concentration of total serum calcium due to hemodilution, which reduces serum albumin levels and results in a significant decrease in the albumin-bound fraction of calcium (Almaghamsi et al., 2018). However, despite this change, serum ionized calcium levels remain constant (Goyal *et al.*, 2022). The fetus requires approximately 30 g of calcium to support healthy physiological changes during pregnancy (Almaghamsi et al., 2018). To meet these needs, calcium intestinal absorption is doubled in early pregnancy, starting from the 12th week of gestation, and reaches its maximum level at term (Heringhausen and Montgomery, 2005). As a result, renal calcium clearance also increases with increased calcium intestinal absorption, and during fasting, calcium in the urine is either normal or low (Jones *et al.*, 2012). However, it is important to note that these changes may contribute to the development of kidney stones during pregnancy (An-Na *et al.*, 1995).

1.14 Diagnosis

Accurately diagnosing CVI necessitates obtaining a comprehensive medical history and performing a thorough physical examination of the lower extremities (Krishnan and Nicholls, 2005). In the assessment of the physical examination, the standing

position is crucial for maximizing venous expansion (Youn and Lee, 2019). Diagnostic methods used to evaluate CVI are categorised as invasive or non-invasive approaches (Eberhardt and Raffetto, 2014). Among the invasive techniques are Contrast Venography and Ambulatory Venous Pressure (AVP), while the non-invasive methods encompass Duplex Ultrasound, Vector Flow Imaging (VFI), Plethysmography, Computed Tomography Venography (CTV), and Magnetic Resonance Venography (MRV). In this study, owing to the specific condition of pregnant women, the utilisation of invasive diagnostic techniques was precluded. Furthermore, non-invasive CTV was also ruled out due to the substantial radiation exposure associated with it, which poses potential risks to the developing fetus. Noninvasive imaging modalities, with a particular focus on duplex ultrasound, are commonly employed for CVI diagnosis and are widely acknowledged as the gold standard when compared to alternative modalities, as highlighted by (Necas, 2010). When selecting an appropriate diagnostic method, it is imperative to consider the specific objectives of the evaluation, as emphasized by (Balogh *et al.*, 2015). Consequently, in certain clinical scenarios, alternative imaging modalities may be employed, as suggested by (Santler and Goerge, 2017). A detailed description of each non-invasive imaging modality is provided below:

1.14.1 Duplex ultrasound:

Duplex ultrasound (DUS) is a widely used and highly accurate imaging modality for the diagnosis of CVI diseases (Gloviczki *et al.*, 2011). DUS provides a comprehensive evaluation of venous anatomy, identifies anatomical variations, detects the etiology of the CVI, assesses blood flow characteristics, and evaluates valve function (Youn and Lee, 2019). Additionally, DUS is a noninvasive, radiation-free, cost-effective, reliable, and easily accessible technique that provides immediate information (Gujja et al., 2014). However, DUS has certain limitations (Santler and Goerge, 2017). These limitations include patient habitus, such as obesity, which may limit the assessment of the deep venous system, particularly when examining it (Uppot, 2018). Inflammation or infection in certain areas, such as open ulcers or severe edema, can also limit the sonographic window and cause suboptimal assessments (Anderson, 2007). Furthermore, patients who are unable to stand for extended periods may not be able to be assessed for venous reflux, leading to suboptimal assessments (Necas, 2010).

Lastly, DUS performance is highly dependent on the quality of the ultrasound machine and the scanning protocol, as well as the experience and expertise of the sonographer performing the examination and interpreting the images (Passman, 2018). Poor-quality ultrasound machines, variable protocols, and insufficient sonographer experience can result in inadequate scanning and interpretation, leading to delayed diagnosis and treatment (Cheung, Singh and Firstenberg, 2021). Therefore, standardising high-quality ultrasound machines, protocols, and interpretations can improve patient care, ensuring accurate diagnoses and better treatments, as suggested by the study conducted by (Cavezzi *et al.*, 2006).

DUS is a diagnostic imaging modality that combines two modes, B-mode and spectral Doppler, to diagnose venous reflux and obstruction in both the superficial and deep venous systems (Lee and Youn, 2019; SVT, 2021). B-mode allows for the assessment of vein patency and the detection of obstructions, such as deep vein thrombosis or venous stenosis, by compressing the veins in the transverse position using an ultrasound probe (Coleridge-Smith *et al.*, 2006) (Fig 1.22). In some cases, changes in natural blood flow characteristics can also be used to diagnose venous obstruction (Morris and Woodcock, 2004). Spectral Doppler, on the other hand, enables the evaluation of venous reflux or the direction of blood flow through the use of provocative maneuvers and the measurement of blood flow velocity (Coleridge-Smith *et al.*, 2006) (Fig 1.23).

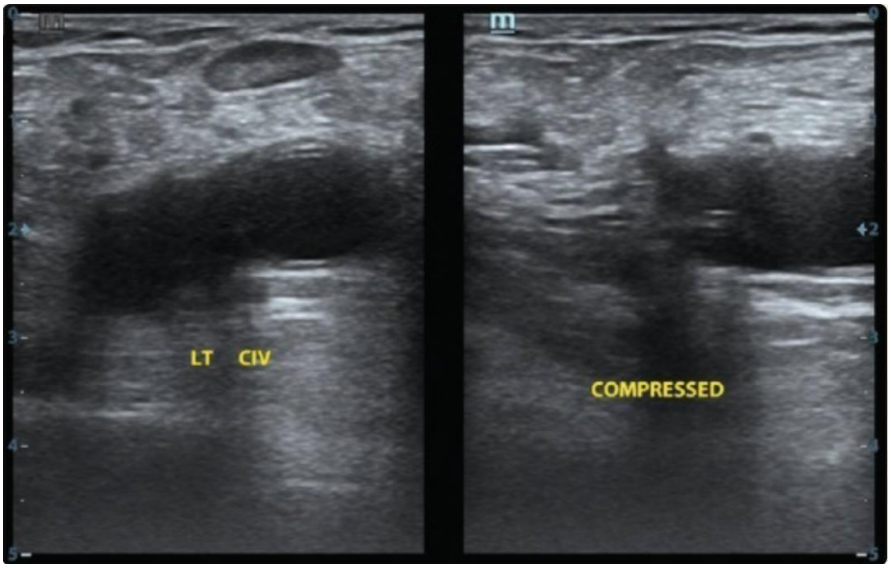


Fig 1.22: B-mode transverse scan showing echogenic (chronic) thrombus in the left common iliac vein with loss of compressibility (Ibrahim *et al.*, 2020).

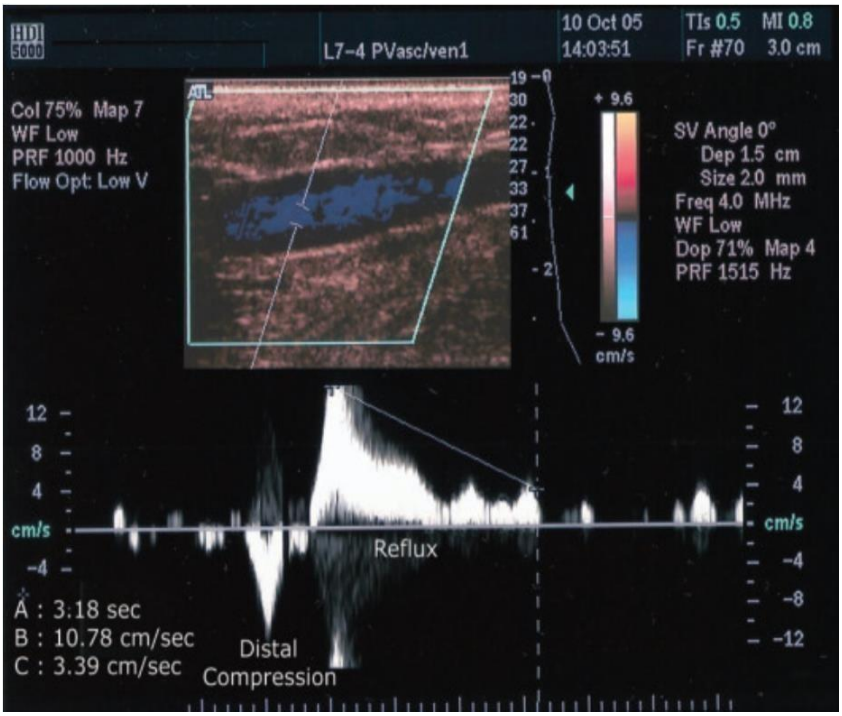


Fig 1.23: Spectral Doppler DUS measuring the reflux in incompetent veins (Pascarella and Mekenas, 2007).

Confirmation of blood flow direction or venous reflux is possible through longitudinal scanning, augmented with limb compression in the reverse Trendelenburg position or in a standing position, with a minimum tilt angle of 300 degrees on an upward tilting table (Necas, 2010b; SVT, 2021). The use of a cuff inflation-deflation tool in a standing position is a more accurate method for detecting venous reflux, as it provides uniform findings compared to other tools (Markel *et al.*, 1994). Venous reflux refers to any prolonged reversed venous blood flow against gravity, with the duration of reflux known as reflux time (Eberhardt and Raffetto, 2014). Pathological reflux occurs when retrograde flow exceeds 0.5 seconds, according to some studies (Min, Khilnani and Golia, 2003), while others state that pathological reflux occurs when retrograde flow exceeds 0.5-1 seconds, depending on the segment being assessed, such as superficial veins (>0.5 sec), perforators (>0.35 sec), and femoral or popliteal veins (>1 sec) (Coleridge-Smith *et al.*, 2006). However, despite its ability to reproduce disease severity, reflux time does not necessarily correlate with clinical signs (Neglén *et al.*, 2004).

The assessment of CVI through DUS examination can be performed unilaterally or bilaterally, based on patients' symptoms, according to (SVT, 2021). The examination typically begins with the patient removing clothing to expose the lower extremities from the groin to the ankle. The examination is carried out while the patient is standing or in the Trendelenburg position to evaluate valve function against gravity, as veins are more distended in a standing position, thereby allowing for more accurate assessment. Due to the intimate nature of the examination, a chaperone should be offered to the patient. The examination usually lasts between 30-45 minutes, and patients may experience dizziness during the examination. To mitigate this issue, patients may be instructed to sit and rest during the examination.

The examination of CVI using DUS is initiated by utilizing a high frequency linear array probe (7.5-13 MHz) to obtain clear images of the superficial and deep veins (Coleridge-Smith *et al.*, 2006). According to this study, curved probes (3.5-5 MHz) are recommended for cases with edema and swollen legs to ensure better penetration and image resolution. The ultrasound probe is applied to the skin overlying the venous blood vessel of interest, using a coupling gel to prevent air from interfering with image quality (Roth and Gafni-Pappas, 2011). Both the B-mode and spectral Doppler modes of DUS are utilized in the evaluation of all venous segments during the examination.The examination begins by assessing the deep and superficial veins systems. The Common femoral vein (CFV) is initially scanned at the groin to evaluate patency and flow. Any anomalies detected in the CFV suggest iliac vein obstruction, thus prompting an examination of the iliac veins and inferior vena cava. The examination is then continued by scanning the length of the femoral vein (proximal to distal), popliteal vein, and calf veins. Thrombus or incompetence findings are documented.

The superficial venous system is assessed next, starting from the groin to evaluate the patency and competency of both the sapheno-femoral junction and the entire length of the LSV. All venous perforators and branches are assessed, and LSV diameters are recorded for potential future treatment purposes. The sapheno-popliteal junction and SSV are subsequently evaluated by repositioning the patient to assess patency and competency. Venous reflux in the LSV or SSV should be assessed for

associated sources of reflux, such as reflux in perforators. The results of the examination are recorded and entered into a research database for further analysis. The use of a coupling gel during the DUS examination and the application of both B-mode and spectral Doppler modes to all venous segments allow for accurate assessment of both the deep and superficial veins in patients with CVI.

1.14.2 Vector flow imaging (VFI)

DUS is a commonly used diagnostic imaging tool for assessing human hemodynamics. However, accurate PRV estimation can be limited due to two factors: its angle-dependency and the anatomical location of the lower extremities superficial veins (Park *et al.*, 2012). Recent advances in ultrasound technology have led to the development of vector flow imaging (VFI), which offers a multidimensional beam steering and angle independence method (Bechsgaard *et al.*, 2018). VFI allows for more accurate estimation of velocity vector amplitude and true blood flow direction at any position in a vessel, resulting in a more realistic representation of flow movements (Goddi *et al.*, 2017). VFI is based on the transverse oscillation (TO) technique and works by emitting standard pulse doppler signals, followed by beamforming and phase shifting samples a quarter of the lateral wavelength (ninety degrees) during receive (Udesen *et al.*, 2015; Brandt, Moshavegh, *et al.*, 2018). The wavelength depends on three components: receive apodization, emit focus, and interrogation depth. Blood flow is visualized in a color box, with the direction of flow indicated by the color wheel and the overlaid arrows inside the color box aiding in the analysis of flow direction and magnitude (Fig 1.24). VFI has shown promise in evaluating the velocity of lower limb veins, as demonstrated by recent studies (Bechsgaard et al., 2016, 2017).

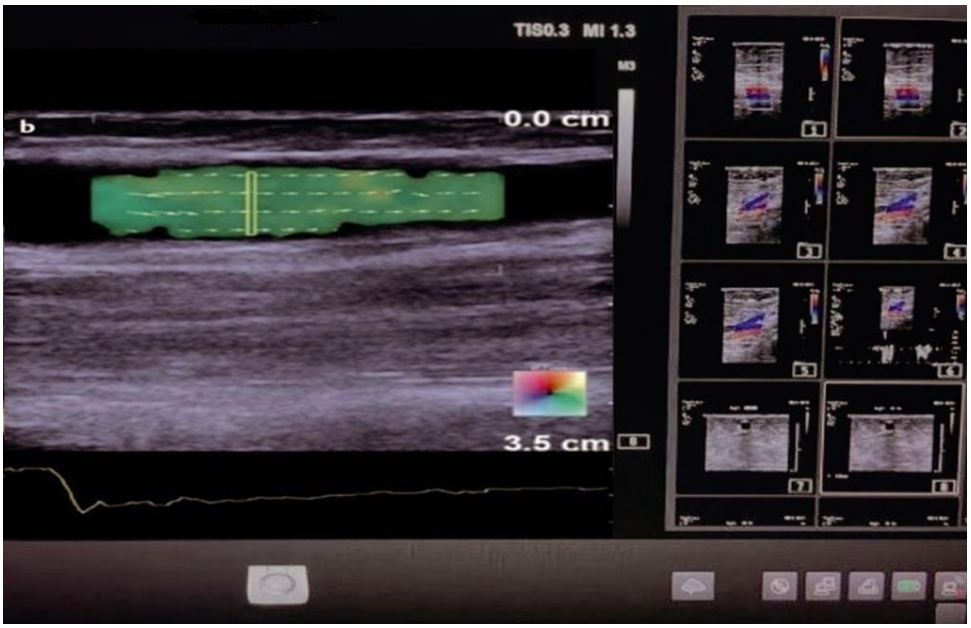


Fig 1.24: The VFI image of the LSV displays a colored box that depicts the flow of blood, and the color wheel indicates the direction of the flow. The arrows overlaid on the colored box assist in assessing both the direction and magnitude of the blood flow.

1.14.3 Plethysmography:

Numerous techniques of plethysmography have been devised to evaluate venous function in the lower extremities (Abu-Halimah and Marston, 2017). These techniques have gained wide attention due to their ability to detect venous reflux (Owens *et al.*, 2000). However, their capacity to identify the anatomical site of reflux is limited (Bays *et al.*, 1994). Consequently, DUS remains the imaging modality of choice for assessing the deep and superficial venous systems (Philip Coleridge-Smith *et al.*, 2006). Despite its superior diagnostic ability, other functional tests, such as plethysmography, may provide supplementary information regarding hemodynamic pathology following a DUS diagnosis (Rosfors et al.,2014). Furthermore, plethysmography can be employed to evaluate the improvement of venous function after intervention (Rosfors et al., 2014). The use of these functional tests in addition to DUS can minimize false-negative diagnoses while ensuring accurate diagnosis in a busy clinical setting (Langford, Tonks and Singh, 2009). Air plethysmography (APG), photo-plethysmography (PPG), strain-gauge plethysmography, and impedance plethysmography are the four types of plethysmography that have been developed using different sensors to measure alterations in blood volume in a limb, with each type being discussed below.

1.14.3.1 Air Plethysmography

Several non-invasive techniques have been developed to examine venous function in the lower limb extremities, including Air Plethysmography (APG). APG involves a tubular air chamber filled with air at 6 mm Hg that encircles the leg and is attached to a pressure amplifier and computer. Leg volume alterations due to filling or emptying of veins cause changes in the air chamber pressure, which can be assessed in millilitres corresponding to the calibration (Christopoulos et al., 1988), APG can examine every component of CVI diseases, including venous reflux, venous obstruction and calf muscle pump dysfunction (Criado *et al.*, 1998a). It can also determine global venous function, such as venous refilling times (VRT), venous volume, ejection fraction (EF) and residual volume fraction (RVF) (Yamaki *et al.*, 2007). APG has other uses such as evaluating the results of venous surgery, diagnosing acute DVT, assessing compression stocking treatment, determining the possibility of ulcer healing, and examining the physiological consequences of wearing high-heeled shoes in healthy women (Dezotti *et al.*, 2016).

Various studies have compared APG to other techniques such as PPG and DUS. In comparison to PPG, APG has been found to be superior in identifying significant reflux, as PPG has low specificity and cannot detect the location of reflux. However, APG is consistent with DUS in its ability to identify limbs with or without reflux, and a combination of APG and DUS together provides the best evaluation of venous reflux. Despite its confirmed implementation in clinical settings, APG is mostly used for research purposes when DUS fails to provide sufficient information about the pathophysiology of CVI, as its use is often limited by its complexity (Bays *et al.*, 1994; Lattimer *et al.*, 2014; Shabani Varaki *et al.*, 2018; Youn and Lee, 2019).

1.14.3.2 Photoplethysmography (PPG)

PPG is a non-invasive technique that can comprehensively assess the pathophysiologic mechanisms of CVI, including muscle pump dysfunction, obstruction, and severity of venous reflux, by measuring VRT (Eberhardt and Raffetto,

2014; Youn and Lee, 2019). VRT refers to the time required for the veins to refill with blood after being emptied by pressure applied at the end of a calf contraction (Eberhardt and Raffetto, 2005). VRT values greater than 25 seconds are indicative of normal veins, while VRT values less than 25 seconds are indicative of significant venous reflux, as the reflux accelerates venous refilling, resulting in shorter refilling time (Saliba Júnior *et al.*, 2014). PPG can also determine other parameters, such as venous volume, ejection fraction, maximum venous outflow, and segmental venous capacitance, providing a comprehensive assessment of CVI (Criado *et al.*, 1998). However, PPG has limited specificity compared to other imaging techniques and is not accurate in identifying the anatomical location of reflux (Saliba Júnior *et al.*, 2014). Nevertheless, PPG can predict treatment outcomes in addition to assessing the degree of reflux (Cheng and Wong, 1995).

The mechanism of PPG, as described in the study conducted by (Nicolaides *et al.*, 2000), involves the use of a small light source and a photoelectric cell that are placed on the skin. The photoelectric cell is positioned opposite or adjacent to the light source to monitor blood perfusion based on transmission and reflection modes. The light produced is transmitted through the skin and scattered by the tissue, and some of it is absorbed. The absorption is highest when the pressure is high, such as when standing or sitting, and the veins are full of blood. During exercise, the venous plexuses become less full, resulting in less light absorption by the red blood cells. The photoelectric cell detects the scattered light that emerges from the skin. Changes in the number of red blood cells in the skin dermis affect the backscatter of light that is detected by the photoelectric cell. Finally, the intensity of the light signals detected by the photoelectric cell is converted into PPG signals (see Fig 1.25).

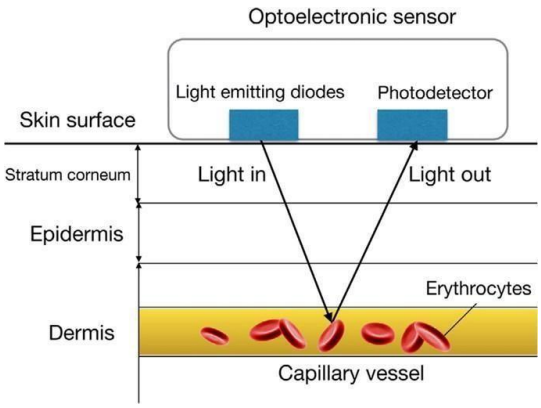


Fig 1.25: The working principle of PPG sensors (Zhao *et al.*, 2017).

1.14.3.3 Strain-Gauge Plethysmography (SGP)

Strain gauge plethysmography (SGP) is a non-invasive and efficient technique that can be employed for diagnosing CVI by assessing the VRT, maximum venous outflow (MVO) rate, and venous capacitance (Rosfors et al., 2014). The method involves a conductive tube that is attached to a plethysmograph by an electrical connection, which is wrapped around the circumference of the leg calf. Any changes in the calf's volume lead to corresponding changes in gauge length and electrical resistance (Kohler and Strandness, 1986). SGP is rapid, easy to perform, and yields quick results (Langford et al., 2009). Although SGP has been commonly employed to diagnose DVT by evaluating the MVO, its use in assessing venous obstruction or reflux is declining due to the potential for errors. This is because changes in posture, cardiac failure, or muscle wasting can lead to inaccuracies in this method. Additionally, the existence of clots in tributary veins or paired veins and the presence of collaterals from prior episodes of DVT may not impact the capacitance or outflow, resulting in normal MVO and venous volume (Abu-Halimah and Marston, 2017).

1.14.3.4 Impedance Plethysmography (IPG)

In the assessment of alterations in skin impedance, the Impedance Plethysmography (IPG) method has been utilised, where changes in limb volume correspond to alterations in electrical resistance (Wells *et al.*, 1995). IPG is considered a non-invasive and safe technique (Golden and Miles, 1986). As stated by (Abu-Halimah and Marston, 2017), the IPG technique involves the use of calf electrodes that measure venous capacitance and outflow. Normal venous function is indicated by high venous capacitance and outflow, while low values indicate abnormal venous function and a higher likelihood of DVT. However, IPG has low sensitivity in detecting DVT as it is only effective in cases of symptomatic DVT. Similar to SGP, IPG is rarely used for the diagnosis of venous reflux due to the influence of posture changes, cardiac failure, and muscle wasting on the technique's accuracy.

1.14.4 Magnetic Resonance venography (MRV):

Magnetic resonance venography (MRV) is a viable diagnostic modality for venous diseases (Silickas *et al.*, 2018) (Fig 1.26), offering several advantages over CTV. Specifically, MRV does not involve ionizing radiation (Abdalla *et al.*, 2015) and employs contrast agents that are generally considered safer than those used in CT (Tombach and Heindel, 2002). Furthermore, MRV exhibits excellent diagnostic accuracy, with reported sensitivity and specificity of 92% and 95%, respectively, for the diagnosis of DVT (Sampson *et al.*, 2007). However, MRV is infrequently utilized for the diagnosis of CVI due to its high cost (Eapen, Hou and Pai, 2018), lengthy examination times related to venous filling (Kim *et al.*, 2012), limited availability (Orbell *et al.*, 2008), and contraindications such as the presence of MR-unsafe implants or claustrophobia (Silickas *et al.*, 2018). Moreover, MRV is restricted in its ability to detect central venous obstruction only, making it an inadequate tool for the diagnosis of various venous diseases (Quan and Gillespie, 2007).

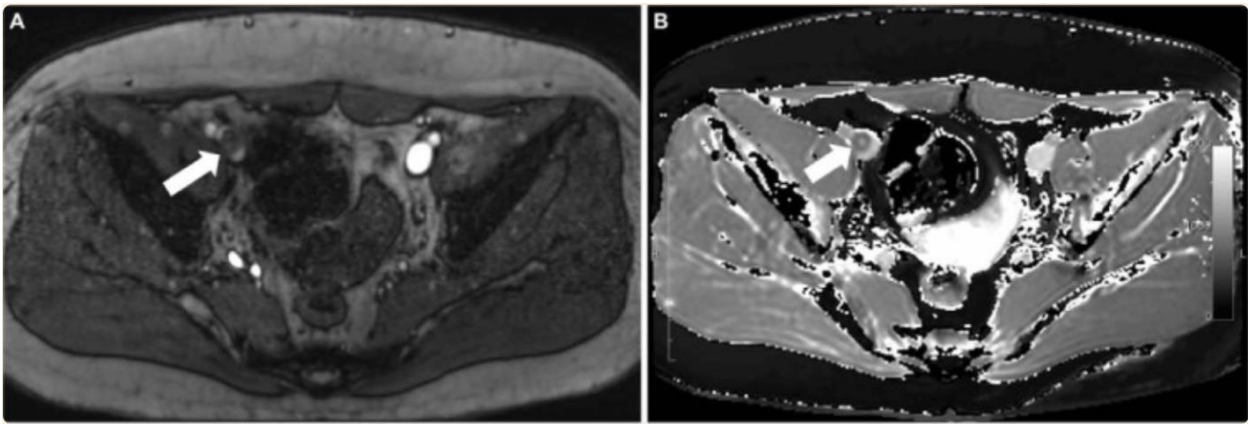


Fig 1.26: (A) Time-of-flight MRV images demonstrating right external iliac vein thrombus (white arrow) and (B) corresponding T_1 map demonstrating low T_1 relaxation time of the thrombus (white arrow) (Silickas *et al.*, 2018).

MRV is a valuable diagnostic tool for venous diseases, and like CTV, it can be performed with or without contrast medium (Orbell *et al.*, 2008). The non-contrast enhanced MRV technique utilizes blood flow characteristics to obtain a signal, known as the 2-D time-of-flight (TOF) technique or phase-contrast venography (Orbell *et al.*, 2008; Arnoldussen and Wittens, 2014). However, this technique has limitations due to its slow acquisition time, making it less effective in scanning peripheral veins that provide poor signals (Yokoyama *et al.*, 2001). Conversely, the new three-dimensional (3-D) technique has decreased acquisition time and is highly sensitive and specific in detecting DVT above the knee, with lower sensitivity below the knee (Cantwell *et al.*,

2006). In contrast enhanced MRV, contrast agents are delivered directly into any peripheral vein to improve the image quality of MRI (Orbell *et al.*, 2008). These contrast agents, such as extracellular gadolinium chelates, are safe and rarely result in nephrotoxicity (Morcos, 2008; Martino *et al.*, 2021). However, contrast enhanced MRV does not provide high-resolution images of calf veins, but this can be overcome by digital subtraction or using a blood pool contrast agent, which enhances the veins and provides better accuracy and high-quality images for comprehensive and faster image acquisition compared to non-contrast enhanced MRV (Jang *et al.*, 2015; Silickas *et al.*, 2018).

1.15 Varicose veins management and treatment

The treatment of VVs presents a significant challenge due to the uncertainty surrounding its pathophysiological mechanisms, which has resulted in numerous studies aimed at identifying the most efficient treatment options (Mansilha and Sousa, 2018). The complexity of VVs is compounded by the difficulty in identifying its signs and symptoms during the early stages of the disease (Pflug *et al.*, 1990; Min et al., 2003), which leads to delayed treatment-seeking behaviors among patients. Consequently, advanced stages of CVI such as eczema, pigmentation, and ulceration may develop (Widmer, 1974). The objectives of VVs treatments are to alleviate symptoms, enhance physical appearance, prevent disease recurrence, and avoid further complications that impact patients' quality of life and cause economic burden to society (Tisi, 2011). VVs treatment options are classified as invasive (surgical interventions), minimal invasive, and conservative treatments (Santler and Goerge, 2017). These therapies may be used alone or in combination to achieve optimal therapeutic outcomes (Smyth et al., 2015).

1.15.1 Invasive treatment (surgical intervention):

1.15.1.1 High ligation (HL) and stripping (S)

The conventional treatment approach for GSV varicosities typically involves saphenofemoral ligation and GSV stripping to the knee level (Sarin et al., 1994; Lin *et al.*, 2015). According to (Böhler, 2016), the high ligation and stripping (HL/S) procedure

is typically conducted under general anesthesia, with the patient placed in a supine position. Two incisions are made, with the first being located in the groin over the femoral pulse and the second near the knee or ankle. The vein is clamped at the first incision site to halt venous blood flow, followed by a cut being made in the vein, which is then sewn at the top, referred to as ligation. A wire is then threaded down through the vein, and the wire along with the incompetent veins is pulled out through the incision below, known as stripping. Finally, the incisions are closed using stitches. Despite its effectiveness in treating varicosities, HL/S treatment has several drawbacks.

The HL/S treatment has both advantages and disadvantages. One of the main advantages of this treatment is its high success rate, which has been supported by previous studies (Pan *et al.*, 2014). This success can be attributed to the fact that HL/S does not result in fibrosis around the vein and does not cause any damage to the saphenous nerve, as explained by (El-Sayed Salem, 1991). Despite its success, HL/S can sometimes result in injury to the saphenous nerve, which can be attributed to the nerve's anatomical location below the knee down to the medial malleolus, near the greater saphenous vein, with branches that run over the vein. This can occur during the stripping procedure (Ramasastry et al., 1987). However, this disadvantage is not considered to be clinically significant as saphenous nerve injury has been reported to have no impact on the patient's quality of life (Holme et al., 1990; Morrison and Dalsing, 2003).

HL/S treatment has several disadvantages including its high cost due to the requirement of general anesthesia (Rautio *et al.*, 2002), which can lead to complications such as nausea, vomiting, and cardiovascular and respiratory issues (Harris and Chung, 2013). Additionally, HL/S can result in thigh hematoma, which is a significant complication that causes postoperative pain and bruising (Rasmussen *et al.*, 2007; Kalteis *et al.*, 2008b). The incidence of thigh hematoma after HL/S is as high as 39% according to the study conducted by (Kent *et al.*, 1999), which can lead to hospital readmission (Millien and Coget, 1993). Moreover, incisions made during HL/S can lead to permanent scars that affect the skin's appearance (Fischer *et al.*, 2001) and may result in wound infections, wound hematoma, and abscess in some cases (Critchley *et al.*, 1997). Additionally, femoral vein or artery injury during surgery can

result in complications such as bleeding (Lin *et al.*, 2015). Finally, patients may need to take a sick leave for up to 2 weeks and mobilize post-surgery, while bruising may last for up to 6 weeks (Nesbitt *et al.*, 2014). Thus, the recovery time post-surgery for HL/S is longer compared to other treatments.

The recurrence of VVs after HL/S surgery is a major concern as evidenced by the high rates reported in numerous studies. The recurrence rate after five years following surgery has been reported to be between 20%-28%(Darke, 1992; Fischer *et al.*, 2002) and 20%-60% (Allaf and Welch, 2005). The high recurrence rate can be attributed to various factors, with incomplete surgery and neovascularization being the most common reasons cited in multiple studies (Stonebridge *et al.*, 1995; Fischer *et al.*, 2002; Winterborn, Foy and Earnshaw, 2004; Gad et al., Hokkam, 2012). Other reasons include incorrect initial diagnosis and inefficient first surgery (Pourhassan *et al.*, 2001; Geier *et al.*, 2005), and the development of VVs alongside changed venous dynamics and neovascularization (Bahk *et al.*, 2012). Although high ligation without stripping can be performed to conserve the saphenous vein, it is not recommended due to its higher recurrence rate compared to HL/S (Rutgers and Kitslaar, 1994).

1.15.1.2 Valvuloplasty

Venous reflux, which involves the destruction of valves, has been identified as a contributing factor in the development of CVI (Ortega et al., 2019). Valvuloplasty is a surgical procedure designed to reconstruct deep vein valves and is often recommended for patients with severe CVI with unhealed or recurrent ulcers (Eberhardt and Raffetto, 2014). When performed in combination with superficial and perforating vein ligation, valvuloplasty has been shown to improve ulcer healing (Hardy, Riding and Abidia, 2004; Tripathi *et al.*, 2004). There are two types of valvuloplasty: external and angioscopy. External valvuloplasty involves the external reconstruction of deep vein valves, while angioscopic valvuloplasty involves reconstruction of the valves using an angioscope (Komai and Juri, 2010). In both cases, the valves are repaired using stitches that are either placed outside or inside the valve to narrow the valve angle or make the valve tighter (NICE, 2007). The goal of the procedure is to improve valve function, thereby reducing venous reflux and venous ulcer recurrence (Wang et al., 2006).

Despite its potential benefits, valvuloplasty has some drawbacks. First, there are risks associated with the surgery, such as wound infections, ulcer recurrence, deep vein thrombosis, bleeding, and pulmonary embolism (Eberhardt and Raffetto, 2014). Second, the effectiveness of valvuloplasty has not been fully validated through large, randomized trials comparing it to gold standard treatments for CVI (Goel et al., 2015). Finally, the procedure has a high failure rate, limiting its clinical use (Eberhardt and Raffetto, 2014).

1.15.2 Minimal invasive treatment

1.15.2.1 Phlebectomy

Phlebectomy, also known as stab avulsion or microphlebectomy, is a commonly used surgical procedure for the treatment of superficial varicose veins (Orhurhu *et al.*, 2021). This technique is known to provide improved cosmetic outcomes and reduced risk of thrombosis (Zhan and Bush, 2014). Phlebectomy can be performed as a standalone procedure or in combination with other treatment methods such as Endovenous Laser Therapy (EVLT). Studies have shown that the combination of phlebectomy and EVLT results in less pain and improved symptoms for patients (Scavée *et al.*, 2003; Aremu *et al.*, 2004; Carradice *et al.*, 2009). However, the role of phlebectomy in conjunction with EVLT is still a subject of debate, with some studies suggesting that it reduces the need for further procedures post EVLT while others finding it unnecessary (Kawai *et al.*, 2020).

Phlebectomy can be performed using different stab techniques, with a new alternative procedure being the Transilluminated Powered Phlebectomy (TIPP) (Franz et al., 2012). TIPP is a safe, minimally invasive and easy to use procedure that uses transillumination light for direct visualization during vein resection. This reduces the risk of incomplete vein resection, decreases operative time, and provides better cosmetic results (Spitz et al., 2016). However, TIPP has been associated with significant bruising, severe pain, and lower quality of life in the early postoperative period (Chetter *et al.*, 2006). In conclusion, phlebectomy is a commonly used surgical technique for the treatment of superficial varicose veins that can be used alone or in

combination with other treatment methods such as EVLT. TIPP is a newer alternative to conventional phlebectomy that offers several advantages but can cause significant postoperative discomfort.

1.15.2.2 Hemodynamic correction procedure (CHIVA) method

The CHIVA method is a minimally invasive surgical procedure that utilises ultrasound to treat VVs by altering the hemodynamics of the leg venous system through the cutting of incompetent tributaries (Bellmunt-Montoya *et al.*, 2013; Orhurhu *et al.*, 2021). This ultimately results in a reduction in the hydrostatic pressure generated by the incompetent deep and superficial venous system in that area (Orhurhu *et al.*, 2021). Despite the CHIVA method's increased rate of superficial thrombophlebitis when performed by inexperienced surgeons (Faccini et al., Franceschi, 2019), it offers several advantages. Firstly, it employs local anesthetics, which is beneficial for patients (Carandina *et al.*, 2008). Secondly, the CHIVA method was found to have a lower recurrence rate of VVs compared to HL/S (Parés *et al.*, 2010). Additionally, this technique was reported to reduce pain and bruising and decrease the risk of nerve injury compared to HL/S (Bellmunt-Montoya *et al.*, 2013). Other studies showed that the CHIVA method was comparable to HL/S and endovenous laser ablation regarding reduced pain outcomes, but at only one-fifth the cost of EVLA (Solís *et al.*, 2009). Nevertheless, further studies are necessary to determine the effectiveness of the CHIVA method in treating VVs and its recurrence rate in comparison to other treatment methods.

1.15.2.3 Chemical ablation

1.15.2.3.1 Foam Sclerotherapy (UGFS)

Sclerotherapy is a recently introduced minimally invasive technique used for the treatment of VVs. The method involves injecting sclerosing agents (liquid or foam) into the incompetent veins, causing inflammation of the endothelium of vein walls, leading to thrombosis (Stücker *et al.*, 2016). Subsequently, thrombus is replaced by connective tissue and absorbed by the body (Albanese and Kondo, 2010). Compared to conventional liquid sclerotherapy, foam sclerotherapy has been reported to be more

effective in treating VVs for various reasons. First, liquid sclerotherapy can cause inflammation and venous occlusion (Orbach, 1950). Second, foam sclerotherapy is more effective in treating larger veins than liquid sclerotherapy (Tessari et al.,2001). The effectiveness of foam sclerotherapy has been reported to be more than double that of liquid (Frullini and Cavezzi, 2002; Ouvry *et al.*, 2008), mainly due to foam's ability to replace venous blood and extend contact time with the venous wall (Stücker *et al.*, 2010). Additionally, the recanalization rate of foam sclerotherapy is lower than that of liquid sclerotherapy (4.5% vs 13.64%) (Bi *et al.*, 2020). Finally, foam sclerotherapy is considered safer than liquid sclerotherapy, as any leakage of foam sclerosing agents is less harmful than that of pure liquid (Frullini and Cavezzi, 2002).

Foam sclerotherapy is a modified version of liquid sclerotherapy, which involves mixing the liquid with a gas, typically oxygen or carbon dioxide, to form a foam (Tessari, 2000). Initially, sclerotherapy was only effective in treating reticular varicosities and telangiectasia, and was used primarily to eliminate residual varicosities post-surgery or as an alternative for those who did not want to undergo surgery. However, with the introduction of duplex ultrasound as a guide for the procedure, ultrasound-guided foam sclerotherapy (UGFS) has emerged as a primary method for treating varicose veins, and is now considered a safer and more effective option that yields superior outcomes (Subramonia and Lees, 2007).

The treatment for UGFS entails the utilisation of tumescent local anesthesia, as noted by (Rasmussen *et al.*, 2011). The procedure involves the positioning of the patient in a reversed Trendelenburg posture. Subsequently, a cannula is introduced into the GVS above the knee under the guidance of duplex ultrasound imaging. Foam sclerosing agents are then injected, and the table is tilted to Trendelenburg position to ensure that the agents are distributed evenly. During this process, duplex ultrasound is employed to monitor the injection of the foam sclerosing agents, ensuring that they travel the full length of the GVS to the saphenofemoral junction and do not spread to the femoral vein. These agents trigger inflammation in the endothelial and subendothelial layers of the vein wall, resulting in luminal destruction and the subsequent reduction of the GVS's entire length. Following treatment, patients are instructed to mobilize, and compression stockings are recommended, as recommended by (Gloviczki and Gloviczki, 2012).

The UGFS treatment approach offers various advantages, but it also has some limitations. Firstly, the procedure exhibits a high success rate ranging between 82% to 100%, as reported by (Lin *et al.*, 2015). Additionally, UGFS is relatively inexpensive as it can be conducted in a medical office without general anesthesia, with low doses and concentrations of drugs administered, as highlighted by (Frullini and Cavezzi, 2002; Subramonia and Lees, 2007). The treatment usually takes about an hour to perform, and patients can immediately return home, providing faster recovery times than surgical treatment, as described by (Rasmussen *et al.*, 2011). Lastly, UGFS is relatively simple to repeat and causes less post-operative pain, according to (Kendler et al., 2007). Nevertheless, Foam Sclerotherapy can result in rare complications such as skin discoloration, bruising, thrombophlebitis, and visual disruption, as noted by (Willenberg *et al.*, 2013). Furthermore, uncommon complications include allergic reactions, necrosis, transient lymphoedema, transient scotoma, local neurological injury, and DVT, as reported by (Cavezzi *et al.*, 2002; Frullini and Cavezzi, 2002). In extremely rare cases, vascular events like stroke and myocardial infarction may occur, as observed by (Jia *et al.*, 2007).

The literature on the recurrence of UGFS yields conflicting results. (Rigby *et al.*, 2004) reported a high recurrence rate of UGFS after five years of follow-up. Their study revealed that while UGFS was successful in the first year of follow-up, its efficacy decreased significantly within three to five years, relative to surgical intervention. Conversely, (Pang *et al.*, 2010) reported a lower recurrence of UGFS treatment than surgery, particularly among patients with clinical stages C5 and C6 of CVI. Furthermore, several studies have supported the effectiveness and lower recurrence rates of UGFS treatment. (Frullini and Cavezzi, 2002; Barrett *et al.*, 2004) reported immediate success rates of approximately 85% in medium and large veins. Additionally, UGFS treatment resulted in symptom improvement and enhanced quality of life for patients after two years, as reported by (Barrett *et al.*, 2004). (Smith, 2005) reported success rates ranging from 80% to 90% after three years of follow-up.

1.15.2.4 Thermal Endo venous ablation:

1.15.2.4.1 Endovenous laser ablation (EVLA)

In recent years, there have been significant advances in the development of endovenous ablation techniques, one of which is endovenous laser ablation (EVLA). EVLA is considered to be a less invasive alternative to high ligation and stripping surgical interventions (Bakker *et al.*, 2013). This procedure typically involves the use of perivascular tumescent local anesthesia (Subramonia and Lees, 2007) and has been described in detail in the literature (Nesbitt *et al.*, 2014). To begin the procedure, an optical laser fiber is inserted into the GSV under the guidance of DUS imaging. The fiber is then passed through the vein from the saphenofemoral junction at the groin to the level of vein insufficiency, during which laser energy is produced to destroy the vein lumen. Alternatively, the GSV can be punctured at the level of the ankle to access the vein. Following this, varicose veins side branches can be removed by phlebectomy. Finally, patients are advised to mobilise immediately after the treatment.

EVLA technique offers both advantages and disadvantages. One major drawback is the high cost associated with the disposable laser equipment used during the procedure (Kalteis *et al.*, 2008). However, other studies have argued that EVLA is a cost-effective alternative to surgical interventions, as it can be performed as a minimally invasive procedure in a medical office without the need for an operating room or general anesthesia (Gohel et al., 2010; Nesbitt *et al.*, 2014). Clinical studies and randomized controlled trials have shown that post-operative pain and outcomes are similar between EVLA and stripping treatments (Bountouroglou *et al.*, 2006; Rasmussen *et al.*, 2007, 2011; Darwood *et al.*, 2008; Kalteis *et al.*, 2008; Theivacumar, et al., 2009). EVLA has additional benefits over stripping, including the avoidance of groin incisions or dissections of the sapheno-femoral junction (SFJ) (Gerard *et al.*, 2002) and smaller hematomas resulting from the procedure (Kalteis *et al.*, 2008). Although EVLA can result in complications such as bruising, skin burn, hematoma, infection, and catheter wounding by laser fiber or a damaged catheter remaining in the body (Liu *et al.*, 2011; Ren *et al.*, 2012), the incidence of these complications is less compared to stripping treatment (Pan *et al.*, 2014). Although these benefits may not

be significant, they can contribute to faster patient recovery. However, clinicians must weigh these benefits against the high cost of the EVLA equipment.

Although short-term benefits have been observed with EVLA treatment, there is a possibility of recurrent VVs in the long term (Mundy *et al.*, 2005). Recanalization, reflux in the midthigh perforator, and reflux in AAGSV are the primary causes of recurrent varicose veins after EVLA (Theivacumar et al., 2009). The development of VVs causes refluxing in AAGSV (Rasmussen *et al.*, 2010). The literature has shown that the recurrence of VVs is similar between surgical and EVLA treatments. For instance, after five years, 73% of limbs had recurrent VVs following surgical treatment, which occurred in the first year (Jones *et al.*, 1996; Dwerryhouse *et al.*, 1999). Similarly, after two or three years, the recurrence of VVs was expected post EVLA treatment, but occurred after nine months (Min et al., 2003). Moreover, the recurrence of VVs was similar between the EVLA and surgery groups after two years of follow-up in the studies by (Theivacumar et al., 2009; Rasmussen *et al.*, 2010). To prevent early recanalization, continuous laser mode should be used during the EVLA procedure instead of pulse laser mode, and laser energy greater than 80 J/cm should be used as recommended by these studies.

1.15.2.4.2 endovenous radiofrequency ablation (RFA)

Endovenous radiofrequency ablation (RFA) is a minimally invasive endovenous ablation technique used in the treatment of VVs. Typically performed under perivascular tumescent local anaesthesia, this procedure provides protection to adjacent tissues by acting as a heat sink and anaesthetic (Do and Kelley, 2007). The process involves the insertion of a catheter electrode into the GSV under duplex ultrasound imaging guidance. Radiofrequency energy is then passed through the catheter electrode along the length of the vein, resulting in thermal changes to the vein wall collagen. This causes vein wall inflammation, fibrosis, and ultimately sealing of

the vein (Subramonia and Lees, 2007). Patients are advised to mobilise immediately following treatment.

RFA has several advantages and drawbacks. Like EVLA, it is a cost-effective treatment that can be performed in a medical office as a minimally invasive procedure, without the need for an operating room or general anaesthesia (Nesbitt *et al.*, 2014). RFA also offers less postoperative pain and faster recovery than other treatments (Lin *et al.*, 2015). Clinical trial studies have reported that RFA is as effective as EVLA and surgical treatments in treating VVs (Almeida and Raines, 2006; Naoum and Hunter, 2007; Rasmussen *et al.*, 2011). RFA has a high vein occlusion rate, with rates of occlusion at 7 days post RFA exceeding 90% (Lurie, Creton, *et al.*, 2003), 85%-90% at 2 years (Weiss and Weiss, 2002), 85% at 4 years and 87% at 5 years (Merchant and Pichot, 2005), and 95% in several studies (Lurie, Creton, *et al.*, 2003; Goode *et al.*, 2010; Rasmussen *et al.*, 2011). RFA also has a low complication rate, with hematoma and infection uncommon post-treatment, although some early and late complications may occur (Anwar, Lane, *et al.*, 2012).

The early and late complications associated with RFA in the treatment of VVs has been investigated in literature. Early complications of RFA include superficial phlebitis, which was reported at rates of 7-9% in studies conducted by studies (Shepherd *et al.*, 2010; Nesbitt *et al.*, 2011; Rasmussen *et al.*, 2011). Endovenous heat-induced thrombosis (EHIT), which refers to thrombus extension to the common femoral vein after RFA treatment, has also been reported (Haqqani *et al.*, 2011). While DVT is a rare complication of RFA (Anwar, Lane, *et al.*, 2012), skin burns and wound issues may occur (Shepherd *et al.*, 2010). Late complications of RFA include skin discoloration, nerve damage, and VVs recurrence. Skin discoloration is caused by detained remaining blood within the veins (Anwar, Lane, *et al.*, 2012), while nerve damage was reported at rates of 4.8-13% in studies conducted by (Leopardi *et al.*, 2009; Shepherd *et al.*, 2010; Rasmussen *et al.*, 2011). The incidence rate of recurrent VVs after RFA was reported to be around 12% at 3 years (Nicolini *et al.*, 2005) and 21% at 4 years (Merchant and Pichot, 2005), and is due to reasons such as recanalization, neovascularization, and technical failure. Recurrence can be prevented in RFA treatment when groin dissection is avoided (Subramonia and Lees, 2007), a theory supported by (Pichot *et al.*, 2004) but disputed by (Lurie *et al.*, 2005).

The incidence rates of recurrent VVs after RFA and surgical intervention were found to be similar, with no differences in recanalization, neovascularization, or technical failure between the two treatment groups (Nesbitt *et al.*, 2014).

1.15.3 Compression therapy:

Compression therapy has emerged as a conservative and fundamental treatment modality for CVI over the course of the past century (Santler and Goerge, 2017). This therapeutic intervention offers a host of benefits, including its cost-effectiveness and suitability for use in outpatient settings (Partsch, 2012). Most significantly, compression therapy is a non-invasive method that addresses the primary pathophysiological mechanisms of CVI, namely hypertension and venous reflux, by compressing the veins and promoting calf muscle pump function (Santler and Goerge, 2017). Furthermore, compression therapy can alleviate the symptoms and signs of early-stage CVI, such as pain, heaviness, and edema (Motykie *et al.*, 1999), and assist in treating the latest stage of CVI, i.e., leg ulcers (Dissemond *et al.*, 2016). Three primary forms of compression therapy are available for the treatment of CVI and VVs: compression stockings, compression bandages therapy, and intermittent pressure compression therapy (Berszakiewicz et al., 2020). These modalities differ in their mechanisms of application, and their characteristics are expounded upon below:

1.15.3.1 Compression stockings therapy

Medical compression stockings are a widely used conservative treatment for CVI, and particularly for VVs (Campos Gomes *et al.*, 2020). These stockings are available in various lengths, models, and compression classes. Studies have discussed the duration and recommended compression class for the stockings to be worn to achieve optimal benefits (Santler and Goerge, 2017). The use of compression stockings offers several advantages, as reported by the International Union of Phlebology (IUP) in certain cases, such as VVs during pregnancy, ulcer healing, and reducing the risk of blood clot formation (Partsch and Baccaglini, 1995). This reduced risk of blood clot formation also decreases the likelihood of recanalization of the treated vessel (Weiss *et al.*, 1999) and post-sclerosis pigmentation in incidents of large VVs (Goldman,

1992). Compression therapy can be used as a standalone treatment or in combination with interventional treatments for VVs, such as post-surgery, post-endovenous ablation, and post-sclerotherapy (Kern *et al.*, 2008; Houtermans-Auckel *et al.*, 2009; Shingler *et al.*, 2013; Al Shakarchi *et al.*, 2018).

Considerable literature has been dedicated to investigating the use of compression stockings therapy following surgery. (Travers and Makin, 1994) traditionally found that the recurrence rate of VVs after surgery decreased by 1-year post-surgery when compression stockings were utilised, as opposed to patients who did not wear stockings. The control group reported a recurrence rate of 71%, while the stocking group reported a recurrence rate of 6%. (Shouler and Runchman, 1989) also reported that wearing low compression stockings (15 mmHg at ankle compared to 40 mm Hg) for 4 weeks post-surgery provided better support for the leg. However, the study by (Houtermans-Auckel *et al.*, 2009) proposed contrasting findings. The study found that using compression stockings four weeks after surgery post elastic bandaging for three days did not offer additional benefits in improving pain, leg edema, complications, and recovery. Literature also discussed the different durations of time for using compression stockings. For instance, the Society for Vascular Surgery and the American Venous Forum recommended the use of compression stockings for up to 7 days after surgery to prevent pain, swelling, and hematoma formation (Gloviczki *et al.*, 2011). This recommendation aligns with the NICE guidelines from 2013, where compression stockings were recommended to be used for no more than 1 week after VVs interventional treatment (Marsden *et al.*, 2013).

In a prospective randomized controlled study conducted by (Bakker *et al.*, 2013), 109 patients who underwent endovenous laser ablation were divided into two groups, with Group A using compression stockings for 2 days and Group B using compression stockings for 1 week. These groups were followed up for 90 days. Results indicated that venous physical function improved, and pain was less severe in the group of patients who used compression stockings for 7 days, as compared to those who used compression stockings for only 2 days. However, no significant difference was observed in physical function and pain after 7 days. These findings were consistent with those reported by (Elderman *et al.*, 2014) in their randomized controlled study, which included 111 patients who were divided into two groups, with the first group

using stockings and the second group not using stockings after venous laser therapy. Results showed that the stocking group had a lower pain score at week 1, with the second group reporting the use of more painkillers to relieve the pain. Moreover, the study found that compression stockings had no effect after 7 days post-venous laser treatment. Thus, these studies highlight the importance of using compression stockings for no more than 1 week after endovenous ablation treatment.

The impact of compression stockings in the treatment of VVs after sclerotherapy has been the subject of investigation in the literature. (Weiss *et al.*, 1999) found that compression stockings improved the results of sclerotherapy treatment, while a randomized control trial by (Hamel-Desnos *et al.*, 2010) reported several benefits in using compression stockings post foam sclerotherapy, including improved satisfaction scores, reduced pain, and better quality of life for patients. The effectiveness of compression stockings post sclerotherapy treatment was found to be directly associated with the level of compression (class of compression) and duration of compression (Cavezzi *et al.*, 2019). (Weiss *et al.*, 1999) found that one week of compression post sclerotherapy showed better results than no compression, while the best results were achieved with three weeks of compression post sclerotherapy. Similarly, (Kern *et al.*, 2007) found that three weeks of class 1 compression (23 to 32 mm Hg) enhanced the clinical disappearance of vessels, thereby improving the sclerotherapy of leg telangiectasias. Additionally, (Nootheti *et al.*, 2009) indicated that three weeks of class 1 compression stockings reduced pigmentation and bruising after sclerotherapy.

1.15.3.2 Compression bandages therapy

Compression bandages come in different designs including multilayer and single layer compression bandages, and are mainly used for the treatment of leg ulcers and acute cases of chronic leg edema (Santler and Goerge, 2017). The efficacy of these different compression bandage designs in the treatment of leg ulcers has been a subject of debate in the literature. Some studies have suggested that both multilayer and single layer compression bandages are equally effective in treating leg ulcers (Castonguay, 2008; Harrison *et al.*, 2011). However, other studies have reported that multilayer

compression bandages provide faster healing of leg ulcers compared to single layer compression bandages (Ukat *et al.*, 2003; O'meara *et al.*, 2012). Compression bandages can also be used in the treatment of venous insufficiency after venous ablation (Joyce *et al.*, 2019). In a survey of the treatment of venous insufficiency, members of the Vascular Society of Great Britain and Ireland reported that compression bandages were commonly used after surgery, with elastic bandages used in 49% of cases, and compression stockings being less commonly used (Edwards *et al.*, 2009).

1.15.3.3 Intermittent compression therapy (ICT).

Intermittent compression therapy (ICT) is a treatment option for patients who are unable to tolerate compression stockings or bandages and has demonstrated similar efficacy to these methods (Kirwan and Pignataro, 2016). It involves the use of an air pump to inflate and deflate a cuff that surrounds the leg, resulting in intermittent pressure compression of the limb (Zhao *et al.*, 2014). The speed at which the cuff is inflated and deflated may vary, and studies have found that fast ICT leads to greater healing of leg ulcers than slow ICT. However, ICT should not be used in patients with peripheral vascular diseases or deep vein thrombosis, and should be carefully monitored in patients with congestive heart failure, as the rapid transfer of a large amount of blood from the lower extremity veins may exacerbate cardiovascular and pulmonary conditions (Kirwan and Pignataro, 2016; Hirsch, 2018). By improving the veno-lymphatic pump, ICT can decrease leg edema and improve microcirculation of the skin, which enhances the normal fibrinolysis process and prevents blood clots (Comerota *et al.*, 1997; Partsch, 2012). While ICT has been shown to improve healing when used alone or in combination with compression bandages, further research is needed to determine if it can replace compression bandages as a primary treatment option (Kirwan and Pignataro, 2016).

1.15.4 Oral pharmacological therapy

Oral pharmacological therapy has been shown to effectively improve symptoms and QoL for patients with CVI, as evidenced in studies by (Nicolaides *et al.*, 2018). Venoactive drugs such as Pentoxifylline, Oxerutins, Calcium dobesilate, Rutosides,

and Daflon/micronized purified flavonoid fraction (MPFF) have been extensively studied for their efficacy in treating CVI. Pentoxifylline has been found to effectively treat venous ulcers with or without compression bandaging (Jull *et al.*, 2012), while Oxerutins and calcium dobesilate have positive outcomes in reducing edema (Akbulut, 2010). Rutosides have been shown to reduce VVs symptoms in pregnancy (Davies, 2019). Among these drugs, Daflon/MPFF is the most widely researched and prescribed. It comprises of 90% diosmin and 10% other active concomitant flavonoids, including hesperidin, diosmetin, linarin, and isorhoifolin (Radhakrishnan, 2022). A double-blinded placebo-controlled randomized controlled trial involving 1692 patients found that Daflon/MPFF improved the signs, symptoms, and quality of life in patients with CVD (Kakkos and Nicolaides, 2018). Additionally, Daflon/MPFF has been found to have several advantages, such as enhancing venous tone and contractility in high-risk VVs patients, improving symptoms of venous ulcers, reducing edema and inflammation, improving microcirculation, decreasing pain, leg heaviness, and night cramps, and reducing leukocyte adhesion and activation in venous inflammation models by venous hypertension or ischemia reperfusion (Ibegbuna *et al.*, 1997; Bush *et al.*, 2017; Tsukanov and Tsukanov, 2017; das Graças C de Souza *et al.*, 2018; Nicolaides *et al.*, 2018). Physicians should refer to these guidelines to identify the most appropriate pharmacological therapy for their CVD patients.

1.16 Rationale for current study

If left untreated, VVs can have significant socioeconomic consequences (Posnett, 2008). The social impact of VVs includes a reduced QoL for patients, which can lead to decreased physical activity, pain, depression, and social isolation (Lindholm *et al.*, 1993; Kaplan *et al.*, 2003). The economic burden of VVs is also considerable. The estimated cost for providing an intervention for VVs was initially set at £780 (Hitchman et al, 2023). This calculation was based on the NICE costing template, which considered the scenario of a single consultant operator performing an endovenous ablation. However, it's essential to note that in the NHS, eligibility for VVs treatment is contingent on approval from the patient's Clinical Commissioning Group (CCG). CCGs are responsible for organising the delivery of NHS services in various regions of England. Typically, only more advanced and severe cases of VVs receive coverage

under the NHS due to certain criteria and considerations. As a result, VVs that are left untreated until they progress to the point of causing skin changes, ulceration, and open wounds can lead to a significant economic burden on the NHS budget. Notably, the NHS reported expenditures of £2.3-3.1 billion between 2005 and 2006 (Posnett and Franks, 2007), which increased to £5.3 billion between 2012 and 2013 (Guest *et al.*, 2015). Subsequently, the costs continued to rise, reaching £8.3 billion between 2017 and 2018 (Guest et al.,, 2020).

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1.17 Research questions

1.17.1 Principle research questions

Are VVs in pregnancy caused by both mechanical and hormonal factors?

1.17.2 Secondary research questions

- Is Vector flow imaging (VFI) tool an accurate diagnostic test to evaluate the mechanical factors such as iliac vein compression which cause VVs in pregnancy?
- Are Estroil (E3) and progesterone serum blood tests sufficient tests to evaluate hormonal factors causing VVs in pregnancy?
- Does lower limb pain during pregnancy related to changes in the lower limb venous system?

1.18 Research hypothesis

The development of post-partum VVs is predictable and caused by both mechanical and hormonal factors.

2 Chapter-2 Literature review (Risk Factors)

Large number of studies have extensively studied the risk factors associated with VVs formation. This proposed study categorised these risk factors into Genomics, metabolomics, biomarkers, histopathology, epidemiology studies including gender, height, pregnancy, hormonal factors, age, occupational factors, obesity, family history, race smoking and alcohol consumption, diet and other potential risk factors.

2.1 Genomics:

Genetic studies have illustrated number of genes and genes polymorphisms linked to VVs formation. Forkhead Box C2 (FOXC2) gene was firstly discovered to be associated with VVs pathology when it was found to be the cause of lymphoedema-distichiasis syndrome and 49% of these patients developed VVs (Brice *et al.*, 2002). Mutations of this gene was reported to play an important role in venous valves damage and reflux in superficial and deep venous system when developing VVs in lower extremities (Surendran *et al.*, 2014). Furthermore, methylenetetrahydrofolate reductase (MTHFR) gene was found to be associated with VVs formation (Krysa, Jones and van Rij, 2012). This gene was reported to be prevalent in patients with VVs compared to controls (Sverdlova *et al.*, 1998). The main function of this gene is to produce an enzyme that regulates homocysteine levels which is involved in blood clotting (Zidan, Rezk and Mohammed, 2013). Deficiency of (MTHFR) gene was hypothesised to increase levels of homocysteine which thus cause damage to inner layer of blood vessel (Varga *et al.*, 2005).

First Genome-wide association studies (GWAS) study highlighted several number of genes polymorphisms and their association with VVs development (Bell *et al.*, 2005). These genes are ABO, CAZ1, ADM, ANGPT1, VEGFA and PIEZO1. Polymorphism in ABO gene represents A blood group which was found to be linked to VVs pathogenesis. Polymorphism in CAZ1 gene regulates blood pressure and circulation as well as stimulates vascular development (Bell *et al.*, 2005). Polymorphisms in ADM gene acts as a vasodilator peptide, stimulates angiogenesis and prevents apoptosis. Polymorphisms in ARHGAP6 gene which elevates the activity of high blood pressure. Polymorphisms ANGPT1 gene plays a role in promoting vascular development.

Polymorphisms in VEGFA gene contains proteins that affects vasculogenesis, angiogenesis and the growth of endothelial cells. Finally, polymorphisms in PIEZO1 gene was found to be involved in cardiovascular function and disease in adults. Therefore, these polymorphisms play an important role in the morphological specification of VVs and therefore can contribute in VVs progression. Second GWAS study reported the strong association between polymorphism in EFEMP1 and CVD aetiology which has a significant role in angiogenesis and vessel development (Ellinghaus *et al.*, 2017). Interestingly, there were no common gene polymorphisms found in these two studies. This may be due to the genetic differences between the populations studied and other non-genetic factors that may change polymorphisms.

Different polymorphisms have also been investigated in the literature. Polymorphism in HFE cause endothelial cell dysfunction and vein wall remodeling (Sokolova *et al.*, 2016). Polymorphism in (MCP1) gene which is characterised by chemokines played an essential role in inflammation (Alexandra S Shadrina *et al.*, 2017). Furthermore, changes in plasma levels in type I plasminogen activator inhibitor PAI-1 4G/5G polymorphism and PAI-1 4G in PAI-1 gene cause abnormalities in fibrin which plays an important role in blood clotting as well as it accelerates inflammation process which damage blood vessels walls (Katrancioglu *et al.*, 2010). Matrix Gla Protein (MGP) gene plays an important role in disturbing proliferation and mineralization processes by decreased carboxylation of MGP (Cario-Toumaniantz *et al.*, 2007). Similarly, polymorphism in Major Histocompatibility Complex (MHC) class III genes was linked to VVs pathogenesis (Shadrina *et al.*, 2018). This polymorphism play an important role in inflammatory and immune response, cell signaling, and heat shock response (Horton *et al.*, 2004). polymorphism in alpha-2 type I collagen gene (COL1A2) (Jin *et al.*, 2013). This case control study found that alterations in COL1A2 gene polymorphism is prevalent in patients with CVI compared to healthy controls.

Remodeling in different types of genes were investigated in literature and found to have a significant the role in VVs formation. These genes include; matrix Gla protein (MGP) gene which plays a significant role in disturbing proliferation and mineralization processes by decreased carboxylation of MGP (Cario-Toumaniantz *et al.*, 2007), circadian locomotor output cycles kaput (CLOCK) which is known to be involved in nucleotide excision repair, destroying Deoxyribonucleic acid (DNA) barriers and

apoptosis in mammalian organisms (Tang *et al.*, 2015) ,This study found higher expression of CLOCK gene in VVs patients compared to healthy controls. This may be due to the effect of hypoxia which increase protein levels of CLOCK gene as reported previously in (Ghorbel, Coulson and Murphy, 2003). Decreased methylation levels of Osteopontin (OPN) and integrin β3 genes and increased levels of proteins of OPN and integrin β3 genes were found in VVs SMC compared to healthy veins (Li, X.-Y. Jiang, *et al.*, 2014). This study reported the role of OPN in stimulating migration and bonding SMC through integrin β3 which these together cause many vascular diseases.

Urbanek and his colleagues pointed out that increased levels of p21, p53, and fas mRNA genotype are associated with VVs formation (Urbanek *et al.*, 2004). Other pathways may be involved in VVs cellular mechanism. For example, Long non coding RNAs (lncRNAs) were found in great saphenous vein (GSV) after pathway analysis which suggested the significant association with VVs incidence (Li, X. Y. Jiang, *et al.*, 2014). A study by Li and his colleages found low expression of lncRNA-GAS5 which is involved in proliferation and migration (Li *et al.*, 2015). Therefore, any change in these genes and pathways may play important role in VVs pathogenesis.

2.2 Metabolomics

Metabolomics studies to assess the cellular biochemical mechanisms of VVs phenotype were illustrated in literature. Multiple studies have found differences in metabolomic status between VVs patients and healthy individuals (Anwar *et al.*, 2017). For example, the role of oxidative stress was significant in the metabolic profile of patients with CVI (Budzyn *et al.*, 2011). This was revealed after investigating high levels of Malo-nyldialdehyde MDA and low levels of uric acid (UA) concentration in CVI patients' blood. Furthermore, high levels of creatine, lactate and myo-inositol metabolites and lower levels of Lipid metabolites were found in VVs group compared to healthy patients (Anwar, Shalhoub, *et al.*, 2012). Patients with VVs showed high levels of glutamate, taurine, myo-inositol, creatine and inosine were found in aqueous extracts and phosphatidylcholine, phosphatidylethanolamine and sphingomyelin in lipid extracts compared with healthy patients (Anwar *et al.*, 2017). Therefore, this study

indicates a relation between phosphatidylcholine and sphingomyelin with inflammation and myo-inositol with cellular proliferation.

2.3 Biomarkers

Several biomarkers have been reported to be associated with VVs development. A significant increase in plasma levels of protein S, vWf, Vascular endothelial growth factor (VEGF) and interleukin-12 (IL-12) were found in patients with VVs (Yasim *et al.*, 2008). Furthermore, the role of haemostatic factor was identified to be linked to VVs formation (Lee *et al.*, 2000). This study reported high levels of plasma fibrinogen, tissue plasminogen activator (t-PA) and von Willebrand factor (vWF) antigens in men and women with CVI and VVs compared to healthy individuals.

2.4 Histopathology

Cellular mechanisms play an important role in the pathophysiology of VVs. These mechanisms include apoptosis, hypoxia, inflammation, extracellular matrix degradation caused by changes in extracellular components.

2.4.1 Apoptosis

It has been argued that VVs formation is associated with venous wall cells dysregulation during the apoptosis process. Apoptosis is a programmed cell death process first reported by (Wide- and Kinetics, 1972). Several studies have reported that apoptotic dysregulation in VVs walls is transmitted by the intrinsic pathway which is maintained by apoptosis proteins (such as Bax or Bcl-2) and certain caspases (Ascher *et al.*, 2001; Ducasse *et al.*, 2008; Kun *et al.*, 2016). These studies found a significant down regulation of Bax pro-optotic protein in VVs segments compared to healthy controls.

2.4.2 Hypoxia

Insufficient amount of oxygen in veins is known as venous hypoxia was suggested to be linked to VVs pathogenesis (Michiels, Arnould and Remacle, 2000b). It was found that cobalt chloride (COCL2) can imitate hypoxia and therefore cause cellular changes which leads to VVs formation (Li and Wang, 2014). Furthermore, it has been illustrated that Increased levels of hypoxia in VVs cause elevated levels of basic fibroblast growth factors (bFGF) and platelet-growth factors (PDGF) which both have an important role in SMC proliferation and cell migration in vein walls (Michiels, Arnould and Remacle, 2000b). These receptors depend on phosphoinositide-3-kinase-protein kinase B/Akt (PI3K/Akt) signalling pathway which is related to hypoxia and considered essential in many vascular diseases (Zhang *et al.*, 2003). Several studies found increased levels of PI3K/Akt/mTOR protein in patients with venous reflux (Lawrence *et al.*, 2014; Rodríguez, 2014; Ortega *et al.*, 2018).

2.4.3 Inflammation

The role of inflammation in the pathogenesis of VVs has been reported in literature (Pocock *et al.*, 2014). The role of leucocyte infiltration in VVs walls is one of the mechanisms which has been suggested to be involved in VVs pathology by causing venous wall remodelling and valve impairment (del Rio Solá *et al.*, 2009). Furthermore, increased levels of molecules involved in inflammation such as cytokines and adhesions molecules have been found in VVs segments compared to normal veins (del Rio Solá *et al.*, 2009). This was due to their significant role in leucocyte migration, fibrosis, angiogenesis and cell proliferation (Sallusto and Baggiolini, 2008).

2.4.4 Extracellular matrix degradation

2.4.4.1 MMPs and TIMPs

Matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs) have been reported to be linked to VVs development (Kucukguven and Raouf A Khalil, 2013).

This is due to the main function of MMPs in regulating extracellular matrix (ECM) components such as proteins, collagen and elastin (Shadrina *et al.*, 2017). Balanced levels of these components are essential to provide elasticity and distensibility of normal veins (Somers and Knaapen, 2006a; Elsharawy *et al.*, 2007). On the other hand, TIMPs control MMPs activation (Slonková *et al.*, 2017). When venous pressure is increased, MMPs activation increases causing changes in ECM, endothelium and smooth muscle cells production which leads to VVs (C S Lim and Davies, 2009; Kucukguven and Raouf A Khalil, 2013). Consequently, imbalance between MMPs and TIMPs may contribute to varicose vein development and studying MMPs and TIMP-2 genotypes provide comprehensive explanation for this imbalance.

2.4.4.2 Collagen changes

It has been suggested that dermal fibroblasts of venous walls in patients with VVs have changes in collagen levels (Sansilvestri-Morel *et al.*, 2003). Clinical investigations have found unbalanced levels of collagen 1 and 3 are associated with VVs formation (Sansilvestri-Morel *et al.*, 2007). Collagen type 3 has a significant role in elasticity while collagen type 1 provides stiffness (Sansilvestri-Morel *et al.*, 2003). The majority of studies have reported decreased levels of collagen 3 and increased levels of collagen 1 are associated with VVs development (Kockx *et al.*, 1998; Sansilvestri-Morel *et al.*, 2003; Kowalewski *et al.*, 2004; Raffetto *et al.*, 2008).

2.4.4.3 Elastin changes

It has been suggested that certain changes in connective tissue indicate changes in MMPS levels which are the enzymes associated with regulating extracellular matrix that breakdown extracellular particles (J. *et al.*, 2000). Besides changes in collagen, reduced amounts of elastic fibers have been found to be associated with venous wall dilatation (Elsharawy *et al.*, 2007; Jeanneret *et al.*, 2007; Demirkiran *et al.*, 2014). Higher elasticity was reported in normal subjects compared to the elasticity in patients with VVs (Clarke *et al.*, 1989). Furthermore, decreased levels of elastin were associated with aging which explains the high prevalence of VVs in elderly patients (Bradbury *et al.*, 1999; T Zahariev *et al.*, 2009).

2.4.4.4 Other extracellular components

Unbalanced levels of other extracellular components including Lipid peroxidation, 8-epi-PGF2alpha and 6-oxo-PGFI have been reported to be associated with VVs formation.

2.4.4.5 Purinergic signalling

The main function of Purinergic signalling is regulating cellular function and changes in endothelial cells and SMC and therefore plays an important role in vasodilation (Ralevic and Burnstock, 2003). Purinergic signalling receptors including P1 and P2 have been found to be potential cause of VVs development (Metcalfe *et al.*, 2007). This study confirmed downregulation of P2X1 receptors and upregulation in P2Y1 and P2Y2 receptors in VVs segments. Lower levels of P2X1 in SMC in VVs leads to decrease in contractile activity (Metcalfe *et al.*, 2007). Furthermore, increased levels of P2Y1 and P2Y2 receptors leads to increase proliferation and synthetic action as well as decrease in contractile activity (Hou *et al.*, 2015). Therefore, alterations in SMC phenotype may result in weakening vein walls and VVs development.

2.4.4.6 Intima, media and adventitia fibrosis

Blood vessels morphology has been discussed in literature. The incident of intima, media and adventitia fibrosishas been investigated in VVs and non VVs veins (Leu, Vogt and Pfrunder, 1979). This study found no association between intima fibrosis and VVs which is a finding in normal veins in all age groups. Furthermore, media and adventitia fibrosis occur only in late stages of VVs.

2.5 Epidemiology

2.5.1 Gender

There are conflicting findings in the literature regarding the prevalence of VVs between genders. Some have found a higher prevalence amongst women (Kontosic *et al.*, 2000; Labropoulos *et al.*, 2001; Carpentier *et al.*, 2004; Makivaara *et al.*, 2004; Chiesa

et al., 2005; T. Zahariev *et al.*, 2009; Tiina M Ahti, Makivaara, Luukkaala, Hakama and Laurikka, 2010).The higher rates in women in these studies was due to mutual risk factors which include pregnancy, obesity, height, smoking, occupational factors and age. On the other hand, others have found a higher prevalence in men (C. J. Evans *et al.*, 1999). Others have suggested that no gender difference exists (Robertson, Amanda J Lee, *et al.*, 2013) .

2.5.2 Height

The association between VVs and increased height has been reported in several studies (Amanda J. Lee *et al.*, 2003; Pappas *et al.*, 2007; Fukaya *et al.*, 2018). (B Komsuoglu *et al.*, 1994; T Sisto, Reunanen, Laurikka, Impivaara, Heliovaara, *et al.*, 1995; F. G. R. Fowkes *et al.*, 2001) found a significant correlation between increased height and VVs development in men only. In contrast, (Beaglehole *et al.*, 1975) reported no relation between height and VVs.

2.5.3 Pregnancy

Large number of studies have found an association between history of pregnancy and the incidence of VVs (Sadick, 1992; Sobaszek *et al.*, 1998; P. Lacroix *et al.*, 2003; Carpentier *et al.*, 2004; Ko *et al.*, 2008; Zahariev *et al.*, 2009; Bihari, Tornoci and Bihari, 2012; Yun *et al.*, 2018). The incidence rate of VVs was higher in women after pregnancy 40.5% (Canonico *et al.*, 1998). Furthermore, Ismail and his colleagues meta-analysis reported an increase incidence of VVs by 82% in women with previous history of pregnancy compared to women with no history of pregnancy (Ismail *et al.*, 2016). Other studies illustrated the influence of multiparity and VVs development (Maffei *et al.*, 1986; T Sisto, Reunanen, Laurikka, Impivaara, Heliovaara, *et al.*, 1995; Labropoulos *et al.*, 2001; Jawien, Grzela and Ochwatt, 2003; Chiesa *et al.*, 2005; Jukkola *et al.*, 2006; Zolotukhin *et al.*, 2017). Although the majority of studies indicated pregnancy as a risk factor. However, some studies found no association between parity and VVs (Beaglehole *et al.*, 1975; F. G. R. Fowkes *et al.*, 2001; C A Engelhorn *et al.*, 2010).

2.5.4 Other hormonal factors

Hormonal changes may also influence VVs formation. This was reported in menopause (Zolotukhin *et al.*, 2017), using oral contraceptive (OCs) (Sadick, 1992; F. G. R. Fowkes *et al.*, 2001) and hormone replacement therapy (HRT) (Bawakid *et al.*, 2005). (Bawakid *et al.*, 2005). In menopause women, the incidence rate of VVs was 31% compared to 40.5% women who developed VVs after pregnancy which considered high in both cases due to hormonal changes (S Canonico *et al.*, 1998). Estrogen and progesterone hormones which are found in OCs and HRT were reported to influence VVs development. Ropacka-Lesiak and his colleagues explained the mechanism of these hormones in their study (Ropacka-Lesiak, Kasperczak and Breborowicz, 2012c). This study reported that estrogen hormone elevates coagulation protein levels which cause thrombosis. Additionally, estrogen dilates venous valves due to its role in relaxation and releasing the chains between collagen fibers. On the other hand, progesterone prevents smooth muscle contraction which therefore increase valvular insufficiency due to vasodilation which cause lack of connection between two valves.There are conflicting findings in literature related to increased progesterone and estrogen levels and VVs formation. Therefore, further studies are required to confirm the relation between these hormones and VVs pathogenesis.

Other hormones may have a significant influence on VVs formation. Androgen hormones receptors were detected in the VVs of all subjects but were found in lower levels in men (García-Honduvilla *et al.*, 2018). Furthermore, high ratio of Serum estradiol/free testosterone (E2/fT) was linked to VVs pathogenesis in men (Ozcan *et al.*, 2015). Relaxin hormone which is related to insulin and insulin- like growth factor found in corpus luteum in pregnant women (Bathgate *et al.*, 2003). Relaxin is involved in many functions such as parturition, regulating sex hormones and renal vasodilator (Baccari and Calamai, 2004). Recently, relaxin has been implicated in the pathogenesis of VVs. For instance, Adams and his colleagues found decreased levels of relaxin-2 and its receptors RXFP1 and RXFP2 in VV (J. *et al.*, 2012). Therefore, the redistribution of these hormones receptors in venous wall may cause VVs which highlights the role of sex hormones in VVs pathogenesis.

2.5.5 Age

The prevalence of VVs increases with increasing patient's age. As explored previously in this review, aging is coupled with reduce in elasticity and changes in diameter size of the deep and superficial venous system (Clarke *et al.*, 1989). The majority of the studies have confirmed the association between increasing age and VVs formation in the general population with no differences between men and women (Dindelli *et al.*, 1993; T. Sisto *et al.*, 1995; Sobaszek *et al.*, 1998; Bradbury *et al.*, 1999; C. J. Evans *et al.*, 1999; Kontosic *et al.*, 2000; Cesarone *et al.*, 2002; P. Lacroix *et al.*, 2003; Kroeger *et al.*, 2004; Bawakid *et al.*, 2005; Ko *et al.*, 2008; I Bihari, Tornoci and Bihari, 2012; Robertson, Amanda J. Lee, *et al.*, 2013; Khan, Chaudhri, Ashraf, Mazaffar, Zawar-ul-Imam, Tanveer, *et al.*, 2013; Ebrahimi *et al.*, 2015; Zolotukhin *et al.*, 2017; M. J. Yun *et al.*, 2018; Mahapatra *et al.*, 2018; G. Aly *et al.*, 2020). (Dimakakos *et al.*, 2013) reported the prevalence rate of CVD increased with age, 23.4% among 45-54 years old and 27% among 55-64 years old. However, other studies have found difference between both genders regarding age risk factor which may be due to hormonal or other potential risk factors.

2.5.6 Occupational factors

Occupational factors have been proposed to have an effect on venous pathology. Professions that require sitting and prolonged standing risk factors were strongly associated with the incidence of VVs (Sobaszek *et al.*, 1998; Carpentier, 2000; Carpentier *et al.*, 2004; Ko *et al.*, 2008). Sitting habits may cause compression of the back side of thighs and therefore interrupt blood flow (Stanhope, 1975). Furthermore, standing position increases blood pressure in lower extremities which therefore cause venous stasis and secondary valvular incompetence (Criqui *et al.*, 2007). The prevalence rate of CVD among population who spend more than 4 hours in prolong standing was accounted for 20.8% (Dimakakos *et al.*, 2013) and 64.8% in (Fokou *et al.*, 2018). Furthermore, prolong standing was also reported in other studies that also took into consideration gender differences. Therefore, occupational risk factors may be subject to gender differences and duration of time that body posturers are maintained.

2.5.7 Obesity

The association between obesity and incidence of VVs has been reported in several epidemiological studies. The prevalence of obese patients who developed VVs was 62.9% in (Dimakakos *et al.*, 2013) and 32.6% in (Fokou *et al.*, 2018). This may be due to excessive fat which increases abdominal pressure (IAP) and femoral vein pressure (Arfvidsson, Eklof and Balfour, 2005). Consequently, increased pressure will demonstrate blood stasis and cause venous dilatation and VVs development (Willenberg *et al.*, 2010). Although large number of studies reported the strong association between high BMI and the aetiology VVs of in both genders (P. Ducimetiere *et al.*, 1981; Sadick, 1992; T Sisto, Reunanen, Laurikka, Impivaara, Heliövaara, *et al.*, 1995; Kontosic *et al.*, 2000; Coughlin *et al.*, 2001; Danielsson *et al.*, 2002; Iannuzzi *et al.*, 2002; P. Lacroix *et al.*, 2003; Jawien, Grzela and Ochwatt, 2003; Kröger *et al.*, 2003; Callejas *et al.*, 2004; Robertson, Amanda J. Lee, *et al.*, 2013; Kohno *et al.*, 2014; Seidel *et al.*, 2015; Sharif Nia *et al.*, 2015; H. H. Kim *et al.*, 2017; Mahapatra *et al.*, 2018). However, other studies reported the significance relation between obesity and VVs development in women (Brand *et al.*, 1988; S Canonico *et al.*, 1998; F. G. R. Fowkes *et al.*, 2001; Amanda J. Lee *et al.*, 2003) and one study in men (F. G. Fowkes *et al.*, 2001).

2.5.8 Family history

Multiple studies agreed that family history is a significant risk factor in developing VVs (Cornu-Thenard *et al.*, 1994; Fiebig *et al.*, 2010; Zöller *et al.*, 2012). The incidence rate of VV caused by heredity was accounted for 42% in patients with VVs compared to 14% in healthy samples (Hirai, Naiki and Nakayama, 1990) and 17.7% in (Fokou *et al.*, 2018). (Kroeger *et al.*, 2004) stated incidence rate of VVs increased when the mother is affected more that the father of both parents. However, some authors suggested that family history information might be biased (Amanda J Lee *et al.*, 2003; T M Ahti *et al.*, 2010). This is because the incidence of family history was self-reported without undergoing clinical examination for parents and offspring (Weddell, 1969). To overcome this issue, risk of bias was minimised in the study by (E. *et al.*, 2018). This study investigated 113 families by examining all patients and parents clinically. The results of this study indicated a strong association between heritability and incidence

of VVs. This result was consistent with large number of studies. Other conflicting studies also exist.

2.5.9 Race

The prevalence of VVs between different races and geographical regions varies in literature. This variation was due to conducting different study types which make it difficult to provide an accurate conclusion. The prevalence of VVs was 5.8% in Egyptian workers compared to 32.1% in English workers in (Mekky, Schilling and Walford, 1969), 49% in white people compared to 36% in people with colour (Maffei *et al.*, 1986), VVs was less common in Caucasian population compared to Asian counterparts in the UK (Subramanian *et al.*, 2007) and CVI was higher in European population compared to black or Asians in San Diego study (Criqui *et al.*, 2007). Therefore, the clear difference in prevalence rate between two groups in these studies highlighted the impact of race differences between two groups.

2.5.10 Smoking and alcohol consumption

Life style factors such as smoking, and alcohol consumption have also been suggested as potential risk factors for VVs among people. Multiple studies have found an association between smoking and VVs development in both genders (P. Ducimetiere *et al.*, 1981; Coughlin *et al.*, 2001; Gourgou *et al.*, 2002; Ko *et al.*, 2008) while others reported the relation between smoking and VVs formation in men (Brand *et al.*, 1988) and other study in women (Lacroix *et al.*, 2003). It has been reported that prevalence of VVs was 33.8% among tobacco smokers in Spain compared to non-smokers (Callejas *et al.*, 2004). On the other hand, this study reported the prevalence of VVs in people who consume alcohol was 25%. Other studies suggested that VVs development was higher in patients who had a long and regular history of alcohol consumption (T M Ahti *et al.*, 2010; H. Kim *et al.*, 2017).

2.5.11 Diet

Diet plays a significant role in regulating healthy veins. It has been suggested that low fiber diet can play a significant role in VVs development (Malhotra, 1972; Richardson and Dixon, 1977; Coughlin *et al.*, 2001). Similar finding was reported in (Beaglehole

et al., 1975). This study found processed carbohydrates with eliminated dietary fiber is a VVs potential risk factor. In the Edinburgh Vein study, lack of fiber was associated with the incidence of VVs in males only (Evans *et al.*, 1999). Low fiber diet may cause constipation and straining at stool which therefore leads to VVs. This hypothesis was explained by (Burkitt, 1972; Coughlin *et al.*, 2001) suggesting that still cecum may cause intra-abdominal pressure against iliac veins and with time may cause veins enlargement and VVs. Furthermore, straining at stool was reported as a risk factor that can contribute to VVs development in men compared to women (Fowkes *et al.*, 2001; Lee *et al.*, 2001).

2.5.12 Other potential risk factors

Small number of studies have reported other potential risk factors may contribute to VVs development. For instance, leg injury history (Lacroix *et al.*, 2003), lack of physical activity (Khan et al.,2013), hypertension (Ducimetiere *et al.*, 1981; Brand *et al.*, 1988; Ebrahimi *et al.*, 2015), high HDL cholesterol level and reduced antithrombin III level (Kim *et al.*, 2017), diabetetis in males (Lionis *et al.*, 2002), Osteoarthrosis (Sisto *et al.*, 1995), Static foot disorder (SFD) (Uhl et al.,2012) and arteriovenous anastomosis (Katsamouris *et al.*, 1989; Murphy and Hands, 2008).

2.6 Literature review summary:

VVs are dilated and tortious veins which are considered an early sign of Chronic venous insufficiency (CVI). The prevalence estimation of VVs varies between studies due to differences in study methodology, study population and geographic location. One study by Canonico reported detailed prevalence estimation in men and women and pregnant and non-pregnant patients which were 17%, 35.2%, 40.5% and 38.2% respectively. This study showed higher prevalence of VVs among women compared to men and pregnant women compared to non-pregnant. Signs and symptoms of VVs depend on the progression stage of the disease which range from pain and heaviness in early stages and ulceration in late stages. VVs related complications have a significant socioeconomical impact which reduce patients' QoL and increase health

care cost. The aetiology of VVs remains unclear in general population and especially in pregnant women despite previously published theories in literature. Large number of studies have studied the risk factors associated with VVs development. This is because the incidence is a good way to study the aetiology of a disease which can help in assessing the cause and effect. These risk factors are genomics, metabolomics, biomarkers, histopathology, epidemiology studies including gender, height, pregnancy, hormonal factors, age, occupational factors, obesity, family history, race smoking and alcohol consumption, diet and other potential risk factors.

Genomics studies have demonstrated number of genes and genes polymorphisms associated to VVs development. These studies reported that any changes in these genes, polymorphisms and pathways may be a potential cause of VVs formation. Moreover, metabolomics studies have reported differences in metabolomic status between patients with VVs and healthy individuals. Other biomarkers were found to be linked with the development of VVs such as increased plasma levels and the role of haemostatic factor in patients with VVs. Histopathological studies which focus on cellular mechanisms play an important role in the pathophysiology of VVs. These mechanisms include apoptosis, hypoxia, inflammation, extracellular matrix degradation caused by changes in extracellular components.

Cross sectional studies have reported conflicting findings regarding other potential risk factors associated with the development of VVs. These risk factors include gender, height, pregnancy, other hormonal factors, age, occupational factors, obesity, family history, race, smoking and alcohol consumption, diet and other potential risk factors. Conflicting findings were reported regarding the association between VVs and gender type. Similarly, diverging findings were found between height and VVs formation. Furthermore, large number of studies have reported an association between pregnancy and VVs development with an incidence rate of 40.5% and 82% in recent meta-analysis. This was due to several hypothesis related to mechanical and hormonal factors. Mechanical factors include increased size of gravid uterus which increases pressure on iliac veins and increased blood volume levels that elevates blood pressure in lower limb vasculature. Hormonal factors include increased levels of estrogen and progesterone in pregnancy. Estrogen hormone elevates coagulation protein levels which cause thrombosis, while progesterone prevents smooth muscle

contraction which cause vasodilation. Although the majority of studies indicated pregnancy as a risk factor. However, some studies found no association between parity and VVs. Besides elevated estrogen and progesterone hormones in pregnancy, other hormonal factors including androgen and serum estradoil/free testosterone in men and relaxin hormone have been implicated in the pathogenesis of VVs.

Furthermore, the prevalence of VVs increases with age due to reduce in elasticity and changes in diameter of both superficial and deep venous system. Occupational factors such as sitting and prolong standing were also found to be connected to VVs development. These occupational factors may be subject to gender differences and duration of time that body postures are maintained. Similarly, obesity risk factor was found to be linked to VVs formation due to excessive fat which may play a role in increasing abdominal and femoral vein pressure. Family history risk factor was reported to be associated with VV formation. The incidence rate of VVs increased when the mother is affected more than the father of both parents. Race risk factor was also reported in literature to be linked to VV development with variation in incidence between studies. This was due to different study types which make it difficult to provide accurate conclusion.

Smoking and alcohol consumption, diet and other potential risk factors were also reported to be associated with VVs development. The prevalence of VVs was 33.8% among smokers compared to non-smokers and the prevalence of VVs among alcohol consumers was 25% compared to counterparts. Moreover, Low fiber diet and processed carbohydrates can play a significant role in VVs development. This is due to its role in causing constipation and straining at stool which thus cause intraabdominal pressure against iliac veins and veins enlargement. Other potential risk factors such as leg injury history, lack of physical activity, hypertension, high HDL cholesterol, diabetes in men, osteoarthritis, static foot disorder and arteriovenous anastomosis were also reported. Although these are contributory factors for the development of VVs, however, the exact mechanism remains unclear. Furthermore, since VVs are more predominant in women compared to men and in pregnant women compared to non-pregnant women. Therefore, pregnant women are at higher risk of developing VVs. The present study will investigate the contribution of both the mechanical and chemical factors in pregnant women.

3 Chapter 3 Methodology & Study Design

3.1 Introduction

This hospital-based prospective longitudinal study received ethical approval from the North of Scotland Research Ethics Committee. The primary objective was to assess the development of VVs among 140 limbs in 70 pregnant women. Recruitment occurred over a one-year period from June 2021 to December 2022, with patients consecutively enrolled at the Maternity Outpatients' Departments of West Middlesex Hospital, Queen Charlotte's and Chelsea Hospital, and St Mary's Hospital. Informed consent was obtained from each patient during their first trimester of pregnancy. They were subsequently followed up during their second and third trimesters and for six weeks post-partum, resulting in a total of four follow-up visits for each participant. The study aimed to analyse venous function during and after pregnancy and evaluate the pathophysiology of VVs when they occur during pregnancy. Patients who developed VVs during the study were compared with healthy patients who did not develop VVs to provide a comprehensive analysis. This study focused exclusively on pregnant women as both the study and control groups are justifiable based on the specific research objectives and the need to maintain the internal validity of the study by maintaining a homogenous control group. It aligns with the study's primary focus on pregnancy-related factors in VVs development among pregnant women.

3.2 Power calculation:

Sample size: we estimate the sample of 140 limbs (70 patients) is required.
Determining the appropriate size for the study was based on two statistical consultations with Joseph Eliahoo from Imperial College London and Paul Basset from (statsconsultancy.co.uk).

Rationale for the sample size calculation:

The sample size was based on detecting the difference in the occurrence of VVs between patients with different characterisitcs (e.g. older age vs. younger age). The calculations assume that the analysis is performed at the limb level (i.e. one observation in the analysis is one limb), and that patients are split into two equally sized groups.

A previous study (Boivin, et al.,2000) suggests that 64% of limbs developed VVs. An absolute difference of 25% in the occurrence of VVs between groups would be of clinical importance. This would assume an occurrence of VVs of 51.5% in one group, and 76.5% in another. With a 5% significance level and 80% power, it is calculated that 57 limbs in each group are required. This would give 114 limbs in total (57 patients).

To allow for the groups not necessarily being of equal size, and to allow for some missing data, it is proposed that the total sample size is increased to 140 limbs (70 patients).

3.3 Inclusion and exclusion criteria

3.3.1 Inclusion criteria

- Pregnant women who are aged between 18 to 47 years old.
- Patients who accepted to be followed up for 1 year and agreed to give informed consent.

3.3.2 Exclusion criteria

- Patients who failed to attend the scans after their first visit.
- Patients who were unable to provide informed consent.
- Patients who were taking part in any other research.
- Patients who did not adequately understand verbal explanations or written information given in English, or who had special communication needs.
- Any incomplete cases such as intrauterine death.
- Patients with congenital vascular abnormalities such as

- syndromes such as Klippel-Trenaunay syndrome (KTS).
- Patients with signs or symptoms of acute DVT.
- Patients with previously diagnosed VVs.
- Patients who have been diagnosed with superficial
- Superficial thrombophlebitis (STP).
- Patient who suffered from severe injury of their great-saphenous vein.

3.4 Study 1: Demographic data

In this study, clinical information was obtained from 70 pregnant women recruited from the maternity outpatient department to facilitate robust analysis. A standard demographic form was used for all patients at each follow-up to record essential information representing the target population's composition. This form recorded patients' details, including their name, day of the visit, hospital name, ID number, and date of birth, as well as potential risk factors associated with the development of VVs, such as race, number of pregnancies, family history of venous disease, BMI (which included weight and height), and occupational factors (Appendix 1).

Recruited patients were asked to complete the demographic form, which took up to 5 minutes. Following this, the BMI risk factor was calculated for each patient by measuring their weight and height. Weight was measured in kilograms to the nearest 0.1 kg, and height was measured to the nearest millimeter. To measure weight, the scale was placed flat on level ground and set to zero before the patient was asked to remove heavy clothing and empty their pockets. The patient was then asked to stand on the scale, and the weight reading was recorded once it stabilised. To measure height, the patient was asked to stand unsupported with their back to the vertical scale, ensuring that their feet were parallel and pointing forward, and their soles were flat on the floor. The horizontal measure was brought down to rest on the top of the participant's head, and the measurement on the vertical scale was recorded. Collecting patients' demographic information took a total of 10 minutes, including measuring weight and height. This information was collected during the first follow-up in the first trimester of pregnancy. In subsequent follow-ups during the second and

third trimesters of pregnancy and the postpartum period, only the BMI calculation was obtained, as the rest of the clinical information remained unchanged.

3.4.1 Statistical analysis of demographic data:

The statistical analysis was conducted using GraphPad Prism version 8.0.0 for Windows, GraphPad Software, San Diego, California USA. The aim was to investigate the association of various risk factors with the incidence of VVs among pregnant women. The age risk factor was compared between two groups (18-34 years and 35-47 years) using the Chi-square test, which examines differences between categorical variables from a random sample (Franke et al., 2011). The number of pregnancy risk factor was compared between first pregnancy and second or more pregnancies using Fisher's exact test, which determines the significance of association between two categorical variables (Kim, 2017). Family history risk factor was compared between patients with previous history of VVs and patients with negative family history of VVs using Fisher's exact test. The BMI risk factor was compared between patients who developed VVs and patients who did not using two-tailed T-tests, which identify significant differences between means of numerical data (Park, 2009). Occupational factors were compared between patients who are in jobs that require more standing and patients who are in jobs that require more sitting using Fisher's exact test. Race risk factor was compared between race groups (White, Arab, Asian, Mixed race, and Black) using the Chi-square test. Continuous data were expressed as mean ± standard deviation (SD). Statistical significance was represented by a P-value less than 0.05. These analyses provided insights into the association of various risk factors with the incidence of VVs among pregnant women, which can inform clinical practice and future research.

3.5 Study 2: Duplex ultrasound study

All 70 patients (140 limbs) underwent an ultrasound color duplex of the lower limb, which was performed by SuperSonic Imagine's Aixplorer ® ultrasound system. Both a curved array transducer (2-5 MHz) and a linear transducer probe of 5-12 MHz with coupling gel between the skin and the probe were used. The examination was performed bilaterally for each patient in the lateral decubitus position and standing

position while holding onto a frame. The standing position was chosen because veins are more dilated while standing, which makes assessment easier and more accurate (Spinedi et al., 2017). During the standing position, patients were instructed to support their weight on the contralateral leg to ensure that the leg being assessed by DUS was relaxed during the evaluation and recordings.

To assess all venous segments, the two primary techniques of DUS were employed. The first technique, B mode, was used in the transverse scan plane to assess vein patency by measuring the diameter of the vein and observing its compressibility to exclude the possibility of blood clots. The second technique, Spectral (Pulse wave) Doppler, was used in the longitudinal scan plane to evaluate venous flow characteristics, including phasicity, spontaneity, and direction of flow, by applying manual distal augmentation to assess reflux duration. The insonation angle of 60o was applied throughout the examination due to 30o beam steering (Bechsgaard *et al.*, 2018).

The deep and superficial venous system were evaluated for three important parameters: the compressibility and diameter measurements of the vein, the reflux time (RT) in seconds (RT exceeding 0.5 seconds was considered pathological), and the peak reflux velocity (PRV) parameter in centimeters per second. PRV is known as the measure of the maximum velocity of blood flow in the retrograde (backward) direction through a valve in a vein (Bechsgaard *et al.*, 2018). In healthy veins, PRV is minimal, and blood flow is unidirectional, flowing from the legs towards the heart. However, in diseased veins with damaged valves, PRV presents higher values indicating severe reflux. Therefore, PRV is used to measure the degree of venous reflux and severity of valve dysfunction (Neglén *et al.*, 2004). PRV was found to be higher in advanced stages of CVI compared to the early stages of CVI in the studies by (Yamaki *et al.*, 2002; Konoeda *et al.*, 2014). Studies have shown that RT was the least consistent parameter in determining superficial or deep venous reflux and suggested the use of only PRV to determine significant reflux (Kasidas, Samuell and Weir, 2004; A. K. Singh *et al.*, 2020). PRV is preferable as it is simpler to measure, less subject to error (a repeatable parameter in various clinical settings), and can differentiate between early and advanced superficial venous insufficiency, unlike RT.

3.5.1 Evaluating the diameters of the RT and LT common iliac veins

In all pregnant women recruited for the study, DUS was used to assess the iliac veins (Figure 3.1), due to the common occurrence of iliac vein compression resulting from the enlarged gravid uterus during pregnancy, which can lead to MTS (Soma-Pillay *et al.*, 2016). MTS is a condition where the right common iliac artery compresses the left common iliac vein against the fifth lumbar vertebra (Figure 3.2), resulting in obstruction of venous drainage from the left lower extremity and potentially leading to DVT in the left leg or lymphedema (Traisrisilp *et al.*, 2021). The risk of DVT is higher in pregnant and postpartum women, with incidence rates 4 to 5 times higher than non-pregnant women of the same age (James *et al.*, 2006). Hence, pregnancy and postpartum periods can exacerbate DVT in patients with MTS. It should be noted, however, that pregnancy may also worsen the compression of the left common iliac vein by the right common iliac artery at the MTS site, resulting in edema in the left leg without DVT. The signs of MTS are expected to resolve post-delivery (Traisrisilp *et al.*, 2021).

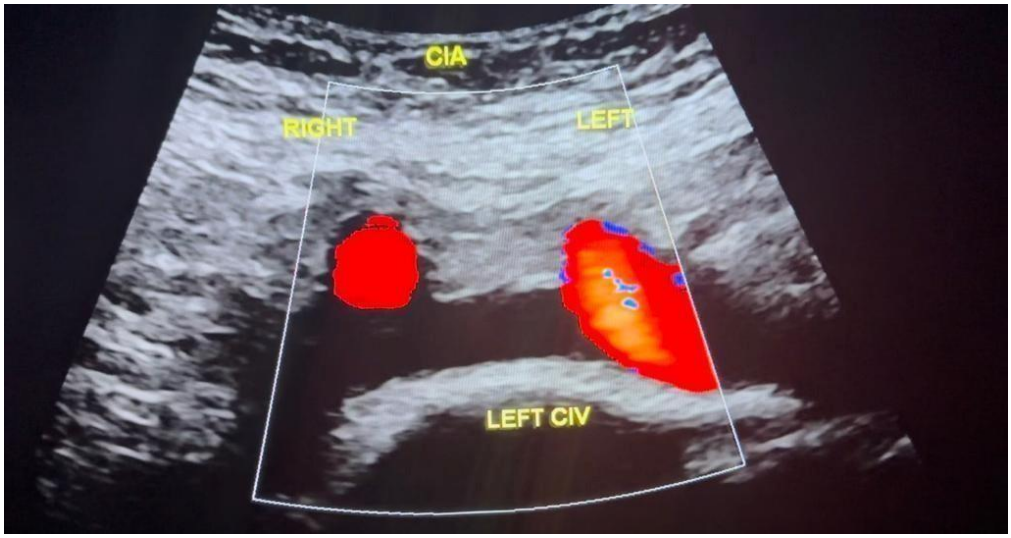


Fig 3.1: The evaluation of Common iliac veins by DUS in this study.

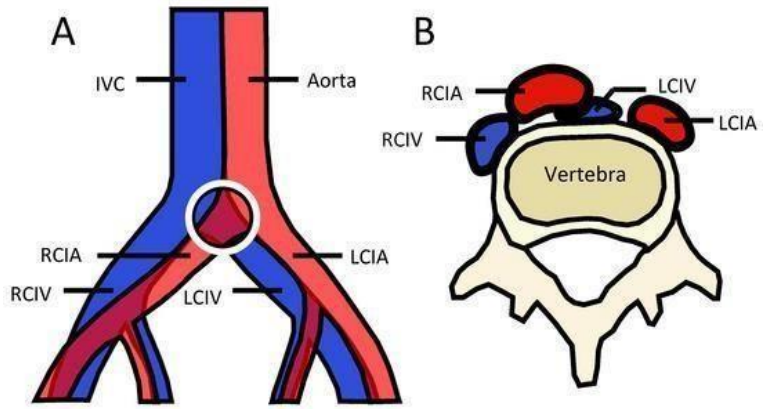


Fig 3.2: The anatomy of MTS, (A) a coronal image (B) a transverse image showing when the right common artery compresses the left common vein against the lumbar vertebra (Florio *et al.*, 2022).

In this study, the scan procedure involved placing the patient in a lateral decubitus position, which is known to improve maternal hemodynamics and gas exchange during pregnancy, and helps prevent the occurrence of supine hypotensive syndrome. A low-frequency, curved array transducer (2-5 MHz) was used, although higher frequencies may be more appropriate for patients with a low body mass index. In non-pregnant patients, the scanning process involves locating the IVC at the level of the xiphoid process, followed by scanning the length of the right and left CIVs. However, in pregnant patients, locating the IVC using DUS is unreliable, particularly in the third trimester, due to the growing size of the gravid uterus and physiological changes during pregnancy. Therefore, in this study, the scan started by placing the probe on the right and left common femoral veins, followed by moving it proximally to the corresponding common iliac veins. The diameters of the right and left common iliac veins were measured during all follow-ups. It is important to note that changes in respiratory waveform over the external iliac vein, common femoral vein, and superficial femoral vein can indicate an obstruction in the common iliac vein (DiVittorio, Bluth and Sullivan, 2002). The sonographer also ensured that the pressure applied by the DUS probe did not affect vein diameter measurements, as excessive pressure can lead to overestimation of the disease. The scan was performed in the first, second, and third trimesters of pregnancy, as well as in the postpartum period, to detect any cases of MTS and assess its impact on venous return and the development of VVs. All measurements were recorded in the study database for subsequent analysis. Finally,

our study involved a comparison of the measured diameter of the left common iliac vein in 20 pregnant women during the third trimester. The findings demonstrated that DUS is accurate, with a mean error of 4%. This study asserts that a mean error percentage of 4% is considered acceptable and does not have practical implications for patient care or decision-making.

3.5.1.1 Statistical analysis of evaluating the diameters of the right and left common iliac veins

Comparisons of numerical data of the right and left common iliac veins between patients who developed VVs and patients who did not develop VVs were made by using two-tailed T-tests, to assess the association of common iliac vein measurements with the incidence of VVs in every trimester of pregnancy and in the postpartum period. Continuous data were expressed as mean ± SD.

3.5.2 Evaluating the lower limb deep and superficial venous system

The examination began with an evaluation of the deep venous system, utilising a linear transducer probe operating at 5-12 MHz. The probe was positioned at the CFV in the groin, then moved along the superficial femoral vein (SFV), popliteal vein (POPV), and calf veins. Three parameters were assessed in these segments, including compressibility and diameter measurements using B-mode technique in the transverse scan plane, detection of any thrombus, and measurement of external vein diameter in a standing position without the Valsalva maneuver. Longitudinal scanning was then employed to perform RT measurements, in which distal manual compression was applied, followed by sudden release, to evaluate reflux (Fig 3.3). All RT recordings for healthy controls less than 0.5 sec and RT recordings for patients with VVs greater than 0.5 were recorded. Additionally, PRV was obtained by manual compression release, and measurements were repeated three times with 60-second intervals between recordings (Fig 3.4).

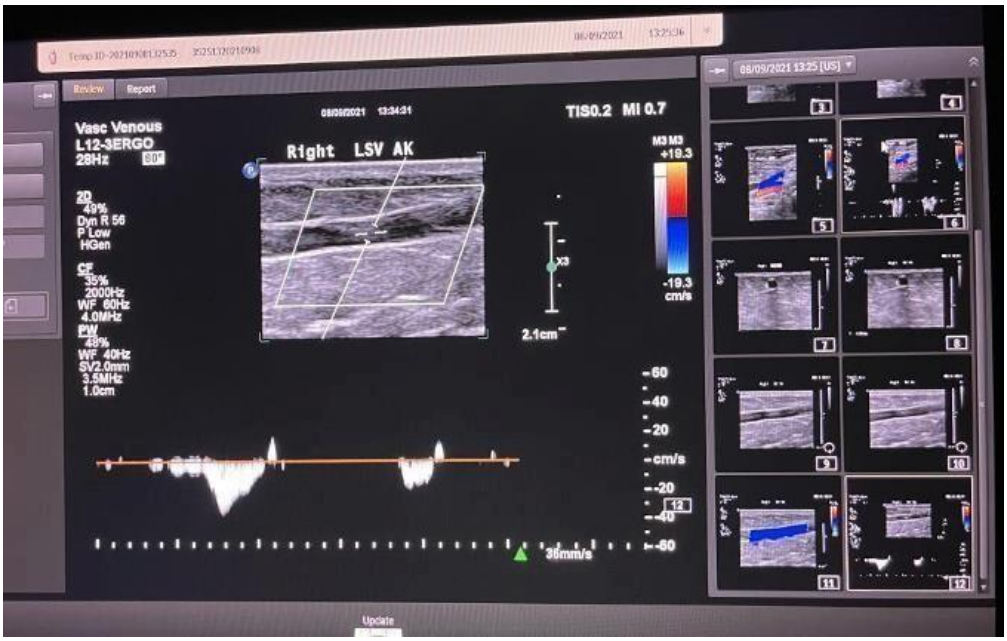


Fig 3.3: Spectral pulse wave doppler technique to evaluate the direction of flow and venous reflux using RT parameter.

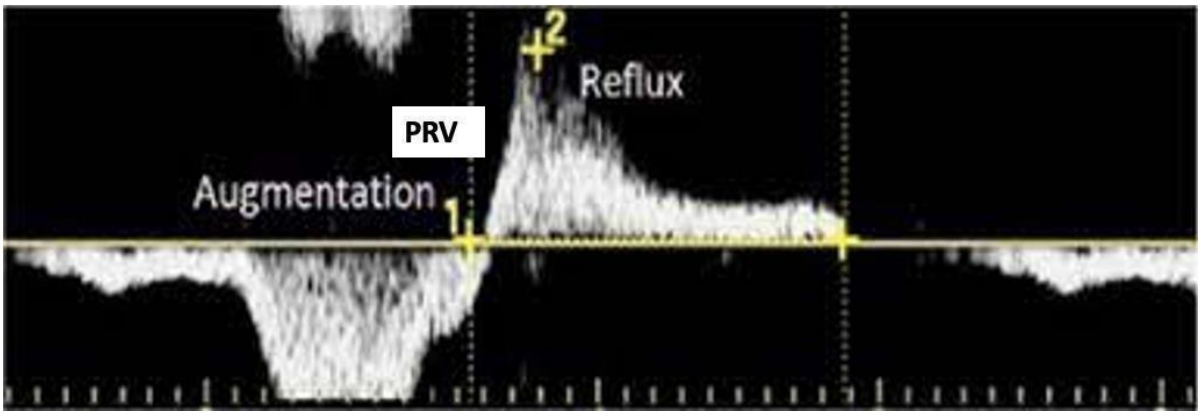


Fig 3.4: Spectral pulse wave doppler technique to evaluate the venous reflux using PRV parameter.

Following the evaluation of the deep venous system, the superficial venous system was assessed with the same transducer probe. The scan began by placing the probe at the groin, including the SFJ, and the entire length of the LSV (Fig 3.5). This was followed by an assessment of the SSV, the anterior accessory saphenous vein (AASV), all venous perforators, and branches. The three parameters of compressibility and diameter measurement, RT, and PRV were also assessed in the superficial veins.

During evaluation of the SFJ, a thigh squeeze followed by sudden release was used to evaluate reflux. An aliasing artifact was observed in the pulse doppler systems due to the Doppler shift of the moving blood scatterer being greater than half the pulse repetition frequency of the ultrasound signal emitted by the doppler transducer corresponding to the Nyquist limit (Terslev *et al.*, 2017). This resulted in the reversal of flow direction and velocity due to the wrap-around effect (Shah and Irshad, 2022). The aliasing artifact was resolved by optimizing the pulse repetition frequency (PRF) to a higher setting, lowering the baseline, and adjusting the gain to distinguish the flow curve from the background noise (Quien and Saric, 2018). All recordings were saved and entered into the research database for analysis. The examination was considered intimate, and a chaperone was offered. The scan duration lasted 30-45 minutes, and patients who felt dizzy during the examination were advised to sit and rest.



Fig 3.5: Evaluating the lower superficial venous system by DUS.

3.5.2.1 Statistical analysis of evaluating the lower limb deep and superficial venous system diameter, RT and PRV:

Initially, the study involved recording the average diameter of venous segments in both limbs at various stages of pregnancy and during the postpartum period. The Friedman

test was utilised to assess the changes in vein diameter for each limb at different stages of pregnancy and post-delivery. Additionally, the Wilcoxon signed-rank test was used to compare the diameter of the same vein segment at the same stage and different stages of pregnancy and postpartum period in each limb, as well as between the two limbs in the same subject. Furthermore, a two-tailed T-test was conducted to compare the numerical data of RT and PRV in the right and left legs of patients who developed VVs and those who did not develop VVs in each trimester of pregnancy and during the postpartum period. The continuous data were presented as mean ± SD.

3.5.3 Measuring the fetal weight in the third trimester

Subsequent to the lower limb venous scan, the fetal weight during the third trimester was estimated by two proficient sonographers. The purpose was to establish any association between increased fetal weight and potential venous pathology. The patients were requested to lie down on their left side, and the SuperSonic Imagine's Aixplorer® ultrasound system was used, along with the low-frequency, curved array transducer (2-5 MHz) abdominal transducer probe, which was the same machine used for the lower limb venous scan. The critical parameters utilised for prenatal fetal weight estimation were head circumference (HC), femur length (FL), and abdominal circumference (AC) (Lanowski *et al.*, 2017) (Fig 3.6). HC was determined by measuring the biparietal diameter and fronto-occipital diameter, whereas AC was estimated by measuring the abdominal transverse and sagittal diameters (Peixoto *et al.*, 2017). FL was calculated by measuring the outer edges of the femur bone (Yeh *et al.*, 1982). These measurements were recorded in the research database, and fetal weight was calculated using Hadlock's formula, which is the most accurate and widely utilised formula for prenatal fetal weight estimation (Esinler *et al.*, 2015). Finally, the average of fetal weight between the two sonographers was computed, recorded, and entered into the research database for subsequent analysis.

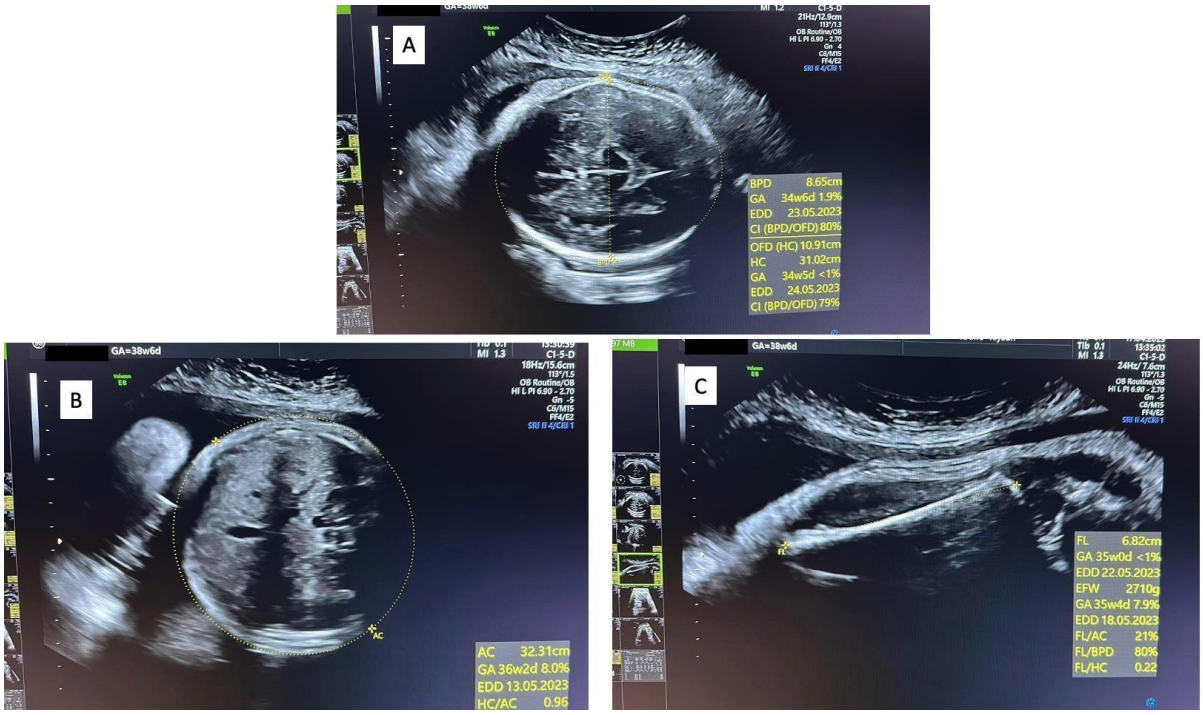


Fig 3.6: Fetal measurements in the third trimester of pregnancy. Image A showing the HC, AC in image B and FL in image C.

3.5.3.1 Statistical analysis for measuring the fetal weight:

Comparisons of numerical data of fetal weight between patients who developed VVs and patients who did not develop VVs was made by using T-test, to assess the association of increased fetal weight with the incidence of VVs. Continuous data were expressed as mean ± SD. DUS recordings of fetal weight acquired from data files were exported and analysed offline using MATLAB (MathWorks, USA) software when recruited patients completed their participation in the research project.

3.6 Study 3: The incidence of VVs in pregnancy using Vector flow imaging (VFI)

Each pregnant women underwent VFI during DUS scanning to evaluate the presence of VVs. The same SuperSonic Imagine's Aixplorer ® ultrasound system was used for both techniques, employing a 5-12 MHz linear transducer probe with the application of coupling gel between the skin and the probe. The evaluation of the deep and

superficial venous systems was conducted for PRV estimation, with the position of the probe on the skin being the same for both techniques. The only difference between the two techniques was the insolation angle, with a 90-degree angle applied in VFI and a 60-degree angle applied in DUS due to the 30-degree beam steering. The PRV measurement were recorded three times, with 60-second intervals between each recording, and the probe was lifted from the skin and the settings adjusted between measurements. This process was carried out for both normal and abnormal cases, with the readings averaged across the three recordings and performed by a single vascular scientist. The optimal machine settings were fixed for both techniques, with a mean PRF of 1.5 kHz for VFI and 1.4 kHz for DUS. The B mode frequency was 7 MHz with a gain of 40%, while the Doppler frequency was 4 MHz with a gain of 50%, and these specifications were recorded for both techniques. The VFI recordings were exported and analyzed offline using MATLAB software upon completion of the patients' participation in the research project.

3.6.1 Statistical analysis for evaluating the PRV between DUS and VFI:

Comparisons of numerical data of PRV in the right and left legs between DUS and VFI between patients who developed VVs and patients who did not develop VVs were made by using two-tailed T-tests, in every trimester of pregnancy and in the postpartum period. Continuous data were expressed as mean ± (SD).

3.7 Study 4: Inter and intra observer measurements study in DUS and VFI

Medical sciences place significant emphasis on the outcome measures of imaging techniques used for diagnoses. Outcome measures refer to tools used to evaluate a patient's status, which measure important outcomes relevant to the patient's current condition (Nelson *et al.*, 2015). Validity, repeatability, and reproducibility are essential characteristics of outcome measures in imaging techniques (Streiner et al., 2015). However, operator dependency is a significant limitation of DUS imaging modality, leading to inadequate repeatability and reproducibility (Zonnebeld *et al.*, 2017). Similarly, operator dependency in VFI imaging modality could also lead to insufficient repeatability and reproducibility. The quality of data and results obtained from

ultrasound imaging modality is highly dependent on the skill and experience of the operator conducting the procedure (Shen *et al.*, 1999).

The concept of reproducibility comprises agreement and reliability. These two concepts determine whether the measurement result is reproducible or not (Gundersen, 2021). The agreement parameter demonstrates the closeness of repeated measurements by calculating the measurement error. It is considered essential in evaluating the usefulness of a measure to monitor health changes over time using repeated measurements (de Vet *et al.*, 2006). On the other hand, reliability determines whether a test can effectively distinguish between study objects, regardless of measurement error. The measurement error is associated with the variability between patients. High reliability is critical in diagnostic practice, where differentiating between healthy and unhealthy people at a single time-point is the primary objective (de Vet *et al.*, 2006). Therefore, agreement depends on the measurement error and is considered a direct feature of the measurement instrument, while reliability relies on the heterogeneity of the study sample. The reliability parameter, such as intraclass correlation coefficients (ICC), measures the variation in study subjects divided by the total variation, which includes the variation in the study subjects and measurement error. The reliability parameter is demonstrated as a ratio between 0 and 1 (Terwee *et al.*, 2007).

Assessing the intra- and inter-observer agreements is crucial for evaluating the reliability and validity of new diagnostic technologies (Buckens *et al.*, 2013). Intra-observer variability refers to the variation in repeated measurements by the same observer, whereas inter-observer variability refers to the variation in measurements between different observers (Meesters *et al.*, 2020). The present study aimed to evaluate the intra- and inter-observer agreements of DUS and VFI measurements in 20 pregnant women during their first trimester. The measurements were selected from routine DUS and VFI protocols, and representative PRV measurements in the left LSV were chosen. The study was conducted post-planned examinations to reduce patient burden, and additional scanning time was approximately 30 minutes. Two repeated measurements were obtained for both intra- and inter-observer agreements, resulting in a total of 40 measurements for each agreement type (20 measurements for 20 patients, in duplicate, by 2 observers for intra-observer agreement, and 20

measurements for 20 patients, in duplicate, by 2 sonographers for inter-observer agreement).

3.7.1 Correlations and DUS Intra and inter observer agreement

Intra and inter observer agreement for DUS measurement was achieved in this study. First, intra-observer agreement assessment of DUS measurements was performed by one trained and experienced sonographer and optimal settings were set for DUS technique. Measurements were repeated two times with 60 s intervals between recordings as suggested by others (Eberhardt and Raffetto, 2014).The probe was released from the skin between each measurement. At the end, reported PRV for each patient was an average estimate over the two replicated recordings. Intraclass correlation coefficients was calculated for intra observer agreement. On the other hand, inter-observer agreement of DUS measurements were performed by two trained and experienced sonographers, blinded to measurement readings. Interclass correlation coefficients was calculated for inter-observer agreement. Finally, linear regression was used to show the correlations and Bland-Altman plots was used to graphically display mean measurement differences and limits of agreement.

3.7.2 Correlations and VFI Intra and inter observer agreements

Intra and inter observer agreement for VFI measurement was performed in this study. Intra-observer agreement of VFI measurements was performed by one trained and experienced sonographer and optimal settings were set for VFI technique. Measurements were repeated two times with 60 s intervals between recordings as suggested by others (Eberhardt and Raffetto, 2014).The probe was released from the skin between each measurement. At the end, reported PRV for each patient was an average estimate over the two replicated recordings. On the other hand, inter-observer agreement of VFI measurements were performed by two trained and experienced vascular technicians, blinded to measurement readings. ICC was calculated for inter-observer agreement. Finally, linear regression was used to show the correlations and Bland-Altman plots used to graphically display mean measurement differences and limits of agreement.

3.8 Study 5: Venous outflow function using (Photoplethysmography) PPG technique

The current study employed photoplethysmography (PPG) to determine venous outflow function, in all enrolled patients. Following DUS and VFI procedures, patients were seated with both legs dangling on the floor, and a PPG sensor was attached to the skin in the medial supra-malleolar area of the leg (Fig 3.7). The resting baseline was set at zero, and patients were instructed to flex and extend one foot five times while the other foot was placed on the floor. Venous emptying was produced through repeated dorsiflexion and plantar flexion of the foot, and a traced graph appeared on the machine's screen (Fig 3.8). The VRT was recorded as normal (>25 s), mild reflux (20-24 s), moderate reflux (10-19 s), or severe reflux (<10 s) based on previously established criteria. Participants with VRT <25 s were classified as patients who developed VVs, whereas participants with VRT >25 s were considered patients who did not develop VVs. The test was repeated to evaluate the VRT of the second leg. Findings were compared with DUS results, and the test was not utilised to determine the anatomic location of the disease. All measurements were recorded promptly in the study database.

It's important to acknowledge that skin tone can potentially influence PPG measurements (Bent et al., 2020). The extent to which skin tone affects PPG readings can vary and is contingent on several factors (Kraft et al., 2023). One critical factor is the sensitivity of the PPG device being used. Different devices may exhibit varying levels of sensitivity to skin tone disparities. While some devices are engineered to function effectively across a spectrum of skin tones, others may necessitate calibration or adjustments to account for these variations. Additionally, the impact of skin tone on PPG measurements may differ among distinct demographic groups (Nowara et al., 2023). The selection of wavelengths of light employed in PPG devices constitutes another factor contributing to variability in the influence of skin tone (Dasari et al., 2021). These devices leverage different light wavelengths to assess blood flow, and the choice of wavelengths can determine the extent to which skin tone affects the measurements. Certain wavelengths may exhibit reduced sensitivity to variations in skin color. Moreover, the anatomical location on the body where PPG measurements are taken can also influence the degree of skin tone's impact (Pedrana et al., 2020).

For instance, measurements obtained from the fingertip may be less susceptible to skin tone-related variations than those taken from the leg.

Despite these considerations, concerted efforts are made in both research and medical device design to mitigate the potential influence of skin tone on PPG measurements. These efforts encompass appropriate device design and, when necessary, calibration processes involving adjustments to settings, light wavelengths, or algorithms. Furthermore, this study had adopted a standardised measurement protocols to ensure consistency during data collection. This entails precise positioning of sensors, consistent body locations for measurements, and uniform measurement durations to minimize variability. In addition, this study has applied a rigorous data analysis techniques to guarantee the accuracy of measurements across diverse populations. Nevertheless, it is crucial to acknowledge that despite these proactive measures, some degree of variability in the effect of skin tone on PPG measurements may persist.



Fig 3.7: PPG sensor attahced to the patient's medial supra-malleolar area of the leg.

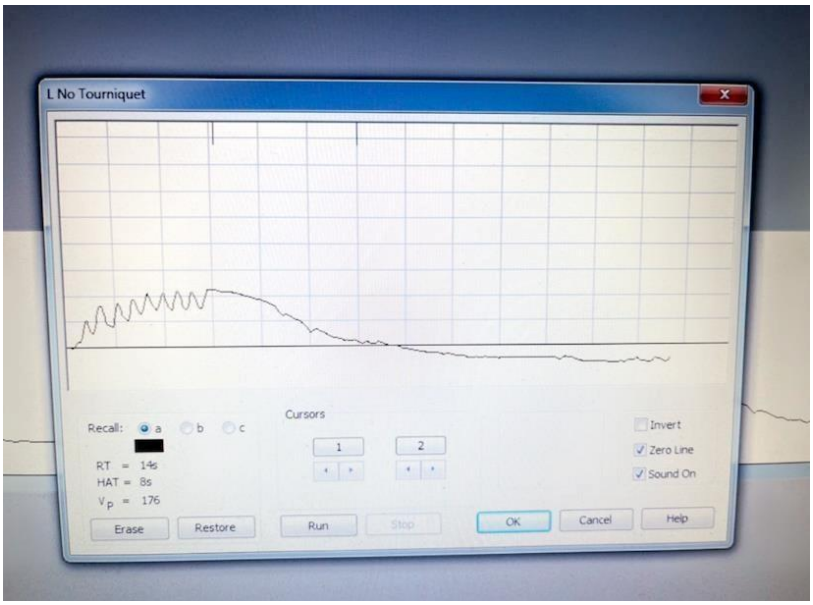


Fig 3.8: PPG showed a VRT < 25 sec indicating a reflux in the left leg.

3.8.1 Statistical analysis for evaluating the venous function using PPG technique:

Comparisons of numerical data of the VRT in the right and left legs between patients who developed VVs and patients who did not develop VVs were made by using two-tailed T-tests, in every trimester of pregnancy and in the postpartum period. Continuous data were expressed as mean ± SD.

3.9 Study 6: Limb volume assessment

Lower leg edema during pregnancy is a common and normal physiological phenomenon, which occurs due to the biochemical changes in blood and the pressure exerted by the gravid uterus on the common iliac veins and vena cava (Reynolds, 2003). Physiological edema, in the absence of serious conditions, is usually observed during the third trimester, resulting in swollen limbs, pain, stiff ankles, and difficulty in walking (Hartmann and Huch, 2005). Accurate diagnostic methods and measurements are necessary to differentiate physiological edema from edema caused by serious conditions such as VVs, DVT, and cellulitis. In this study, limb volume was evaluated during pregnancy to investigate its correlation with potential VVs incidence and its association with CIVs measurements. Several methods,

including water displacement volumetric measurements and girth measurements, have been used to assess limb volume (Tierney *et al.*, 1996). The water displacement method is considered the gold standard as it has high reliability and sensitivity with an error rate of less than 1% (Waylett-Rendall and Seibly, 1991). However, this method is time-consuming and requires special equipment for patients with large limbs, limited mobility, or open wounds (Mayrovitz *et al.*, 2005).

On the contrary, various studies have indicated that girth measurement methods, such as the cylinder and truncated cone methods, have been found to be favorable (Kaulesar Sukul *et al.*, 1993; Casley-Smith, 1994; Sander *et al.*, 2002). These techniques are easy to perform, require simple equipment such as a tape measure, and are clinically useful in determining changes in limb size. Unlike water displacement methods, which are complex and difficult to identify changes in limb size, girth measurement methods divide the limb into segments, each with a cylinder or cone shape, and the volume of the limb is calculated by adding the volumes of these segments (Karges *et al.*, 2003). The truncated cone method, also known as the frustum method, has been found to be more accurate than the cylinder formula as the limb is more conical in shape. Additionally, advanced imaging techniques, such as DUS, MRI, and CT scans, have been used to evaluate limb volume measurements. Some attempts have been made during pregnancy to assess skin edema using portable ultrasound methods to measure skin thickness (Yanagisawa *et al.*, 2019a; Banba *et al.*, 2022). MRI or CT scans have been used to create a three-dimensional model of the limb and calculate its volume (Angelhed *et al.*, 2008; Sommer *et al.*, 2012; Yang *et al.*, 2014). Although these advanced techniques have high accuracy, CT scans were not utilised in this study due to potential harm from radiation exposure on pregnant women, while MRI is costly and time-consuming.

In this study, the lower limb volume of all enrolled pregnant women was evaluated using the Truncated cone method to provide accurate and efficient measurements for clinical evaluation. This method entails measuring the highest circumference (lower part of the knee patella) and lowest circumferences (above the ankle joint) of leg segments, along with the distance between them, which is then calculated using a mathematical formula (Perrin and Guex, 2000). This method has the advantage of measuring bilateral limb volume edema, where no comparator limb is available

(Smoot, Wong and Dodd, 2011). Furthermore, the Truncated cone method formula's sum of volumes provides a highly accurate estimate compared to water displacement (correlation coefficient = 0.98) (Stranden, 1981). All the pregnant women were seated with their knees flexed at a 90-degree angle, and limb volume was measured using manual perimetry with a tape measure to determine limb circumference (Fig 3.9). However, tape measures' tension may be inconsistent, leading to changes in limb circumference measurement of up to 3% (Cheah *et al.*, 1989). Therefore, this study employed a spring-loaded controlled tension device to ensure consistent tension to the skin without compressing the underlying tissue to provide accurate and repeatable measurements, as previously (Mayrovitz *et al.*, 2005). Our study entailed a comparison of the measured circumference of the lower part of the knee patella (highest circumference) in 20 pregnant women during the first trimester. The results demonstrated that tape measure tension is accurate, with a mean error of 3%. This study asserts that a mean error percentage of 3% is deemed acceptable and does not have practical implications for patient care or decision-making. Limb circumferences were measured every 10 cm from the sole up to the seventh measurement, as reported by (Karges *et al.*, 2003). From those measurements, the data were entered into a computer, and the volume was calculated based on the truncated-cone formula (Campanholi, Duprat Neto and Fregnani, 2011):

$V= h (C1´C2 +C1^{2}+C2^{2)}/ 12 \pi$

V: Volume of each segment (final segment of the limb).

C1 and C2: Circumference measured between the points (at either end of each segment length)

H: Distance between the circles (C1 and C2 in each segment), measured in centimeters.

Total limb volume is determined by the sum of the segment volumes.

π : 3.14159



Fig 3.9: Measuring the highest point of the leg circumference using a tape measure for the Truncated cone method.

3.9.1 Statistical analysis for limb volume assessment:

Three analyses were performed for the limb volume assessment: First, a comparison of numerical data of limb volume in the right and left legs. Second, a comparison of limb volume between patients who developed VVs and patients who did not develop VVs groups. These two analyses were achieved by using two-tailed T-tests, in every trimester of pregnancy and in the postpartum period. Continuous data were expressed as mean ± (SD). Thirdly, a comparison between the right and left common iliac veins and limb volume in the two groups was also expressed as mean ± (SD).

3.10 Study 7: Hormonal blood test assessment

The present study aimed to investigate the potential association between the levels of serum progesterone and estrogen hormones and the development of VVs in pregnant women. Exclusively, Estriol (E3) and progesterone were the focus of assessment within the scope of this study. This selective focus was guided by the well-established

understanding that increased levels of estrogen and progesterone induce relaxation in smooth muscle and collagen fibers within the veins, resulting in vasodilation. This physiological response potentially predisposes individuals to the development of valve reflux and VVs, as substantiated in earlier research studies (Mashiah, 1999; Raffetto and Khalil, 2021). Estriol (E3) and progesterone serum blood tests were conducted during each follow-up visit, which included the first, second, and third trimesters, as well as the three months following delivery, to assess hormonal changes and their possible correlation with venous pathology incidence. Blood samples were drawn via venepuncture in the median cubital vein of each participant, and venous blood samples of a maximum of 5 ml were collected in test tubes, centrifuged, and aliquoted, with the serum stored at -70°C until analysis was carried out. Upon completion of the study, the collected samples were transferred to a research tissue bank for storage and future research purposes.

3.10.1 Statistical analysis for hormonal blood test assessment:

The values of progesterone and E3 hormones were expressed as mean values and standard deviation for normally distributed data. T-tests were used to assess the association of increased levels of progesterone and E3 in pregnancy with the incidence of VVs by comparing the levels of these hormones in patients who developed VVs and patients who did not develop VVs in every trimester of pregnancy and in the postpartum period.

3.11 Study 8: Quality of life assessment in pregnancy and limb symptoms

In this study, the Chronic Venous Disease Quality of Life Questionnaire (CIVIQ-20) was utilized due to the specific features of CVD. The CIVIQ-20 is a valid, reliable, and internationally accepted questionnaire that was originated and authorized in France in 1996. It has been used in many countries and languages and has been shown to be sensitive and stable in studies of different populations. Furthermore, it covers the main areas of QoL in CVD patients and includes 20 questions covering four QoL dimensions: pain, physical, psychological, and social. The questionnaire is scored

from 0 to 100, with a higher score indicating a lower QoL and vice versa (*CIVIQ users' guide*, 2015). This study aimed to assess the perceptions of pregnant women with VVs regarding their QoL, specifically to determine if lower limb pain during pregnancy is related to changes in the lower limb venous system.

In this study, all 70 pregnant women in their first trimester who presented in maternal outpatient clinics and provided informed consent, were asked to complete the CIVIQ-20 questionnaire to assess their QoL. The questionnaire was administered at baseline in the first trimester of pregnancy, followed by the second and third trimesters of pregnancy and in the postpartum period. Participants were given the questionnaire to complete by themselves in the waiting room, and no assistance was provided to avoid any interference from an investigator. The CIVIQ-20 questionnaire comprises 20 questions designed to evaluate four quality of life dimensions: pain (four items), physical (four items), psychological (nine items), and social (three items). Each question corresponds to a possible answer ranging from 1 to 5. The overall scores of the CIVIQ-20 range from 20-100, with lower scores indicating greater patient comfort.

3.11.1 Statistical analysis for QoL assessment:

The scores of the CIVIQ-20 QoL assessment questionnaire was expressed as mean values and standard deviation for normally distributed data. T-tests were used to assess the association between QoL questionnaire scores and the incidence of VVs by comparing these scores between patients who developed VVs and patients who did not develop VVs in every trimester of pregnancy and in the postpartum period.

4 Chapter 4 Results:

4.1 Overview of the results

This study presents findings on various factors that are associated with the incidence of VVs during pregnancy, as well as the discrepancies between diagnoses made by DUS and VFI. The results showed that older age, multiparity, positive family history of VVs, higher BMI, prolonged standing, white race, and increased fetal weight in the third trimester were all significantly associated with the incidence of VVs. The study also found that there was a significant discrepancy between DUS and VFI in diagnosing VVS in pregnant women. While DUS detected VVs in 64% of patients in the third trimester, VFI detected VVs in 70% of patients which showed that VFI is more sensitive in detecting venous reflux. The results of the study further indicated that patients with VVs had smaller common iliac vein diameters, and that the difference in limb volume measurements between patients with VVs and those without VVs was statistically significant. Moreover, the study revealed that patients who developed VVs had higher hormonal levels and lower QoL assessment scores compared to those who did not develop VVs. Additionally, the study observed that 8 out of the 28 patients who developed VVs in the third trimester had MTS, which continued into the postpartum period. Overall, the findings of this study could advance our comprehension of the risk factors linked to VVs during pregnancy. These results have significant implications for clinical practice and may facilitate the enhancement of care for pregnant women who experience VVs.

4.2 Results of study 1: Demographic data

At the baseline, the participants in this study were from diverse racial backgrounds, with 46% identifying as white, 20% as Asian, 11% as Arabs, 10% as black, and 13% as mixed race. Age was stratified into two categories: a younger group (18-34) and an older group (35-47), with mean ages (standard deviation) of 28.7 ± 2.3 and 37.6 ± 1.9, respectively. Parity was also categorized as first pregnancy or second or more

pregnancies, with 44% of participants in their first pregnancy and 56% in their second or more pregnancies. Family history of VVs was categorized into positive or negative, with 57% of participants having a positive history of VVs and 43% having a negative history. Occupational factors were also stratified into two groups: prolonged standing or sitting, with 57% of participants in jobs requiring prolonged standing and 43% in jobs requiring more sitting. Mean (± standard deviation) BMI values for patients during pregnancy were 23 ± 1.7 in the first trimester, 25.1 ± 1.8 in the second trimester, 27.2 ± 2 in the third trimester, and 24.1 ± 1.72 in the postpartum period. The variance in BMI values between the first trimester of pregnancy, the second trimester, the third trimester, and the postpartum period exhibited pronounced statistical significance, as evidenced by a remarkably low p-value of <0.0001 for each comparison. A detailed summary of patients' characteristics is presented in (Table 4.1).

Item	Charactristics
Total number	70 (140 limbs)
Age Younger age group (18-34) Older age group (35-47)	 27 (39 %) 43 (61 %)
Family history of VVs Positive Negative	 40 (57%) 30 (43%)
Number of pregnancies Second or more pregnancy First pregnancy	 39 (56%) 31 (44%)
Occupational factors Standing Setting	 40 (57%) 30 (43%)
Race White Arab Asian Mixed race Black	 32 (46%) 8 (11%) 14 (20%) 9 (13%) 7 (10%)
BMI, kg/m2, mean ± SD First trimester Second trimester Third trimester Post-partum	 23 ± 1.7 25.1 ± 1.8 27.2 ± 2 24.1 ± 1.72

Table 4.1: Patients characteristics at baseline.

4.2.1 The association between age risk factor and the incidence of VVs

The current study demonstrated a positive correlation between older age and the incidence of VVs during pregnancy. Specifically, in the younger age group (18-34 years), only 15% of patients developed VVs while 85% did not. Conversely, in the older age group (35-47 years), 95% of patients developed VVs while only 5% remained free from VVs. These results were statistically significant (P value <0.0001), indicating that the incidence of VVs was significantly higher in older pregnant women in comparison to younger pregnant women (Fig 4.1). This study has identified that the observed outcomes were subject to influence by additional risk factors, namely, a positive family history of VVs and occupational factors. Within the older age group, specifically, it was ascertained that a substantial proportion of patients, accounting for 56%, possessed a positive family history of VVs. Furthermore, within the same age group, 61% of patients were engaged in occupations that necessitated prolonged periods of standing. Consequently, these findings underscore the conclusion that age, when considered as a risk factor, cannot be regarded in isolation but rather as a multifactorial risk element, with its influence contingent upon the interplay with other variables, such as familial predisposition and occupational demands.

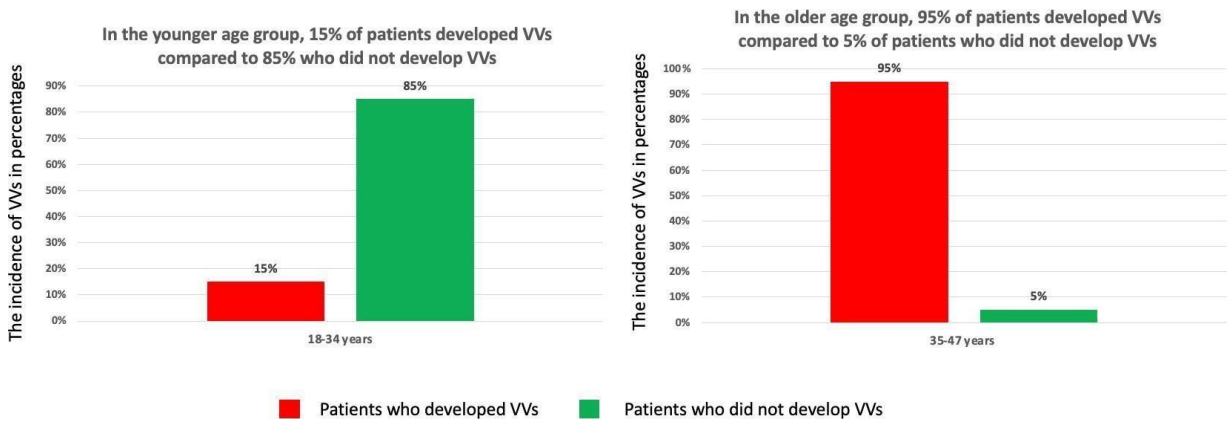


Fig 4.1: The incidence of VVs was higher in patients from the higher age group compared to younger age group patients (*p*-value<0.0001).

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4.2.2 The association between the multiparity risk factor and the incidence of VVs.

The findings of this study demonstrate a positive correlation between the incidence of VVs and parity. Specifically, the results indicated that among participants experiencing their first pregnancy, 39% developed VVs while 61% did not. In contrast, among participants experiencing their second or subsequent pregnancies, 85% developed VVs while only 15% did not. These differences were determined to be statistically significant (p-value<0.0001). Our finding is consistent with the results of a prior study conducted by (Ismail *et al.*, 2016). In their meta-analysis, they demonstrated a significant 82% increase in the likelihood of VVs development among women with a history of pregnancy. Thus, this study provides evidence that a greater number of pregnancies is associated with an increased likelihood of developing VVs (Fig 4.2).

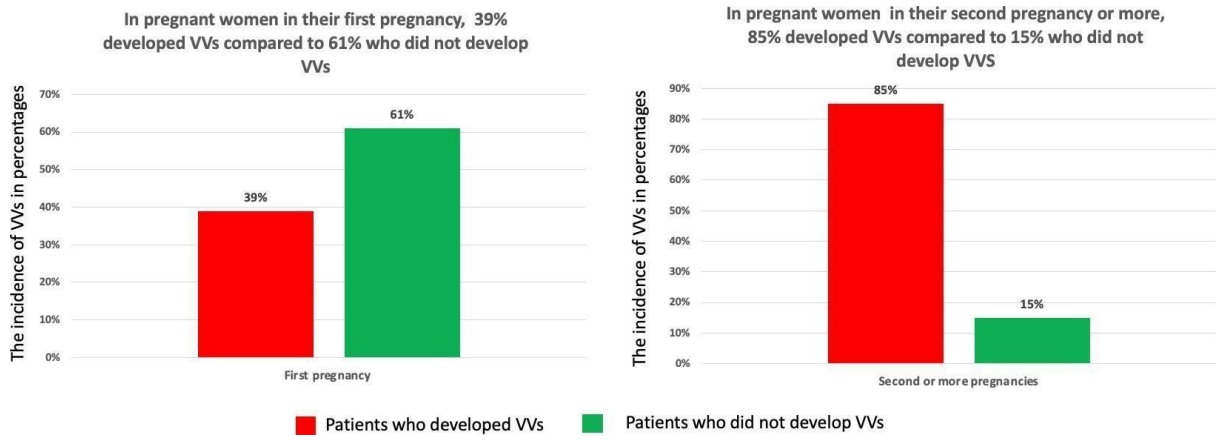


Fig 4.2: The incidence of VVs was higher in patients with second or more pregnancies compared to patients in their first pregnancy (*p*-value<0.0001).

4.2.3 The association between family history of VVs and the incidence of VVs.

This study indicates that pregnant women with a positive family history of VVs are at a higher risk of developing VVs during pregnancy. The results revealed that among patients with a positive family history of VVs, 92% developed VVs, while only 8% did

not. In contrast, among patients with negative family history of VVs, 27% developed VVs, while 73% did not. These differences were statistically significant (p-value < 0.0001). Thus, the findings suggest that having a positive family history of VVs is associated with an increased incidence of VVs during pregnancy (Fig 4.3).

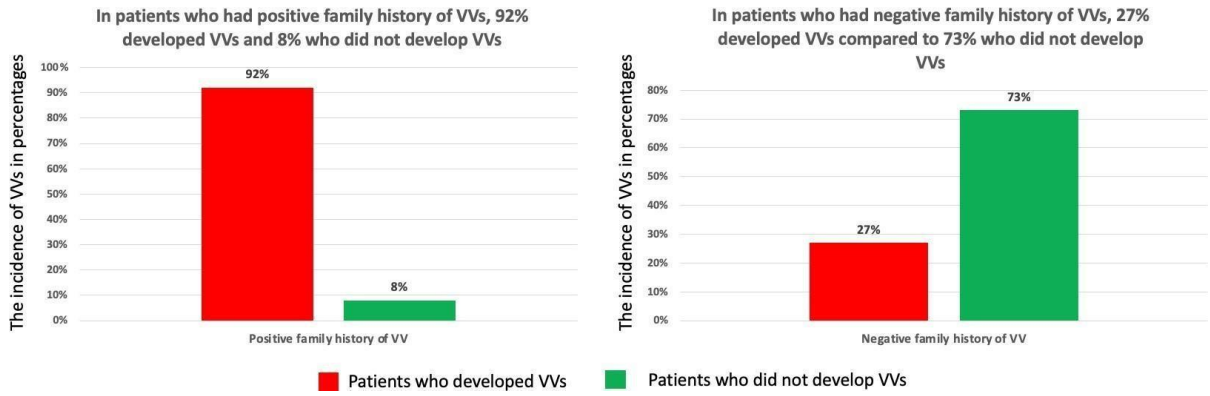


Fig 4.3: The incidence of VVs was higher in patients who had a positive family history of VVs compared to patients with a negative family history of VVs (*p*-value <0.0001).

4.2.4 The association between higher BMI and the incidence of VVs.

This study investigated the changes in BMI throughout all trimesters of pregnancy and the postpartum period, and their association with the development of VVs. The results indicated that BMI increased during pregnancy, with the highest measurements observed in the third trimester, followed by a return to normal in the postpartum period. Moreover, patients who developed VVs had a significantly higher BMI compared to those who did not develop VVs, as evidenced by statistical significance (P<0001) in the first, second, third trimesters, and postpartum periods (Fig 4.4).

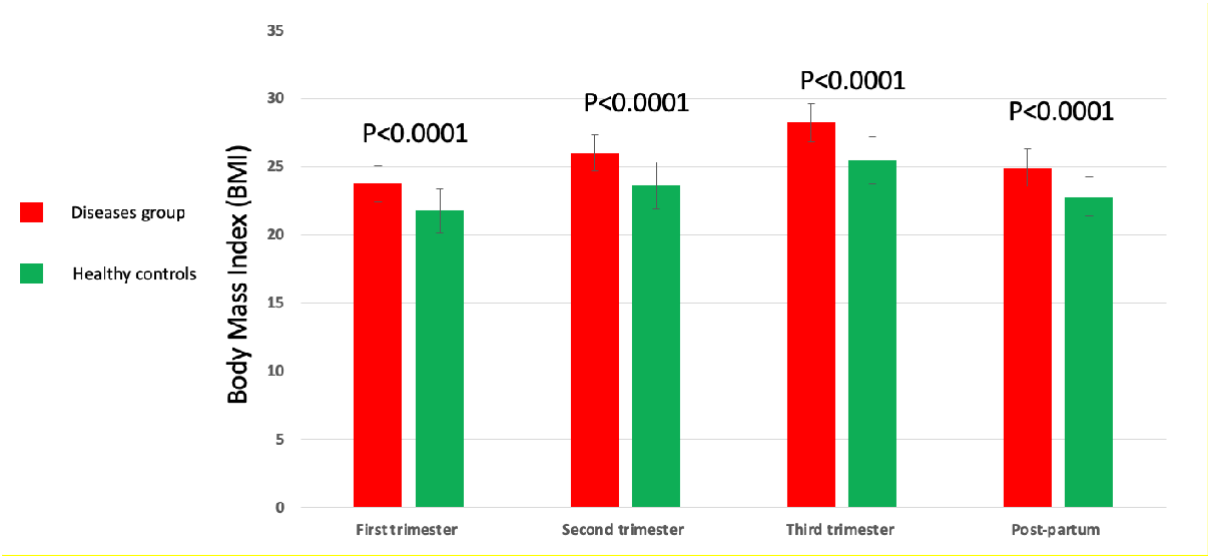


Fig 4.4: The incidence of VVs increased in patients with higher BMI compared to healthy controls who had lower BMI.

4.2.5 The association between occupational risk factors and the incidence of VVs

The present study revealed a positive correlation between VVs incidence and prolonged standing risk factor. Specifically, the findings demonstrated that participants in occupations that required prolonged standing (more than 4 hours) had a significantly higher risk of developing VVs during pregnancy, with 85% of these patients developing VVs compared to 15% who did not develop VVs. On the other hand, patients in jobs that involved prolonged sitting had a lower risk of developing VVs, with 37% of these patients developing VVs compared to 63% who did not develop VVs. These results were statistically significant (p-value < 0.0001) (Fig 4.5).

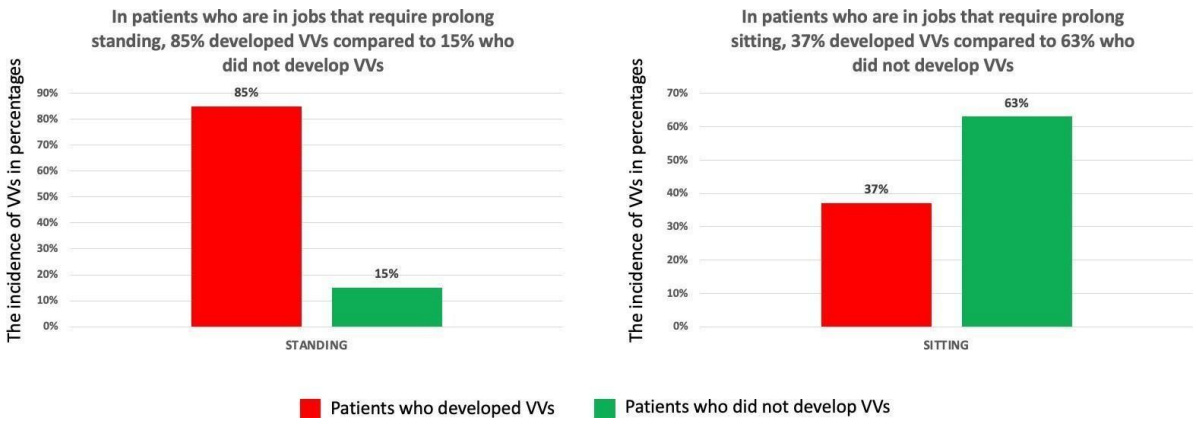


Fig 4.5: The incidence of VVs was higher in patients who were in jobs that require more standing compared to patients who were in jobs that require more setting (*p*-value <0.0001).

4.2.6 The association between race risk factor and the incidence of VVs

This study investigated the association between race, lifestyle factors, and the development of VVs. The study categorised participants into five different races, including White, Arab, Asian, Mixed race, and Black. The results indicated that the incidence of VVs was significantly higher among White race patients (40%, n=28) compared to other races (Arab 6%, n= 4), (Asian 8%, n=6), (Mixed race 6%, n=4), and (Black race 4%,n=3), with a p-value of <0.007 (Fig 4.6). This discrepancy may be attributed to a combination of environmental and genetic factors. Among White race patients with VVs, 75% (n=21) had a positive family history of VVs, and 64% (n=18) had jobs that required prolonged standing. Additionally, 89% (n=25) of White race patients with VVs had a low-fiber diet, which may contribute to constipation and increased intra-abdominal pressure. This pressure, in turn, may enlarge the iliac veins and lead to VVs, as suggested by previous studies (Lawson *et al.*, 1972; Coughlin *et al.*, 2001). Straining during bowel movements has also been identified as a risk factor for VVs (Fowkes *et al.*, 2001; Lee *et al.*, 2001). The study findings underscore the need for further research on the role of lifestyle factors and genetic predisposition in the development of VVs, particularly among White race patients.

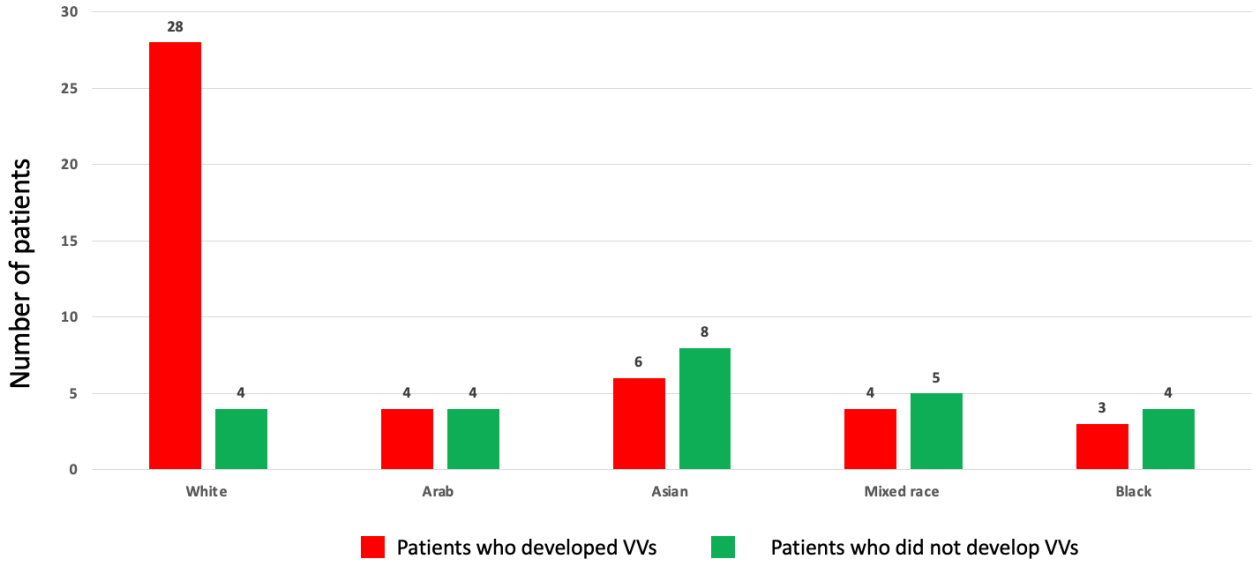


Fig 4.6: The incidence of VVs was higher in patients from the white race compared to other races (*p*-value=0.007).

4.3 Results of study 2: Duplex ultrasound (DUS) study

4.3.1 Results of evaluating the diameters of the RT and LT common iliac veins

The present study investigated the effect of pregnancy on the diameter of the right and left common iliac veins. Results revealed a significant decrease in diameter during pregnancy, particularly in the third trimester when the gravid uterus reaches its maximum size. However, the diameter returned to normal in the post-partum period among patients who did not develop VVs. Mean diameters and standard deviations at the origin of the left and right common iliac veins were compared between patients who developed VVs and those who did not, and the results are presented in (Table 4.2). The decrease in diameter of the right common iliac vein between the two groups was found to be statistically significant during the third trimester and post-partum periods (P=0.0132 and P=0.0379, respectively), while the mean percent stenosis of this right vein due to compression by the artery was 5.9%. Similarly, the decrease in diameter of the left common iliac vein was found to be statistically significant during the second and third trimesters as well as the post-partum period (P=0.0055, P<0.0001, and P<0.0001, respectively), with a mean percent stenosis of the left vein

due to compression by the artery of 20.4%. Stenosis percentage was calculated by using the Percentage decrease formula $= \frac{\text{Starting value} - \text{Final value}}{\text{staring value}} \times 100$.

	Patients group	Vein diameter in first trimester	Vein diameter in second trimester	Vein diamater in third trimester	Vein diamater in post-partum
LT common iliac vein	Patients who developed VVs	8.35 ± 0.911	7.77 ± 1.14	5.94 ± 1.47	6.6 ± 1.4
	Patients who did not develop VVs	8.47 ± 0.83	8.46 ± 0.86	8.26 ± 0.835	8.47 ± 0.83
RT Common iliac vein	Patients who developed VVs	8.42 ± 1	8.2±1	7.62±1	7.93±1
	Patients who did not develop VVs	8.48 ± 0.88	8.46 ± 0.9	8.27 ± 0.88	8.48 ± 0.88

Table 4.2: Measurements of RT and LT common iliac vein diameters (mm) during pregnancy and postpartum presented by the (mean and ± standard deviation).

According to the results of this study, 29% (8 out of 28) of patients who developed VVs in their left leg during the third trimester of pregnancy also developed (MTS), as shown in Table 4.3. The incidence of MTS persisted in these cases into the post-partum period. The study also found that the left common iliac vein measurements in patients with MTS had a mean and standard deviation of 8.1mm±1 in the first trimester, 7.2mm±1.1 in the second trimester, 4.2mm±0.63 in the third trimester, and 4.9mm±0.64 in the post-partum period. The mean percent stenosis of the left common iliac vein due to compression by the artery was 39.5%.

	First trimester	Second trimester	Third trimester	Post-partum
Patient 1	7.4 mm	5.2 mm	2.8 mm	4.3 mm
Patient 2	9.7 mm	7.3 mm	4.8 mm	6.2 mm
Patient 3	7.2 mm	6.9 mm	4.5 mm	5.2 mm
Patient 4	8.4 mm	8 mm	4.6 mm	5.1 mm
Patient 5	9.8 mm	9.3 mm	4.4 mm	5.2 mm
Patient 6	7.9 mm	7.1 mm	4.5 mm	4.7 mm
Patient 7	7.9 mm	7.3 mm	3.9 mm	4.2 mm
Patient 8	7.2 mm	6.8 mm	4.1 mm	4.5 mm

Table 4.3: Measurements of the left common iliac vein diameters (mm) in patients with MTS during pregnancy and postpartum.

4.3.2 Evaluating the superficial and deep veins venous system results

All venous segments in the superficial and deep system diagnosed by DUS were patent in all trimesters of pregnancy and in the postpartum period.

4.3.2.1 Superficial and deep venous diameter measurements results

According to the results obtained from DUS, there was a progressive rise in the diameters of all segments of the superficial and deep venous systems during the second and third trimesters of pregnancy (as indicated in Table 4.4). However, these changes tended to normalise in the postpartum period. The observed alterations in both deep and superficial venous diameter measurements were found to be statistically significant ($P<0.0001$) across different stages of pregnancy and after delivery, based on the Friedman test. Moreover, there was no statistically significant difference observed between the legs of the same patient ($P > .05$), as determined by the Wilcoxon signed-rank test.

	Vein diameter (mm)	First trimester	Second trimester	Third trimester	Post-partum
Superfecial veins	LSV (RT leg)	2.42 ± 0.4	2.71 ± 0.44	3.04 ± 0.49	2.52 ± 0.44
	LSV (LT leg)	2.46 ± 0.41	2.77 ± 0.46	3.1 ± 0.48	2.5 ± 0.45
	SSV (RT leg)	2.33 ± 0.3	2.59 ± 0.29	2.88 ± 0.27	2.38 ± 0.31
	SSV (LT leg)	2.28 ± 0.25	2.56 ± 0.24	2.94 ± 0.23	2.34 ± 0.27
	AASV (RT leg)	3.64 ± 0.37	3.87 ± 0.41	4.13 ± 0.44	3.68 ± 0.41
	AASV (LT leg)	3.72 ± 0.41	3.88 ± 0.41	4.14 ± 0.47	3.74 ± 0.42
Deep veins	CFV (RT leg)	12.4 ± 0.63	12.68 ± 0.70	13.1 ± 0.68	12.51 ± 0.66
	CFV (LT leg)	12.7 ± 0.57	13 ± 0.63	13.37 ± 0.64	12.65 ± 0.64
	SFV(RT leg)	7.07 ± 0.65	7.4 ± 0.63	7.67 ± 0.72	7.14 ± 0.62
	SFV (LT leg)	7.22 ± 0.61	7.51 ± 0.60	7.82 ± 0.65	7.29 ± 0.64
	POPV (RT leg)	6.59 ± 0.81	6.92 ± 0.80	7.23 ± 0.82	6.59 ± 0.81
	POPV (LT leg)	6.57 ± 0.73	6.88 ± 0.74	7.23 ± 0.75	6.61 ± 0.77

Table 4.4: Superficial and deep venous diameter throughout pregnancy and in the postpartum period presented by the (mean and ± standard deviation).

4.3.2.2 *Venous reflux time (RT) results*

In this study, the presence of reflux was assessed in all deep and superficial venous segments using RT parameter (Fig 4.7). The findings revealed no reflux in the first and second trimesters of pregnancy in any venous segments. However, in the third trimester, 64% of patients who developed VVs demonstrated venous reflux (>500 ms) unilaterally, with 62% in the left and 38% in the right (as depicted in Fig 4.8). Notably, all refluxes were found in the LSV . Of the patients evaluated, 64% were categorised as having developed VVs, while 36% did not. In the post-partum period, 17% of patients continued to exhibit VVs in the LSV unilaterally, with 67% in the left and 33% in the right, while 83% did not have VVs (as demonstrated in Fig 4.9). Importantly, the study revealed that patients who developed VVs had significantly higher reflux (>500 ms) in comparison to those who did not develop VVs in the third trimester of pregnancy and the post-partum period, with P-values of less than 0.0001 in both instances.

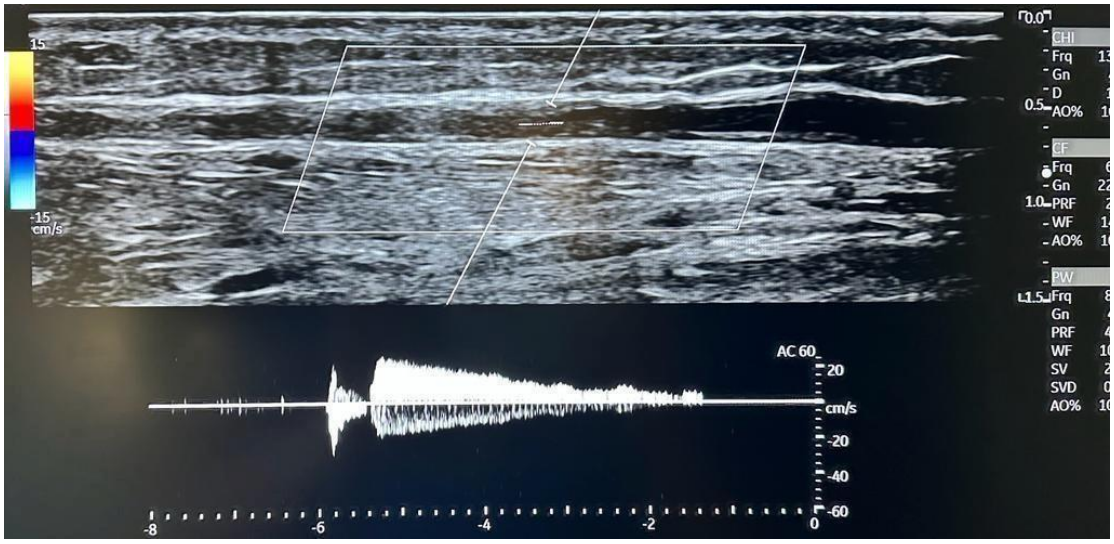


Fig 4.7: DUS showing RT= 2 sec (RT>0.05 sec) in the LSV.

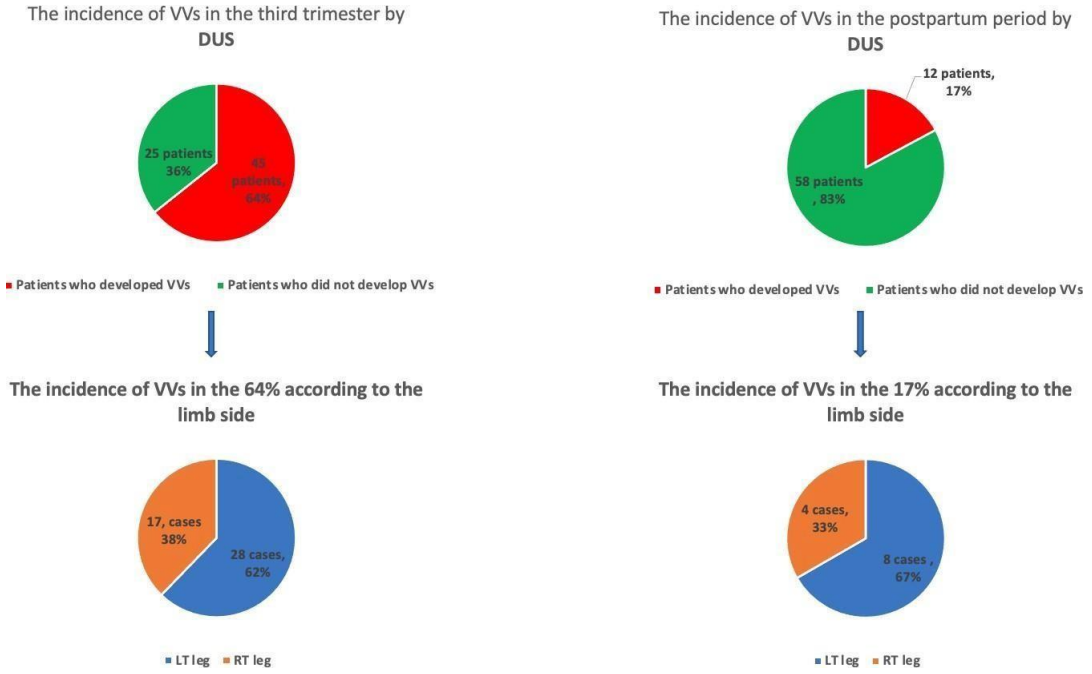


Fig 4.8: Pie charts showing the incidence percentage of VVs according to the period and limb side diagnosed by DUS.

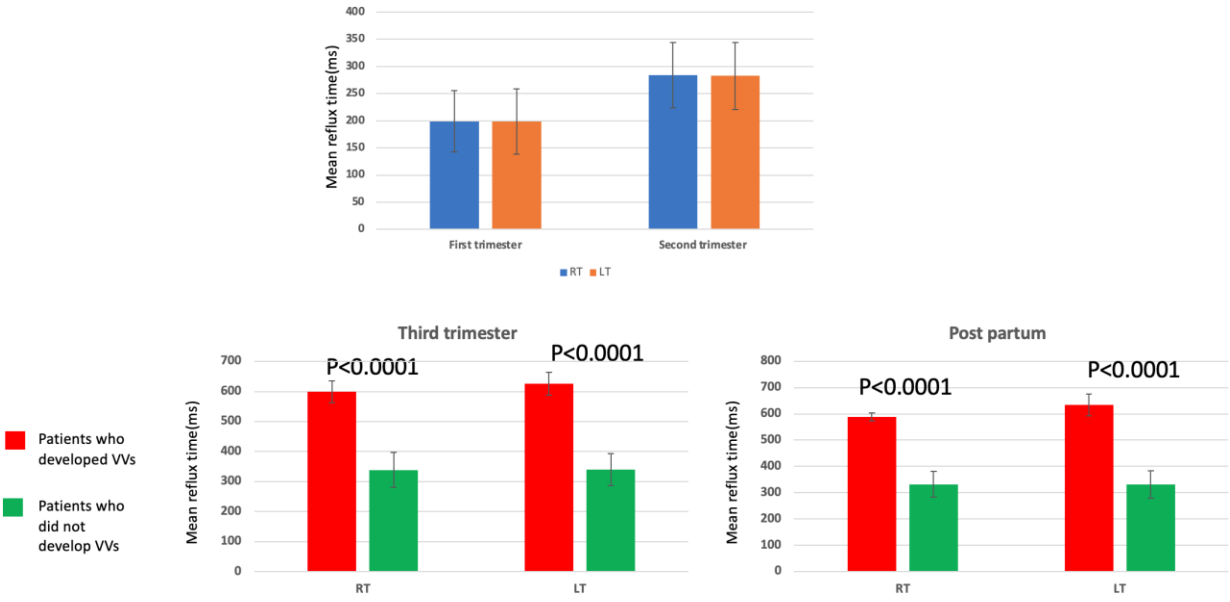


Fig 4.9: Reflux time (RT) was normal (< 500 ms) in the first and second trimesters and longer (>500 ms) in patients who developed VVs compared to patients who did not develop VVs in the third trimester and post-partum periods.

4.3.2.3 *Peak reflux velocity (PRV) results*

The present study evaluated PRV in all examined deep and superficial venous segments. The findings revealed that a PRV cut-off point of 48.2 cm/sec at the LSV has a significant discriminatory power between limbs with VVs and non-VVs. No reflux was detected in any venous segment during the first and second trimesters of pregnancy (PRV<48.2 cm/sec). However, in the third trimester of pregnancy, 64% of patients who developed VVs had PRV values ≥48.2 cm/sec unilaterally, with the LSV being the most commonly affected segment. The remaining 36% did not develop VVs. In the post-partum period, 17% of patients who developed VVs with PRV values ≥48.2 cm/sec continued to have VVs unilaterally (67% in the left and 33% in the right), and the LSV was also the most commonly affected segment. The remaining 83% of patients did not develop VVs. Notably, limbs in patients who developed VVs had significantly higher PRV values (≥48.2 cm/sec) compared to those in patients who did not develop VVs in both the third trimester of pregnancy and the post-partum period (P< 0.0001 and P<0.0001, respectively) (Fig 4.10).

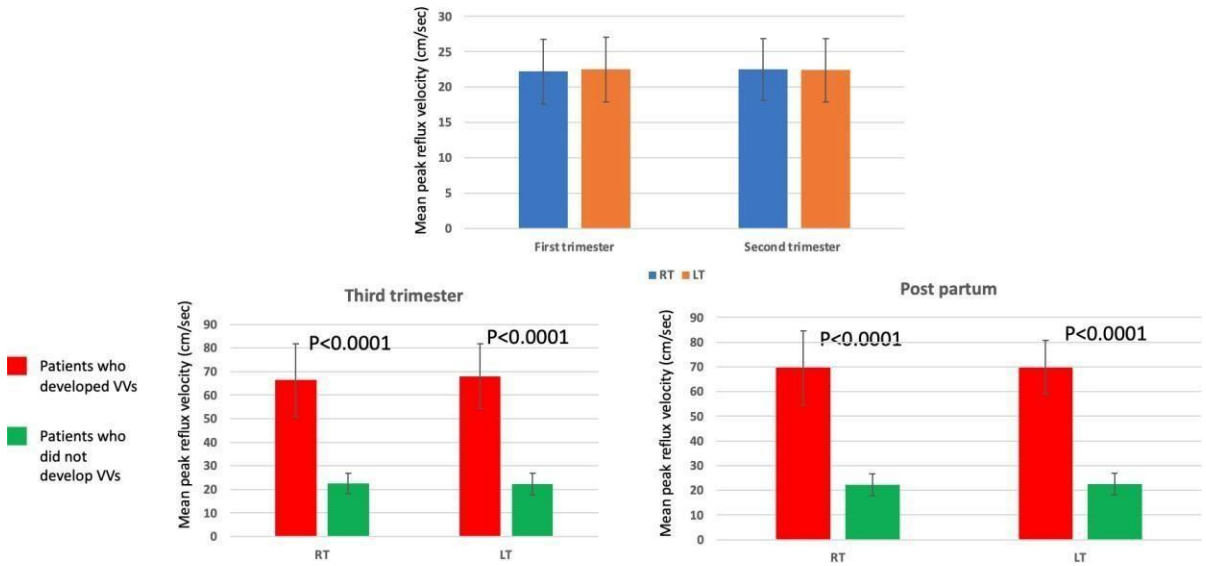


Fig 4.10: Peak reflux velocity (PRV) was higher in patients who developed VV compared to patients who did not develop VVs in the third trimester and post-partum periods.

4.3.2.4 *Measurements of fetal weight in the third trimester*

Results of this study indicated that the incidence of VVs increased directly with increased fetal weight in the third trimester of pregnancy. This study has found that patients who developed VVs in this study had higher fetal weight in the third trimester compared who did not develop VVs and the difference was statistically significant (P value <0.0001) (Fig 4.11). Further, this study examined the relationship between fetal weight and maternal BMI during the third trimester of pregnancy. The findings revealed that the mean maternal BMI for mothers with larger babies (higher fetal weight) was 27.5 ± 1.4, whereas the mean maternal BMI for mothers with smaller babies (lower fetal weight) was 26.6 ± 1.6. Importantly, a statistically significant difference was observed between these two groups (P value = 0.0069). These results suggest that increased fetal weight during the third trimester cannot be attributed solely to isolated factors contributing to the development of VVs. Instead, it appears to be influenced by the higher BMI risk factor in expectant mothers.

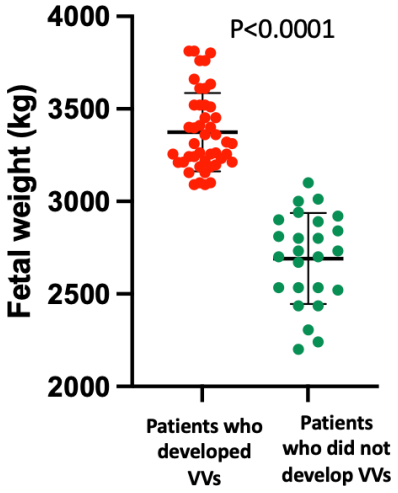


Fig 4.11: The incidence of VVs was higher in patients who had higher fetal weight compared to patients who had lower fetal weight in the third trimester of pregnancy.

4.4 Results of study 3: The incidence of Varicose veins in pregnancy using VFI

This study demonstrated that both VFI and DUS can identify pathological retrograde venous blood flow in patients with VVs. However, VFI was found to be superior to DUS

in detecting venous reflux, as it identified four additional patients with reflux in the third trimester (70% for VFI vs 64% for DUS) and four more patients in the postpartum period (23% for VFI vs 17% for DUS) (Fig 4.12 and Fig 4.13). Furthermore, the study compared the PRV obtained using VFI and DUS in patients with and without VVs in all trimesters of pregnancy and the postpartum period. The results revealed that the mean PRV was higher in DUS compared to VFI and the mean PRV differed significantly between the two methods in all trimesters of pregnancy and the postpartum period, as shown in Table 4.5, Fig 4.14, Fig 4.15, and Fig 4.16.

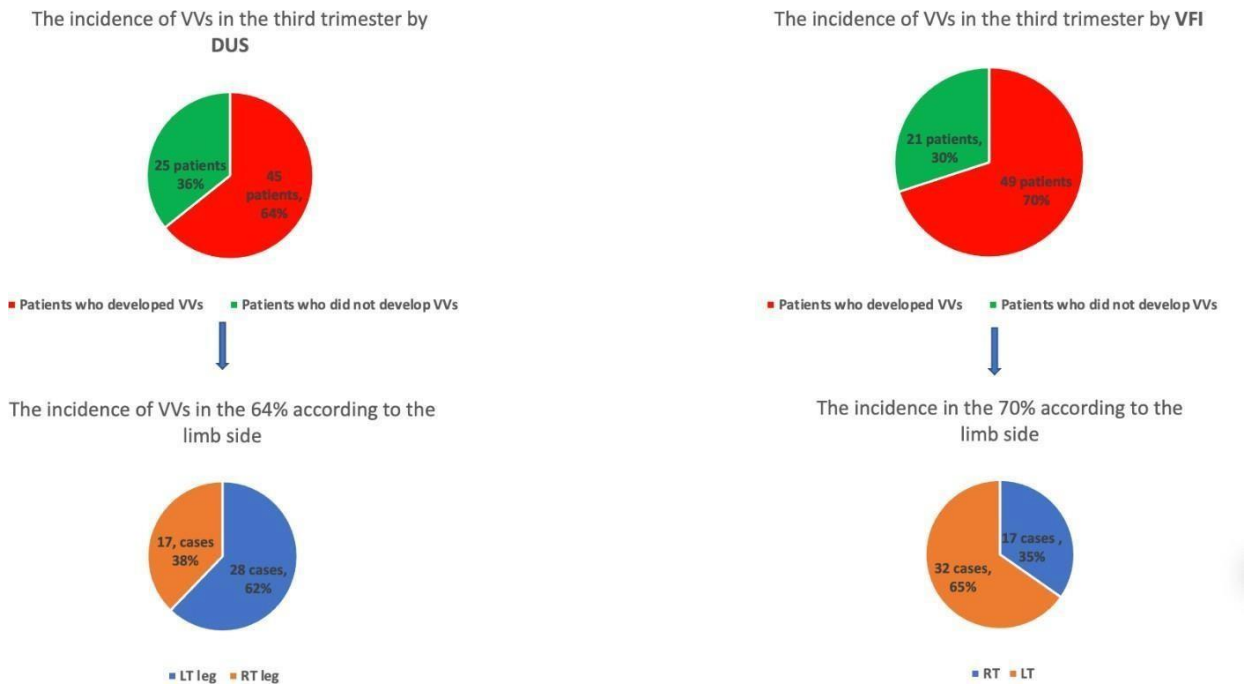


Fig 4.12: The incidence of VVs diagnosed by VFI was higher in the third trimester of pregnancy compared to DUS.

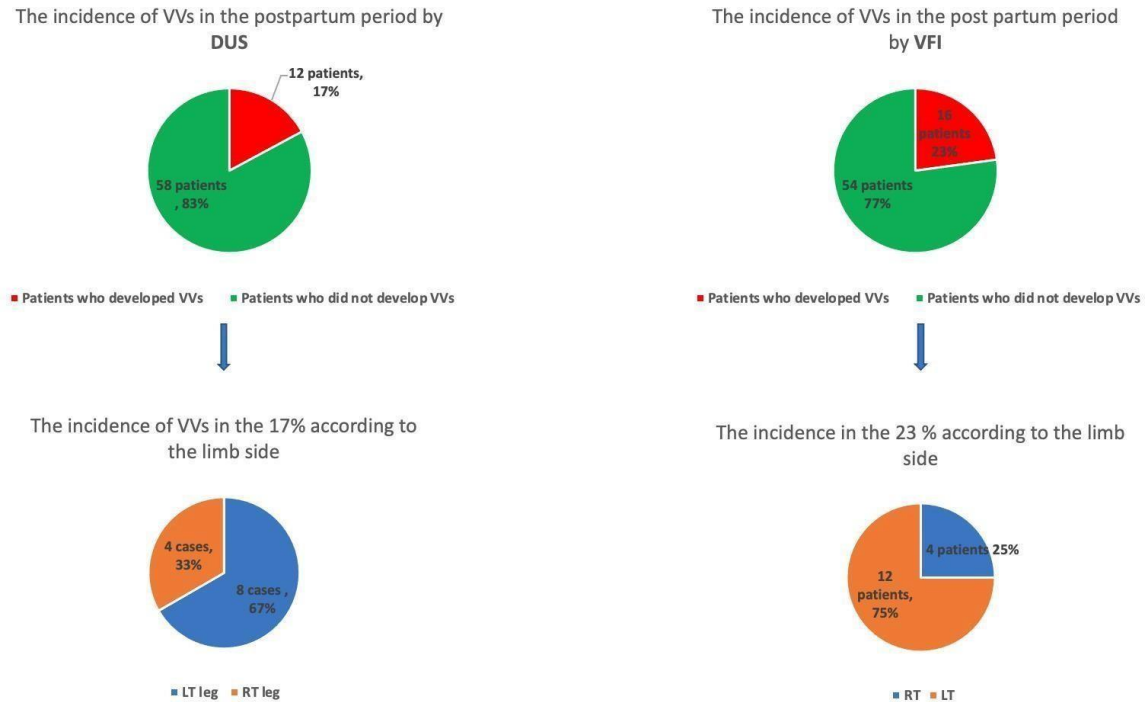


Fig 4.13: The incidence of VVs diagnosed by VFI was higher in the post-partum period of pregnancy compared to DUS.

	First trimester	Second trimester	Third trimester	Post-partum
Right leg	VFI: 16.4 ±5.2 cm/s. DUS: 22.2 ± 4.6 cm/s **(P<0.0001)**	VFI: 16.2 ±5.8 cm/s. DUS: 22.5 ±4.4 cm/s **(P<0.0001)**	In patients who developed VVs: VFI: 52.4 ± 6 cm/s. DUS: 66.4 cm/s ± 15.8 **(P<0.0001)** In patients who did not develop VVs: VFI: 16.7 ± 5.9 cm/s. DUS: 22.6 ±4.4 cm/s **(P=0.0018)**	In patients who developed VVs: VFI: 50.4 ±1.2 cm/s. DUS: 69.6 ± 15.1 cm/s **(P<0.0001)** In patients who did not develop VVs: VFI: 16.7 ±5.1 cm/s. DUS: 22.2 ±4.5 cm/s **(P<0.0001)**
Left leg	VFI: 16.3 ±5.3 cm/s. DUS: 22.5 ± 4.6 cm/s **(P<0.0001)**	VFI: 16.3 ±5.6 cm/s. DUS: 22.4 ±4.57 cm/s **(P<0.0001)**	In patients who developed VVs: VFI: 52.7 ±3.3 cm/s. DUS: 67.9 ± 13.8 cm/s, **(P<0.0001)** In patients who did not develop VVs: VFI: 16.3 ±5.4 cm/s. DUS: 22.2 ± 4.6 cm/s **(P<0.0001)**	In patients who developed VVs: VFI: 52.2 ±2.8 cm/s. DUS: 69.8 ±11.0 cm/s, **(P<0.0001)** In patients who did not develop VVs: VFI: 16.4 ±6.0 cm/s. DUS: 22.5 ± 4.4 cm/s **(P<0.0001)**

Table 4.5: The difference between the mean PRV in DUS and VFI in the right and left legs in all trimesters of pregnancy and postpartum.

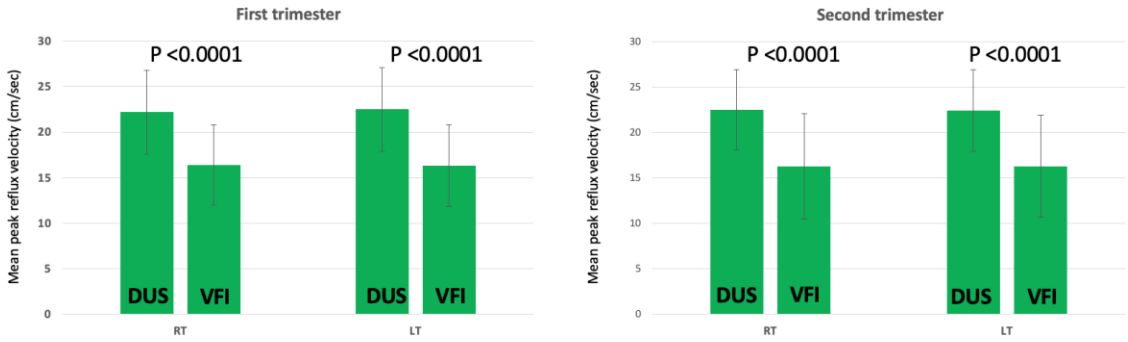


Fig 4.14: The mean PRV was higher in DUS compared to VFI in the first and second trimesters.

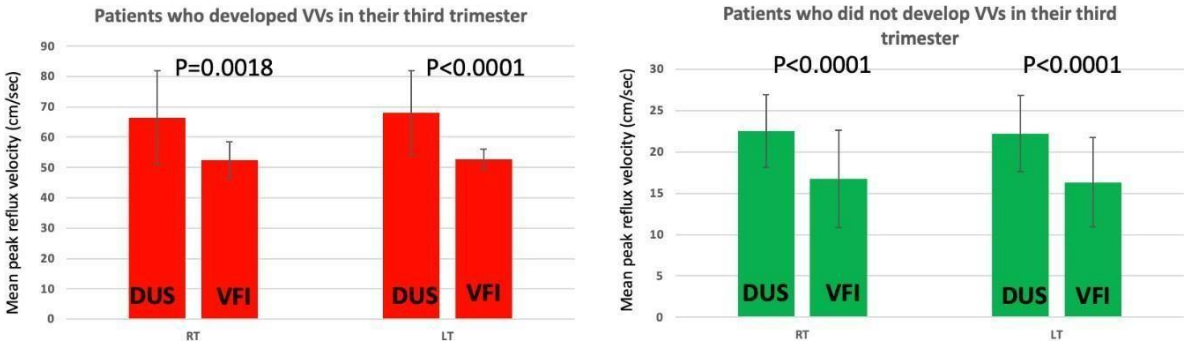


Fig 4.15: The mean PRV was higher in DUS compared to VFI in patients who developed VVs and in patients who did not develop VVs in the third trimester.

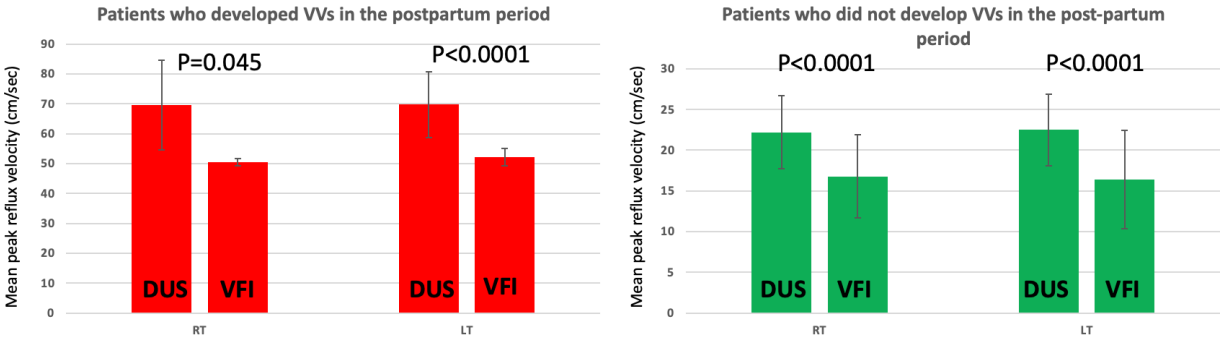


Fig 4.16: The mean PRV was higher in DUS compared to VFI in patients who developed VVs and in patients who did not develop VVs in the post-partum period.

4.5 Results of study 4: Inter and intra-observer measurements study in DUS and VFI

DUS Intra- and inter-observer agreements were assessed by ICCs using Microsoft Excel for Mac (version 16.54 (21101001). ICCs were calculated using the "C-1" model (i.e. consistency in single measures) with a 95% CI (Liljequist, Elfving and Roaldsen, 2019). A p-value <0.05 was considered to be statistically significant. ICCs were interpreted as poor (<0.40), fair (0.40-0.60), good (0.60-0.75), or excellent (0.75-1.00) agreement (Cicchetti, 1994). Bland-Altman plots were used to present the absolute measurement errors graphically. Simple linear regression was used to evaluate zero slopes to calculate any proportional bias.

4.5.1 Correlations and DUS Intra and inter-observer agreements

Intra and inter-observer agreement in DUS for PRV measurements of the left LSV was achieved in this study. Results of ICC for the intra-observer agreement assessment of DUS ranged from 0.941 (0.259-0.986) (i.e., excellent) (Fig 4.17). This means that DUS has clinically excellent limits of intra-agreement. On the other hand, the results of ICC for inter-observer agreement assessment for DUS ranged from 0.822 (0.068-0.95) (i.e., Good) (Fig 4.18). This means that DUS has clinically good limits of inter-agreement. Regarding correlations, DUS was found to have excellent intra-correlation (96%) and good inter-correlation (86%). Finally, linear regression was used to show the correlations and Bland-Altman plots were used to graphically display mean measurement differences and limits of agreement.

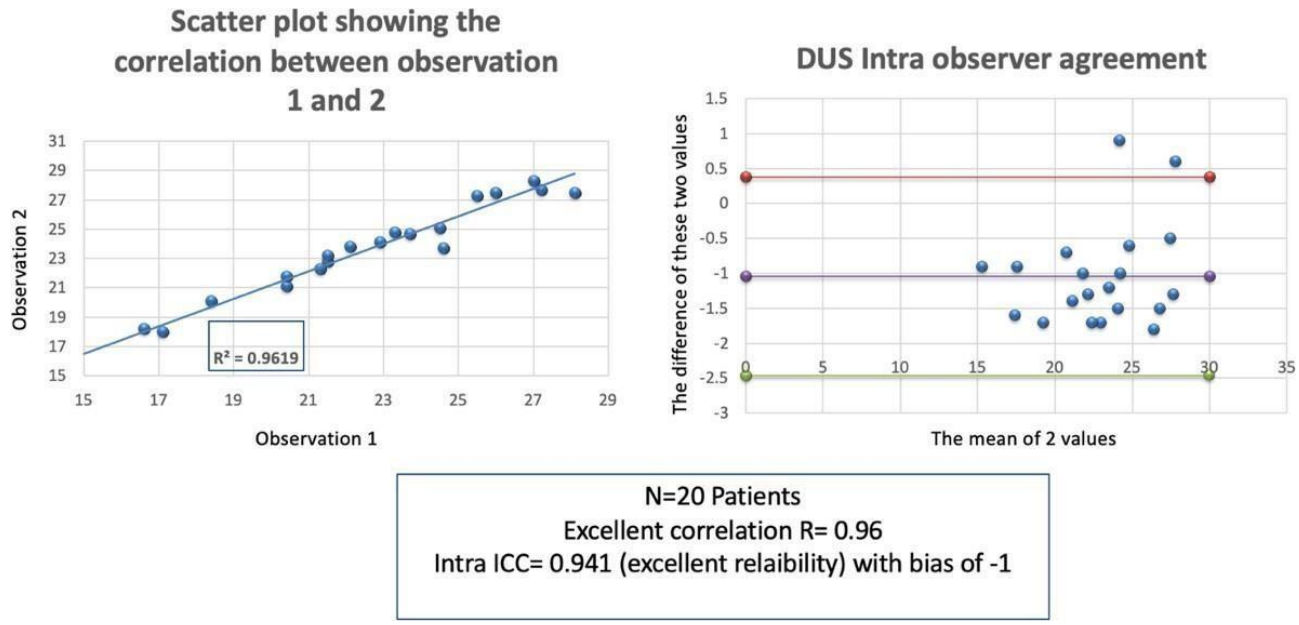


Fig 4.17: DUS had excellent intra-correlation (96%) and excellent intra-agreement (0.941).

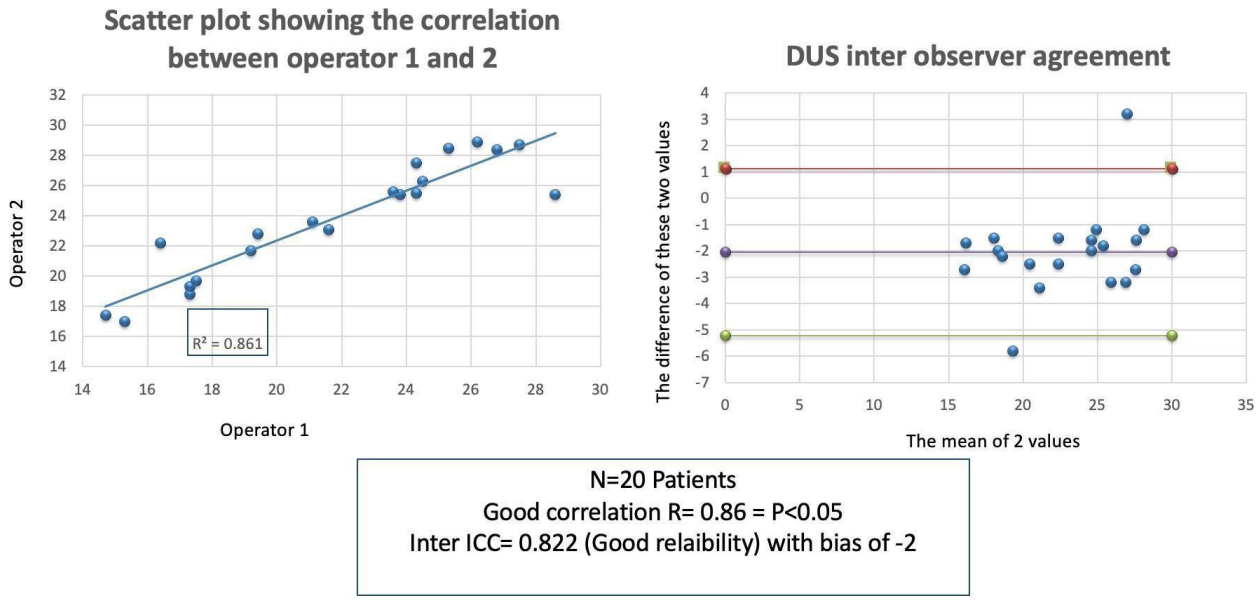


Fig 4.18: DUS had good inter correlation coefficient (86%) and good inter agreement (0.822).

4.5.2 Correlations and VFI Intra and inter observer agreements

Intra and inter-observer agreement in VFI for PRV measurements of the left LSV-was achieved in this study. Results of ICC for the intra-observer agreement assessment of

VFI ICC ranged from 0.937 (0.093-0.986) for the PRV at the left LSV (i.e., excellent) (Fig 4.19). This means that VFI has clinically excellent limits of intra-agreement. On the other hand, the results of ICC for inter-observer agreement assessment for VFI ranged from 0.79 (0.225-0.935) for LT LSV PRV (i.e., Good) (Fig 4.20). This means that VFI has clinically good limits of inter agreement. Regarding correlations, VFI has been found to have excellent intra-correlation (97%) and good inter-correlation (78%). Finally, linear regression was used to show the correlations and Bland-Altman plots were used to graphically display mean measurement differences and limits of agreement.

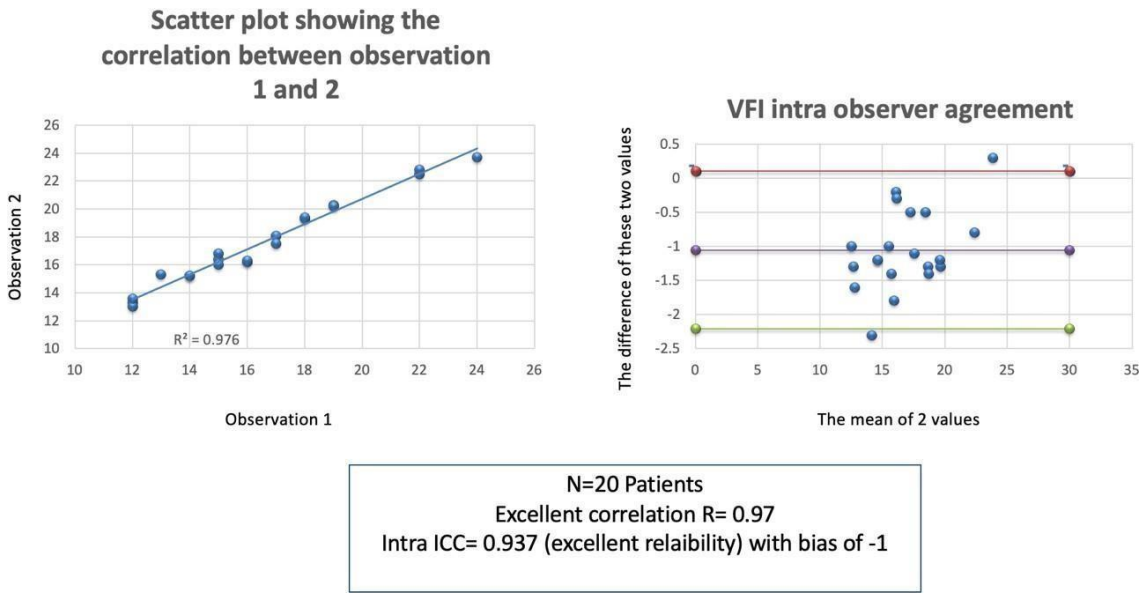


Fig 4.19: VFI had excellent intra correlation (97%) and excellent intra agreement (0.937).

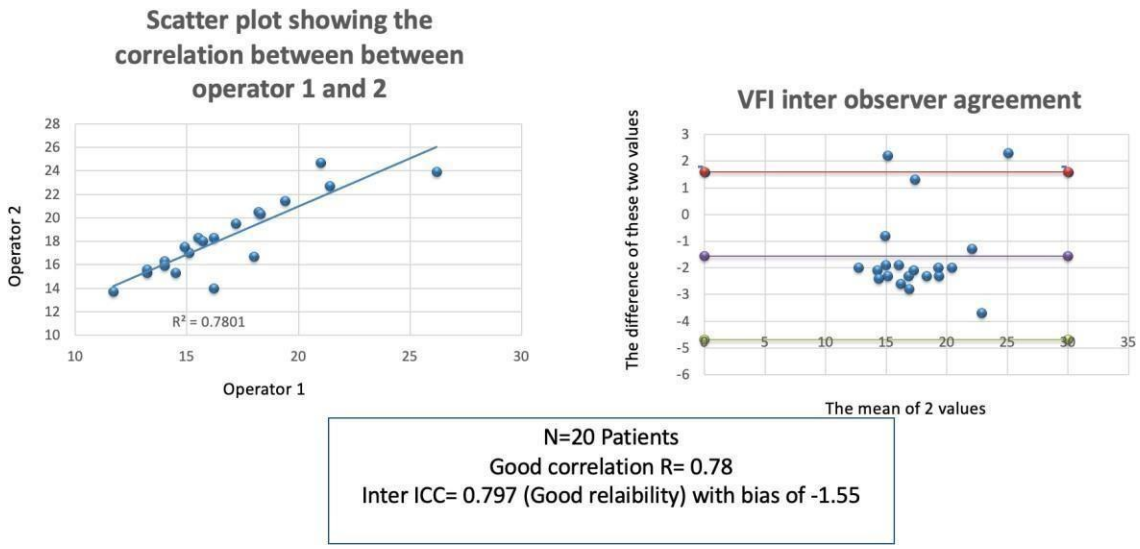


Fig 4.20: VFI has good inter correlation (78%) and good inter agreement (0.797).

4.6 Results of study 5: Venous outflow function using the PPG technique

The present study employed PPG to detect pathological retrograde venous blood flow in patients who developed VVs and to determine the location of venous reflux. Results showed that no reflux was detected in the first and second trimesters of pregnancy, as indicated by VRT exceeding 25 seconds. However, 64% of patients developed venous reflux unilaterally (62% in the left and 38% in the right) in the third trimester of pregnancy, as evidenced by VRT below 25 seconds. Additionally, 17% of patients continued to exhibit venous reflux in the post-partum period (67% in the left and 33% in the right). Notably, diseased limbs exhibited significantly lower VRT (<25 sec) than healthy limbs in patients who did not develop VVs in the third trimester of pregnancy and post-partum period (P<0.0001and P<0.0001, respectively) (Fig 4.21). This finding indicates that VRT measured by PPG can effectively differentiate patients who developed VVs from those who did not. Furthermore, patients who developed VVs in the third trimester of pregnancy and post-partum period exhibited moderate reflux at 10-19 seconds. The results of the PPG technique were found to be consistent with

those obtained by DUS in all trimesters of pregnancy and the post-partum period (Fig 4.22).

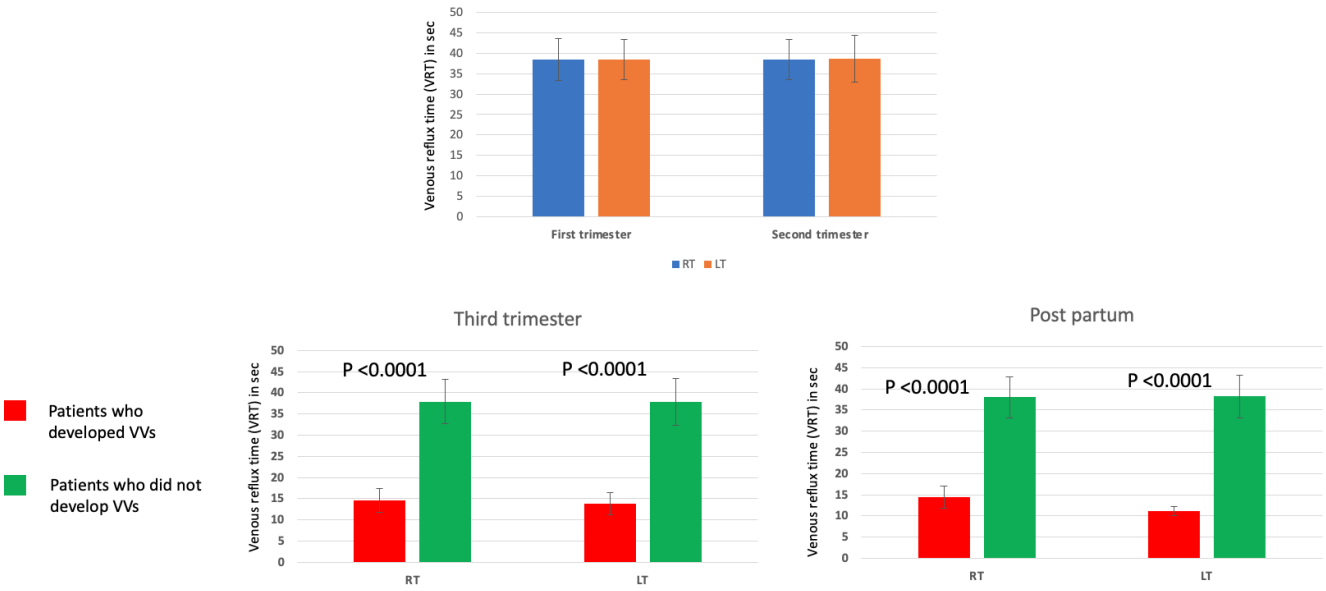


Fig 4.21: PPG measurements were normal (VRT >25 sec) in the first and second trimesters. In the third trimester and post-partum periods, PPG measurements were (VRT < 25 sec) in patients who developed VVs and who did not develop VVs.

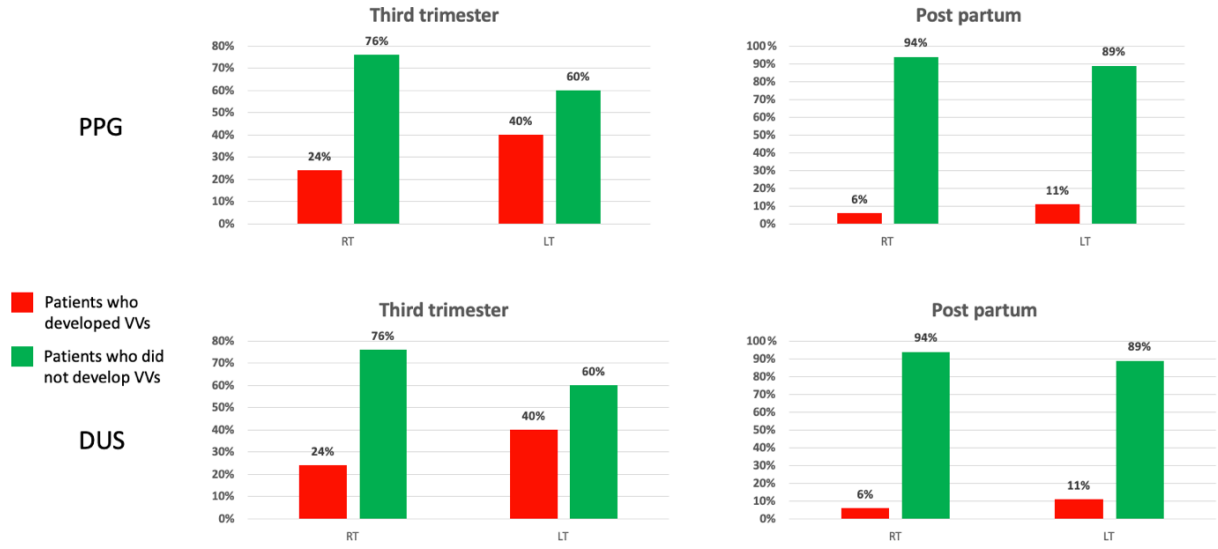


Fig 4.22: The incidence of VVs diagnosed by PPG was 100% consistent with the incidence of VVs diagnosed by DUS.

4.7 Results of study 6: Limb volume assessment

The present study investigated the relationship between limb volume measurements during pregnancy and the development of VVs. The findings revealed a significant increase in limb volume measurements during pregnancy, with the highest volume observed in the third trimester, followed by a decrease in the post-partum period. Notably, left limb volume was found to be higher than the right limb volume. The results also indicated statistically significant changes in limb volume measurements between trimesters and in the post-partum period (Fig 4.23). Furthermore, limb volume measurements were consistently higher in patients with VVs compared to those without, with statistically significant differences observed in the third trimester and post-partum period (Fig 4.24). Finally, the study found that the compression of right and left common iliac veins was associated with larger lower limb volumes in patients with VVs, while patients without VVs had less compressed common iliac veins and decreased lower limb volumes (Fig 4.25)(Fig 4.26). These findings suggest the potential utility of limb volume measurements as an indicator of VVs development during pregnancy and the importance of monitoring iliac venous compression in patients with VVs.

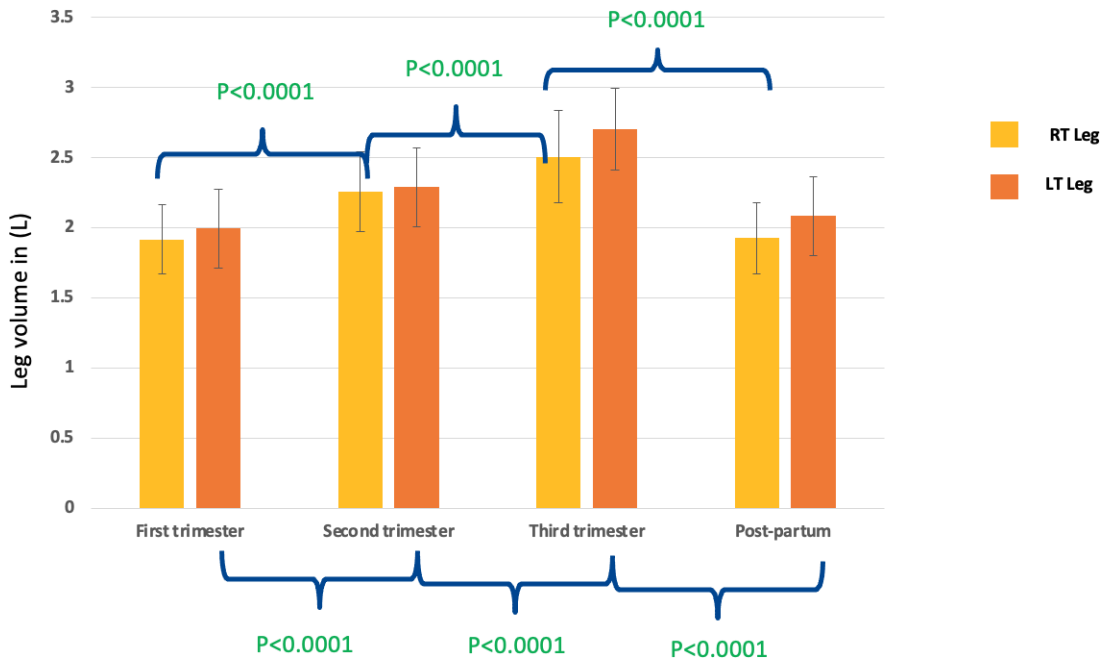


Fig 4.23: Limb volume measurements increased throughout pregnancy, reaching the highest measurements in the third trimester, then returning to normal post-partum. Also, limb volume measurements were higher in left legs compared to right legs.

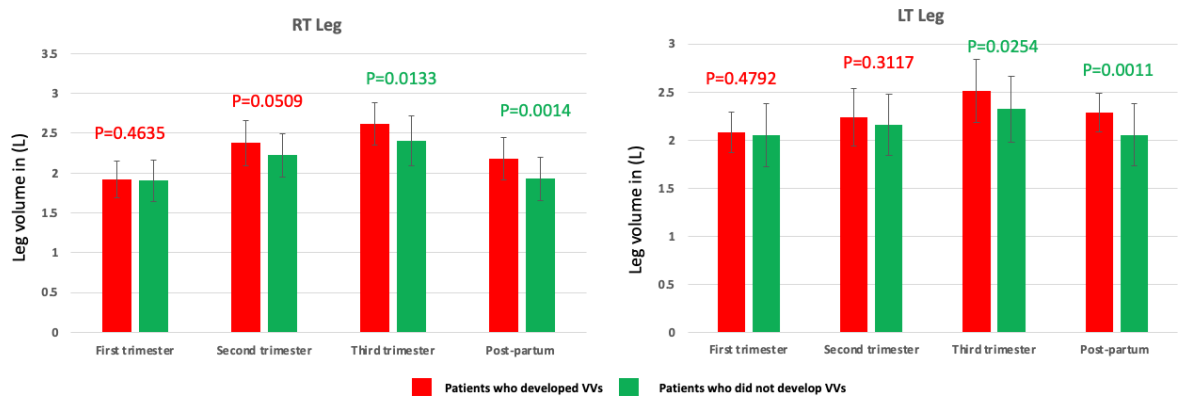


Fig 4.24: Limb volume measurements were higher in patients who developed VVs compared to patients who did not develop VVs during pregnancy and in the postpartum period. However, it was statistically different in the third trimester and in the post-partum period only in both limbs.

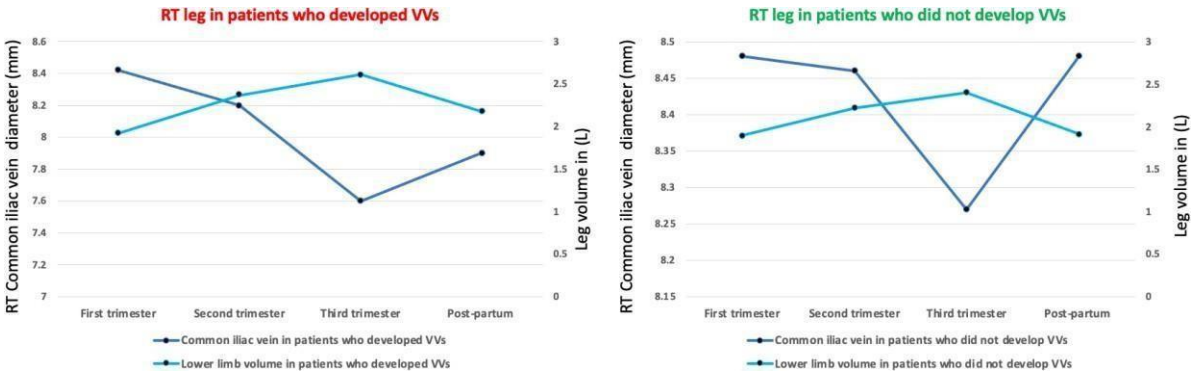


Fig 4.25: RT common iliac veins in patients who developed VVs were compressed and associated with larger lower limb volumes. Conversely, in patients who did not develop VVs, RT common iliac vein was less compressed and associated with decreased lower limb volumes.

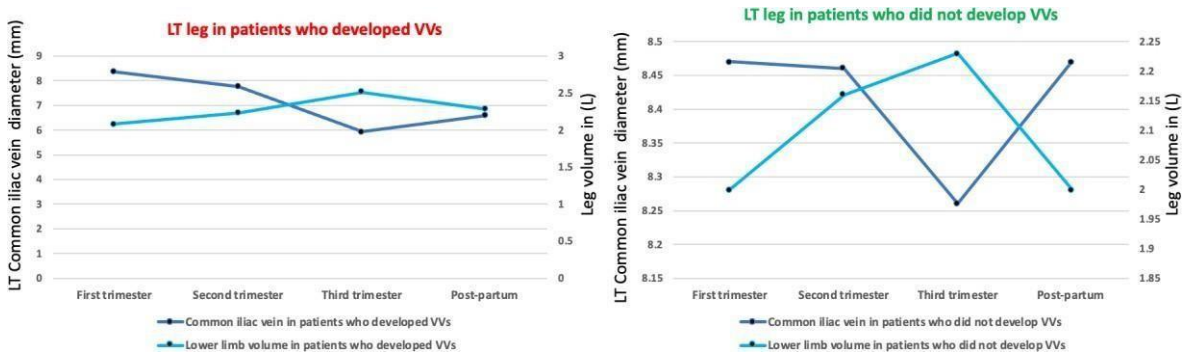


Fig 4.26: LT common iliac veins in patients who developed VVs were compressed and associated with larger lower limb volumes. Conversely, in patients who did not develop VVs, LT common iliac vein was less compressed and associated with decreased lower limb volumes.

4.8 Results of study 7: Hormonal blood test

Results of this study showed a general increase in the levels of serum progesterone and Estroil (E3) hormones during pregnancy in all 70 recruited pregnant women, reaching the highest levels in the third trimester followed by reduced levels to their normal values in the post-partum period. Moreover, results of this study showed that levels of progesterone were higher in patients who developed VVs compared to patients who did not develop VVs, and the difference was statistically significant in all trimesters of pregnancy and in the post-partum period (first p=0.0036, second p= 0.0036 and third p <0.0001) and in post-partum period p=0.0026 as shown in (Fig 4.27). Further, E3 hormone was also higher in patients who developed VVs compared to patients who did not develop VVs, and the difference was statistically significant in all trimesters of pregnancy and in the post-partum period (first p=0.0025, second p <0.0001 and third p <0.0001) and in post-partum period p=0.0102 as shown in (Fig 4.28).

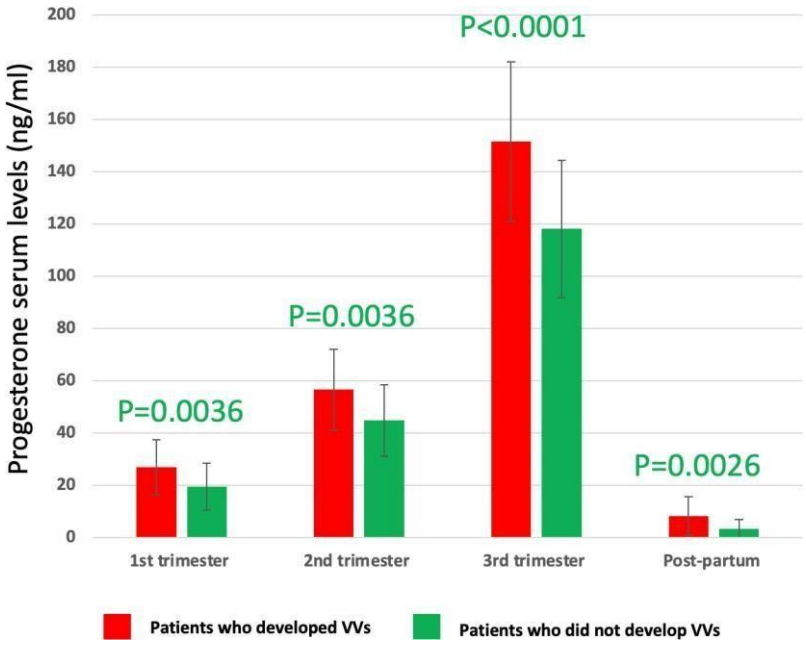


Fig 4.27: Progesterone hormone levels were higher in patients who developed VVs compared to patients who did not develop VVs.

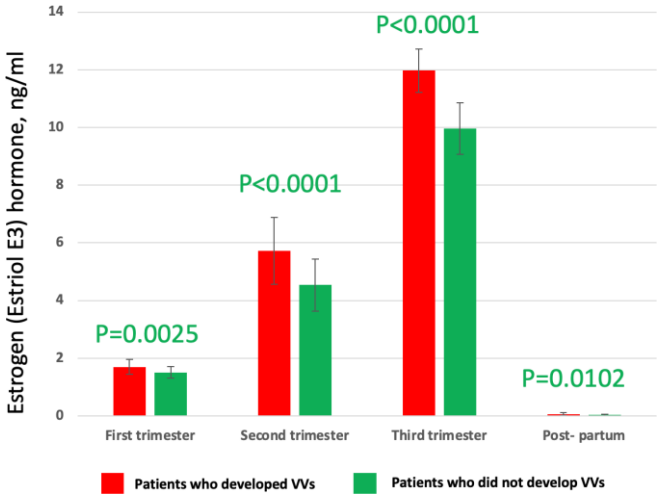


Fig 4.28: Estoril hormone levels were higher in patients who developed VVs compared to patients who did not develop VVs.

4.9 Results of study 8: Quality of life assessment in pregnancy and limb symptoms

Results of the QoL assessment tool (CIVIQ-20 questionnaire) in this study showed that QoL worsen from baseline reaching higher CIVIQ-20 scores (poorer QoL) in the third trimester followed by lower scores (better QoL) post-partum. Furthermore, higher scores (poorer QoL) were seen in the group of patients who developed VVs throughout

pregnancy and in post-partum periods compared to lower QoL scores in the healthy controls group. The difference was statistically significant in the third trimester of pregnancy only (P<0.0001) as seen in (Fig 4.29). The present investigation explained the correlation between the poor QoL of pregnant women who manifested the onset of VVs in the third trimester of pregnancy and various associated risk factors. The study revealed that among these participants, 23 individuals, constituting 71% of the sample, were engaged in occupations necessitating extended periods of standing, while 28 individuals, amounting to 62% of the cohort, exhibited elevated BMI values in contrast to their counterparts who did not exhibit the development of VVs.

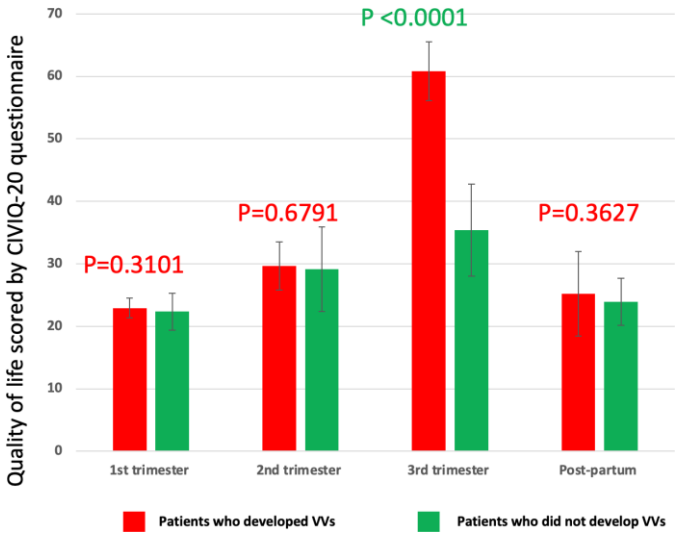


Fig 4.29: QoL scores were higher in patients who developed VVs (poorer QoL) compared to scores in patients who did not develop VVs (better QoL).

5 Chapter 5 Discussion and conclusion

5.1 Discussion

The present study has established that demographic variables such as older age (35-47 years), multiparity, positive family history of VVs, higher BMI, occupations that require prolonged standing, and white race are associated with the development of VVs. Prior studies have also reported an association between these risk factors and the development of VVs. For instance, studies have identified older age (Dimakakos *et al.*, 2013; Ebrahimi *et al.*, 2015; Zolotukhin *et al.*, 2017; G. Aly *et al.*, 2020), multiparity (T Zahariev *et al.*, 2009; Imre Bihari, Tornoci and Bihari, 2012; M.-J. Yun *et al.*, 2018), positive family history of VVs (Cornu-Thenard *et al.*, 1994; Fiebig *et al.*, 2010; Zöller *et al.*, 2012), higher BMI (Kohno *et al.*, 2014; Sharif Nia *et al.*, 2015; H. Kim *et al.*, 2017), prolonged standing (Ko *et al.*, 2008; Dimakakos *et al.*, 2013; Fokou *et al.*, 2018),and white race (Maffei *et al.*, 1986; Criqui *et al.*, 2007; Subramanian *et al.*, 2007) as risk factors for VVs.

Conversely, some studies did not find an association between age and VVs (Silvestro Canonico *et al.*, 1998), or between multiparity and the progression of VVs (C. A. Engelhorn *et al.*, 2010). The development of VVs in this study could be attributed to factors specific to the study population, such as a higher prevalence of pre-existing VVs before pregnancy or difficult pregnancies exacerbating pre-existing VVs. Moreover, some studies have reported no association between positive family history and VVs (Coughlin *et al.*, 2001), which may be due to self-reported data being subject to bias (Ahti, *et al.*, 2010). Similarly, some studies have found no correlation between higher BMI and VVs (B. Komsuoglu *et al.*, 1994; Coughlin *et al.*, 2001) or between prolonged standing and VVs (Correa Posada, Contreras Correa and García Vélez, 2022). However, a significant incidence of C5 or C6 ulcer was observed in this study among patients in occupations requiring more than 4 hours of standing. These divergent findings may be attributed to differences in study design, sample size, and population characteristics across studies.

In this study, DUS assessment was employed to examine changes in the diameter of the right and left common iliac veins during pregnancy. The results indicate that there is a decrease in the diameter of these veins, particularly in the third trimester when the gravid uterus is at its maximum size. This is a common finding in pregnancy due to the compression effect of the gravid uterus on the common iliac veins (Soma-Pillay *et al.*, 2016b). These decreased diameters tend to return to normal in the post-partum period in some cases, which is consistent with previous research findings (De Stephano *et al.*, 2014; Dronkers *et al.*, 2016; Traisrisilp *et al.*, 2021b).

Furthermore, the study found that the decrease in the diameter of the right and left common iliac veins in patients who developed VVs was statistically significant during the third trimester and post-partum periods. These findings support the relationship between the compression of common iliac veins during pregnancy, particularly in the third trimester, and the development of VVs. This is because the compression of the common iliac veins leads to reduced blood flow return to the heart, which results in blood pooling and increased pressure in the legs, ultimately leading to the formation of VVs (Bong, 2001). Although the compression of common iliac veins may improve after delivery as the pressure on the veins is reduced, this study found that the compressed common iliac veins in patients who developed VVs remained compressed in the post-partum period. This may be due to prolonged periods of common iliac compression during pregnancy, which can lead to damaged vein walls and scar tissue formation. This perspective is consistent with previous research findings (Morris *et al.*, 2020).

Additionally, this study observed a statistically significant decrease in the diameter of the left common iliac vein during the second trimester between patients who developed VVs and those who did not. It is noteworthy that the compression of the left common iliac vein occurred earlier and at a higher degree of compression compared to the right common iliac vein. The mean percent stenosis of the left common iliac vein due to compression was 20.4%, while the mean percent stenosis of the right common iliac vein was only 5.9%. This finding can be attributed to the anatomical variation in which the right common iliac artery passes anterior to the left common iliac vein, while the right common iliac vein usually remains in its normal anatomical position, running alongside the right common iliac artery (Harward, 2011).The aforementioned

observation holds substantial clinical significance as it implies that the left common iliac vein is more vulnerable to compression during pregnancy and could potentially play a more prominent role in the development of VVs. This aligns with the findings of this study, which reported a higher incidence of VVs in the left leg (62%) as compared to the right leg (38%). As such, it is imperative for healthcare providers to take into account this anatomical variation while assessing pregnant patients with existing VVs or those susceptible to their development, as it could influence their management and overall prognosis.

Iliac vein compression represents a frequent discovery in individuals presenting with lower limb VVs, as documented in extant literature (Yang et al., 2018; Li et al., 2021). Historical reports have indicated that iliac vein compression was discerned in 2-5% of patients afflicted with CVD (Cockett and Thomas, 1965). In the context of this study, it is noteworthy that iliac vein compression during pregnancy not only influenced the onset and progression of VVs but also led to the manifestation of MTS within the left lower extremity. It is imperative to clarify that while "iliac vein compression" is a broader term that can refer to any form of iliac vein constriction or narrowing, MTS specifically refers to the compression of the left common iliac vein by the right common iliac artery.

This research underscores a salient finding—during the third trimester of pregnancy, a substantial subset of patients (29%, or 8 out of 28) who developed VVs in the left lower extremity concurrently developed MTS, without concurrent evidence of DVT. Notably, this MTS persisted into the postpartum period, exhibiting a mean percent stenosis of 39.5% within the left common iliac vein, attributable to extrinsic compression by the overlying artery. The etiology of MTS in these patients may be attributed to the substantial enlargement of the gravid uterus during pregnancy, thereby significantly compressing the left iliac vein and impeding venous return in the left lower limb against gravitational forces. An alternative explanation pertains to the elevated pressure in the lower extremities of individuals with VVs, which may contribute to iliac vein compression, particularly during pregnancy when the uterus exerts additional pressure on the left common iliac vein.

To augment the existing body of knowledge, it is pertinent to note that prior literature has previously reported the association between iliac vein compression, a more

overarching term, and VVs (Cockett and Thomas, 1965) (Yang et al., 2018; Li et al., 2021). Furthermore, the association between pregnancy and the occurrence of MTS has been substantiated in previous studies (Traisrisilp et al., 2021). Notably, earlier research has also explained the increased risk of developing DVT during pregnancy (Traisrisilp et al., 2021; Mei et al., 2020). Nevertheless, it is appropriate to underscore that our current study establishes the first attempt to present the relationship between MTS during pregnancy and the development of VVs.

The present study's results revealed a significant association between compressed common iliac vein and the development of VVs, which was observed alongside the finding that patients with VVs had higher fetal weight in the third trimester compared to those who did not develop VVs. Specifically, in this study, patients with VVs had a higher fetal weight in the third trimester compared to those without VVs, as assessed by DUS (3374 kg ± 212 vs. 2629 ± 246.2, respectively). These findings are consistent with those reported in a study by (Dustova, Babadjanova and Ikhtiyarova, 2019), which found that fetal weight at delivery was higher in pregnant women with VVs compared to healthy pregnant women (3650.0 ± 0.54 g vs. 3435.5 ± 0.52 g, respectively). The elevated fetal weight in the third trimester, particularly in the growing womb, is believed to compress the inferior vena cava, thus impeding the venous blood return from lower extremities to the heart. This compression leads to an increase in blood pressure in the lower leg veins, contributing to the development of VVs (Smyth, Aflaifel and Bamigboye, 2015). Although fetal weight was associated with the development of VVs in this study, however, this study suggests that increased fetal weight during the third trimester cannot be attributed solely to isolated factors contributing to the development of VVs. Instead, it appears to be influenced by the higher BMI risk factor in expectant mothers.

In this study, deep and superficial vein diameters in the lower extremities of pregnant women were found to gradually increase during pregnancy, with most cases returning to normal values in the post-partum period as determined by DUS evaluation. Statistically significant variations in diameter were found in each limb at different stages of pregnancy and after delivery, but not between legs. These findings are

consistent with those of previous studies by (Boivin, Cornu-Thenard and Charpak, 2000; Goulart *et al.*, 2013; Asbeutah *et al.*, 2015). The first evaluation was performed during the first trimester of pregnancy, when increased levels of estrogen and progesterone hormones can weaken venous wall tone and decrease valve function (Somers and Knaapen, 2006b). It is noteworthy that vein behavior regarding diameter increase did not differ between competent and non-competent veins.

In the present study, assessment of venous reflux by DUS revealed 64% of refluxes occurred exclusively in the LSV in the third trimester of pregnancy and 17% in the post-partum period. This finding aligns with the work of (Min, Khilnani and Zimmet, 2003), which identifies refluxes in the LSV as the primary underlying cause of VVs. To distinguish between competent and incompetent veins, the current study utilised the RT and PRV parameters. Results indicate that patients who developed VVs had significantly higher reflux (>500 ms) than those who did not develop VVs during the third trimester of pregnancy and the post-partum period. This observation has been supported by previous studies, including (Labropoulos *et al.*, 2003; Asbeutah *et al.*, 2006; Zygmunt, 2014). Specifically, patients who developed VVs during pregnancy had a higher mean RT than those who did not. In the third trimester, the mean RT in the right leg was 599.1 ms in patients with VVs compared to 338.8 ms in patients without VVs. Similarly, the mean RT in the left leg was 626 ms in patients with VVs compared to 339 ms in patients without VVs. In the post-partum period, the mean RT in the right leg was 588.5 ms in patients with VVs compared to 332 ms in patients without VVs. The mean RT in the left leg was 634 ms in patients with VVs compared to 330 ms in patients without VVs

Previous studies (Callam, 1994; Welch *et al.*, 1996; Valentín and Valentín, 1999) have demonstrated that while RT is a commonly utilized parameter for determining venous reflux, it is also the least reliable in evaluating venous reflux. In contrast, (Yamaki *et al.*, 2007) have noted that PRV can be used to detect both reflux and obstruction simultaneously, in addition to assessing the magnitude and clinical severity of reflux. Furthermore, difficulty in instructing patients on the proper Valsalva maneuver has led to inconsistencies in RT recordings, even in incompetent veins, as the maneuver modifies the time graph prior to RT (Ricci *et al.*, 2018). Therefore, some researchers, such as (A. K. Singh *et al.*, 2020b), suggest using only PRV to assess significant reflux,

as it provides the highest peak and is thus least altered. Consistent with previous findings, the current study also advocates for the use of PRV as the most consistent parameter for detecting venous reflux, unless a larger patient sample size and better patient understanding of the Valsalva maneuver are employed to improve estimation parameters.

The present study found that the mean PRV was higher in patients who developed VVs during pregnancy than in those who did not. Specifically, in the third trimester, the mean PRV was 66.4 cm/sec in the right leg and 67.9 cm/sec in the left leg in patients who developed VVs, compared to 22.5 cm/sec and 22.2 cm/sec, respectively, in patients who did not develop VVs. In the post-partum period, the mean PRV was 69.9 cm/sec in the right leg and 69.8 cm/sec in the left leg in patients who developed VVs, compared to 22.2 cm/sec and 22.5 cm/sec, respectively, in patients who did not develop VVs. These findings are consistent with those of (Singh *et al.*, 2020b), however, this current study provided detailed information on PRV means calculated for each limb side in both competent and incompetent veins. The comprehensive information and consistent results support the conclusion that PRV was higher in incompetent veins compared to competent veins, indicating significant reflux. Such reliable knowledge and strong evidence can inform clinical decision-making and treatment for patients with venous insufficiency.

This study aimed to determine the PRV cut-off point for pathological reflux based on the PRV values obtained from the LSV where the reflux occurs. The results showed that the discriminatory power between limbs with and without VVs was best achieved when the cut-off value for PRV was set at 48.2 cm/sec at the level of the LSV. This finding was close to a previous study by (Konoeda *et al.*, 2014), that suggested the cut-off point 47.8 cm/s at the LSV. Different recommendations have been presented regarding the optimal cut-off points for PRV in different studies. For example, a study by (A. K. Singh *et al.*, 2020b) suggested that the optimal cut-off point is 27.4 cm/s for the PRV at the SFJ, while other studies recommended a PRV of more than 30 cm/sec as significant reflux (Callam, 1994; Valentín and Valentín, 1999). These discrepancies in recommendations may be due to scanning different parts of the venous system

because the flow dynamics and anatomy vary in different regions of the venous system. Other reasons may be due to operator-dependent factors that can affect velocity evaluation, such as insonation angle, manual angle correction, spectral broadening, beam steering, sample volume placement, and Doppler gain. Furthermore, the lack of structured and defined ultrasound examination protocols for estimating peak velocity with DUS may also contribute to these differences in optimal cut-off point recommendations.

Although DUS is widely used for diagnosing venous diseases, its accuracy in estimating PRV is limited by its angle-dependency. To overcome this limitation, this study used VFI, which is a multidimensional beam steering and angle-independent method to assess venous reflux in patients with VVs. The study found that both DUS and VFI were able to detect venous reflux by measuring venous velocities, but VFI was superior to DUS in estimating PRV and detecting venous reflux. Specifically, in the third trimester of pregnancy and in the post-partum period, VFI detected venous reflux in 70% of cases compared to 64% for DUS, and in 23% of cases compared to 17% for DUS, respectively, with no difference in the two imaging modalities' velocity settings. The mean PRV for VFI was lower than that for DUS, suggesting that VFI was more sensitive in detecting changes in blood flow velocities. This finding is consistent with previous studies that compared PRV measurements by VFI and DUS in various lower limb veins (Bechsgaard *et al.*, 2016, 2017, 2018). The higher mean PRV for DUS may indicate that this technique overestimates peak velocity, which is a drawback caused by spectral broadening with increasing angle of insonation, resulting in inaccurate flow velocity estimation (Eicke *et al.*, 1995).

Although VFI may be misunderstood as it underestimated blood velocities in this study, a lower standard deviation for VFI compared to DUS suggests that VFI is more accurate. A smaller standard deviation indicates values that are closer to the mean and less dispersed, indicating higher accuracy (Seem, Nachmias and Nachmias, 1988). In assessing the LSV, this study found that VFI underestimated PSV by 6% compared to DUS. This finding is consistent with previous studies that reported different percentages of underestimation. For example, (Pedersen *et al.*, 2012a) reported that VFI underestimated PSV by 8% when assessing the carotid artery compared to DUS. Additionally, when evaluating the ascending aorta, VFI

underestimated PSV by 22.9% compared to transesophageal echocardiography (Hansen *et al.*, 2009). Furthermore, the study demonstrated that VFI accurately differentiated between patients who developed VVs and those who did not, and patients who developed VVs had significantly reduced common iliac vein diameters compared to those who did not develop VVs. This highlights the potential of VFI to diagnose and evaluate venous reflux caused by mechanical factors, such as iliac vein compression during pregnancy.

The current study's key finding indicates that in experienced hands, DUS and VFI examinations are reliable imaging techniques for detecting venous reflux during pregnancy. Findings of this study indicated that PRV measurements of DUS and VFI showed excellent intra- and good inter-observer agreement (inter-observer ICC: VFI 0.79, DUS 0.82; intra-observer ICC: VFI 0.93, DUS 0.94). It is common for inter-observer variability to be lower compared to the intra-observer variability meaning that different observers tend to disagree more compared to a single observer (Ismail *et al.*, 1990). This can occur for several reasons as described by (Coggon, Rose and Barker, 2003); each observer may have different interpretation of the criteria being measured; they may use slightly different techniques when making the measurement , systematic differences between observers due to factors such as experience or training and there is always some degree of random variability in any measurement which all these factors lead to differences in the results. Previous studies have assessed intra and inter observer agreement of DUS; for measuring lower limb venous reflux by reflux time parameter with the support of manual cuff compression (Broholm *et al.*, 2011), evaluating the uterine vein diameter (Amin, Wong and Jurkovic, 2019), measuring the cephalic vein diameter (Zhang *et al.*, 2022), in the planning of arteriovenous fistula surgery by quantifying the venous and arterial diameter (Zonnebeld et al., 2017). Likewise, the assessment of intra and inter observer agreement of VFI were demonstrated earlier in the studies by (Brandt, Hansen, *et al.*, 2018; Dong *et al.*, 2023) to measure the dimeter of the carotid artery.

Despite the extensive research on the usefulness of DUS and VFI in clinical decision-making, their reproducibility has not been fully established, especially in studying the reducibility for the estimation of PRV in veins. As far as we know, one study by (Brandt,

Moshavegh, *et al.*, 2018b) examined the intra- and inter-observer agreement in the assessment of PRV in the portal vein using VFI. This study showed that VFI was more reliable for assessing venous flow in the portal vein compared to DUS and the Intra- and inter-observer agreement was highest for VFI compared to DUS (inter-observer ICC: VFI 0.80, DUS 0.3; intra-observer ICC: VFI 0.90, DUS 0.69). The finding of this study was supported by the fact that VFI relies less on the operator than DUS, as it involves fewer parameter settings. This reduced operator dependence could be a reason why VFI yields better precision and consistency among both individual and multiple observers as previously mentioned by (Hansen *et al.*, 2011; Pedersen *et al.*, 2012b). As a comparison with our study, DUS was slightly more reproducible compared to VFI, it is possible that the operators were not familiar with VFI, as it is a more recent technique compared to DUS. Additionally, the PRV parameter utilized in evaluating venous reflux in the LSV is more difficult to assess due to the requirement of manual distal compression and release, unlike the assessment of portal vein. This study is believed to be the first of its kind to examine intra- and inter-observer agreement in evaluating the PRV in the LSV while pregnant, using DUS and VFI techniques.

Besides the non-invasive imaging modalities DUS and VFI, PPG technique was performed in this study to assess venous reflux by using the VRT which provides an objective parameter of venous reflux estimation (Saliba Júnior *et al.*, 2014c). Although PPG lacks the capability of detecting the anatomical location of reflux (Gohel *et al.*, 2007). However, in this study, PPG was 100% consistent with DUS findings in the ability of detecting lower limb venous reflux. The studies by (Yamaki, Nozaki and Sasaki, 2000; Beraldo, Satpathy and Dodds, 2007) reported similar results. Additionally, PPG was able to determine the severity of reflux and correlate these findings with clinical outcomes. This correlation has been validated as VRT of PPG was shorter in patients in CEAP clinical stages C2-C3 suggesting a moderate venous reflux compared to VRT in healthy patients with clinical class of C0. The association between CEAP clinical class of primary CVD and the parameters of venous PPG has been supported by (Khorev *et al.*, 2017; Shaydakov and Sannikov, 2021).

The discriminatory VRT value between patients with reflux and patients without reflux in this study is VRT of 25 seconds. This was consistent with previously published

studies (Iafrati *et al.*, 1994; Stücker *et al.*, 2005). On the other hand, other studies indicated the VRT of 20 seconds for a significant venous reflux (Dunn *et al.*, 1994; Kelechi and Bonham, 2008). Other studies reported different values of VRT for a pathological reflux. For instance, <18 seconds in the study by (Tucker *et al.*, 1998) and VRT ≤ 15 seconds in the study by (Sarin *et al.*, 1993). VRT between 20 to 25 seconds is often used in literature as a cut-off point to assess the presence of venous reflux. However, the variability in VRT between studies may be due to several factors including: using different measurement techniques to evaluate the VRT such as using different types of PPG sensors or using different methods for processing the data which may lead to variation in the reported values. Another reason may be due to differences in the study population demographics which can also change VRT values. For instance, VRT values differs according to the age of the patient as healthy children have normally shorter VRT compared to adults as reported in the he Bochum study (Stücker *et al.*, 2005). This is due to the developing small mass of muscle in children compared to adults (Davies, White and Young, 1983). Therefore, this study suggested using PPG in a conjunction with other imaging modalities such as DUS to provide reliable results in regard of the presence of significant venous reflux.

Previously published studies on the assessment of limb volume using the grith measurement Truncated cone method have been reported in patients with Lymphedema in lower extremises (Mayrovitz *et al.*, 2007; Campanholi, Duprat Neto and Fregnani, 2011; Katch and Katch, 1974) lymphedema in upper extremities (Megens *et al.*, 2001; Karges *et al.*, 2003) and lymphedema in arms and legs (AWB Stanton, JW Northfield, 1997). The truncated cone method has been shown to yield valid outcomes in the assessment of limb volume, as demonstrated in the majority of previous studies. To our knowledge, this study was the first study to use Truncated cone method to assess the limb volume in pregnancy to correlate the findings with VVs incidence and to associate these findings with right and left common iliac vein measurements.

Findings of this study showed that limb volume measurements increased during pregnancy reaching the highest volume in the third trimester then in some cases returned to its normal values in the post-partum period. These findings were supported by (Yanagisawa *et al.*, 2019b; Morimoto and O'Rourke, 2021). This increase was

related to leg odema in pregnancy which is a common sign that occur due to either water retention or due to increased pressure on the common iliac veins due to growing uterus causing venous stasis and pooling in the lower legs (Reynolds, 2003). In addition to the expected increase of leg volume during pregnancy, this study found that limb volume measurements were higher in patients who developed VVs compared to patients who did not develop VVs. These findings were statistically significant in the third trimester of pregnancy and in the post-partum period only which showed the significant effect of VVs on increasing the limb volume since VVs developed in this study during these periods. The main reason for this may be because edema caused by VVs can be more severe and persistent and it can cause pain, itchiness, heaviness, and night cramps compared to edema caused by pregnancy alone. This suggests that the pain experienced by pregnant women with VVs is related to changes in the lower limb venous system, rather than the onset of pregnancy itself. Similar findings were demonstrated in the studies by (Bamigboye and Hofmeyr, 2006; Smyth, Aflaifel and Bamigboye, 2015).

Additionally, there was a slight increase in limb volume in the left limbs compared to the right limbs, but this difference was not statistically significant. The reason of this variation may be due to the increased size of gravid uterus which can contribute to the compression of the right common iliac artery which result in compressing the left common iliac vein causing reduced blood flow, elevated pressure in the pelvis and lower leg and therefore increased left limb volume was higher in cases of MTS. Several studies have observed the significant increase in left leg volume compared to the right leg in cases of MTS only (Traisrisilp *et al.*, 2021b; Florio *et al.*, 2022b). This study also found that the measurement of leg volume was associated with common iliac vein measurements. Patients who developed VVs had larger limb volumes that were correlated with compressed common iliac veins, while patients who did not develop VVs, had smaller limb volumes and larger common iliac veins. This observation was mentioned earlier in (Traisrisilp *et al.*, 2021).

Progesterone and estrogen hormones during pregnancy have a significant effect on the venous system (Lenkovic *et al.*, 2009; Serra *et al.*, 2016; Taylor, Hicks and Heller, 2018). According to previous epidemiological research, women are more likely to develop VVs in their lower extremities venous system (J. Beebe-Dimmer *et al.*, 2005;

Kucukguven and Raouf A. Khalil, 2013), and pregnancy increases the frequency of occurrence (Boivin, Cornu-Thenard and Charpak, 2000; Rabhi *et al.*, 2000b), with the highest occurrence in multiparous women (Coughlin *et al.*, 2001; Ismail *et al.*, 2016). The current study findings showed a general increase in the levels of serum progesterone and Estroil (E3) hormones during pregnancy in all pregnant women, reaching the highest levels in the third trimester followed by reduced levels to their normal values in the post-partum period. This increase in hormones is an important physiological change that occur during pregnancy to provide necessary support both mother and fetus (Kumar and Magon, 2012; Mandalà, 2020). Besides the normal increase in hormonal levels, the study findings also highlight those levels of progesterone and Estroil (E3) hormones were higher in patients who developed VVs compared to patients who did not develop VVs, and the difference was significant. This is because estrogen and progesterone relaxes smooth muscle and collagen fibers in the vein leading to vasodilation and the potential development of valves reflux and VVs (Mashiah, 1999; Raffetto and Khalil, 2021). Our research outcomes are in agreement with prior studies that detected a connection between the increased levels of estrogen and progesterone and the development of VVs (Mashiah, 1999; Ropacka-Lesiak, Kasperczak and Breborowicz, 2012a). Therefore, this study emphasises that progesterone and Estroil (E3) hormones are sufficient tests to evaluate hormonal factors causing VVs in pregnancy.

The increased levels of estrogen and progesterone hormone in pregnant women with VVs in comparison to their counterparts without VVs can be attributed to a multifaceted interplay of factors. Firstly, the observed hormonal increase may be associated with the onset of gestational diabetes, a condition that typically initiates during the second trimester of pregnancy and is concurrent with the escalation of progesterone and estrogen levels (Nadal et al., 2009). However, it is noteworthy that our study did not encompass the assessment of gestational diabetes, thereby representing a limitation in our analysis. Furthermore, genetic predisposition emerges as another possible factor contributing to these hormonal variations. Genetic factors can exert influence over hormone metabolism and receptor sensitivity, thereby leading to individual variations in hormone levels. Some individuals may possess a genetic predisposition characterised by elevated hormone levels, which, in turn, may contribute to the development of VVs.

In the last two decades, there have been multiple specialized scales created to measure CVI diseases. This could be because it's challenging to find an instrument that can apply to the broad range of CVD.The scales should be able to identify significant changes in QoL not only after the intervention but also during the physiological changes that occur during pregnancy. Therefore, this study used the CIVIQ-20 questionnaire to analyse the difference in the QoL between pregnant women who developed VVs and those who did not. This is because CIVIQ-20 is the only scale designed for CVD. Although CIVIQ-20 questionnaire lacks the stability in the social dimension which was reported in (Zappadu *et al.*, 2016; Sinožić *et al.*, 2017; Wu and Ma, 2021). This was because the social dimension in this questionnaire may not be relevant to all cultural or socioeconomic groups thus can not capture the social impact of venous disease on patients (Launois *et al.*, 2012). However, this study tried to overcome this issue by providing further explanation to patients for each question to adapt to different cultural contexts.

CIVIQ-20 questionnaire is designed to capture patients' subjective experiences and provide valuable insights into the patients' perspective. CIVIQ-20 has high degree of reliability as it can effectively differentiates the severity of symptoms and clinical CEAP classes (de Almeida *et al.*, 2022). CIVIQ-20 demonstrates good responsiveness and a high level of sensitivity to change, and it is acceptable and easy to complete by patients (Launois, Mansilha and Jantet, 2010). This study also explained that CIVIQ-20 is a suitable QoL scale for longitudinal studies due to its brevity, five-point Likert scale format that enhances sensitivity, and absence of items that show a highest or lowest effect leading to limited or no differentiation among individuals who score at those extremes. Its ability to detect changes effectively makes it an ideal QoL scale for randomized clinical trials. The extensive use of the CIVIQ-20 in recent years provides confirmation of this fact. Studies have been published previously on the use of CIVIQ-20 questionnaire to evaluate the effect of CVI diseases on patients (Launois, Mansilha and Jantet, 2010; Radak *et al.*, 2013; Özdemir *et al.*, 2016; Xia *et al.*, 2022), to evaluate the effect of compression therapy (Vayssairat, Ziani and Houot, 2000; Martínez-Zapata *et al.*, 2008) to differentiate between the effect of surgical methods for VVs (Gavrilenko *et al.*, 2006; Kalteis *et al.*, 2008a; Alghamdi *et al.*, 2021) and post venous stenting (Neglén *et al.*, 2007), drug therapy (Martínez-Zapata *et al.*, 2008;

Guex *et al.*, 2009), to evaluate the effect of of Neuromuscular Electrical Stimulation (NMES) on women with VVs (Abu *et al.*, 2020) and physical therapy (Carpentier and Satger, 2009). These previous studies have demonstrated that CIVIQ-20 scale produced valid results when used to assess the QoL in most cases.

Results of the CIVIQ-20 questionnaire in this study showed that QoL worsen from baseline reaching the highest CIVIQ-20 scores (poorer QoL) in the third trimester followed by lower scores (better quality of life) post-partum. This is because pregnancy is a time of change that brings about significant physical physiological, mental, and emotional transformations (Setse *et al.*, 2009). Although normal pregnancies may be free from complications, however, the alterations that occur can impact the well-being of expectant mothers and have consequences for the health of both the mother and baby. One instance of this is that increased size of gravid uterus especially in the third trimester may impede movement of the chest or create an extra burden on the cardiac load resulting in chest constriction (Ramlakhan, Johnson and Roos-Hesselink, 2020). As a result, the physical and mental well-being of pregnant women alters throughout the duration of pregnancy. Our research results align with earlier studies that evaluated the QoL in uncomplicated pregnancies, even when employing different QoL scales. The study by (Wu *et al.*, 2021) used EuroQoL Group's visual analog scale (EQ-VAS) to evaluated QoL in pregnant women with no complications and found that the QoL was at its peak (good QoL) during the initial stages of pregnancy, which remained consistent throughout pregnancy but declined to the lowest levels (poor QoL) towards the end of term because of various physical and mental changes like pain and discomfort. Also, the study by (Haas *et al.*, 2005) used the (SF-36) assessment tool in pregnancy and found that QoL worsen during pregnancy and improved in the post-partum period. Low quality of sleep along with poor QoL were demonstrated in the third trimester compared to early pregnancy in the study by (Sut, Asci and Topac, 2016).

Moreover, the present study revealed that the group of patients who developed VVs during pregnancy and post-partum periods had higher scores indicating poorer QoL, as compared to the patients who did not develop VVs. The statistically significant difference in QoL scores was observed only in the third trimester of pregnancy. Not only are women with VVs in the legs susceptible to experiencing intense discomfort,

pain, and swelling especially in the third trimester of pregnancy, which can have adverse effects on their physical and emotional health, but the physical and physiological changes that come with pregnancy can further impact their well-being. These symptoms may limit their ability to perform everyday activities and lead to a lower QoL compared to pregnant women who do not have VVs. This fact reinforces the notion that pregnant women with VVs experience a greater burden of lower limb pain, which is associated with alterations in the lower limb venous system, in addition to the challenges of pregnancy. There is lack of studies that assessed QoL using CIVIQ-20 questionnaire in patients with VVs during pregnancy. Although a previous study by (Khryshchanovich *et al.*, 2022) utilized the CIVIQ-20 scale to assess QoL in pregnant women with VVs, its main objective was to evaluate the effectiveness of elastic compression stockings. To our knowledge, this study was the first study to use CIVIQ-20 questionnaire to assess the QoL in each trimester of pregnancy and in the post-partum to correlate the findings with the development of VVs.

5.2 Conclusion

In conclusion, VVs are a common indication of CVI that manifests as an abnormal twisting and dilation of the superficial veins in the lower limbs. Although VVs are prevalent during pregnancy, the underlying pathophysiology of this condition remains poorly understood. The signs and symptoms of VVs in pregnancy are similar to those observed in the general population and vary based on the stage and severity of the disease. Complications of VVs can significantly affect patients' QoL and pose a substantial economic burden on society. This study comprehensively assessed venous function in pregnant patients throughout each trimester and post-partum period to investigate the pathophysiology of VVs during pregnancy. These findings have important implications for improving our understanding of VVs and informing the development of effective preventative and therapeutic strategies.

This study has identified several demographic factors that are associated with the development of VVs, including older age, multiparity, positive family history of VVs, higher BMI, occupations involving prolonged standing, and white race. It is crucial to

emphasise that these risk factors do not operate in isolation but rather interact in a multifactorial manner. Additionally, this study utilised DUS assessment to investigate changes in the diameter of the common iliac veins during pregnancy, demonstrating that healthy patients return to normal measurements post-partum. However, in patients with VVs, a statistically significant decrease in diameter was observed, particularly on the left side with more severe compression, which can lead to the development of MTS in some cases. These findings were consistent with the observation that patients who developed VVs had higher fetal weight with higher maternal BMI in the third trimester compared to those who did not develop VVs. These insights contributed to a better understanding of the pathophysiology of VVs during pregnancy and highlighted the importance of monitoring venous function in pregnant patients to prevent the development of complications such as MTS.

This study also provides valuable insights into the changes in venous changes in pregnant women. The findings suggest that due to hormonal changes, the diameter of both deep and superficial veins gradually increases during pregnancy, with a return to normal values in the post-partum period. Moreover, the use of DUS and VFI revealed that venous reflux occurs predominantly in the LSV during the third trimester of pregnancy and the post-partum period, with higher RT and PRV measurements observed in patients who developed VVs. The study also highlights the limitations of DUS in estimating PRV due to its angle-dependency and advocates for the use of PRV as the most consistent parameter for detecting venous reflux. The use of VFI, which is angle-independent, was found to be more sensitive in detecting changes in blood flow velocities than DUS. This highlights that VFI is a precise diagnostic tool used to assess the mechanical factors responsible for the development of VVs during pregnancy. Nevertheless, both DUS and VFI examinations were found to be reproducible and reliable imaging techniques for detecting venous reflux during pregnancy.

Furthermore, this study employed the PPG technique to evaluate venous reflux using the VRT parameter. While the PPG technique does not have the ability to pinpoint the anatomical location of reflux, it showed 100% consistency with DUS findings in detecting lower limb venous reflux and was also able to accurately assess the severity of reflux, indicating a moderate venous reflux in patients who developed VVs in this

study. These findings highlighted the potential of PPG as a reliable alternative for assessing venous reflux, albeit with certain limitations. This study also revealed that limb volume measurements were significantly higher in pregnant women who developed VVs compared to those who did not. This finding can be attributed to the more severe and persistent edema resulting from VVs, which exacerbates the expected increase in leg volume during pregnancy. This implies that the pain experienced by pregnant women with VVs is primarily due to changes in the lower limb venous system rather than the pregnancy itself. Additionally, the study found that patients with VVs had larger limb volumes that were associated with compressed common iliac veins, while patients without VVs exhibited smaller limb volumes and larger common iliac veins.

In addition to the expected increase in serum progesterone and Estroil (E3) hormone levels during pregnancy, this study has revealed that higher levels of these hormones were observed in patients who developed VVs compared to those who did not. The study findings suggest that estrogen and progesterone induce relaxation of smooth muscle and collagen fibers in the veins, leading to vasodilation and the potential development of venous reflux and VVs. Thus, the study emphasizes the significance of progesterone and Estroil (E3) hormone levels as reliable markers for evaluating hormonal factors associated with the development of VVs during pregnancy. Moreover, this study demonstrated by using CIVIQ-20 questionnaire a decline in QoL from baseline, with the highest scores observed during the third trimester and lower scores post-partum. The study also revealed that pregnant women who developed VVs had significantly higher scores, indicating a poorer quality of life compared to those who did not develop VVs. These results emphasize the importance of early detection and management of VVs during pregnancy to improve the QoL of affected women. Overall, the results of this study this study confirmed that VVs in pregnancy caused by both mechanical and hormonal factors. These results contributed to the growing body of literature on the pathophysiology of VVs on pregnant women, providing valuable insights into the that VVs in pregnancy can negatively impact the overall well-being of pregnant women. This highlights the need for accurate and early detection, prompt treatment, and appropriate preventive measures to mitigate the negative impact of VVs on pregnant women's QoL.

5.3 Limitations and Future Research:

The primary constraint encountered in this investigation pertains to the limited sample size employed. Given the multifaceted nature of this study, wherein the development of VVs is influenced by a confluence of multiple risk factors, the necessity for a substantial sample size becomes evident. A larger cohort also is important to facilitate a robust comparison and contrast among the disparate risk factors. This includes, but is not confined to, the capacity to discern distinctions among participants stemming from the same risk factor, as exemplified by the suboptimal representation of patients within the racial risk factor subgroup due to limited numbers. The small sample size in this study can be attributed to the challenges associated with enrolling and retaining pregnant women for follow-up visits across various stages of pregnancy and post-partum. Each follow-up visit involved a comprehensive set of six assessments, including gathering demographic data, performing DUS and VFI scans, conducting PPG examinations, evaluating lower limb volume, administering hormonal blood tests, and assessing QoL. These assessments can be time-consuming, and given the demands of pregnancy and the routine antenatal care, some pregnant women may not have adequate time to participate in all of the evaluations. Future studies can address the issue of small sample size by first, adopting a multi-center approach, which would involve collaborating with multiple hospitals or clinics in different geographic locations. This would serve to increase the number of participants and ensure greater representation of the target population, resulting in a more diverse and inclusive sample of pregnant women.

Secondly, to address the issue of time-consuming study procedures and minimize the burden on participants, potential solutions could include streamlining the assessment process and prioritising the most critical assessments. For example, certain assessments could be given higher priority based on their relative importance, and conducted first to maximize participation and reduce attrition. Furthermore, leveraging technological advancements such as automation and mobile health solutions could simplify the data collection process and minimize the time and effort required from the participants. Finally, to address the difficulties of enrolling and retaining pregnant women in the study, offering incentives or rewards could be beneficial. These may consist of cash incentives, vouchers for necessary items or services, or non-monetary

incentives like educational resources. These methods may help overcome the challenges associated with a small sample size and improve the study's chances of achieving its research goals.

One additional limitation when using questionnaires to collect data on family history, there is a possibility of recall bias as individuals with venous disease may have greater awareness of the prevalence of the disease among their family members or they may not have complete knowledge of their family members' medical conditions. Therefore, relying solely on self-reported family history may not provide a complete and accurate picture of an individual's risk for certain diseases. However, in this study, we minimised the potential for recall bias by gathering family history information at the beginning of the study. Therefore, based on the self-reported data collected in this study, there is evidence of a genetic predisposition for developing VVs. To minimise bias, future studies may need to incorporate other factors like genetic testing or medical examinations.

Another constraint of our study is that we utilised manual compression of the vein to determine venous reflux, which is comparatively more convenient and comfortable to administer than automated cuff compression. However, the latter method yields more consistent results and is easier to reproduce. Therefore, it is recommended to use automated cuff compression in future studies to ensure consistent and replicable results. A further limitation of this study pertains to the challenge of ensuring patients comprehend the correct technique for performing the compression and release (augmentation) which was employed in conjunction with manual compression of the vein to assess venous reflux. Such difficulty may potentially impede the accurate estimation of the RT parameter in DUS. For future studies, it is necessary to implement measures to ensure that patients have a better understanding of the compression and release (augmentation), as this can lead to improved estimation of the RT parameter. Moreover, as the current investigation is the pioneering attempt at employing the Truncated cone method for assessing lower limb volume during pregnancy, it is imperative to corroborate the efficacy of this technique in future research endeavors to corroborate the current study's findings.

Further, prospective research future could investigate the use of PPG with other imaging modalities to overcome its limitations, such as the incapability to determine

the anatomical location of reflux and facilitate a more comprehensive evaluation of venous reflux. Also, the PRV parameter in DUS and VFI is an important measure used to detect the severity of venous reflux in patients. This parameter is determined based on certain cut-off point values, which may vary depending on the specific study or clinical setting. However, despite its clinical utility, there is no universally agreed upon or fixed guidance regarding the cut-off points for determining the severity of venous reflux using this parameter. This can potentially lead to inconsistencies in the interpretation and diagnosis of venous reflux, and may impede the development of standardized diagnostic criteria and treatment protocols. Additional research is necessary to establish more conclusive and evidence-supported recommendations for the PRV parameter when evaluating venous reflux. Additionally, research should focus on developing more effective diagnostic tools for identifying VVs in pregnant patients, such as the use of imaging techniques and other non-invasive methods. This would allow for earlier detection and intervention, which could improve outcomes and reduce the risk of complications.

While the present study, along with some prior investigations, has shown an association between the emergence of MTS and VVs in pregnant patients, additional research is necessary to confirm this correlation. Such research may entail gathering data from a more substantial cohort of pregnant patients with MTS and VVs, utilising more robust research methodologies, and employing statistical analyses to assess the magnitude and statistical significance of the relationship. Further, since the augmented levels of estrogen and progesterone hormones in pregnant women with VVs are underpinned by a complex interplay of factors, including the potential influence of gestational diabetes and genetic predisposition. Nevertheless, it is imperative to acknowledge that the absence of gestational diabetes assessment in our study serves as a notable limitation that warrants consideration. Future studies should aim to build upon the findings of our research and investigate additional factors that may contribute to the development and progression of VVs in pregnant women. This could include examining the role of genetics, lifestyle factors, and other medical conditions that may increase the risk of VVs. Future studies should also prioritise prevention of VVs in pregnant women by identifying effective interventions. These interventions may include lifestyle modifications, such as physical activity and dietary changes, as well as prophylactic measures such as the use of compression stockings. The ultimate

objective of future research should be to enhance the management of VVs in pregnancy and decrease the impact of this condition on both pregnant women and the healthcare system.

6 References

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7 Appendix:

Patient demographic form

Patient's surname: _____	**Patient's first name:** _____
Date of birth: ____/____/_____ dd mm yyyy	**Patient's hospital number:** _____
Hospital's name: _____	**Visit date:** ____/____/_____ dd mm yyyy

Varicose veins risk factors:

1. **Race:**
   - ☐ White
   - ☐ Asian
   - ☐ Black or African-American
   - ☐ Arab
   - ☐ More than one race
   - ☐ Unknown or not reported

2. **The number of pregnancies:**
   - ☐ First pregnancy
   - ☐ Second or more pregnancies

3. **Is there any family history of varicose veins?**
   - ☐ No
   - ☐ Yes (specify which parent _____).

4. **Height=**_____cm

   Weight= _____kg

   BMI=_____(kg/M^2).

5. **Occupational factors: In daily basis, do you usually spend your time:**
   - ☐ Mostly standing
   - ☐ Mostly setting

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Appendix1: The demographic form used in this study