The evaluation of the pathophysiology of Varicose veins in pregnancy

Version 2

16/05/2021

Main Sponsor: Imperial College London

Funders: Saudi Cultural Bureau Study coordination centre: Vascular Laboratory

Sponsor's/protocol number: 20HH5790

NRES reference: XXXX

NCT number: IRAS Project ID: 277754

Protocol authorised by:

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This protocol describes the evaluation of the pathophysiology of Varicose veins in pregnant women by taking blood samples for genomic and hormonal testing. Venous blood reflux and patency will be evaluated by using Duplex ultrasound and vector flow imaging. This proposed project will also assess the limb volume using Truncated cone tool. Following this, iliac vein compression will be evaluated using Photoplethymography (PPG) technique. Finally, patient's quality of life will be assessed by using Aberdeen Varicose Veins Questionnaire (AVVQ). Every care was taken in its drafting, but corrections or amendments may be necessary. These will be circulated to investigators in the study. Problems relating to this study should be referred, in the first instance, to the Chief Investigator. This study will adhere to the principles outlined in the UK Policy Framework for Health and Social Care Research It will be conducted in compliance with the protocol, the Data Protection Act and other regulatory requirements as appropriate.

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List of Abbreviations

VV	Varicose veins
CVI	Chronic venous insufficiency
NHS	National Health Service
VFI	vector flow imaging
PPG	Photoplethymography
AVVQ	Aberdeen Varicose Veins Questionnaire
DUS	Duplex ultrasound
CFV	common femoral vein
FV	femoral vein
LSV	long saphenous vein
SSV	short saphenous vein
TO	Transverse oscillation
C1	Circumference at the end of segment length 1
C2	Circumference at the end of segment length 2
L	Segment length
VRT	venous refiling time
E3	Estriol
CEAP	Clinical-Etiology-Anatomy-Physiology

Study summary

Title: The evaluation of the pathophysiology of Varicose veins in pregnancy.

Design: Prospective longitudinal study.

Aim: The primary aim of this proposed study is to better understand the pathophysiology of VV in pregnant women.

Outcome measures: Venous patency, blood flow direction, peak reflux velocity, circumferences (C1, C2) at the end of each segment length (L), BMI, VRT, Genomic DNA blood test, Estroil (E3) levels, progesterone serum levels, outcome of quality of life.

Population: 70 Random pregnant women.

Eligibility: Pregnant women aged between 18-47 years old.

Duration: 1 year, follow up period will be conducted at the end of first, second and third trimester and three months after giving birth.

1 Introduction

1.1 Background

Varicose veins (VV) are the early sign of Chronic venous insufficiency (CVI) which are characterised by abnormal tortuosity and dilatation of superficial veins of lower limb extremities (Naoum *et al.*, 2007). Symptoms of VV are variable and depend on the stage and severity of disease. In early stages, symptoms of VV include pain, leg heaviness and mild swelling (Adhikari *et al.*, 2000). As the disease advances, symptoms include skin changes such as pigmentation, lipodermatosclerosis and ulceration (Kendler *et al.*, 2010; Shadrina *et al.*, 2018). Pregnancy is an established risk factor for the development of VV (Ismail *et al.*, 2016). Three mains hypotheses for this observation have been proposed. First, it was hypothesised that increased size of gravid uterus during pregnancy may result in increased venous pressure on iliac veins resulting in a disruption of lower limb venous outflow (Mashiah *et al.*, 1991). However, VV may develop in the first trimester before the onset of these mechanical factors (Dindelli *et al.*, 1993). Secondly, pregnancy results in an increased blood volume which may lead to elevate pressure in lower limb vasculature (Ismail *et al.*, 2016). Both theories suggest that high pressures may result in venous valve impairment (Bergan *et al.*, 2006). Thirdly, VV in pregnant women were found to be associated with increased estrogen and progesterone levels (Mashiah *et al.*, 1991; Ortega, Asunsolo, Alvarez-Rocha, *et al.*, 2018). 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 (Ropacka-Lesiak, et al., 2012). Moreover, large number of studies have discussed the associated risk factors that contribute to VV development. However, there are lack of studies that consider VV risk factors and pathophysiology in pregnant women. Understanding the aetiology of VV in pregnancy may allow us to better identify those at risk and manage this risk accordingly.

1.2 Rationale for current study

Regardless of the underlying pathophysiological cause, VV may have a negative social impact on patient's life and negative economic impact on health care budget. VV if left untreated can progress to late stages of chronic venous insufficiency (CVI) which include skin changes and ulceration (Adhikari *et al.*, 2000). Ulcers lower patient's quality of life and may result in social isolation, depression, reduce the ability to work and patients will be dependent on others (Evans *et al.*, 1994; Cesarone *et al.*, 2002). Furthermore, venous disease is costly to manage for healthcare service. For example, the cost of managing chronic wounds caused by ulcers in the UK National Health Service (NHS) during 2005 to 2006 was estimated from 2.3 to 3.1 billion pounds which was equivalent to 3% of total UK health care budget (Posnett, J. and Franks, 2007). Interestingly, the cost was increased between 2012 to 2013 when cost was estimated at 5.3 billions of pounds annually which consume 4% of the total health UK health care budget (Guest *et al.*, 2015).

1.3 Research hypothesis:

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

2 Study aim and objectives:

The primary aim of this proposed study is to better understand the pathophysiology of VV in pregnant women. This aim will be achieved by meeting the following objectives:

Primary objective:

To develop an antenatal test to predict the risk of developing VV post-partum.

Secondary aims:

1. To evaluate mechanical factors causing VV in pregnancy
2. To evaluate hormonal factors causing VV in pregnancy.
3. To determine whether lower limb pain during pregnancy is related to changes in lower limb venous system.

3 Methods

3.1 Study design:

A prospective longitudinal study will be undertaken to assess the development of VV among pregnant women. This study will follow up patients for one year, starting by following up pregnant women from the first trimester of pregnancy to the end of the second and third trimester. Last follow up will be performed three months after giving birth. Consequently, this proposed prospective study will be conducting four follow ups for each participant.

3.2 Recruiting pregnant women in their first trimester:

Initially, patients will be approached by a member of the direct care team in the Maternity outpatients' departments in the following hospitals: West Middlesex hospital, Queen Charlotte's and Chelsea hospital and St Mary's hospital. This will be managed by experienced research professionals in the area of vascular surgery. If a patient is prepared to discuss their potential role in this research, then the researchers will discuss the study and recruitment details at the patient's convenience. Recruitment is completely voluntary and will not affect their routine care, this will be made clear during the discussion regarding the research, and again during the consenting process. Time will be made available for any questions and for the patient to consider recruitment in their own time. Patients will be required to consent before any study-related procedures are carried out. Then her personal identifiable data will be coded and pseudonymised.

The recruited patient will be interviewed to obtain clinical information that is essential for robust analysis. This clinical information will include standard demographic form which will be used for all patients in each follow up in order to record and gather important information which represents the composition of the target population. Gathered data will include patients' details; patients name, day of visit, hospital name, ID number, date of birth. Furthermore, this demographic form will record potential risk factors which may associate with VV development such as race, number of

pregnancies, family history of venous disease, BMI which include weight and height, occupational factors, smoking, alcohol consumption and diet. Any complications arising from pregnancy will be also recorded (appendix 12.1). Finally, this proposed study will only record fetal weight in second and third trimester to correlate increased fetal weight with potential venous pathology.

First, patients will be asked to fill the demographic form then will be asked to stand on a scale to measure the weight. Height will be recorded by bringing the horizontal measure down to rest on the top of the participants head. Following this, patient will be asked to sit while measurements are recorded in a relevant source data sheet.

Next, the patient will be asked to stand on a step facing the sonographer to assess the incidence of varicose veins using Duplex ultrasound (DUS) and vector flow imaging (VFI). While patient is standing, Truncated cone tool will be performed immediately by measuring leg circumferences in each segments of each limb to produce accurate measurements of lower limbs volume. Following this, patient will be asked to sit on a chair for perform Iliac vein compression test (venous outflow function) using photoplethysmography (PPG) technique. Finally, patient's quality of life assessment will be evaluated by using Aberdeen Varicose Veins Questionnaire (AVVQ) check list (Klem, Sybrandy and Wittens, 2009). Thereafter, the patient will be thanked for their participation and excused.

Clinical Laboratory tests will be managed and performed by experienced research professionals in the area of vascular surgery. Four appointments will be arranged for each patient to investigate DNA genomic testing and Estriol (E3) and progesterone hormonal testing. First appointment during first trimester will include both genomic DNA and hormonal blood tests. The three remaining appointments in the second trimester, third trimester and three months after giving birth will include hormonal blood samples only. Patients will be entitled to exit the study at any time without providing a reason, this is clearly stated on our information sheet and consent form. Verbal consent will be requested before all follow-up ultrasound scans.

Researchers will ensure that demographic data, ultrasound data, lower limb volume and iliac vein compression measurements, histopathological results and quality of life

assessment results will be collected and stored in such a way that confidentiality is preserved. All data will be pseudonymised by replacing names with research codes and by keeping any identifying information separate from the main dataset.

The data will be analysed with the help of the Imperial College statistics department. The results of this study may be presented at local, national or international meetings or submitted for publication in a peer-reviewed journal. From a patient's perspective, all data will be pseudonymous before the statistical analysis phase of the study and plans to publish the results of the study are made clear in our patient information sheet. Data and all appropriate documentation will be stored for a minimum of 10 years after the completion of the study, including the follow-up period.

3.3 Study group protocol

3.3.1 Protocol for gathering patient's demographic information

Recruited pregnant women will be asked to take a seat to fill a demographic form which contains patients' personal details (name, date of birth, hospital number, date of visit and hospital name) and other varicose veins risk factors (see appendix 12.2), filling this form may take up to 5 min. Following this, BMI risk factor will be calculated by measuring patients' weight and height.

First, weight scale will be placed flat on the level ground. Scale will be ensured is at zero before commencing. Patient will be asked to remove heavy clothes and empty pockets. Preferably, shoes should be removed. Following this, patient will be asked to stand, and weight reading will be recorded when it has stabilised. Weight will be recorded in kilograms and to the nearest 0.1 kg. Second, height will be measured by asking the patient to remove their shoes and stand unsupported with their back to the vertical scale. Patients feet should be parallel to each other while standing, toes pointing forward and soles flat on the floor. Also, patient should be ensured that she is standing as tall as possible looking straight ahead. Horizontal measure will be brought down to rest on the top of the participants head. The measurement on the vertical scale to the nearest millimetres will be recorded on the relevant source data

sheet. Measuring weight and height will take 5 min, which means collecting patients' demographic factors will take 10 min in total.

3.3.2 Protocol for Duplex ultrasound scan (DUS)

All recruited pregnant women be scanned by duplex ultrasound scan (DUS) to investigate the evidence of VV. The examination will be bi lateral for each patient. The patient will be asked to remove their clothes to expose the lower limb from groin to ankle. Patient will be examined standing to evaluate valves competency against gravity. This is because veins are more dilated while standing which make it easier and more accurate to assess. This examination is considered intimate; therefore, a chaperone will be offered. The scan duration will take 30-45 min and patients may sometimes feel dizzy during the examination. To overcome this issue, patients will be asked to sit and take a rest during the examination.

There are two main techniques which will be applied to all venous segments. Firstly, B mode technique. B mode technique will be used to evaluate vein patency in transverse scan plane by observing the compressibility of veins to rule out any possibility of blood clots. Secondly, Spectral (Pulse wave) doppler technique will be used to evaluate venous flow characteristics (phasicity, spontaneity and direction flow) in longitudinal scan plane by applying manual distal augmentation to assess the reflux duration. All scans and measurements will be performed on SuperSonic Imagine's Aixplorer ® ultrasound system. Following this, 9 MHz linear ultrasound probe will be placed on the patient skin over the venous blood vessel to be scanned, with the use of coupling gel between the skin and the probe.

The scan will start by evaluating the deep and superficial veins system. Firstly, deep veins will be assessed by scanning the Common femoral vein (CFV) at the groin for patency and flow. Any abnormality in CFV indicates iliac vein obstruction therefore iliac veins and inferior vena cava should be examined. The examination will be continued by scanning the length of femoral vein (FV) (proximal to distal), popliteal vein and calf veins. Any thrombus or incompetence (Incompetent veins are defined as a reflux time more than 0.5 seconds) will be recorded.

Secondly, superficial venous veins will be assessed after completing the deep venous system examination. This will be achieved by starting again from the groin to evaluate the patency and competency of both sapheno-femoral junction and the entire length of long saphenous vein (LSV). This will be coupled with assessing all venous perforators and branches. LSV diameters will be recorded in case of any required future treatment. After that, examining the sapheno-popliteal junction and short saphenous vein (SSV) will be performed by repositioning the patient to assess patency and competency. Therefore, VV found in LSV or SSV should be evaluated to detect linked sources of reflux such as reflux in perforators. Finally, all recordings will be saved and entered in the research database for analysis.

3.3.3 Protocol for Vector flow imaging (VFI):

Ultrasound machines currently use conventional Spectral (Pulse wave) doppler to assess the reflux duration in varicose veins protocols. Although spectral doppler is widely used, however, it is limited in detecting accurate peak reflux velocity estimation due to two main reasons. Firstly, its angle-dependency which means it can only detect blood velocities along the direction of ultrasound beam. Secondly, due to the anatomical location of lower extremities superficial veins. To overcome this issue, Vector flow imaging (VFI) which is a new ultrasound technique can be used. This technique is a beam steering and angle independent technique which can detect accurate peak reflux velocity estimation. VFI has been used recently in evaluating the velocity of lower limb veins and obtained promising results (Bechsgaard *et al.*, 2016, 2017).

While the recruited patient is standing, she will be scanned by VFI which is based on transverse oscillation (TO) ultrasound method. VFI software will be installed in the same SuperSonic Imagine's Aixplorer ® ultrasound scanner. Again, 9 MHz linear ultrasound probe will be placed in longitudinal scan plane on the patient's skin over the venous blood vessels to be scanned, with the use of coupling gel between the skin and the probe. The scan will start by emitting conventional Pulse wave doppler estimation. Following this, by using special estimator and changing apodization in receive, both axial and transverse velocity components will be found at the same time.

Then, blood flow will appear in a colour box, a colour wheel will indicate the direction of the blood flow and overlaid arrows in the colour box will make the interpretation of flow direction and magnitude easier. Finally, the patient will be asked to rest and take a seat. All recordings will be saved and entered in the research database for analysis.

3.3.4 Protocol for Limb volume assessment

Weight gain during pregnancy is well known. This occur not only due to fetal growth and uterus enlargement, but also due to water retention and increased blood volume which are required for fetal circulation. This excessive water retention especially in the third trimester is coupled with accumulated fat cause swelling (odema) especially in both lower limbs. Therefore, measuring limb volume during pregnancy is considered significant to estimate water and fat amounts gained during pregnancy and correlate these findings with potential VV incidence. This proposed study will assess the lower limb volume based on girth measurements using Truncated cone method. This method has the advantage of measuring bilateral limb volume oedema where no comparator (unaffected) limb is available. This method measures limb volume by manual perimetry using tape measure to find limb circumference. Tape measures tension may be inconsistent which can change the limb circumference measurement by up to 3% (Cheah *et al*., 1989). Therefore, this study will use spring-loaded controlled tension device to keep the tape in closer contact with the skin without compressing the underlying tissue.

Measurements will be taken while the patient is standing. Circumferences will be measured every 10 cm from the sole up to the seventh measurement. A single researcher will measure all patients to prevent differences in tension on the tape. The data will be processed using the truncated-cone formula (Campanholi, Duprat Neto and Fregnani, 2011):

$$V = h\,(C1 \times C2 + C1^2 + C2^2) / 12\pi$$

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 centimetres.

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

Finally, Patient will be asked to rest and take a seat on a chair, circumferential measurements will be entered into computer software programmes or pre-programmed calculators to calculate limb volume of each recruited patient.

3.3.5 Protocol for Iliac vein compression test (venous outflow function)

This proposed study will examine iliac vein compression (venous outflow function) in pregnancy using photoplethysmography (PPG) technique. This is due to the difficulty of examining iliac veins using ultrasound in pregnancy due to increased size of gravid uterus. Large size of gravid uterus in pregnancy causes iliac veins compression which makes iliac veins hard to investigate. PPG is a non-invasive method used to evaluates the severity venous reflux by measuring the venous refiling time (VRT). VRT is considered to be abnormal when the refilling time is measured (less than 20 seconds). PPG technique has low specificity compared to other imaging tests and it is considered not accurate in locating the areas of reflux. However, alongside with its capability of evaluating the degree of reflux, it can also predict the treatment outcomes (Cheng and Wong, 1995).

The principal of PPG works by using a small light source and a photoelectric cell attached to the skin. Produced light will transmit the skin and will be scattered by the tissue and partially absorbed. Scattered light will emerge out passing the skin to be detected by photoelectric cell. Photoelectric cell will be attached on the side or opposite the source of light to monitor blood perfusion based on transmission and reflection modes. Following this, the intensity of the light signals detected by photoelectric cell will be converted to PPG signals.

In this proposed study, patient will be seated with her both legs dangling on the floor. PPG sensor will be attached to the patient's skin in the medial supramalleolar area of the leg (the distal lower medial area of the leg). The exam will start by asking the patient to flex and extend one foot (five times), while the other floor placed on the floor.

Following this, a traced graph will appear on the machine's screen. When the trace is stable, it means that it is the end of the VRT. The exam will be repeated to evaluate the filling time of the second leg. All measurements will be recorded immediately.

3.3.6 Protocol for blood tests (genomic and hormonal blood tests)

Genetic risk factors are reported to play an important role in the aetiology of varicose veins. In this proposed study, we aim to estimate the heritability of varicose veins among recruited pregnant women by taking a 30-ml blood sample for genomic DNA testing. This will be performed once in the first trimester. Results will be entered to the research database for analysis and correlation with the UK bio bank study (Fukaya *et al.*, 2018).

Along with the genomic blood samples, hormonal blood samples will be taken from patients in each trimester and three months after giving birth. This is because elevated estrogen and progesterone hormones levels were linked to VV development (Mashiah *et al.*, 1991; Ortega, Asunsolo, Leal, *et al.*, 2018). Progesterone serum blood test will be conducted to evaluate the progesterone levels during pregnancy and correlate the result with the incidence of VV. Normal progesterone serum levels varies according to the trimester of pregnancy. For instance, according to the NHS, progesterone levels in the first trimester estimates between 6.9 to 470 nmol/L, 71.6 to 300 nmol/L in the second trimester and 88 to 770 nmol/L in the third trimester. Therefore, progesterone hormone reaches the highest levels in the third trimester which suggests the potential effect of this hormone on venous pathology in late pregnancy.

In addition, estrogen hormone blood test will be tested to associate the effect of estrogen with VV incidence. Estrogen hormone has three main types which are estrone, estradiol and Estriol and each type has different function. Estrone (E1) is the main hormone in females which is found after menopause. Estradiol (E2) is a female hormone found in non-pregnant women and involved in regulating menstrual cycles. Estriol (E3) is a type of estrogen hormone produced with higher amounts in pregnancy. This proposed study will investigate Estriol (E3) and progesterone serum blood test in each follow up which include first, second, third trimesters and three months after

giving birth in order to correlate hormonal changes with any potential venous pathology incidence.

Therefore, four appointments will be arranged for each patient to investigate DNA genomic testing and Estriol (E3) and progesterone hormonal testing. First appointment during first trimester will include both genomic DNA and hormonal blood tests. The three remaining appointments in the second trimester, third trimester and three months after giving birth will include hormonal blood samples only. At the end of the study, these samples will be transferred to research tissue bank to store for future research.

3.3.7 Protocol for Quality of life assessment

Several quality assessment tools are used to measure the functional impact of a disease on patients' quality of life as perceived by the patient. Both generic and disease specific measures tools are used to estimate patient's quality of life status. This proposed study will use the Aberdeen Varicose Veins Questionnaire (AVVQ) quality of life assessment tool. AVVQ is a disease specific quality of life assessment tool which is developed in specific for patients with VV. Patients will be asked to answer 13 questions which assess patient symptoms such as pain, swelling, itching and ulcers. This tool also assesses the impact of VV on social and daily life activities and the use of support stocking. Perceived cosmetic impact is taken into consideration in AVVQ tool. Each question is designed to reflect the severity of the issue referred to in the question. AVVQ questionnaire is scored from 0-100, where 100 represents the most severe issues related to VV and lowest quality of life while zero represents the best quality of life. Finally, patients will be thanked for their participation and excused.

3.4 Clinical intervention and procedures

Clinical intervention and procedure	Time
- Consent - Clinical data collection - Blood samples (genomic+ hormonal) - DUS - VFI - Truncated cone tool - PPG - AVVQ	First trimester (0-13 weeks)
- Clinical data collection - Blood sample (hormonal only) - DUS - VFI - Truncated cone tool - PPG - AVVQ	Second trimester (14-26 weeks)
Same as second trimester	Third trimester (27-40 weeks|)
Same as second trimester	Three months after giving birth

4 Participant entry

4.1 Pre-registration evaluation

Blood test for pregnancy conformation.

4.2 Inclusion criteria

- Pregnant women who are aged between 18 to 47 years old
- Patients who are willing to be followed up for 1 year and agreeing to give the informed consent will be included in the inclusion criteria.

4.3 Exclusion criteria

- Any incomplete cases such as intrauterine death.

- Participants who fail to attend the scans after their first visit will be excluded.

- Patients with congenital vascular abnormalities/syndromes such as Klippel Klippel-Trenaunay syndrome.

- Patients who are unable to provide consent.

- Anyone who are taking part in any other research.

- Potential participants who might not adequately understand verbal explanations or written information given in English, or who have special communication needs.

- Patients with signs of acute deep vein thrombosis.

- Patient suffered from severe injury of their great-saphenous vein.

4.4 Recruitment and follow up period

Recruitment period will take up to three months. Follow up period will be conducted at the end of first, second and third trimester and three months after giving birth.

4.5 Withdrawal criteria

Participants choice

5 Adverse events

5.1 Definitions

Adverse Event (AE): any untoward medical occurrence in a patient or clinical study subject.

None are expected during this study.

Serious Adverse Event (SAE): any untoward and unexpected medical occurrence or effect that:

- **Results in death**

- **Is life-threatening** – refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe

- **Requires hospitalisation, or prolongation of existing inpatients' hospitalisation**
- **Results in persistent or significant disability or incapacity**
- **Is a congenital anomaly or birth defect**

Medical judgement should be exercised in deciding whether an AE is serious in other situations. Important AEs that are not immediately life-threatening or do not result in death or hospitalisation but may jeopardise the subject or may require intervention to prevent one of the other outcomes listed in the definition above, should also be considered serious.

5.2   Reporting Procedures

All adverse events should be reported. Depending on the nature of the event the reporting procedures below should be followed. Any questions concerning adverse event reporting should be directed to the Chief Investigator in the first instance.

5.2.1 Non-Serious AEs

All such events, whether expected or not, should be recorded.

5.2.2 Serious AEs

An SAE form should be completed and faxed to the Chief Investigator within 24 hours. However, death due to eclampsia and any health complications worsened during pregnancy does not need reporting as SAEs.

All SAEs should be reported to **the North of Scotland Research Ethics Committee** where in the opinion of the Chief Investigator, the event was:
- 'related', ie resulted from the administration of any of the research procedures; and

- 'unexpected', ie an event that is not listed in the protocol as an expected occurrence

Reports of related and unexpected SAEs should be submitted within 15 days of the Chief Investigator becoming aware of the event, using the NRES SAE form for non-IMP studies. The Chief Investigator must also notify the Sponsor of all SAEs.

Local investigators should report any SAEs as required by their Local Research Ethics Committee, Sponsor and/or Research & Development Office.

> **Contact details for reporting SAEs**
> **jrco@imperial.ac.uk**
> **m.aslam@imperial.ac.uk**
> **Fax: 02083832083 attention Dr Mohammed Aslam**
> **Tel: 02083831541 (Mon to Fri 09.00 – 17.00)**

6 Incidental findings:

A lay summary of aggregated results will be provided to all participants. If there is an incidental finding such as DVT, which likely have a major impact on patient's medical management then the doctor treating her will be informed and participant will be removed/ withdrawn from the study.

7 Assessment and follow up

All previously mentioned interventions and procedures including (DUS, VFI, Truncated cone tool, PPG, hormonal blood test, AVVQ questionnaire) will be performed four times (in first, second, third trimester once and three months after giving birth). DNA genomic blood test will be performed once only in the first trimester of pregnancy. No

further follow-up required after this time point. The study expected time for each participant is 12 months in total.

7.1 Study closure

The study will be closed after one year (9 months pregnancy + 3 months after giving birth) the last 12 months appointment of the last recruited patient is completed, and all data have been received.

7.2 Study outcome measures

	Intervention/procedure	Outcome measures
Primary outcome	DUS	- Patency - Blood flow direction(antegrade/retrograde) - Peak reflux velocity
Secondary outcomes	VFI	- Patency - Blood flow direction(antegrade/retrograde) - Peak reflux velocity
	Truncated cone tool	- C1 - C2 - h
	PPG	Severity of venous reflux by measuring Venous refiling time (VRT) (Abnormal less than 20 sec)
	Genomic blood test	Genomic DNA
	Hormonal Blood test	Estroil (E3) levels, progesterone serum levels
	AVVQ questionnaire	Outcome of quality of life (0-100). 0 least severe (good quality of life), 100 most severe (low quality of life).

8 Statistics and data analysis

8.1 Sample size

An exact binomial test with a nominal 5% two-sided significance level will have 91.27% power to detect the difference between the null hypothesis proportion, π_0 of 0.25 and the Alternative proportion, π_1, of 0.1 when the sample size is **70**.

8.2 Analysis plan

All analyses will be performed based on the intention of follow-up. The analysis population will include all participants enrolled in the study, including those who developed varicose veins during pregnancy and those who do not.

Analysis of primary and secondary outcomes:

Venous patency and Peak reflux time recordings from VFI scan will be correlated with Venous patency and peak reflux time recordings from DUS scan. This will be performed using descriptive summary statistics. Mixed multivariable logistic regression will be used to model venosus patency and peak reflux velocity in each scan.

Secondary outcome which include measuring the circumferences (C1, C2) and length of segments (h) using Truncated cone method. Calculated volume will be determined by summing segment volumes derived from the truncated cone formula. Pearson product moment correlations, paired t tests, and linear regression tests will be used to assess relative association and absolute differences between calculated volumes in each follow up.

Secondary outcome which include PPG will be analysed by comparing the mean value for the venous refilling time (VRT) by Student's *t* test, agreement between DUS and PPG classification was expressed as a kappa (***K)*** value.

Genomic DNA blood samples results will be correlated with the UK biobank study. Test statistics will be used to identify genetic variants that influence varicose vein susceptibility.

Each hormonal blood samples results including Estroil (E3) and progesterone serum levels will be correlated with the results of the next follow up to provide comprehensive information about increased hormonal levels in pregnancy and varicose veins development.

Outcome of quality of life statistical analysis will be performed using the Statistical Package for the Social Sciences (SPSS 23.0) for Windows. Evaluation of AVVQ consisted of quality of life in first trimester, second trimester, third trimester and three months after giving birth will be done using the Friedman test.

8.3 Data analysis:

The data obtained will be subjected to statistical analysis by an independent biostatistician. SPSS Version 23.0 or/ and STATA Version 10.0 will be used for statistical analysis. Measures such as the mean and standard deviation or median and interquartile range will be used to summarise continuous variables such as age and BMI. Tables reporting the numbers and percentages will be used for categorical variables such as varicose veins and smoking. Chi square or Fisher's exact test will be used to compare categorical variables such as varicose veins and smoking at the final trimester. The t-test or Mann-Whitney test will be used to compare continuous variables such as age with varicose veins at the final trimester. The paired t-test or Wilcoxon signed-rank test will be used to compare BMI between first and third trimester. The Kappa statistic will be used to compare DUS and VFI regarding direction and velocity. Longitudinal analysis will be used to look at factors associated with varicose veins. Factors include age, BMI, smoking, ethnicity, pregnancies and family history. A significance value (p-value) less than 0.05 is considered statistically significant.

9 Regulatory issues

9.1 Ethics approval

The chief investigator has obtained approval from the Research Ethics Committee and HRA. This study must also receive confirmation of capacity and capability from each participating NHS trust before accepting participants into the study or any research activity is carried out. The study will be conducted in accordance with recommendations for physicians involved in research on human subjects adopted by the 18th World Medical Assembly, Helsinki and later revisions.

9.2 Consent

Consent to enter the study must be sought from each participant only after a full explanation has been given, an information leaflet offered, and time allowed for consideration. Signed participant consent should be obtained. The right of the participant to refuse to participate without giving reasons must be respected. After the participant has entered the study the clinician remains free to give alternative treatment to the specified in the protocol at any stage if she feels it is in the participant's best interest, but the reasons for doing so should be recorded. In these cases, the participants remain within the study for the purposes of follow- up and data analysis. All participants are free to withdraw at any time from the protocol treatment without giving reasons and without prejudicing further treatment.

9.3 Confidentiality

The Chief Investigator will preserve the confidentiality of participants taking part in the study and is registered under the provisions of the Data Protection Act 2018.

9.4 Indemnity

Imperial College London holds negligent harm and non-negligent harm insurance policies which apply to this study.

9.5 Sponsor

Imperial College London will act as the main Sponsor for this study. Delegated responsibilities will be assigned to the NHS trusts taking part in this study.

9.6 Funding

Saudi Arabian Cultural Bureau in London is funding this study. No payment will be made to participants and to researchers.

9.7 Audits

The study may be subject to inspection and audit by Imperial College London under their remit as sponsor and other regulatory bodies to ensure adherence to GCP and the UK Policy Framework for Health and Social Care Research.

10 Study management:

The day-to-day management of the study will be co-ordinated through Dr Mohammed Aslam (CI).

11 Publication policy:

The results of this study may be published in peer-reviewed scientific journals or conference presentations.

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13 Appendix:

13.1 Complications arising from pregnancy (this is a general information and won't be given to the participant).

Some pregnant women may experience complications during pregnancy. These complications may affect the mother's health or fetal health or both. The most common pregnancy complications include high blood pressure, gestational diabetes, preeclampsia placenta previa and infections. Pregnancy complications will be recorded in this study for each participant in order to correlate any potential complication with the incidence of VV and to generate comprehensive analysis at the end of this study.

Preeclampsia is one of the main pregnancy complications which cause fetal morbidity and mortality. Early signs of preeclampsia include high blood pressure and accumulated amounts of proteins in urine which is known as proteinuria after 20 weeks of pregnancy (Oettinger-Barak *et al.*, 2005). Untreated preeclampsia may lead to eclampsia which involves seizures that may result in coma, brain damage and fetal death. Preeclampsia is aggravated by several risk factors including older age, nulliparous, chronic hypertension, high BMI>30 and gestational diabetes during pregnancy.

High blood pressure and Gestational diabetes mellitus (GDM) are serious pregnancy which result in maternal and fetal complications. Hypertension arises when arteries carrying blood from the heart to body organs are narrowed which thus cause high blood pressure in arteries. In pregnancy, hypertension reduce amount of blood flow containing nutrition and oxygen to the placenta. Consequently, this will affect fetal growth and further complications such as preterm delivery and preeclampsia. On the other hand, GDM is identified as glucose intolerance developed during pregnancy

period at any grade and whether insulin or diet adjustment is used for treatment (Spaight *et al.*, 2016). Pregnant women with GDM are at higher risk of developing diabetic 2 after pregnancy (Berger *et al.*, 2016). Furthermore, GDM is coupled with high rates of caesarean delivery, increased obesity, fetal macrosomia which may cause birth complications such as stillbirth and shoulder dystocia (Lefkovits, Stewart and Murphy, 2019).

Placenta previa, placenta accreta and vasa previa are also critical pregnancy complications. Placenta previa occur when the placenta lies low in the uterus less than 2 cm from the cervix or covers the cervix completely (Oyelese and Smulian, 2006). This can lead to further complications such as bleeding at labour and premature delivery. Placenta accreta is known as abnormal implantation of the placenta in the uterus (Silver, 2015). This condition is vital and cause severe bleeding at labour (Oyelese and Smulian, 2006). Vasa previa arise when fetal umbilical cord blood vessels is located near or across the cervix internal opening. At labour, umbilical cord blood vessels will rapture causing fetal bleeding and critical neonatal mortality and morbidity (Ruiter *et al.*, 2015).

Infections may develop during pregnancy and cause further maternal and fetal complications. These infections may be caused due to several factors including immune system changes during pregnancy, hormone fluctuations, stress and mycobiome such as viral, bacteria and fungus (Azwa, Marsh and Hawkins, 2012). Infections during pregnancy may be passed from the mother to her fetus causing critical complications such as preterm labour, congenital anomalies and maternal sepsis (Lauretti and McKay, 2008).