PREVALENCE OF ASYMPTOMATIC THROMBOPHILIA AMONG PREGNANT WOMEN ATTENDING ANTENATAL CARE CLINIC AT MPIGI HEALTH CENTER IV

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DECLARATION

I, Sekeba James, declare that this work is original, and to the best of my understanding it has never been submitted to any other university or other institution of higher learning for an academic award. Where such information pertinent to the subject has been cited, the source has been acknowledged.

Signature: …… Date: ……

APPROVAL

This is to certify that this dissertation has been done under my supervision and is ready for university examination.

Signature:……. Date: …….

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DEDICATION

This dissertation report is dedicated to my family for the financial assistance, untiring support and encouragement whenever I felt low, for the great love and care. Special thanks goes out to you Nakkazi Hajarah for standing with me throughout the entire process, my children Nabukko Elizabeth, Namakula Hannah and Bijja Nathan, you have been of great help to me. May the almighty God keep you and bless you all the days of your life.

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LIST OF OPERATIONAL DEFINITIONS

Thrombophilia is a condition described by the body's tendency to form clots inaptly. It presents as either hereditary or acquired.

Hereditary thrombophilia is a condition that arises from antithrombin deficiency, or protein C or protein S deficiency.

Acquired thrombophilia occurs due to anti-phospholipids, and is characterized by thrombosis and presence of lupus anticoagulant as well as phospholipid-binding antibodies.

Pulmonary embolism is the sudden blockage of a major blood vessel (artery) in the lung, usually by a blood clot. In most cases, the clots are small and are not deadly, but they can damage the lung. But if the clot is large and stops blood flow to the lung, it can be deadly.

LIST OF ABBREVIATIONS

APCR	Activated Protein C Resistance
APL	Anti-phospholipid
DVT	Deep Vein Thrombosis
FVL	Factor V Leiden
MTHFR	Methylenetetra hydrofolatereductase
PAI	Plasminogen Activator-Inhibitor
VTE	Venous Thromboembolism
DIC	Disseminated Intravascular Coagulation

ABSTRACT

Background: Shortened coagulation tests (Thrombophilia) are a marker of hypercoagulability observed in pregnancy and immediate postpartum period. While this is beneficial for preventing maternal hemorrhage, at the same time also predisposes the women for multiple complications. This study determined the prevalence of thrombophilia, the associated socio-demographic factors among pregnant women attending antenatal care clinic at Mpigi Health Center IV.

Methods: This was a cross sectional study in which adult consented pregnant women attending Mpigi Health Centre IV were enrolled. Four milliliters of citrated blood were taken off, and spun to obtain hemolysis free plasma which was later analyzed for coagulation tests of prothrombin time (PT), activated partial thromboplastin time (aPTT) and thrombin time (TT). In addition, socio-demographic characteristics of the participant were recorded using a questionnaire. Data were analyzed, and presented as tables and a pie-chart. Logistic regression was used to establish the associated factors of thrombophilia, and variables with a p-value ≤ to 0.05 was considered statistically significant.

Results: The study enrolled 136 pregnant women, their mean age was 29.7 years (range; 18 to 39 years). Most (50.9%, N=69) of the women were in different relationships, and a single participant (0.74%) reported a history of thrombophilia. The coagulation parameters of the participants varied by gestation period. The mean PT was 9.27 seconds with SD of ±1.13, while the mean a PTT was 35.59 ± 4.95 seconds. The mean TT was 27.51± 3.78 seconds. Thrombophilia was observed in 17 out of 136, giving a prevalence of 12.5% (95% confidence interval: 9.10 – 14.92). Age category, gestation period and history of thrombophilia showed a significant association ($p<0.05$).

Conclusion: This study has found a high prevalence of thrombophilia, and there cases were typically characterized by delayed diagnosis. This necessitates the need to establish early diagnosis and establish the need for anticoagulation therapy to prevent adverse pregnancy outcomes.

CHAPTER ONE: INTRODUCTION

1.0 Overview

This chapter introduces the study topic, problem statement, significance of the study, objectives of the study, research questions, justification of the study and conceptual frame work.

1.1 Background

Thrombophilia is the affinity of the body to easily form blood clot (Greer, 2015). It is linked to a varied group of coagulation disorders mostly, deep vein thrombosis (DVT) and pulmonary embolism (Kamel*et al*., 2014)**.**Amongst the known thrombophilias is antithrombin III deficiency, prothrombin G20210A gene mutation, proteins S and C deficiency, activated protein C resistance and the ant phospholipid syndrome(Bates*et al*., 2012)**.**

Thrombophilia can also result from genetic mutations, acquired or an interaction of the two (Zotz *etal*., 2008; James, 2011). Hereditary thrombophilia is as a result of antithrombin, proteins C and S deficiency and prothrombin gene variant 20210A. There is also substantialimpact by genetic defects like activated protein C resistance (APCR) due to factor V Leiden, G 20210 A polymorphism on the prothrombin gene, increased factor VIII plasma levels or hyper-homocysteinemia (McLintock *et al*., 2012; Chan*et al*., 2014). On the other hand, the acquired thrombophilia is due to anti-phospholipid syndrome(Green-top Guideline, 2015). Other causes are linked to coagulation factor deficiencies, such as factors XI, VIII, IX, and fibrinogen.

Pregnancy is a hyper-coagulable state that is associated by a 4 – 5 times hypercoagulable trend as compared to the normal state (Greer, 2015). The established pathophysiological mechanism is that during pregnancy, clotting factors I, VII, VIII, IX, and X rise; protein S and fibrinolytic activity diminish; and resistance to activated protein C develops (Zotz *et al.*, 2008; Springel *et al.*, 2016). Thrombophilia is known to affect between 0.8 – 2.0 per 1000 pregnancy, at the same time, it accounts for 1.1 deaths per 100,000 pregnancies(Brenner and Aharon, 2007; Di Minno *et al*., 2015). Owing to this, thrombophilia is described as an independent predictor of morbidity and mortality among women of child bearing age (Holzhauer*et al*., 2012; WHO, 2014). It is associated with an increased risk of thrombo-

embolism, pregnancy loss and adverse obstetric outcomes (Mahmoodi*et al*., 2010; Connors, 2017).

Though it is under reported, thrombophilia is a significant cause of global peripartum morbidity and mortality(Elisabeth*et al.,* 2015).In a surveillance study bySpringel *et al*., (2016), it was revealed that three hundred and thirteen women died of thrombophilia associated risks between the years 2006 to 2010,signifying 9.3% of all pregnancy related death. Further, various thrombophilias have been reported; for example, Factor V Leiden (FVL) mutation affects 2-10%, Methylenetetrahydrofolatereductase (MTHFR) mutation is reported among 8-16%, prothrombin gene mutation affects 2-6%, proteins C and S deficiencies affects 0.2-1% while anti-cardiolipid antibodies are reported among 1-7% (Brenner and Aharon, 2007; Di Minno*et al*., 2015).

According to World Health Organization (2014), as the risk of thrombophilia remains high, laboratory screening and confirmation of the at-risk pregnancies is recommended. In Uganda, although fetal maternal complications are reportedly high, it remains unknown whether thrombophilia is implicated in these, as the present practice neither indicates nor screens for thrombophilia.

1.2 Problem statement

Thrombophilia in pregnancy is linked to severe preeclampsia, intrauterine growth retardation, abruption placentae and still birth. It is progressively on the rise mainly as a result of altered life style. Women with severe thrombophilia reveal a high outright risk for pregnancy-associated venous thromboembolism regardless of family history(Zotz *et al*., 2008; Chan*et al*., 2014). Owing to this, it has been widely recognized that pregnant women be screened for thrombophilia, and those affected be considered for routine antenatal thromboprophylaxis (Bucciarelli*et al*., 2013).

At Mpigi Health Centre IV, the review of 2016 and 2017 antenatal records indicated that out of 1803 and 2611 pregnant women attended to respectively, a significant number presented with one or more complaints related to the risk of thrombophilia (Hospital records, unpolished). A personal talk with the attending in-charge of antenatal clinic indicated that on a weekly basis, at least 4 referrals are made to specialized hospitals in Kampala, and these are

symptomatic of thrombophilia. At one of the continuing medical education (CME) session, it was brainstormed that majority of the antenatal care attendees exhibited obvious signs, some required obstetric emergence (Hospice CME records, 2017, unpublished). To this, it is evident that the risk of thrombophilia in pregnancy is high, however, there is paucity of data on the exact magnitude of thrombophilia in terms of its prevalence. This has impeded the development of evidence-based risk stratification to guide thromboprophylaxis as a potential intervention. Whereas it is recommended that all patients with a history of prior venous thrombotic events and those with these characteristic adverse pregnancy events should be evaluated for thrombophilia, in Uganda, this is neither indicated, nor done. Thus, results of this research will assist in providing knowledge and documentation of thrombophilia in our set up.

1.3 Objectives

1.3.1 Main objective

To determine the prevalence of asymptomatic thrombophilia among pregnant women attending antenatal care clinic at Mpigi Health Center IV.

1.3.2 Specific objectives

i) To determine the prevalence of thrombophilia among pregnant women attending antenatal care clinic at Mpigi Health Center IV

ii) To explore the associated socio-demographic factors and the most affected age group of thrombophilia among pregnant women attending antenatal care clinic at Mpigi Health Center IV.

1.4 Research questions

i) What is the prevalence of thrombophilia among pregnant women attending antenatal care clinic at Mpigi Health Center IV?

ii) What are the associated socio-demographic factors and the most affected age group of thrombophilia among pregnant womenattending antenatal care clinic at Mpigi Health Center IV?

1.5 Study justification

The results from the study will be used to put forward the associated socio-demographic factors and the burden to acquiring thrombophilia, so that subsequent interventions are put forward towards reducing the associated morbidity and mortality among pregnant women.

The study will be useful in providing timely obstetric care and intervention to such at risk women before the pregnancy effects are passed on

The study report shall still benefit future researchers by providing reference information to those interested in the similar study.

The findings of this study will be compiled into a dissertation as a partial fulfillment of the requirements for the award of a Bachelor's in Medical Laboratory Science of Clarke International University (CIU).

1.6 Conceptual frame work

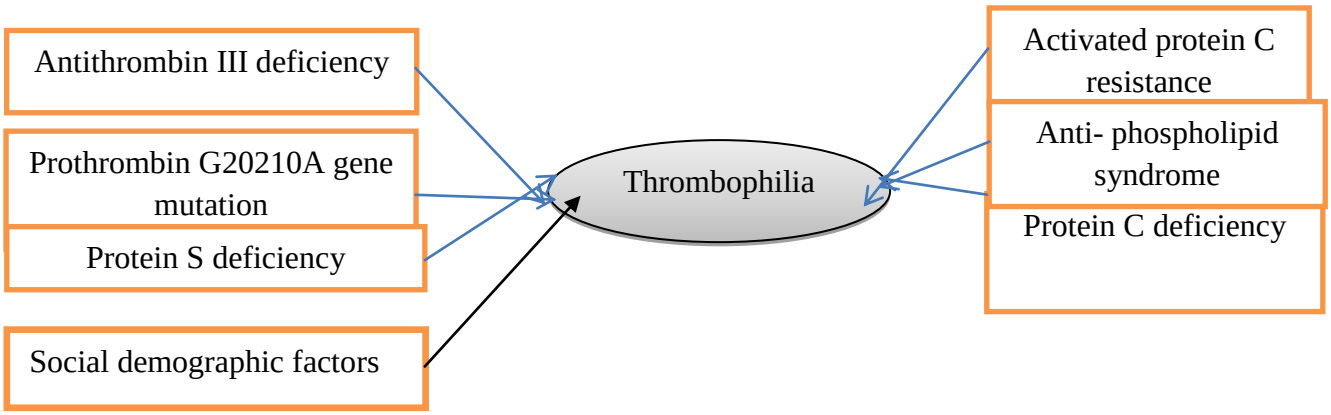


Figure 1:Conceptual frame work

Figure 1 shows the conceptual fame work. It denotes the cause effect relationship, in other terms exposure variable linked to the outcome predisposition (thrombophilia). Accordingly, the conceptual frame shows the interplay between dependent variable (thrombophilia) and the independent variables. The independent variables includes: antithrombin III deficiency, prothrombin G20210A gene mutation, proteins S and C deficiency, anti-phospholipid syndrome, activated protein C resistance and socio-demographic factors.

CHAPTER TWO: LITERATURE REVIEW

2.0 Introduction

This chapter presents the review of the related literature under the following titles of objectives; physiological changes in pregnancy, prothrombotic, genetic and inflammatory pathophysiology, hereditary and acquired thrombophilias, prevalence of thrombophilia among pregnant women, thromboprophylaxis to prevent obstetric complications and diagnosis of thrombophilias.

2.1 Physiological changes in pregnancy

Physiological changes occur in pregnancy that synergistically creates a hypercoagulable state and thus favors clot formation. Within the hemostatic system there is increased activity of coagulation factors: von Willebrand, factors V, VII, factor X and a gradual increase in factor VIII C. Increases in the levels of fibrinogen factors II, VII, X and XII may also be as high as 20-200% (Mahmoodi*et al*., 2010). While levels of antithrombin III and protein C remain constant, there is a fall in the free and total protein S (Connors, 2017). Major changes occur within the fibrinolytic system to meet the hemostatic challenges that is an increase in the levels of plasminogen, plasminogen activator antigen and tissue plasminogen activator(Kupferminc *etal*., 2011). The concentration and activity of plasminogen activator-inhibitor increases five-fold (PAI) (Kupferminc *et al*., 2011). These plasminogen activators ensure successive depression of fibrinolytic activity(Rubio-Jurado *et al*., 2012).

2.2 Prothrombotic, genetic and inflammatory pathophysiology

Numerous studies have indicated an association between the presence of a thrombophilia and adverse obstetric complications such as placental abruption, stillbirth, preeclampsia and recurrent miscarriage(Coumans *et al.,* 2009). The hypothesis is that the pre-existence of a thrombophiliaexaggerates the physiologically induced state of hypercoagulation causing micro-thrombi that disrupt the utero-placental perfusion(Kamel*et al*., 2014; Greer, 2015).

2.3 Hereditary Thrombophilia

2.3.1 Antithrombin deficiency

Antithrombin is an anticoagulant synthesized in the liver and endothelial cells. It has an inhibitory effect on thrombin, clotting factors X, IX, XI, XII and tissue factor bound VIIa(Bates, 2010; Bucciarelli*et al*., 2013). Antithrombin type Ideficiency is inherited as an autosomal dominant trait, and is associated with 235 mutations according to the human gene mutation (Stenson *et al*., 2009). Regarding obstetric complications, one study reported a significant increase of miscarriage in association with antithrombin deficiency compared to controls (22.3% versus 11.4% in controls)(Luxembourg *et al*., 2011). Another study revealed a fetal loss of between 28 to 32% in women with antithrombin III deficiency compared with 23% in unaffected controls(Benedetto*et al*., 2010; Middeldorp, 2013).

2.3.2 Prothrombin gene mutation

The prothrombin gene mutation is as a result of a defect in clotting factor II at position G20210A the G→A transition at nucleotide 20210 in the prothrombin gene. It is inherited as an autosomal dominant trait. The amount of plasma prothrombin is increased by 30% in heterozygous carriers and as much as 70% in homozygosity(Bafunno and Margaglione, 2010).The reported prevalence in Europe is around 2% to 6%(Bates, 2010).

2.3.3 Activated protein C resistance (APCR)

APCR causes extension of the activated partial thromboplastin time by interfering with the protein C pathway(Bates, 2010; Rubio-Jurado *et al*., 2012).Proteins C and S are key components of the anticoagulation pathway. Protein C is a natural anticoagulant and limits the conversion of fibrinogen to fibrin through the degradation of factors Va and VIIIa and activated protein C adopts a major role in the coagulation cascade. Activated protein C is only effective when bound to its cofactor protein S (Kocher *et al*., 2007).

2.3.3.1 Congenital/Hereditary APCR

The molecular basis for the hereditary defect is a point mutation in the factor V gene located on chromosome 1 (1691 G→A). This mutation has been coined the factor V Leiden mutation.The mutation causes the replacement of an amino acid arginine by glycine, resulting in diminished APC cleavage of factor Va and continued formation of thrombin by the

prothrombinase complex rendering the activated form of factor V, factor Va, less susceptible to proteolysis by activated protein C (Kocher *et al*., 2007; Rodger*et al*., 2010).

2.3.4 Factor V Leiden mutation

The presence of the factor V Leiden mutation produces a protein that is intrinsically resistant to activated protein C, causing the pathological phenotype. The factor V Leiden mutation accounts for a significant percentage of people with a thrombotic event or a family history of thrombosis. A prevalence as high as fifty percent has been quoted in familial studies of venous thromboembolism(Silver*et al*., 2010).

2.3.5 Protein C deficiency

The hereditary pattern of protein C deficiency is generally a pattern of autosomal dominance but exhibits with varying degrees of penetrance. Protein C is a naturally occurring vitamin K dependent protein that is produced in the liver. It is a key component of the protein C system. Upon activation by thrombin, a complex is formed between thrombin, thrombomodulin, proteins C and S. Protein S functions as an important cofactor in the inhibitory effect of protein C.Deficiency of protein C primarily causes a loss of function in the protein C gene and the majority of mutations result from single nucleotide substitutions(Stenson *et al.,* 2009; American College of Obstetricians and Gynecologists Committee, 2010).

2.3.6 Methylenetetrahydrofolatereductase deficiency and hyperhomocystinaemia

Increased homocysteine is an independent risk factor for venous thrombo-embolism. The 667 C→T MTHFR mutation results in a thermo labile enzyme with reduced activity for the remethylation of homocysteine. The homozygous form of the mutation induces a state of hyperhomocysteinaemia. Hyperhomocysteinaemia has a reported prevalence of around 5 % to 16 % in the general population(Rodger*et al*., 2010).

2.4 Acquired thrombophilia

2.4.1 Acquired hyperhomocystinaemia

Acquired hyperhomocystinaemia may result from dietary and lifestyle factors such as a reduced intake of folate, vitamin B6 or vitamin B12, excessive caffeine consumption and excessive coffee intake. Certain medical conditions such as hypothyroidism or renal

impairment may also cause the acquired form of hyperhomocystinaemia. The Homocysteine Lowering Trial Collaboration has suggested that endothelial dysfunction, alteration of platelet reactivity and disruption of prostacyclin pathways, may be some of the mechanisms responsible for the reported venous thrombosis risk as well as the theoretical risk of pregnancy loss. A meta-analysis of ten studies of recurrent pregnancy loss concluded that acquired hyperhomocystinaemia is a risk factor (Fogerty and Connors, 2009; Bates, 2010).

2.4.2 Acquired APCR

Although ninety-five percent of cases of activated protein C resistance are due to the factor V Leiden mutation, *in vitro* resistance to activated protein C may occur in the absence of the factor V Leiden mutation. This phenomenon is the entity of acquired activated protein C resistance (APCR) and may be induced by several factors. The various physiological alterations of the clotting factors during pregnancy may potentiate the development of acquired APCR (Dargaud*et al.,* 2009; Luxembourg *et al*., 2011).

2.5 Prevalence of thrombophilia among pregnant women

Global estimates of venous thromboembolic events in pregnancy is 200 per 100,000 deliveries (WHO, 2016) and the burden variesacross different continents. The risk of thrombophilia in Africa is almost 2.5-fold and is estimated at 500 per 100,000 (American College of Obstetricians and Gynecologists Committee, 2010; Greer, 2015). Thrombophilia remain a leading cause of death which has been estimated to range from 1.2 to 4.7 per 100,000 pregnancies in sub Saharan Africa (WHO, 2016). The wide burden is ascribed to both inherited and acquired risks. A multicenter study in Porland indicated that among 396 women with unexplained loss of at least one pregnancy 36 (9.1%) were carriers of inherited thrombophilia. Factor V Leiden mutation was present in 29 women (73%), prothrombin gene mutation G20210A in 6 (1.5%) and in 1 (0.3%) patient both mutations were detected (Skrzypczak*et al*., 2012).Another study among African women revealed a risk of between 28 to 32% (Mantha, Bauer, and Zwicker, 2010). In India, twenty-three of the 108 patients (21.3%) had thrombophilia markers (Luxembourg *et al*., 2011). The different thrombophilia among the Caucasians are: APCR 20%, prothrombin G20210A mutation 8%, antithrombin deficiency 4%, protein C deficiency 5% and protein S deficiency 3% (Luxembourg *et al*., 2013).

2.6 Associated socio-demographic factors and the most affected age group of thrombophilia among pregnant women

Pregnancy is an acquired and independent risk factor for the development of VTE. Acquired risk determinants can significantly increase the thrombotic risk further during pregnancy and the puerperium. The risk of thrombophilia is 5 times higher in pregnant women than in non-pregnant women of similar age (Bucciarelli *et al.,* 2013). These include maternal age (≥35 years), caesarean section, obesity, high parity (≥4), infection, and a personal or family history of VTE (Silver *et al*., 2010; Middeldorp, 2013; Greer, 2015). Other factors that increase the risk of thrombophilia among pregnant women includes; trauma or fracture, immobilization, long distance travel, hospitalization, catheterization, obesity, use of oral contraceptive or hormone therapy use, corticosteroid use, statin use, diet, physical activity, sedentary time, and acute infection (Di Minno *et al*., 2015; Connors, 2017).

2.6.1 Age and race/ethnicity.

Although thrombophilia affects all ethnicities and age category, its risk is morecommonin older individuals (Holzhauer*et al*., 2012). Although the role of aging to the risk of thrombophilia is not well understood, it has been proposed that blood coagulability may increase with age (Green-top Guideline, 2015). In addition, age effects are likely mediated by a higher prevalence of provoking risk factors for thrombophilia like immobility, hospitalization, and surgery (Chan*et al.,* 2014; Greer, 2015). The risk is further amplified by theincrease in hormone levels that impact women in their childbearing years (Greer, 2015).

2.6.2 Ethnicity

The risk of incident thrombophilia has been reported highest among the black individuals, then white individuals, and the lowest risk among Asian or Hispanic individuals (Mahmoodi*et al*., 2010). This is attributed to the genetic differences, and the role of environmental co-founding to disease pathogenesis.

2.6.3 Medication use

In a 2014 systematic review and meta-analysis, agents such as ethinyl estradiol with levonorgestrel were associated with a 50 to 80% lower risk of thrombophilia than those with gestodene, desogestrel, cyproterone acetate, or drospirenone (Bates*et al*., 2012). Further, a

population-based case-control study reported that among oral hormonal therapy users, current use of conjugated equine estrogens was associated with a 2-fold greater risk of incident thrombophilia as compared with current estradiol use (OR=2.08; 95% CI: 1.02, 4.27) (Greer, 2015).

2.6.3 Diet

Although the role of diet in the risk of thrombophilia is commonly considered, the relation of diet remains controversial. Some studies have evaluated the relation between specific nutrients or food groups, and some evidence has suggested that fruits and vegetables (Kamel*et al*., 2014) and alcohol (James, 2011).

2.7 Thromboprophylaxis to prevent obstetric complications

Despite conflicting evidence and lack of convincing data supporting the use of anti-thrombotics to prevent adverse obstetric outcome in women with thrombophilic disorders, the prescription of these is pervasive. Proponents favoring antithrombotic therapies tend to acquire results from small observational studies(Rodger*et al*., 2010). Others have simply extrapolated evidence from studies involving anti-phospholipid syndrome.The treatments that are usually favored are predominantly, various dosages of low dose aspirin, unfractionated heparin and low molecular weight heparin.

2.8 Diagnosing thrombophilia

Thrombophilia is diagnosed by having blood tests. The tests look for anticoagulant deficiencies. Laboratory diagnosis is based on investigation of the plasmatic anticoagulant pathways to detect prothrombin, thrombin and activated partial thromboplastin time. Other assays based on use of proteins C andS deficiencies, and anti-phospholipid antibodies/lupus anticoagulants have been documented. More tests include activated protein C (APC) resistance, factor V Leiden mutation and other mutations.

CHAPTER THREE: METHODOLOGY

3.0 Introduction

This chapter discusses the study design, study area, scope of the study and content scope, study population, sample size, sampling techniques, selection criteria, study variables, sample analysis, statistical analyses, ethical consideration and dissemination plan.

3.1 Study design

A cross sectional study was carried out among pregnant women of all age groups attending antenatal clinic. The study was conducted in the period of September and November, 2018.

3.2 Study population

The population included all pregnant women attending antenatal clinic at the time of study.

3.3 Study area

This study was carried out in Mpigi Health Centre IV and the study participants were recruited from the antenatal clinic in the outpatient department. This facility was chosen because no such study has been carried out there, yet 857 expectant mothers visit the site for antenatal services every month but coagulation studies are not part of the investigations carried out.

3.4 Sample size

The sample size was determined using the formula of (Kish and Leslie, 1965).

$n = \frac{Z^2pq}{d^2}$ where;

n = desired sample size; Z = standard normal deviate; usually set at 1.96 which corresponds to 95% confidence level; P= proportion in the target population estimated to have thrombophilia, in this case, a prevalence of 34.6% as reported from United States of America will be used; q= 1-p (proportion in the target population not having the particular characteristics); d= the degree of accuracy, usually set at 0.05 level; As there are no studies that have reported the prevalence of thrombophilia, 34.6% prevalence was considered, thus;

$n= 1.96^2 \times 0.346 \times 0.654 / 0.05^2 = 148$ pregnant women

3.5 Sampling technique

Consecutive enrollment sampling was the sampling strategy for use during sample collection for the research. This type of sampling involved choosing the pregnant women from the population until when the required sample size was attained. With this technique, participants had an equal chance of being selected. It was more feasible and less time consuming for the researcher thus reducing bias by estimating sampling errors easily leading to provision of accurate results thus representing a realistic magnitude of the problem.

3.6 Selection criteria

3.6.1 Inclusion criteria

All pregnant women attending antenatal clinic in Mpigi Health Centre IV and had consented to the study. We considered such women who had been on anticoagulation therapy for less than 30 days (Greer, 2015). In addition, the study enrolled participants who were willing to consent.

3.6.2 Exclusion criteria

Previously diagnosed patients with thrombophilia and on anticoagulation therapy for greater than 30 days were not be enrolled in the study.

3.7 Data collection

Data was collected using a researcher-administered questionnaires (Appendix ii) addressing the demographic factors of thrombophilia. Laboratory testing for coagulation defects were done to determine the percentage of thrombophilia among pregnant women.

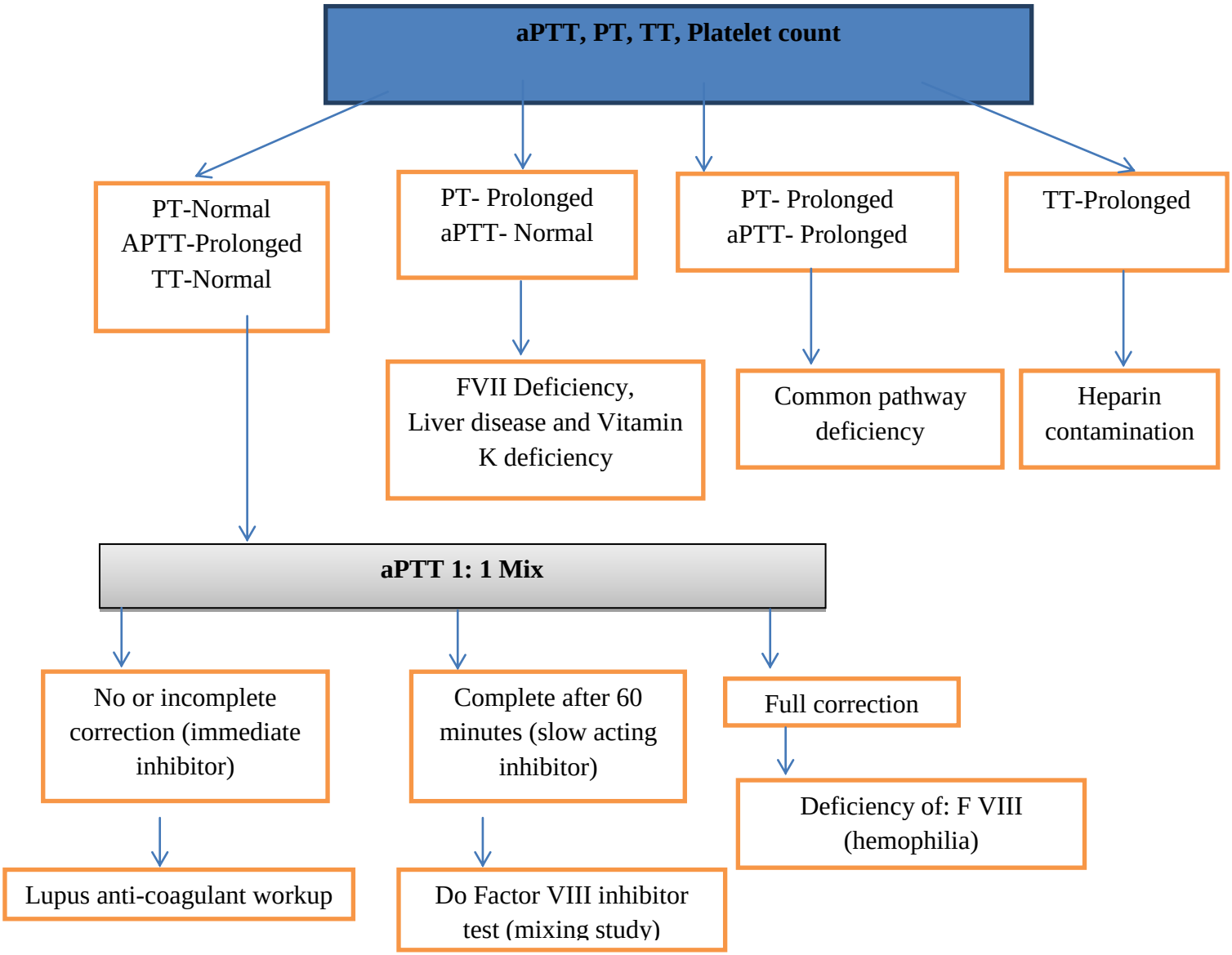
3.7.1 Sample collection

Blood samples were collected from the patients by venipuncture procedure (see appendix iii) and placed in citrates vacutainers labelled with patient name and hospital number to avoid mix ups. The blood draw procedure were carried out by a laboratory technician attached to Mpigi Health centre IV, with a diploma in Laboratory technology, and licenced by the Allied Health Professionals Council to legally practice. The laboratory technician is well qualified and competent enough as he had performed this activity for greater than 10 years. The obtained blood was then centrifuged at 1800 rpm for 5 minutes to obtain plasma. This was

used for batch analyses of differential tests, namely: prothrombin time (PT), activated partial thromboplastin time (aPTT) and thrombin time (TT).

3.8 Laboratory diagnosis

A basic coagulation workup (BCW) was performed to screen for asymptomatic thrombophilia. This included prothrombin time (PT), activated partial thromboplastin time (aPTT), platelet count and thrombin time (TT).The platelet count were done using an automated hematology analyzer. The coagulation profile was done using mixing study approach to identify factor deficiencies as illustrated in the study flow below;



3.9 Quality control

The test kits with coagulation reagents were stored at 2-8^{0}C, the plasma was stored at -20^{o}c analyzed as a batch. In addition, tests were done in duplicates, and an average value taken.

3.10 Data management

Data was primarily entered in Microsoft excel sheets for cleaning and sorting before importation to SPSS ver.16.0 for statistical analysis. Differences in proportions was assessed by chi-square test. P-value <0.05 was considered statistically significant at 95% confidence interval. Results obtained were presented using bar graphs, frequency table, and pie charts.

3.11 Dissemination plan

Copies of findings will be compiled into a report and submitted for marking to the institute of allied health sciences, IHSU for the award of a Bachelor's of Medical Laboratory Science. Copies of the research report will be submitted to the district health office, Mpigi district and a manuscript will be prepared and submitted in a peer reviewed journal.

3.12 Ethical consideration

The ethical approval was sought from Clarke International University Research and Ethics committee. Permission to carry on research was obtained from Mpigi Health Centre IV. Written informed consent was obtained from each study participant and results were handled with confidentiality (See appendix IV and V).

CHAPTER FOUR: RESULTS

4.0 Introduction

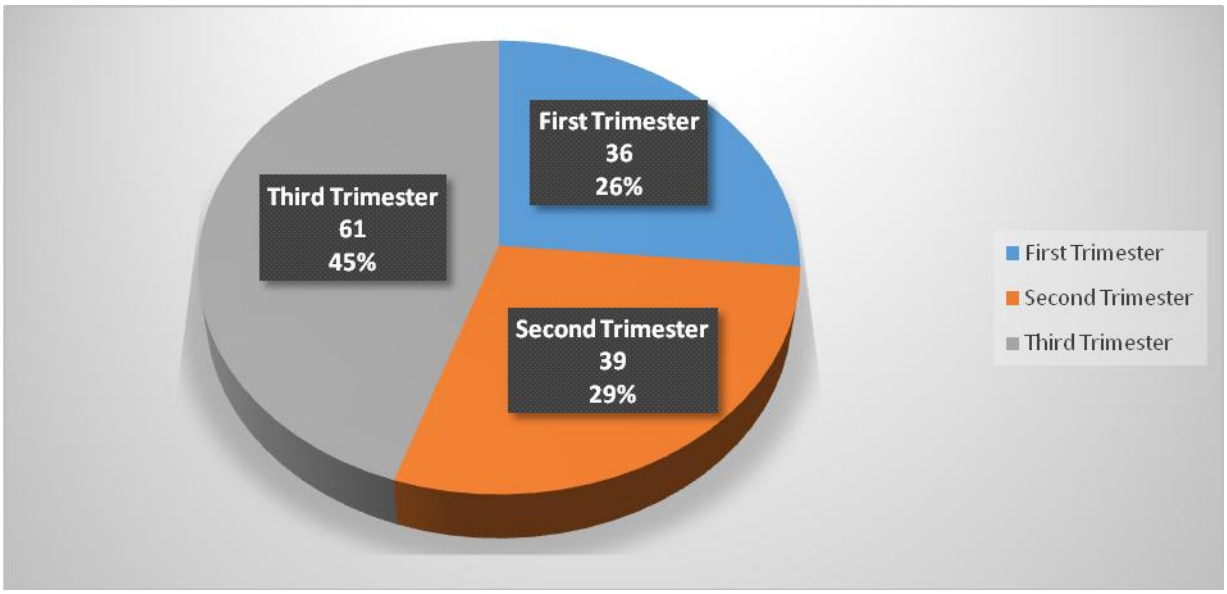
The study enrolled 136 pregnant women, their mean age was 29.7 years (range; 18 to 39 years). Majority (50.9%, N=69) were in a relationship, and only one participant (0.74%) reported a history of thrombophilia. Their demographic factors are given in table 1.

Table 1: Demographic characteristics of study participants

Variable	Frequency (n=136)	Percentage
Age category (Years)		
18-20	24	17.65
21-25	17	12.50
26-30	59	43.38
31-35	16	11.76
Above 35	20	14.71
Religion		
Catholic	59	43.38
Protestant	46	33.82
Muslim	3	2.21
Pentecostal	26	19.2
Others	2	1.47
Education level		
None	17	12.50
Primary	47	34.56
Secondary	63	46.32
Tertiary	9	6.62
Occupation		
Employed	69	50.73
Unemployed	67	49.26
Marital status		
Married	78	57.35
Divorced	21	15.44
Widowed	8	5.88
Others	29	21.32
Gestation period		
1st trimester	36	26.47
2nd trimester	39	28.68
3rd trimester	61	44.85
History of Thrombophilia		
Yes	1	0.75
No	135	99.25

Participants were in various gestational periods, as summarized in figure 1.

Figure 2:Distribution of study participants per gestation period



The coagulation parameters of the participants varied by gestation period. The mean PT was 9.27 seconds with SD of ±1.13, while the mean aPTT was 35.59 ± 4.95 seconds.The mean TT was 27.51± 3.78 seconds.

Thrombophilia defined by lowered coagulation test parameters(PT, aPTT and TT) was observed in 17 out of 136, giving a prevalence of 12.5% (95% confidence interval: 9.10 – 14.92). The coagulation pattern is given in table 2.

Table 2: Coagulation test parameters of the study participants

Coagulation test	**Frequency (n=136)**	**Percentage**
Normal PT	129	94.85
Shortened PT	7	5.15
Normal aPTT	134	98.53
Shortened aPTT	2	1.47
Normal TT	128	94.12
Shortened TT	8	5.88

Participants with multiple shortened coagulation tests were 7, as indicated in the table 3.

Table 3: Participants with shortenedmultiple coagulation tests

Coagulation tests	**Frequency (n=7)**	**Percentage**
PT and aPTT	4	57.14
PT and TT	2	28.57
aPTT and TT	1	14.29

The pattern of shortened PT, aPTT and TT according to gestation period is given in table 4.

Table 4: PT, aPTT and TT according to gestation period

Gestation period	Shortened PT (n=7)	Shortened TT (n=8)	Shortened aPTT (n=2)	Shortened TT and aPTT (n=1)	Shortened TT and PT (n=2)	Shortened PT and aPTT (n=4)
1st trimester	6	4	1	1	1	1
2nd trimester	1	2	0	0	1	2
3rd trimester	0	2	1	0	0	1

We used multivariate regression analysis to determine the sociodemographic factors associated with thrombophilia as summarized in table 5.

Table 5:Multivariate analysis of sociodemographic factors associated with thrombophilia

Variable	Crude Odds Ratio	Adjusted Odds Ratio	P-value
Age category (Years)			
18-20	1.00	1.00	0.002
21-25	1.23	0.78	
26-30	1.83	1.04	
31-35	2.81	2.02	
Above 35	2.88	2.31	
Religion			
Catholic	1.00	1.00	0.511
Protestant	0.19	0.11	
Muslim	1.01	1.07	
Pentecostal	0.21	0.71	
Others	1.19	1.02	
Education level			
None	1.00	1.00	0.781
Primary	0.99	1.03	
Secondary	0.87	0.87	
Tertiary	1.02	0.65	
Occupation			
Employed	1.00	0.91	0.812
Unemployed	0.87	0.38	
Marital status			
Married	1.00	1.00	0.382
Divorced	0.31	0.63	
Widowed	0.84	0.28	
Others	0.74	0.19	
Gestation period			
1st trimester	1.00	1.00	0.003
2nd trimester	1.96	1.81	
3rd trimester	2.42	2.46	
History of Thrombophilia			
Yes	1.00	1.00	0.001
No	0.97	0.89	

From table 5, age category, gestation period and history of thrombophilia showed a significant association ($P<0.05$) while religion, education level, occupation and marital status showed no association ($P>0.05$).

CHAPTER FIVE: DISCUSSION

5.0 Introduction

Pregnancy is a prothrombotic process, and is associated increase of procoagulant factorsand may aggravate inherited as well as acquired thrombophilia. Women with thrombophiliaought to be counseled as their condition may increase the risk of adverse pregnancy outcomes.The prevalence of thrombophilia was 12.5%. This value is higher than that from multicenteric study in Porland that reported a prevalence of9.1%(Skrzypczak *et al*., 2012).It is however lower than the 32% reported from Malawi (Mantha, Bauer, and Zwicker, 2010), and 21.3% from India(Luxembourg *et al*., 2013). The difference is explained by the variation in study populations for these studies.

The mean PT was 9.27 seconds with SD of ±1.13, while the mean aPTT was 35.59 ± 4.95 seconds. The mean TT was 27.51± 3.78 seconds. The coagulation parameters of the participants varied by gestation period.The associated factors for thrombophilia were age category, gestation period and history of thrombophilia. Age has been assessed as a factor of thrombophilia. In previous studies, the risk of thrombophilia was 5 times higher in older women, specificallymaternal age greater than ≥35 years (Holzhauer *et al*., 2012; Bucciarelli *et al.,* 2013). This is in agreement with a previous report by Sanka *et al*. (2017). Although the role of aging to the risk of thrombophilia is not well understood, it has been proposed that blood coagulability may increase with age (Green-top Guideline, 2015). In addition, age effects are likely mediated by a higher prevalence of provoking risk factors for thrombophilia like immobility, hospitalization, and surgery (Chan*et al.,* 2014; Greer, 2015). Also, thrombophilia showed a statistical association with the third trimester, as earlier reported (Hellgren, 2013; Lloyd R *et al.,* 2013). The explanation for this is the hypercoagulability seen towards term (Lloyd R *et al.,* 2013). History of thrombophilia is a genetic or acquired defect, which in this study showed a statistical association. This is similar to previous reports (Bucciarelli *et al.,* 2013; Lloyd R *et al.,* 2013; Chan*et al.,* 2014). Pregnant women with thrombophilia ought to be monitored, which was not the case. To this, it is clear that further studies are needed to address the risk of increasing and unknown thrombophilia in preventing pregnancy complications.

5.1 Conclusion

This study has found a high prevalence of thrombophilia, and the cases were typically characterized by delayed diagnosis. The study has also found that thrombophilia is associated with age category, gestation period and history of thrombophilia; this necessitates the need to establish early diagnosis and establish the need for anticoagulation therapy to prevent adverse pregnancy outcomes.

5.2 Recommendations

Based on this study, we recommend routine screening of pregnant women for the risk of thrombophilia, and those affected to be managed early to avoid pregnancy complications. In addition, for women with known risk factors for thrombophilia, they ought to be referred early to an obstetrician with expertise in thrombophilia. Based on these findings, we propose an extensive laboratory thrombophilia screening when a family history of thromboembolism has been recorded.

5.3 Limitations

This study did not establish specific gene abnormalities associated with the cases of thrombophilia. In addition, the study did not establish the genetic and acquired risks of thrombophilia among the study participants.

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APPENDICES

APPENDIX I: INFORMED CONSENT FORM (English Version)

Informed Consent Template

Informed Consent to Participate in Research

We are asking you to take part in a research study called: 'Assessment of asymptomatic thrombophilia among pregnant women attending antenatal care clinic at Mpigi Health Centre IV'

The person who is in charge of this research study is Sekeba James. The research will be conducted in Mpigi Health Centre IV, Mpigi district of Uganda.

Purpose of the study

The purpose of this study is to:

- The study will seek to determine the prevalence of thrombophilia and assess laboratory markers of thrombophilia among pregnant women attending antenatal care clinic at Mpigi Health Center IV.
- To determine the prevalence of thrombophilia among pregnant women attending antenatal care at Mpigi Health Centre IV.
- To determine that is by thrombophilia among pregnant women attending Mpigi Health Centre IV.
- To explore the associated socio-demographic factors and the age group most affected by thrombophilia among pregnant women attending antenatal care at Mpigi Health Centre IV.
- To disseminate information to local population on the burden of thrombophilia among pregnant women, and support campaigns to timely screening in regards to improved pregnancy outcomes.

Study Procedures

You are being asked to participate in this study, as you are a Ugandan woman who can help us to better understand the risk of thrombophilia during pregnancy.

If you take part in this study, you will be asked to:

- Provide about three milliliters of blood for laboratory analyses of coagulation tests

- Take part in a one-time, one-on-one, semi-structured interview; the interview will take approximately 25 minutes;
- The interview will take place at a location most convenient to you as the participant;
- The blood draw procedure will be carried out by a laboratory technician attached to Mpigi Health centre IV, with a diploma in Laboratory technology, and licenced by the Allied Health Professionals Council to legally practice. The laboratory technician is well qualified and competent enough as he has performed this activity for greater than 10 years;
- The questionnaire will be administered by the principal investigator, and he will ensure that all terms are as simplified as possible to smoothen the flow of discussion.

Benefits

There are no direct benefits in terms of treatment but participants will be given results for free especially if a participant has prolonged coagulation factors.

This will also provide a better understanding in regard to the burden of thrombophilia. In addition, this research will provide timely obstetric care and intervention to at risk participants before the pregnancy effects are passed on to the unborn child.

Risks or Discomfort

Pain may be incurred during blood collection but samples will be collected in a professional way to reduce pain.

Compensation

No form of compensation will be provided to the participant.

Privacy and Confidentiality

We will keep your study samples and records private and confidential. Certain people may need to see your study records. By law, anyone who looks at your records must keep them completely confidential. The only people who will be allowed to see these records are:

The research team, including the Principal Investigator and those involved with the study**.**

I may publish what I have learnt from this study. If l do, l will not include your name. l will not publish anything that would let people know who you are.

Voluntary Participation / Withdrawal

You should only take part in this study if you want to volunteer. You should not feel that there is any pressure to take part in the study. You are free to participate in this research or

withdraw at any time. There will be no penalty or loss of benefits you are entitled to receive if you stop taking part in this study.

You can get the answers to your questions, concerns, or complaints

If you have any questions, concerns or complaints about this study, or experience an adverse event or unanticipated problem, contact the researcher on 0712531186/0706460629.

If you have questions about your rights as a participant in this study, general questions, or have complaints, concerns or issues you want to discuss with someone outside the research, call the CIUREC Chairperson Dr. Samuel Kabwigu on (0779610100) & the executive secretary of UNCST on (0414 -705500) respectively.

Assessment of understanding

Please check which box best describes your assessment of understanding of the above informed consent document:

□ I have read the above informed consent document and understand the information provided to me regarding participation in the study and benefits and risks. I give consent to take part in the study and will sign the following page.

□ I have read the above informed consent document, but still have questions about the study; therefore I do not give yet give my full consent to take part in the study.

_____ _____

Signature of Person Taking Part in Study Date

_____

Printed Name of Person Taking Part in Study

_____

Thumb print of Person Taking Part in Study

_____ _____

Signature of Person Obtaining Informed Consent / Research Authorization Date

_____

James Sekeba / Research Authorization

CONSENT ON SPECIMEN STORAGE

CONSENT PAGE – USE OF SPECIMEN FOR FUTURE RESEARCH

INFORMED CONSENT FOR STORAGE OF BIOLOGICAL RESEARCH MATERIALS

1.Please list the names of research materials you would like to store. Plasma……

2. State the reason(s) why you wish to store the research materials.

At the end of the study, we will use the plasma to perform other investigations including prothrombin time, activated partial thromboplastin time, and thrombin time.

3. How long will you store the research materials?

The research material will be stored for up to 2 months.

4. Where will you store the research materials?

Mpigi Health centre IV, P.O. BOX 111, Mpigi district.

0706460629, sekebaj@yahoo.com.

……

5. Describe the disposal plan for the research materials after the expiry of the storage period.

All specimens collected will be destroyed in accordance to the laboratory procedures following Good Clinical and Laboratory Practice.

PARTICIPANT'S DECLARATION:

(Tick only one of the options)

I, ……. accept/do not accept that the research materials obtained from my body as a research participant be stored confidentially for future use.

Participant	**Principal Investigator**
Name……	Name…….....
Signature…… ..	Signature……..
Date……	Date……..
Thumbprint of the Participant	**……..**

APPENDIX II: QUESTIONNAIRE

Dear participants, I request you to provide the information in regard to this study about the burden of Thrombophilia among pregnant women attending ANC in Mpigi Health Centre IV.

Section A: Socio-demographic data

1. Study number: …….
2. Age: ……. Years
3. Religion:

   Catholic [ ] Protestant [ ] Muslim [ ] Pentecostal [ ]

   Others, specify ……
4. Education level:

   Primary [ ] Secondary [ ] Tertiary [ ] University [ ]

   None [ ]
5. Occupation: Employed [ ] Unemployed [ ] Self employed [ ]
6. Marital status: Married[ ] Divorced [ ] Widowed [ ]

   Others specify: …….

History of Thrombophilia (prolonged bleeding at home): ……

Section B: Result Slip

Test (Tick appropriately)	**Time (Minutes' Seconds'')**	**Result Interpretation**
PT		
aPTT		
TT		

Okuwebwa olukusa okwetaba mu kunonyereza kuno (Luganda version)

Tukusaba okwetaba mukunonyereza kuno okuyitibwa: 'Obungi bwe ekilwadde kya Thrombophilia mu bakyala be'mbuto abanyera eddagala ku dwaliro lye Mpigi Health Centre IV'

Omunonyereza omukulu ye Sekeba James era okunonyereza kujja kukolebwa mu dwaliiro lye Mpigi Health Centre IV, Mawokota mu Mpigi district mu Uganda.

Ekigendelerwa kyo kunonyereza kuno

Ebigendelerwa byebino wamanga:

- Okunonyereza kuno kugenderedwa okumanya obanga ddala ekilwadde kino wekiri mu bakyala abanyera edagala mu Mpigi Health Center IV.
- Okumanya obanga ddala ekilwadde wekiri na bakyala bameka abakirina nga banywera edagala mu Mpigi Health Centre IV.
- Okumanya eneyesa na myaka ki ekilwadde kino gyekisinja okukosa mu bakyala abanywera eddagala mu Mpigi Health Centre IV.
- Okubunyisa amawulire mubantu nadala abo bekikwatako ku bunji bwekilwadde basobole okubaawo kyebakola okulaba nti abakyala be'mbuto bakebelebwa kulwobulungi bwabwe. .

Kinakolebwa kitya

Osabidwa okwetaba mu kunonyereza kuno, nga bwoli omukyala omunayuganda asobola okutuyamba okutegera obulabe bwe ekirwadde kino mu bakyala be'mbuto.

Bwonetaba mu kunonyereza kuno, ojjakusabibwa:

- Okutuwa omusayi mls 3 okusobola okunonyereza mu laboratory
- Ojja kubuzibwayo nekubibuuzo, ekyo kijja kutwala nga eddakika 25;
- Ebibuuzo bino bijja kukubuzibwa mukifo ekyekusifu eri gwe, osobole okweyabuluza obulungi;
- Omusayi gujja kukugibwako omusawo omutendeke obulungi nga akolera kudwaliiro lino era nga ayina ne layisinsi emukiriza okukola omulimu guno okuva mukitongole kyo bulamu nga era alina obumanyirivu mu mulimu guno obutaaka wansi wa myaka kumi;
- Ebibuzo bijja kubuzibwa omunonyereza omukulu era ajja kukakasa nti byangu gyoli okusobola okukwanguyiza.

Emigaso

Tewali mugaso gyoli nadaala mubujjanjabi bwonafuna naye nga ojja kuwebwa ebinava mukukeberebwa omusayi gwo kubwerere naddala nga ozulidwamu ekirwadde kino.

Ebinazulibwa mu kunonyereza kuno, bijja kuwa bekikwatako okumanya ku bunji bwabantu abalina ekilwadde kino basobole okuyambibwa nga ekilwadde tekinakola bulabe kumwana atanazaliibwa.

Obulaabe

Ojjakulumizibwa naddala nga ojibwako omusayi, naye omusayi gujja kugibwako no'bukuggu obwenjawulo okukendezza obulumi.

Okuliyirilwa

Tewari kuliyiyirwa kwona kunawebwa oyo eyetabye mukunonyereza kuno.

Okukuma ebyaama

Omusayi gwona ogunakozesebwa mukunonyereza kuno ne biwandiko byona bijja kukumibwa nga byakyaama. Mumateeka oyo yena anabikozesa alina okubikuuma nga byakyaama. Abanakirizibwa okubilaaba bebano:

Abanonyereza, omuli omunonyereza omukulu nabo bakolara nabo**.**

Njinza okufulumya ebivudde mukunonyereza kuno era bwenaabanga nkyikoze, elinya lyo telijja kufulumizibwa era sijja kufulumya ebyo ebinaretera abantu okukumanya.

Oliwadembe okwetaba mukunonyereza / nokukuvamu

Oliina kwetaba mukunonyereza kuno nga wesimidde. Towuliira nga omuntu akakindwa okwetaba mukunonyereza kuno. Olina edembe okukwetaba mu oba nokukuvamu esaawa yona. Tojja kutekebwako musingo gwona oba okugibwako byoliina okufuna bwonaaba nga osazewo okuvamu.

Osobola okuyambibwa singa obeera nekibuuzo kyona oba okwemulugunya kwona

Bwobanga olina okwemulugunya kwona, ebibuzo oba amagezi gona kuba kusimu zino wamanga 0712531186/0706460629.

Bwobanga olina ebibuzo ku dembe lyo nga eyetabye mu kunonyereza, okwemulugunya oba esonga yona byoyagala okwogerako wabweru wokunonyereza kuba kusimu eno okutukirira CIUREC Chairperson Dr. Samuel Kabwigu kusimu (0779610100) no'muwandisi omukulu UNCST kusimu (0414 -705500).

Okukebeera okumanya

Soma olabe akabokisi okalaga bwotegedde okuwebwa olukusa:

- □ Nsomye byona ebiri wagulu era nembitegera bulungi nga bwebitwata ku kwetaba mukunonyereza, ebigendelerwa, ebilungi. Nzikiriza okwetaba mukunonyereza kuno era ngenda kusako ekinkumu oba sayini.
- □ Nsomye byona wagulu naye nkyalina ebibuuzo kukunonyereza kuno era sinabba kukiriza kukwetabamu.

_____ _____

Sayini eyoyo eyetabye mukunonyereza Enaku zomwezi

_____

Elinya lyoyo eyetabye mukunonyereza

_____

Ekinkumu kyoyo eyetabye mukunonyereza

_____ _____

Sayini yoyo akirizidwa okunonyereza Enaku zomwezi

_____

James Sekeba / Research Authorization

OKUKIRIZIBWA OKUTEREKA OMUSAYI

OMUSAYI OGUNAKOZESE JEBUJJA

1. Wandika amanya gebyo byoyagala okutereka. Musayi

……

2. Lwaki wandiyagadde okubitereka?

Okunonyeereza nga kuwedde, tujja kukozesa omusayi guno okunonyereza kubintu ebirara.

3. Omusayi onagutereka okumala ebanga ki?

The research material will be stored for up to 2 months.

4. Omusayi guno onagutereka wa? (Give the full physical address, telephone contact, and email address of the location)

Mpigi Health centre IV, P.O. BOX 111, Mawaokta, Mpigi district.

0706460629, sekebaj@yahoo.com.

……

5. Ekiseera kyo kugutereka nga kiwedeko, onagutekawa?

Omusayi gwona gujja kusanyizibwawo nga amateeka laboratory gegobelera nga esanyawo omusayi.

Okukiriza kwoyo eyetabye mukunonyereza:

Nze, ……. Nzikiriza/sikiriza nti omusayi ogunzigidwako okukozesa mukunonyereza okuterekebwa okukozesebwa jebujja.

Eyetabye mukunonyerezebwa	**Omunonyereza omukulu**
Amanya……	Amanya…….....
Sayini…… ..	Sayini……..
Enaku zomwezi……	Enaku
zomwezi……..	

Ekinkumu kye'tabye mukunonyereza……..

QUESTIONNAIRE (LUGANDA)

Nyabo nkusaba okumpa ebikukwatako kulwo kunonyereza kuno kwewetabyemu okutwata kubilwadde bwa thrombophilia mu bakyala be mbuto abanywera edaggala mu dwaliro lye Mpigi Health Centre IV.

Section A: Oyo eyetabye mukunonyereza

1. Number ekuweledwa: …….
2. Emyaka gyo: …….
3. Eddini gyo ssoma:

   Mukatuliki [ ] Muprotestant [ ] Musilamu [ ]

   Mulokole [ ]

   Endala etayogedwako wandika wano ……
4. Ekibiina kwewakoma mu mukusoma:

   Primary [ ] Secondary [ ] Tertiary [ ] University [ ]

   Sisomangako [ ]
5. Omulimu: Nkola [ ] Sikola [ ] Nekozesa [ ]
6. Ndi mufumbo[ ] Silimufumbo [ ] Ndinamwandu [ ]

   Ekirara kyona wandika wano: …….
7. Mukika kya mwe waliyo abantu bona bwebabeera bafunye ekiwundu nga kirwawo nga kikyareta omusayi.: ……

Section B: Result Slip (ya munonyereza)

Test (Tick appropriately)	**Time (Minutes' Seconds'')**	**Result Interpretation**
PT		
aPTT		
TT		

APPENDIX III: PROTHROMBIN TIME

Principle: This procedure detects a deficiency of those factors involved in prothrombin conversion of the coagulation scheme.

Specimen: A blue top vacutainer containing 3.2% sodium citrate wasused. The sample were obtained by non-traumatic venipuncture. A one to nine ratio of anticoagulant to blood in the tube was considered. The testwas run as soon as possible or up to 24 hours at room temperature.

Reagents: Thromboplastin reagent and controls.

Procedure:

If test plasma(s), and/or Thromboplastin are at refrigerator temperature, they were placed on an aliquot mixer (reagents) or in a test tube rack until they reach room temperature.Mix the normal control thoroughly and pipet a small amount (approximately 0.3 ml.) into a 12 X 75 mm. test tube placed in the heating block. Allow to warm at 37°C for at least 1 minute but no longer than 5 minutes.Mix the Thromboplastin thoroughly. Pipet 0.2 ml of the Thromboplastin into a pre-warmed labeled fibrocup. Set a timer and allow to warm for 1 minute.Move the fibrocup into the reactor well position.Replace the tip on the automatic pipet and then fill with 0.1 ml. normal control. Depress pipet to dispense sample into the fibrocup containing the Thromboplastin while depressing Timer bar to start timer.The probe arm will drop into place in the reaction well. When a fibrin network has formed, electrode and timer stop, and the result is registered on the digital readout in seconds and tenths.Record the time; depress the readout reset button until zero position registers, dip the probe electrode into deionized water, and wipe with a lint-free absorbent tissue and reposition the probe arm to resting position.Repeat procedure on normal control until duplicate results that agree within 1 second are obtained. Record the average of the two results as the normal control value for the Prothrombin Time.

Interpretation: Prolonged values are associated with deficiencies of Factors V, VII, and X and severe prothrombin deficiency.

APPENDIX IV: ACTIVATED PARTIAL THROMBOPLASTIN TIME

Materials**:**12 x 75 test tube, pipettes 100 µL, pipette tips, Calcium chloride reagent, 0.025M, Partial thromboplastin: consists of phospholipids and a contact activator, water bath, incubator, or heat block at 37˚C, test tube rack, stop watch, controls Level I and III (normal and abnormal) and citrated plasma specimen

Principle**:** The activated partial thromboplastin time (aPTT) is also known as the partial thromboplastin time (PTT). The PTT result (in seconds) is the time required for plasma to clot when maximal surface contact activation, optimal phospholipid, and calcium concentration are provided. Calcium is removed from the plasma of the test specimen during collection in a sodium citrate tube. Because calcium is a coagulation co-factor clotting is inhibited by calcium chelation. The specimen is centrifuged to obtain platelet-poor plasma (PPP) with all available coagulation factors except for calcium and platelets. Under carefully controlled conditions and with properly prepared reagents, calcium, partial thromboplastin and an activator or agonist (such as kaolin, celite or ellagic acid) are added to the plasma to be tested. The partial thromboplastin reagent stimulates activated platelet surfaces by providing a phospholipid surface on which enzymatic reactions can occur. The activator (agonist) provides a negatively charged surface for activation of factor XII. After a specific incubation time of citrated plasma with the APTT reagent which allows for optimum activation of contact factors, calcium chloride is added. The calcium chloride activates the clotting cascade. The time required for the plasma to clot is the activated partial thromboplastin time. Clot formation can be detected by optical or electromechanical methods, using manual, semi-automated or automated devices.

Procedure**:**Working in pairs, pre- warm a sufficient quantity of calcium chloride reagent to 37˚C for the number of tests to be performed.Pipette 100 µL or 0.1 mL of normal control into a labeled test tube.Into each test tube, add 100 µLor 0.1 mL of prewarmed, partial thromboplastin reagent.Incubate the control/ partial thromboplastin mixture at 37^{o}C for a minimum of three (3) minutes. Add in100 µL or 0.1mL of calcium chloride into the control/ partial thromboplastin mixture and start the stop watch immediately.Mix the tube once,

immediately after adding the calcium reagent. Allow the tube to remain in the heat block, approximately 20 seconds, mixing occasionally.

After 20 seconds, remove the tube from the water bath/heat block. Wipe off the outside of the tube. Gently tilt the tube back and forth until a visible clot forms.

Stop the stop watch immediately and record the time in seconds. Record the time and average the two results if they match appropriately. If not, repeat a third time and average the two that match within acceptable limits. Be sure and cross out any values you aren't using for the final calculation. Include measurement unit of seconds on report sheet.

PTT Reference Range: 28.0-35.0 seconds

Clinical Significance: A long partial thromboplastin time indicates a significant defect in at least one of the plasma procoagulants. When the partial thromboplastin time is used in combination with the prothrombin time, most procoagulant disorders can be classified. The PTT time is abnormal with deficiencies XII, XI, IX, VIII, PK, HK (intrinsic pathway) and X, V, II, I (common pathway)

Prolonged PT	**Prolonged PTT**	**Potential Factor Deficiency**
√		VII
√	√	X,V, II, I , Vitamin K deficiency, DIC, Liver Disease
	√	XII, XI, IX, VIII

APPENDIX V: BLOOD COLLECTION PROCEDURE

Required

Citrated vacuum tubes, tourniquet, alcohol swabs, cotton swabs, gloves, syringes and sharps container

Procedure

1. Explain the procedure of sample collection to the patient.
2. Make the patient in a comfortable position.
3. Select tube appropriate for type of samples desired, needle and syringe.
4. Label the tube with the patient's laboratory identification number.
5. Put on gloves.
6. Select site for venipuncture.
7. Apply the tourniquet 3-4 inches above the selected puncture site.
8. Cleanse venipuncture site with alcohol preparation in a circular fashion, beginning at the site and working outward and allow site to dry.
9. Remove needle shield and Perform venipuncture
10. Remove tourniquet as soon as blood appears in the syringe, and draw about 2ml of blood into the syringe.
11. Place a cotton swab or gauze over the site and withdraw the needle in a smooth and cautious manner so as not to bruise the vein.
12. After withdrawing the needle fully, apply pressure to the cotton swab over the puncture site and ask the patient to apply pressure for 3 to 5 minutes until the bleeding stops.
13. Introduce the blood into the tube, mix the well and discard the needle and syringe into the sharps container.

APPENDIX VI: INTRODUCTORY AND CORRESPONDENCE LETTER

IHSU INTERNATIONAL HEALTH SCIENCES UNIVERSITY
sanitas per scientiam

making a difference to health care

Dean's Office-Institute of Allied Health Sciences

Kampala, Friday 28th September 2018

TO THE FACILITY INCHARGE
MPIGI HEALTH CENTER IV
P.O. BOX 111, MPIGI.

Dear Sir/Madam,

RE: ASSISTANCE FOR RESEARCH

Greetings from International Health Sciences University.

This is to introduce to you **Sekeba James** Reg. No. **2014-BMLS-PT-010** who is a student of our University. As part of the requirements for the award of a Bachelors Degree of Medical Laboratory Sciences of our University, the student is required to carry out research in partial fulfillment of his award.

His topic of research is: **Assessment of asymptomatic thrombophilia among pregnant women attending antenatal care clinic at Mpigi Health Center IV.**

This therefore is to kindly request you to render the student assistance as may be necessary for his research.

I, and indeed the entire University are grateful in advance for all assistance that will be accorded to the student.

Mr. Sekeba is allowed to carry out research in Mpigi

Sincerely Yours,

INTERNATIONAL HEALTH SCIENCES UNIVERSITY
28 SEP 2018
INSTITUTE OF ALLIED HEALTH SCIENCES
P. O. Box 7782, Kampala - Uganda

MPIGI HEALTH CENTRE
04 OCT 2018
MPIGI DISTRICT COUNCIL

for Dr. Okiria John Charles (PhD)
Associate Professor / Dean IAHS
(0772409126 / 0752409126)

The International Health Sciences University
P.O. Box 7782 Kampala - Uganda
(+256) 0312 307400 email: deanahs@ciu.ac.ug / jokiria@ciu.ac.ug
web: www.ciu.ac.ug

APPENDIX VII: APPROVAL LETTER FROM RESEARCH ETHICS COMMITTEE

CLARKE INTERNATIONAL UNIVERSITY
LEAD · INNOVATE · TRANSFORM

RESEARCH ETHICS COMMITTEE

(+256) 0312 307400
rec@ciu.ac.ug
www.rec.ciu.ac.ug

21st November, 2018

UG-REC-015

IHSU-REC/0115

CLARKE INTERNATIONAL UNIVERSITY
21 NOV 2019
APPROVED / VALID UNTIL
RESEARCH ETHICS COMMITTEE
P. O. Box 7782, Kampala - Uganda

Mr. Sekeba James
Principal Investigator
Clarke International University
P.O Box 7782
Kampala, Uganda

Category of review
[x] Initial review
[ ] Continuing review
[ ] Amendment
[ ] Reactivation
[ ] SAEs

RE: "PREVALENCE OF ASYMTOMATIC THROMBOPHILIA AMONG PREGNANT WOMEN ATTENDING ANTENATAL CARE CLINIC AT MPIGI HEALTH CENTRE IV".

Reference is made to the above mentioned protocol which was submitted to Clarke International University Research Ethics Committee (CIUREC) for initial review and approval.

You have addressed all the issues earlier raised and the committee is satisfied with the responses submitted in version 2.0 dated 09th November, 2018.

l am glad to inform you that your study has been approved for a period of one year from 21st November, 2018 to 21st November, 2019.

The documents approved include the following;

Document	Language	Version	Submission Date
Protocol	English	Version 2.0	09th November, 2018
Consent forms			
Informed Consent	English	Version 2.0	09th November, 2018
Informed Consent	Luganda	Version 2.0	09th November, 2018
Data collection tools			
Semi-structured questionnaire	English	Version 2.0	09th November, 2018

Please note that any problem of a serious nature as a result of this study to the participants should be reported to CIUREC and Uganda National Council of Science and Technology (UNCST) immediately.

MPIGI HEALTH CENTRE IV
21 NOV 2018
MPIGI DISTRICT COUNCIL

#Make a Difference

St. Barnabas Road, Kampala-Namuwongo
3rd Floor, International Hospital Kampal
P.O. Box 7782 Kampala, Uganda

Also note that annual report and request for renewal where applicable should be submitted at least one month before the expiry date of approval. In addition, you are also required to submit copies of the stamped approved documents to the Uganda National Council for Science and Technology (UNCST) before the study can commence.

We would like to congratulate you and wish you a successful conduct of the study.

Yours Sincerely,

CLARKE INTERNATIONAL UNIVERSITY
21 NOV 2019
APPROVED / VALID UNTIL
RESEARCH ETHICS COMMITTEE
P. O. Box 7782, Kampala - Uganda

21 NOV 2018

Dr. Samuel Kabwigu
CIUREC Chairperson

Date