PREVALENCE AND TYPES OF ABNORMAL COAGULATION PARAMETERS IN PREECLAMPSIA WITH SEVERE FEATURES AT KENYATTA NATIONAL HOSPITAL, 2019; A CROSS SECTIONAL STUDY.

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DEPARTMENT OF OBSTETRICS AND GYNAECOLOGY

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2021

DECLARATION

I, **Dr. Chege Rahab Njeri,** do declare that, this research was undertaken in part fulfillment for the award of a Masters of Medicine in Obstetrics and Gynecology from the University of Nairobi and was my original work and has not been undertaken and presented for a degree in any other University.

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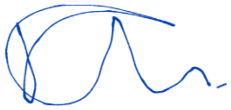

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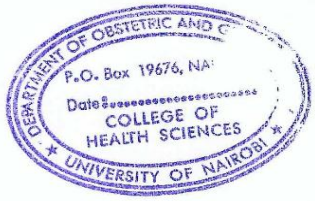

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LIST OF ABBREVIATIONS

APTT- Activated Partial Thromboplastin Time

BT- Bleeding Time

CMEs- Continuous Medical Education

CT- Clotting Time

DIC- Disseminated Intravascular Coagulopathy

ED- Endothelial Dysfunction

ERC- Ethics and Research Committee

FDP- Fibrin Degradation Products

GH- Gestational Hypertension

HB- Hemoglobin Levels

HELLP- Hemolysis, Elevated Liver Enzymes, Low Platelet Count

ICU- Intensive Care Unit

INR- International Normalized Ratio

IUFD- Intra Uterine Fetal Demise

ISO- International Organization for Standardization

KNH- Kenyatta National Hospital

LBW- Low Birth Weight

LDH- Lactate Dehydrogenase

LFTs- Liver Function Test

NICU- Neonatal Intensive Care Unit

PE- Pre-Eclampsia

PGI2- Prostacyclin I2

PI- Principal Investigator

PIGF- Placental Growth Factor

PPH- Post Partum Hemorrhage

PT- Prothrombin Time

RAs- Research Assistants

RFTs- Renal Function Test

Sflt1- Soluble fms-Like Tyrosine Kinase-1

SOP- Standard Operating Procedure

SPE- Preeclampsia with Severe Features

SPSS- Statistical Package for Social Sciences

TXA2- Thromboxane A2

TT- Thrombin Time

UECs- Urea, Electrolytes, Creatinine

UoN- University Of Nairobi

VEGF- Vascular Endothelial Tyrosine Kinase

WHO- World Health Organization

OPERATIONAL DEFINITIONS

Pre-eclampsia: A disease in pregnancy characterized by the presence of a systolic blood pressure greater than or equals to 140 mm Hg or a diastolic blood pressure greater than or equal to 90 mm Hg or higher, on two occasions at least 4 hours apart in a previously normotensive patient.

Pre-eclampsia with severe features: Is a disorder of pregnancy characterized by high blood pressure and significant proteinuria after 20 weeks gestation. Preeclampsia with severe features is defined as the presence of one of the following symptoms or signs in the presence of preeclampsia: Systolic Blood Pressure of 160 mm Hg or higher or Diastolic Blood Pressure of 110 mm Hg or higher, on two occasions at least 4 hours apart while the patient is on bed rest (unless antihypertensive therapy has previously been initiated); impaired hepatic function as indicated by abnormally elevated blood concentrations of liver enzymes (to double the normal concentration), severe persistent upper quadrant or epigastric pain that does not respond to pharmacotherapy and is not accounted for by alternative diagnoses, or both; progressive renal insufficiency (serum creatinine concentration >1.1 mg/dL or a doubling of the serum creatinine concentration in the absence of other renal disease); new onset cerebral or visual disturbances; pulmonary edema; thrombocytopenia (platelet count <100,000/μL).

Pregnancy Outcome: The results of conception and ensuing pregnancy. It can be further classified as good maternal, fetal and neonatal outcomes and poor maternal, fetal and neonatal outcome based on the ensuing pregnancy.

Prothrombin time: Time taken for plasma to clot when exposed to tissue factor. Assesses the extrinsic pathway. (11.8 to 15.1 seconds).

Activated partial thromboplastin time: Time taken for plasma to clot when exposed to substances that activate contact factors. Assesses both the intrinsic and common pathways. (24.3 to 35 seconds).

Fetal growth restriction: Estimated weight below the 10^{th} percentile for gestational age in the second half of pregnancy.

Intra uterine fetal death: delivery of a fetus above 20 weeks with no signs of life.

Coagulation profile: A coagulation profile includes INR, APTT, platelets , bleeding and clotting times and fibrinogen. It is a screening test for abnormal blood clotting because it examines the factors most often associated with a bleeding problem.

Abnormal coagulation parameters: In this study, abnormal coagulation parameters were defined as APTT more than 35 seconds, PT more than 15.1 seconds and platelets less than 150,000/L.

ABSTRACT

Introduction: Pre-eclampsia with severe features is a major medical and public health concern. Participants with preeclampsia with severe symptoms often present with abnormal coagulation parameters. Dysfunction of the blood coagulation system, especially the fibrinolysis is a salient feature of preeclampsia. This varies in severity and therefore necessitates different treatment modalities. Endothelial dysfunction being the baseline of preeclampsia pathogenesis activates tissue factor that in turn begins a sequence that leads to abnormal coagulopathy.

Objective: To determine the prevalence and types of coagulation parameters among women with preeclampsia with severe features from 34 weeks till delivery at KNH, 2019.

Methodology: This was a cross-sectional study conducted at Kenyatta National Hospital (KNH) labor and antenatal wards, involving women with preeclampsia with severe features from 34 weeks gestation till delivery. The sample size was ninety eight (98). Participants meeting the inclusion criteria had blood samples taken for a coagulation screen- Activated partial thromboplastin time (APTT) and prothrombin time (PT), full blood count with emphasis on platelet counts. Assessment of liver function tests- Alanine transaminase (ALT) and Aspartate transaminase (AST) was done for case definition. All samples were analyzed under controlled processes at KNH, which is ISO accredited laboratory.

Data was extracted from questionnaires and uploaded into an SPSS spreadsheet (version 25) for analysis. Frequencies and proportions of demographic data were calculated and presented in a table. Prevalence estimates with corresponding 95% confidence intervals of abnormal coagulation parameters, types of abnormal coagulation parameters, and proportions of adverse maternal and neonatal outcomes in adverse coagulation parameters were calculated using the exact Clopper-Pearson method.

Results: Ninety eight (98) women with preeclampsia with severe features of legal age were recruited with 96 consenting for enrollment into the study. Majority were aged below 35 years (79%) and65% of the participants were unemployed. The prevalence of abnormal coagulation parameters was 43 % (95% CI: 32.8-52.6%). Most of the participants had more than one abnormal coagulation parameter. Postpartum hemorrhage (PPH) and intrauterine fetal demise (IUFD) were more prevalent in participants with prolonged PT levels at 14% and 43% respectively.

Conclusion: The prevalence of abnormal coagulation parameters in patients with preeclampsia with severe features is high and more studies are needed to determine if abnormal coagulation parameter increases the risk of adverse maternal and fetal outcomes.

CHAPTER 1: INTRODUCTION

Hypertension is the most common medical problem encountered during pregnancy, complicating 2-3% of pregnancies (1). It is among the major causes of mortality and morbidity among pregnant women. Most maternal mortality are more attributable to eclampsia, than preeclampsia and therefore perinatal mortality is higher following eclampsia, than preeclampsia (2). Preeclampsia is a unique disease that occurs only in pregnancy.

World Health Organization (WHO) found that its incidence is seven times higher in developing countries (2.8% of live births) than in developed countries (0.4%) (18). It is responsible for almost 50,000 maternal deaths worldwide. Vata PK et al showed that preeclampsia occurs in about 10% of all pregnancies, setting the African prevalence higher than the global prevalence. In Ethiopia, a study by Kumar Vata et al 2015 showed that the incidence of preeclampsia at Dilha University Referral Hospital was 2.23%, with a 65.68% having severe preeclampsia (SPE) and 17.42% having mild pre-eclampsia (mPE).

Preeclampsia is a condition that affects multiple systems in the body. It is defined as new-onset hypertension with systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg, measured on two occasions at least four hours apart, and proteinuria of > 0.3 g per 24 hours or ≥ 1+ proteinuria, detected by urine dipstick after 20 weeks of pregnancy, or in the absence of proteinuria, new-onset hypertension with new onset of any one of the following: thrombocytopenia (platelet count < 100 000/µl), renal insufficiency (serum creatinine concentration > 1.1 mg/dl or a doubling of the serum creatinine concentration in the absence of other renal disease), impaired liver function (raised concentrations of liver transaminases to twice normal concentrations), pulmonary edema, or cerebral or visual problems (4).Globally, pre-eclampsia complicates about 2%-10% of pregnancies.

Normal coagulation in the body is a balance between the clot formation systems and the mechanisms that inhibit the clot formation beyond the injury site (19). Pregnancy is a hypercoagulable state that is involved with changes in hemostasis. This leads to an increase in most clotting, reduction in anticoagulants and the fibrinolytic activity. Among the factors affected physiologically include:

Platelets: which tend to decrease due to haemodilution and possibly increased destruction.

Coagulation factors where: - some factors tend to increase in pregnancy while others either remain the same or decrease. Factors VIII, X, X11 increase. Factor XIII increases during the first trimester, but goes back to 50% of its pre-pregnant levels by the third trimester. Factor V increases in the first trimester, but later stabilize, while factor II may remain the same throughout the entire pregnancy (5).

Dysfunction of the blood coagulation fibrinolysis system is a salient feature of preeclampsia. This varies in severity and therefore necessitates different treatment modalities. Endothelial dysfunction being the baseline of preeclampsia pathogenesis, activates tissue factor that in turns begins a sequence that leads to abnormal coagulopathy(6).

The greatest clotting activity is experienced in the third sage of labor, during placental expulsion (6). This is made possible by the release of thromboplastic substances hence ensuring maternal blood clotting reducing the incidences of postpartum hemorrhage.

Among the tests used to assess the coagulopathy are:- platelet counts which are normally decreased in normal pregnancy and have been used as an indicator for abnormal coagulation parameters(5). Other tests are prothrombin time (PT), activated partial thromboplastin time (APTT), which are normally shortened in normal pregnancy.

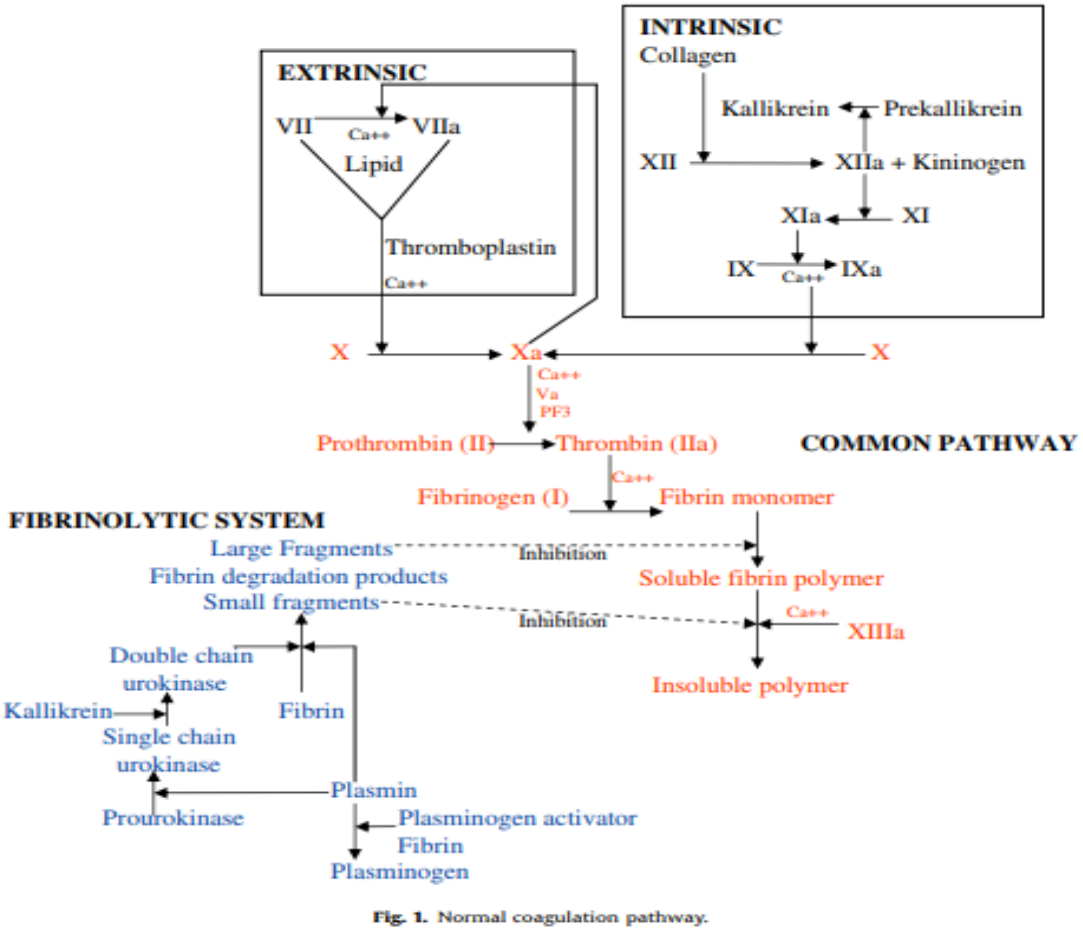


Fig. 1. Normal coagulation pathway.

Figure 1: Coagulation Cascade (adapted from Thorton and Douglas (2010))

Pre-eclampsia is associated with adverse maternal, fetal and neonatal effects. Among the maternal adverse outcomes are progression into eclampsia, HELLP syndrome, PPH that can lead to DIC and AKI. Fetal outcomes: FGR, IUFD, and neonatal outcomes: stillbirth, prematurity with low birth weight, NBU/NICU admission and early neonatal deaths(7). PE accounts or more than 50,000 maternal deaths annually(3). A study done in KNH between March and July 2004 by Karanja showed the prevalence of hypertensive disorders to be 6.5%, 55% of which were preeclampsia (20). Between 1995 and 1999, Obore reviewed maternal mortalities at KNH and found that 84% of deaths were due to eclampsia in participants with hypertensive disorders, while preeclampsia accounted for the remaining 16% (21).

A vital balance is necessary between the coagulation and anticoagulation system in order to maintain the utero placental perfusion and organ perfusion in pregnancy (6).In PE, the coagulation – fibrinolytic system is among the major systems that are adversely affected by maternal inflammatory reactions and immune dysfunction(6). The hypercoagulable state is exaggerated and the coagulation – anticoagulation balance is down regulated leading to blockage of the placenta and other organs by micro thrombi(8). Coagulopathy is a dreaded complication of PE and can occur as a result of primary disease or it can result from complications e .g. IUFD, abruption placentae or postpartum hemorrhage(9). Coagulopathy affects up to 15% of cases with preeclampsia with severe features and approximately accounts for 15% of maternal death(9). These mortalities were linked mostly to the development of DIC and progressive thrombocytopenia, as encountered in preeclampsia (9). This prothrombotic state may end up in development of chronic DIC and this leads to changes in the placenta and kidney, thereby increasing the risk of bleeding (10).DIC accounts for 25% of maternal mortalities (17). DIC follows a sequence of events. Tissue factor which can be released from fetal tissue, maternal decidua, trophoblastic tissue or endothelium initiates the sequence.

CHAPTER 2: LITERATURE REVIEW

A prospective cohort study conducted in 2009 at Shiraz University of medical sciences in Iran sought to compare the coagulation parameters in participants with sPE and those with normal pregnancies. It was also to determine whether having a normal platelet count would reassure the physician that no other significant clotting anomalies were present in participants with severe preeclampsia. Assessed coagulation parameters included platelet count, APTT, PT, total fibrinogen levels and fibrin degradation products (FDP). Out of 50% of the participants with sPE, 24% met the criteria for HELLP syndrome, 12% had eclampsia and 4% had HELLP and eclampsia simultaneously. This study showed that APTT combined with platelet count were found to be useful parameters for detection of an early on-going coagulopathy in participants with sPE who may develop DIC(11).

In Sudan, a prospective case control study done in 2016 at a tertiary hospital investigated coagulation parameters in pregnant women with preeclampsia. PT, aPTT and fibrinogen levels were analysed. There was significant difference between the PT (P; 0.01, 0.06) and APTT (P; 0.02, 0.04) among the PE group and normal pregnant women. PT and APTT were significantly higher in PE group, with hypofibrinogenemia observed in 16.6% of cases (8).

In India, a study was set to compare the coagulation profile in preeclamptic and eclamptic women with normotensive pregnant women. The analysed parameters included bleeding time (BT), clotting time (CT), PT, APTT and platelet count. It revealed that sPE and eclampsia were highly associated with thrombocytopenia and coagulation abnormalities. Platelet count and APTT had a predictive value in screening for DIC in cases of severe preeclampsia. Thrombocytopenia was observed in 23.33% of participants with SPE and in 30% of participants with eclampsia. PT was not found to be significantly prolonged. APTT increased significantly in severe preeclampsia than in normal pregnancy. Platelet count was found to decrease with disease severity, bleeding time was not significantly prolonged with increased severity and clotting time increases slightly in preeclampsia and eclampsia than in normal pregnancies (10).

A prospective self-controlled cohort study carried out in Nigeria in 2011, found that thrombocytopenia predicts the risk of coagulopathy. It showed that abnormal PT is common as mean platelet count reduce and APTT abnormalities occurred when the platelet count were much lower. Low levels of platelets (<80,000/L) can be used as a predictive value and deter the

progression in severity of disease when promptly detected and managed (9). This is supported by the findings that platelet count below 100,000 leads to an increased risk of prolonged PT and APTT. It has therefore been recommended that coagulation evaluation should be carried out in preeclamptic participants with platelet count less than 100,000 (12).

Several studies have also been carried out, showing a correlation between abnormal coagulation parameters and maternal-foetal outcomes. A hospital based observational study carried out in India looked at platelet count as a prognostic marker for feto-maternal outcomes in preeclampsia and eclampsia. It showed that mean platelet count was significantly lower in the preeclampsia and eclampsia groups than in the women with normal pregnancies. The bleeding time was also found to be significantly increased in these two groups than in normal pregnancies. Clotting time also showed significant increase in the case groups than control group. This led to the conclusion that, early detection of coagulopathies have a big role in reducing the mortality and morbidity of both the woman and the foetus, and platelet count being a low cost rapid and easily available test can be used as a predictor of disease severity(13). In 2014 a study looked at maternal and foetal outcomes in pregnancy induced hypertension. Among the parameters assessed in the study was, Hb, platelet count, bleeding time, clotting time, PT and APTT. The results showed that out of 32 participants with abnormal coagulation parameters, 27 of them (84.37%) had adverse outcomes ,(HELLP, PPH, DIC, ascites, acute renal failure and maternal death) whereas 30(93.75%), had unfavourable foetal outcomes(FGR, and perinatal death) (15).

A hospital based observational study conducted in India in 2017 by(13), showed that foetal outcomes among thrombocytopenic participants (those with preeclampsia and eclampsia), showed a strong negative correlation between platelet count and the outcomes (these were not specified in the study). Another study conducted in New Delhi in 1991, set to look at nature and extent of coagulation defects in neonates born to mothers with PIH. Coagulation parameters analysed included PT, APTT, TT and serum fibrin/fibrinogen degradation products (FDP) assay. Among the participants with severe preeclampsia, DIC, aspiration pneumonia, septicaemia and gastrointestinal haemorrhage were among the complications observed. Prolongation of PT, APTT and TT was found in neonates born to mothers with PIH as compared to those from normotensive women(14).

A hospital based study that looked at maternal and foetal outcomes in pregnancy induced hypertension, done in 2014, showed that abnormal coagulation parameters were significantly associated with foetal mortality and morbidity, including IUGR and even perinatal death(15).

In 2014, a retrospective case control study by Han L et al, found that APTT decreased from 29 in early pregnancy to 27 in late pregnancy, while PT shortened from 9.9 in early pregnancy to 9.6 in late pregnancy.TT did not appear to significantly change.

Problem Statement

Preeclampsia is common in our set up. Most participants with preeclampsia with severe features develop coagulation abnormalities. These abnormalities have been associated with severe blood loss predisposing women at childbirth to postpartum hemorrhage. No study has been done to assess the prevalence and types of abnormal coagulation parameters in preeclampsia with severe features and proportions of adverse maternal and fetal outcomes in those with abnormal coagulation parameters. More so, these participants are not routinely fully investigated for coagulopathies, save for the total blood count that includes the platelet count.

Conceptual Framework

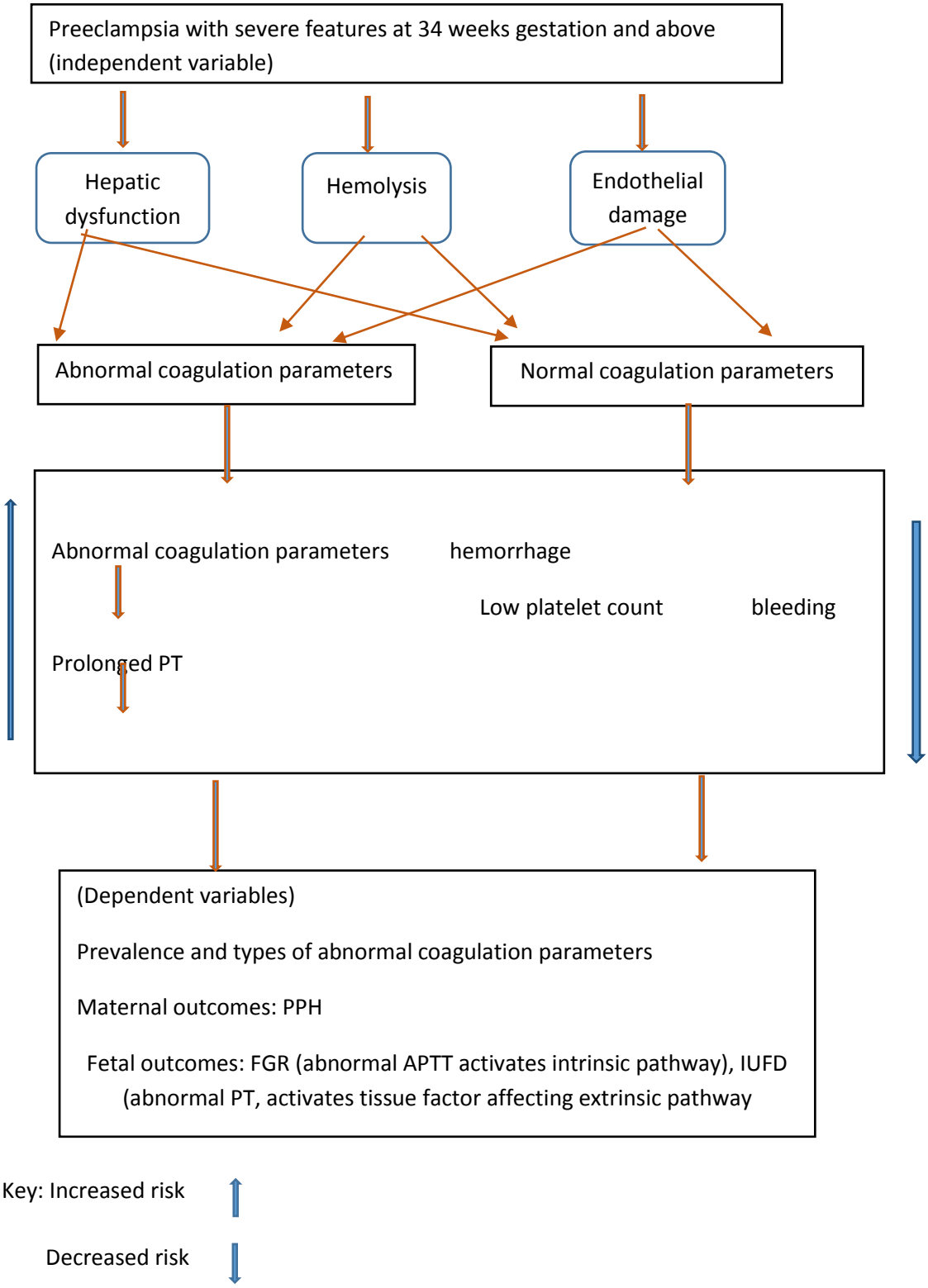


Figure 2: Conceptual Framework

Justification

Preeclampsia still presents a major challenge to perinatology more so when it presents with severe features and abnormal coagulation parameters. The aim is to deliver women with preeclampsia with severe features by the 34^{th} week of gestation, in absence of evidence of target organ damage. At this gestation, the estimated fetal weight is about 2000 grams, and the fetal lungs have been presumed to have matured thus enhancing the chances of survival. Coagulation profile is not among the baseline investigations routinely done for these pregnant women. Studies have shown quite a high prevalence of abnormal coagulation parameters among women with preeclampsia with severe features, with some of the studies showing a relationship between these abnormal coagulation parameters and feto maternal outcomes. No local studies had been done in this field. This study then investigated the prevalence and types of abnormal coagulation parameters among these women and proportion of adverse maternal outcomes (PPH) and fetal outcomes (FGR, IUFD) can be used to inform policy.

Research Question

What is the prevalence and types of abnormal coagulation parameters among women with preeclampsia with severe features from 34 weeks gestation till delivery in KNH, November 2019 to January 2020?

Study Objectives

Broad Objective

To determine the prevalence and types of coagulation parameters among women with preeclampsia with severe features from 34 weeks till delivery at KNH.

Specific Objectives

Among the women with preeclampsia with severe features from 34 weeks gestation till delivery in KNH, November 2019 to January 2020, the study will:

Determine the prevalence of abnormal coagulation parameters.

Describe the types of abnormal coagulation parameters.

Determine the proportion of adverse feto-maternal outcomes in those with abnormal coagulation parameters.

CHAPTER 3: METHODOLOGY

Study Design

The study adopted a cross- sectional study design. The study population consisted of women at 34 weeks gestation, already diagnosed with preeclampsia with severe features. Their coagulation parameters results were assessed from the files, and those without results had samples drawn for testing. In this study, the parameters assessed were: APTT, PT and platelet levels. The prevalence and types of the abnormal coagulation parameters were then analyzed. In this study abnormal coagulation parameters were defined as platelet levels less than 150,000/L, PT more than 15.1 seconds and APTT more than 35 seconds. Combined abnormal parameters were defined as abnormality of more than one of these 3 coagulation parameters evaluated.

This study was not powered to look at proportion of adverse obstetric outcomes, however those with abnormal coagulation parameters were followed until delivery and the proportion of maternal and fetal outcomes reported. Maternal outcome included PPH through estimation of blood loss after delivery. Fetal outcomes included fetal growth restriction (FGR) and intra uterine fetal death (IUFD).

Study Site and Setting

The study was conducted at the department of Obstetrics and Gynecology, Kenyatta National Hospital (KNH). KNH is the largest teaching and referral hospital in Kenya and the East and Central Africa region. It has a bed capacity of 1,800 beds with a high patient turnover. It is the main hospital for the College of Health Sciences, University of Nairobi and conducts an average of 12,000 deliveries annually.

KNH is a teaching hospital for the University of Nairobi, Faculty of Medicine and visiting students from other institutions. The hospital has obstetrics and gynecology department, with a maternity wing that conducts approximately 10,000 deliveries per year. The department has one labor ward,

three antenatal and post-natal wards, two maternity theatres, 2 gynecological wards, which also double as oncology wards. The hospital also has a blood transfusion laboratory and is capable of providing comprehensive obstetric care. The facility has a number of specialists who offer specialized services. The KNH labor ward is the initial place of management for participants with preeclampsia with severe features, after which they are transferred to the wards after delivery or for observation once they stabilize.

On average, four patients are managed for PE with severe features from the acute rooms in the maternity unit daily. Routinely, the tests conducted for all participants admitted with PE include total blood count, urea, electrolytes, and chloride levels (UECs) renal function tests (RFTs), liver function tests (LFTs). Some important non-routine tests include uric acid levels, coagulation profile and lactate dehydrogenase levels (LDH).

The tests carried out were coagulation profile, with emphasis on PT, APTT and total blood count with emphasis on the platelet counts. The KNH/UON lab that conducted these tests is ISO certified. The turnaround times for each of these tests were approximately one hour. For total blood count, the machine used was the SYSMEX XN 1000, while for coagulation profile, the machine used was the ACL ELITE PRO. Both the machines are automated.

Study Population

The study population was pregnant women with a confirmed diagnosis of PE

Inclusion Criteria

- Pregnant women who were of legal age (18 years and above)
- Women from 34 weeks gestation
- Participants with preeclampsia with severe features

Exclusion Criteria

- Participants with known bleeding disorders who had a high propensity to bleeding regardless of their coagulation profile status.

Sample Size Determination and Sampling Procedure

Sample size

A single proportion formula for sample size determination by Fishers (1981) was used, assuming a prevalence of prolonged PT of 6.6% (Meshram et al. (2014) and 5% margin of error at 95% Confidence Interval (CI).

Formula

$$n = \frac{\mathrm{Z}^2 \mathrm{xp}(1-\mathrm{p})}{\mathrm{d}^2}$$

Assumptions:

Precision (d) = 5%

Prevalence (p) = 6.6%

Estimated sample size:

$$n = \frac{1.96^2 \mathrm{x} 0.053(1-0.053)}{0.05^2} = 95$$

Required sample size, n = 95

Data variables

	Independent	**Dependent**
	Socio-demographic characteristics: maternal age, parity, marital status, occupation, education level	
Objective 1		Prevalence of abnormal coagulation parameters among women with preeclampsia with severe features
Objective 2		Types of abnormal coagulation parameters among women with preeclampsia with severe features.
Objective 3		Proportion of adverse maternal outcomes in abnormal coagulation parameters among women with preeclampsia with severe features
Objective 4		Proportion of adverse fetal outcomes in abnormal coagulation parameters among women with preeclampsia

with severe features.

Sources and Methods of Recruitment

Recruitment began in the labor ward from the admissions book. The same was done for the mothers admitted in the antenatal wards. Files for all women with an entered diagnosis of PE with severe features were retrieved. A diagnosis of PE with severe features was confirmed by looking at the BPs at admission and after 4 hours, as well as other features of severity including platelet count, liver enzymes and creatinine levels from the filed results in the file prior to recruitment. A confirmation of the gestation age of 34 weeks was done by looking at the date of the last normal menstrual period, extrapolation from the ANC book, history on quickening and morning sickness, and or an ultrasound scan done prior to recruitment. The women meeting this criterion were prepared for recruitment and subsequent enrollment. The principal investigator with the help of the research assistants took the identified women through the study and consent sought. Informed, signed consent was obtained from the participants before being subjected to an interviewer guided questionnaire. After consent was administered, participants were enrolled into the study. This was done by a specially trained study assistant and the principal investigator from a private place in labor ward or ante natal ward. The first part of the questionnaire collected information about the socio demographic characteristics of the participants. The second part defined the features of severity. The third part captured the results of the coagulation screening and the fourth obstetric outcomes.

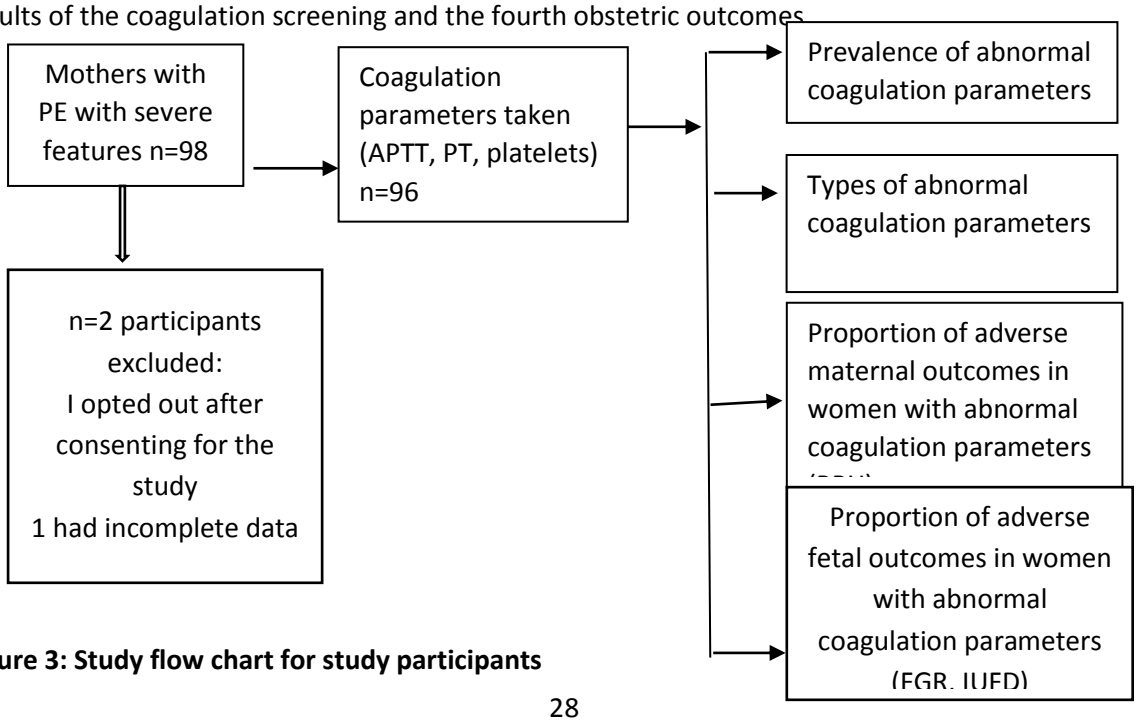


Figure 3: Study flow chart for study participants

Training for the Study Team

Two research assistants, a clinical officer and a nurse in the reproductive health unit, and the data manager were trained by the principal investigator. The team was trained on the study protocol, study tools, data collection procedures and of the confidentiality clause. Training was scattered to accommodate different shifts and availability of the trainees prior to the commencement of data collection at a selected area within the study site. The training also entailed piloting the questionnaire to 6 to 10 eligible participants in the maternity unit. The PI and the RAs worked closely with the data management teams and abided by the laid down standard operating procedures for data handling and security.

Data Collection Procedures

After ethical approval, data was collected using a structured questionnaire (annex II).The questionnaires were administered to participants who were consented by the principal investigator aided by trained research assistants. All participants with a diagnosis of PE with severe features were enrolled into the study and an interviewer administered questionnaire executed. Convenient sampling procedure was used to enroll the participants until the appropriate sample size was arrived at. The data was collected prospectively using a single questionnaire per participant. This was done over time from the time of enrollment, till delivery. Blood was collected at enrolment into the study.

The collected data was identified by assigning study specific unique identifiers to the participants. All electronic data was stored in an external hard drive and password protected after encryption. The standard KNH laboratory request form was used to collect data on the coagulation profiles. The collected data was verified by the PI on a daily basis before uploading to the excel sheet for cleaning and coding. All data sets were secured using a password, only known and accessible by the PI and the data manager. The decision to collect blood at one point was influenced by the observation that

most women come to us at around 34 weeks, when delivery is imminent within 48 hours within which they received corticosteroids to help in maturation of the fetal lungs. This gestation was thus optimal for survival of the fetus after delivery.

Laboratory Procedures

After the administration of the study questionnaire, files were reviewed to check for coagulation profile and platelet count results. Those who did not have results had their blood drawn for coagulation profile and total blood count tests- with emphasis on the platelet count. The samples were drawn, either by the principal investigator or trained research assistant, with the help of the doctor covering the respective wards for analysis in the laboratory. The samples were collected in a refrigerated light blue vacutainer tube containing sodium citrate for coagulation profiles and an EDTA containing purple vacutainer tube for total blood count, and taken to the KNH laboratory, accompanied by the usual KNH laboratory requisition form, within an hour. The blood was analyzed using automated ACL ELITE PRO and SYSMEX XN 1000 respectively. The results were received from the laboratory, filed and the files retrieved to analyze the results.

Quality Assurance

The questionnaires were pre-tested in labor ward, where the pilot study was conducted and analyzed before a final draft was administered to the study participants. The research assistants were trained on appropriate interview techniques, filling the questionnaires and principles of good clinical practice. Recording of clinical findings was done after thorough scrutiny. Unique identifiers were assigned to all the study participants. If double entries were discovered, one of the questionnaires was withdrawn, discarded and serialization rectified. Information filled on the questionnaires was checked for any errors and corrected.

Study samples were taken under aseptic conditions by trained research assistants who were either qualified clinical officers or nurses. In case of spillage or misplacement of the sample, this was

reported to the principal investigator for recollection. At the laboratory, the samples were stored and processed under strict biosafety standard operating procedures.

The normal range of platelet count is between 150,000-450,000/L. Thrombocytopenia is one of the criteria for preeclampsia with severe features and was a study variable. For preeclampsia the cut off is <100,000/However, not all participants with preeclampsia with severe features had this degree of thrombocytopenia. In this study, platelet count below 150,000/L was classified as abnormal.

Data Analysis and Presentation

Data was extracted from questionnaires and uploaded into an SPSS spreadsheet (version 25) for analysis. Frequencies and proportions of demographic data were calculated and presented in a table. Prevalence estimates with corresponding 95% confidence intervals of abnormal coagulation parameters, types of abnormal coagulation parameters, and proportions of adverse maternal and neonatal outcomes in adverse coagulation parameters were calculated using the exact Clopper-Pearson method.

Ethical Considerations

Permission was sought from the KNH and UON Ethics Research Committee (ERC) to carry out this study as part of the UON thesis dissertation. Permission was also sought from the KNH research committee and the department of Obstetrics and Gynecology, KNH. All the study participants were subjected at will to opt out consenting procedure, and were enrolled upon voluntarily signing the consent form. The procedure for blood collection was explained to the participants by the PI or research assistant. No pain management medications were provided during the blood collection process.

The participant's personal details were de-identified by use of an assigned unique identifier, only applicable to the study. This coded information was uploaded to the excel sheet and password protected. Back up data were kept in a password encrypted external hard drive, only known to the PI. No extra cost was charged to the patient.

Study Results Dissemination Plan

The results of the study were presented to the department of Obstetrics and Gynecology for inputs from the faculty and as part of the fulfillment of the master in Obstetrics and Gynecology. Following revisions by both the internal and external examiners, the findings will be disseminated to the KNH maternity in form of CMES and a report submitted to the hospital management.

Study strengths and limitations

Strengths:

This was the first study of its kind to be carried out locally. It therefore gives some back ground and room for future studies.

The study analyzed the different pathways of the coagulation cascade: APTT (intrinsic and common pathways), PT (extrinsic pathway).

The study also assessed immediate maternal neonatal outcomes.

Limitations:

The study entailed collecting data from the study participants and their records. This might have introduced information bias, which was reduced by collaborating information from the study participants and from the records before uploading to the excel software for cleaning and coding.

Only one time assessment of coagulation profile was done before delivery.

PPH was evaluated by visual estimation of maternal blood loss, and may have introduced bias in accurate measurement.

Serum fibrinogen and D-dimer tests were not done due to financial constraints, hence DIC was not excluded.

Other aspects of the coagulation cascade were not assessed as further factor studies were not done.

A detailed obstetric ultrasound for fetal surveillance looking at resistive indices, biophysical profile, estimated fetal weights was not done.

Study Closure and Plan

The study involved recruitment and data collection, followed by data analysis and presentation to the department of obstetrics and gynecology for review. The final stage will entail feedback to the key stakeholders. Recommendations made will be incorporated in the final report before publication.

CHAPTER 4: RESULTS

Ninety eight (98) women with preeclampsia with severe features of legal age were recruited with 96 consenting for enrollment into the study. Prevalence and types of abnormal coagulation parameters were analyzed.

Demographic and clinical characteristics

Table 1. Socio-demographic and clinical characteristics of women with preeclampsia with severe features at the Kenyatta National Hospital, 2019-2020

			N (98)	
			n	%
Demographic characteristics	Age	(Median (IQR))	29 (10)	
		<35	77	79
		35+	21	21
	Gestation	(Median (IQR))	36 (4)	
	Marital status	Single	21	21
		Married	77	79
	Education level	Tertiary	28	29
		Secondary	39	40
		Primary	31	32
	Occupation	Employed	34	35
		Unemployed	64	65

Clinical characteristics	Parity	Nulliparous	39	41
		1	23	24
		2	14	15
		3+	20	21
	Hist. prolonged bleeding	Yes	1	1
		No	97	99
	Hist. bleeding after delivery	Yes	1	1
		No	85	99
	AST	Normal	71	72
		Elevated	27	28
	ALT	Normal	68	69
		Elevated	30	31
	Creatinine	Normal	60	62
		Elevated	37	38
	Symptoms	Headache	70	71
		Visual disturbances	22	22
		Epigastric pain	25	26
		Respiratory distress	3	3
	Mode of delivery	Vaginal	27	28
		Caesarean section	68	72
	Gestation at delivery	(Median (IQR))	36.5 (4)	
		<37 weeks	49	50

	37+	49	50

In this study, majority of women with preeclampsia with severe features were less than 35 years of age with a prevalence of 79%. The median age was 29 years. Majority of these women were married (79%) and unemployed (65%). Most of these women were nulliparous with a prevalence of (41%), and 99% did not have a history of prolonged bleeding during menses or trauma.

(28%) of the participants had elevated AST levels, while 31% had elevated ALT levels. The median gestation at delivery was 36 weeks, with the main mode of delivery being caesarean section in 68 (72%) participants.

PREVALENCE OF ABNORMAL COAGULATION PARAMETERS AMONG WOMEN WITH PREECLAMPSIA WITH SEVERE FEATURES FROM 34 WEEKS TILL DELIVERY AT KNH

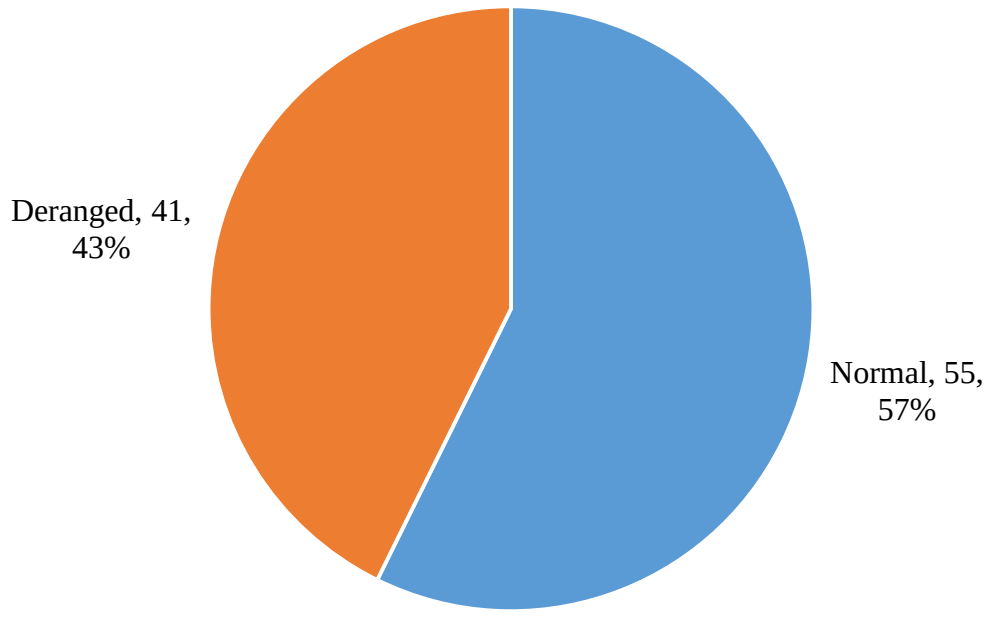
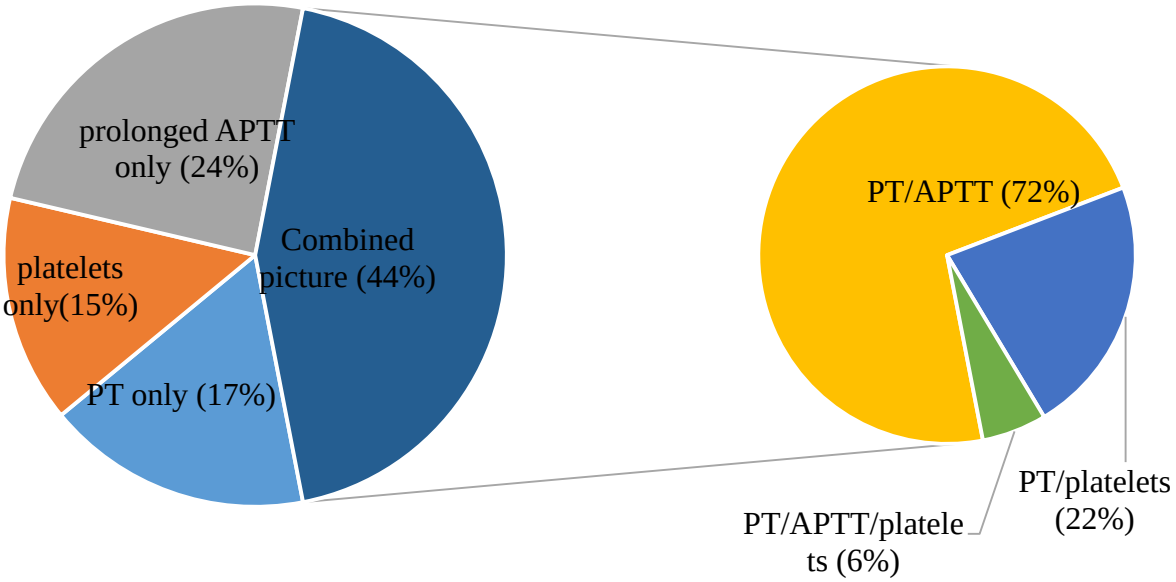


Figure 4. Prevalence of abnormal coagulation parameters among women with preeclampsia with severe features from 34 weeks gestation until delivery at KNH 2019-2020.

The prevalence of abnormal coagulation findings was **43% (95% CI = 32.8-52.6%)**

TYPES OF ABNORMAL COAGULATION PARAMETERS AMONG WOMEN WITH PREECLAMPSIA WITH SEVERE FEATURES FROM 34 WEEKS TILL DELIVERY AT KNH

Figure 5. Types of abnormal coagulation parameters among women with preeclampsia with severe features from 34 weeks gestation until delivery at KNH 2019-2020



According to Fig. 5 above, Majority of the participants had combined abnormal coagulation (44%), followed by those with prolonged APTT only (24%). The least abnormal parameter was platelets with a prevalence of 15%.

Among those with combined abnormal coagulation parameters, most of the participants had both PT and APTT abnormal (72%). Those with prolonged PT and low platelet counts were 22%, with the least combination being those with all three abnormal coagulation parameters 6%.

Table 2.Types and confidence intervals of abnormal coagulation parameters among women with preeclampsia with severe features from 34 weeks gestation till delivery at KNH 2019-2020

Abnormal coagulation parameter	Prevalence % (95% CI)
APTT	24 (11.2-37.5)
Platelets	15 (3.8-25.5)
PT	17 (5.6-28.6)
Combined abnormalities	44 (28.7-59.1)
PT/APTT	72 (51.2-92.9)
PT/Platelets	22 (3.0-41.4)
PT/APTT/Platelets	6 (0.0-16.1)

PROPORTION OF ADVERSE MATERNAL AND NEONATAL OUTCOMES IN ABNORMAL COAGULATION PARAMETERS

Table 3. Proportion of adverse maternal and fetal outcomes in abnormal coagulation parameters among women with preeclampsia with severe features from 34 weeks until delivery at KNH 2019-2020

Abnormal coagulation parameter	proportion, % (95% CI)		
	PPH	FGR	IUFD

PT	14 (0-40.2)	0 (0)	43 (6.2-79.5))
APTT	0 (0)	30 (1.6-58.4)	20 (0-44.8)
Platelets	0 (0)	17 (0-46.5)	0 (0)
Combined abnormal coagulation parameters	0 (0)	12 (0-27.1)	12 (0-27.1)

Most cases of PPH were encountered in participants with prolonged PT levels (14%). Cases of IUFD were also more prevalent in patients with prolonged PT at 43%. FGR cases were more prevalent amongst participants with prolonged APTT levels (30%). The incidence of IUFD and FGR were similar amongst participants with combined abnormal coagulation parameters.

CHAPTER 5: DISCUSSION, CONCLUSIONS, AND RECOMMENDATIONS

Discussion

The aim of this study was to look at the prevalence and types of abnormal coagulation parameters among women with preeclampsia with severe features. The prevalence of abnormal coagulation parameters was 43%. Most of the patients had more than one abnormal coagulation parameters. The proportion of adverse maternal outcome was more amongst the participants with prolonged PT levels. FGR was more common amongst the participants with prolonged APTT as compared to those with combined abnormal coagulation parameters. IUFD was most prevalent in those with prolonged PT levels.

The study showed that combined abnormal coagulation parameters were more common in women with preeclampsia with severe features (44%), evidenced by prolonged PT and APTT (72%). This was comparable to a study done by OO Awolola et al, who found a prevalence of 46% of prolonged PT and 23% of prolonged APTT in relation to thrombocytopenia. OO Awolola found the prevalence of combined abnormal coagulation parameters to be 6.7%, which was lower than the prevalence in this study of 72%. This prevalence was higher than that of Pritchard et al (50%) (6). The varying combinations and differences in prevalence could be attributed to the different populations studied (sample size 90 vs. 98 in our study, inclusion of eclampsia), individual predisposition to coagulopathy, or interpretation of the results.

Abnormal APTT was found to have a prevalence of 24%, comparable by a study by Lei Han et al, who found significantly prolonged levels of APTT among women with preeclampsia with severe features. This result shows that a degree of coagulation dysfunction occurs in the intrinsic coagulation pathways of patients with preeclampsia with severe features (6).

The prevalence of low platelets in this study was 15% that compared to a study by OO Awolola et al with a prevalence of 14.4% (prospective cohort study of 90 patients with a platelet count below 100,000/L).This difference was attributed to different cut off levels for definition of thrombocytopenia by different researchers (6)

The prevalence of PPH in this study was more among patients with combined abnormal coagulation parameters and prolonged PT 17% and 14% respectively. A retrospective study done by Gayat E et al showed that a PT more than 1.5 times the normal may predict the need for advanced intervention to control PPH (27). However the findings were in contrast to studies that have shown PT and APTT

do not have a predictive value in PPH (28,29). This study lacked power to adequately relate the abnormal coagulation Parameters to adverse maternal outcomes (PPH).

IUFD was more prevalent amongst participants with prolonged PT levels (43%). This was comparable to studies by Meshram et al (47%) and Maslow AD et al (15, 19). The mechanism behind this was due to release of tissue thromboplastin from the fetal circulation into the maternal circulation, activating maternal coagulation system, leading to intravascular consumption of coagulation factors and platelets (19).

FGR was more prevalent in participants with abnormal APTT levels (30%). This was comparable to a study by Venilla et al whose prevalence of FGR in prolonged APTT was 45% (21). The mechanism behind this is that the fetus is more sensitive to hypoxia and reduced blood flow induced by vasoconstriction and micro thrombi formed as a result of compromised coagulation status.

Conclusion

- The prevalence of abnormal coagulation parameters (43%) was significant.
- The most common abnormality was combined abnormal coagulation parameters at 44%.
- PPH and IUFD were more prevalent in patients with abnormal PT at 14% and 43% respectively, whereas FGR was more prevalent amongst participants with abnormal APTT at 30%.

Recommendations

- Coagulation profile should be integrated in routine screening of women with pre-eclampsia with severe features due to the high prevalence of abnormal coagulation parameters.
- A total blood count alone for detection of thrombocytopenia is inadequate in assessment of abnormal coagulopathy in patients with preeclampsia with severe features
- More studies required to assess the role of serial coagulation profile testing and the association between abnormal coagulation parameters and adverse maternal and fetal outcomes.

TIMELINES

	2019					
	July	**August**	**October**	**November2019-January 2020**	**February 2020- April 2020**	**May 2020**
Proposal Development						
Proposal Presentation						
Ethics Review						
Data Collection						
Data Analysis						
Report writing and presentation						
Submission to the department						

BUDGET

Item	Description	Amount in Ksh.
Personnel	A) 1 research assistants' allowances @ ksh. 5,000/month X 3 months b) Data clerk/ Statistician @ Ksh. 30,000	Ksh. 15,000 Ksh. 30,000
Supplies	Draft proposals printing: 60pages, 3 copies @ Ksh. 10 per page	Ksh. 1,800
	Final proposal printing; 60 pages, 3 copies @ Ksh.10 per page	Ksh. 1,800
	Questionnaire Printing, 4pages @ Ksh. 10 per page	Ksh. 40
	Questionnaires photocopying, 4 pages, 98 copies @ Ksh. 2 per page	Ksh. 790
	Airtime @ Ksh.2000	Ksh. 2000
Transport costs	-	Ksh. 1000

KNH/UoN ERC	Submission to ERC (twice)	Ksh. 4,000
Miscellaneous costs	-	Ksh. 10000
TOTAL		**Ksh. 66,430**

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ANNEXES

Annex I: Informed Consent (English)

PREVALENCE AND TYPES OF ABNORMAL COAGULATION PARAMETERS IN PREECLAMPSIA WITH SEVERE FEATURES AT KENYATTA NATIONAL HOSPITAL, 2019; A CROSS SECTIONAL STUDY.

Principal investigator: Dr. Chege Rahab Njeri.

Introduction:

I Dr. Rahab Chege, a postgraduate student at the Department of Obstetrics & Gynecology, University of Nairobi, am conducting a study on **Prevalence and Types of Abnormal Coagulation Parameters In Preeclampsia with Severe Features at Kenyatta National Hospital, Maternity department from34weeks gestation until delivery**. You are hereby requested to participate in the study.

This information will help you make a decision on whether to participate in the study or not. You may ask any questions about the study or anything in this form that is not clear.

Purpose of the study:

In participants with preeclampsia with severe features, the coagulation system is affected and derangements in these factors may have a key role in both maternal and fetal outcomes.

This study has not been done locally, and therefore, this study can help in policy formulation regarding management of abnormal coagulation parameters in women with preeclampsia with severe features.

Benefits:

Your participation will help us identify if indeed there is an association between abnormal coagulation parameters in women with preeclampsia with severe features. This information will go a long way in helping women locally, regionally and in the world.

Possible risks:

This study will be slightly invasive in that, a small amount of blood (2mls), might be drawn from your vein for testing in the lab. Slight pain will be experienced during the process. There will be no added risks to your standard care as that accorded to other participants.

Voluntarism:

This is a voluntary exercise and you can withdraw at any point during the study with no repercussions. The management you receive at the hospital will be standard and not influenced by your decision.

Compensation:

No compensation will be offered for participation in the study.

Procedure:

As a study participant, the researcher and research assistant will obtain some information from your medical records and conduct a short interview with you and your responses filled in a questionnaire. The questions asked include: your age, number of children, marital status, education level, prior history of hypertension, history of bleeding disorders.

Blood, approximately 2mls will be drawn for testing in the lab. You will be followed up daily until delivery and immediate outcomes documented. The complications that occur will be handled in KNH by respective doctors.

Confidentiality:

The information from you and from the medical records will be confidential. No names or any information identifying you will be included in the questionnaires and the final report.

Contact information:

If you have any questions regarding the study, you can contact Dr. Rahab Chege through telephone number 0723241102. You may also contact the KNH/UoN/ERC Commitee-0735-274288/0721-665077.

Or

The chairperson,

KNH/UON Ethics and Research Committee

P.O. Box 20723-00202, Nairobi.

Telephone number: (254-020) 2726300-9 Ext 44355

Email: uonknh_erc@uonbi.ac.ke

Your participation in the study will be highly appreciated.

Consent:

I_____ hereby voluntarily consent to participate in the study. I acknowledge that a thorough explanation of the nature of the study has been given to me by Dr./Mr./Mrs._____. I clearly understand that my participation is completely voluntary.

Signature of Participant _____ Date _____

Signature of Researcher/ Assistant _____ Date _____

Annex II: Informed Consent (Swahili)

Fomu ya ithini

kichwa cha utafiti:

MAAMBUKIZI NA AINA ZA VIGEZO VYA KUGANDA DAMU VISIVYO VYA KAWAIDA KATI YA WAJAWAZITO WALIO NA PREECLAMPSIA YA SIFA KALI KATIKA HOSPITALI KUU YA KENYATTA, 2019.

Mtafiti Mkuu: Dk. Chege Rahab Njeri

Utangulizi:

Jina langu ni Rahab Chege, mwanafunzi wa shahada ya kwanza katika Idara ya Obstetrics, Chuo Kikuu cha Nairobi. Ninafanya utafiti ili **maambukizi na aina za vigezo vya kuganda damu visivyo vya kawaida kati ya wajawazito walio na preeclampsia ya sifa kali wiki 34 za ujauzito.**

Taarifa hii itakusaidia kufanya uamuzi juu ya kushiriki katika utafiti au la. Unaweza kuuliza maswali yoyote kuhusu utafiti au chochote katika fomu hii ambacho hukielewi.

Kusudi la utafiti:

Kwa kujiunga na utafuti huu, utasaidia kulinganisha matokeo ya ujazito miongoni wa wamama walio na vigezo vya kukata damu visivyo vya kawaida na vilivyo vya kawaida baina ya wale walio na ugonjwa wa shinikizo la damu iliyo na vipengele kali. Hii itasaidia kuboresha maisha ya wamama wajawazito walio na shida hii, pamoja na watoto wao.

Faida:

Kwa kushiriki katika utafiti huu, utatuwezesha kutarajia matatizo yoyote ambayo yanaweza kutokea katika ujauzito wako wa baadaye ikiwa una hali hii na kujaribu kuyazuia. Utapata pia habari kuhusu hali hii inayoathiri wanawake wengi kama vile dalili zake na jinsi inavyoweza kutibiwa, na maswali yoyote unaweza kuwa nayo kuhusu hali hii yatajibiwa.

Hatari zilizowezekana:

Hakuna hatari zinazohusika na ushiriki wako katika utafiti huu. Utapokea kiwango cha utunzaji kama wagonjwa wengine katika hospitali. Utatolewa damu kidogo tu takribani 2mls. Utasikia uchungu kidogo tu damu ikitolewa. Matibabu unayoyapata yataendelea vile vile hata ukijihisi kutoka katika utafuti huu.

Ushiriki:

Kushiriki katika utafiti huu ni kwa hiari. Ikiwa unachagua kushiriki katika utafiti huu, unaruhusiwa kuondoka wakati wowote ikiwa unataka kufanya hivyo. Utunzaji unaopokea kutoka hospitali hauwezi kuathiriwa na uamuzi unaofanya

Fidia:

Hakuna fidia itatolewa kwa kushiriki katika utafiti huu.

Utaratibu:

Ikiwa unakubali kushiriki katika utafiti huu, mchunguzi mkuu au msaidizi wake wa utafiti atawahoji wewe na kujaza majibu katika maswali. Msaidizi anaweza pia kupata maelezo ya ziada kutoka kwa kumbukumbu zako za matibabu. Baina ya maswali utakayo ulizwa ni: miaka yako, watoto ulionao, kama umeolewa, kiwango chako cha masomo, kama umekua na shida ya kuoanda damu hapo awali, kama ukona shida yoyote ya kuvuja damu.

Baada ya hapo, damu itatolea kiasi cha millilita mbili na kutumwa kwenye maabara kwa uchambuzi, Timu ya utafiti itafuatilia kila siku mpaka siku tatu baada ya kujifungua ili kuangalia shida zozote zitakazo tokea kwako ama kwa mtoto wako na hizi zitaandikwa. Shida ambazo zitatokea, zitashughulikiwa na madaktari papa hapa KNH.

Usiri:

Taarifa utakayatoa itahifadhiwa kwa siri. Majina au maelezo yoyote ya kukutambulisha hayatakuwa kwa ripoti ya mwisho

Maelezo ya mawasiliano:

Kwa habari zaidi juu ya utafiti unaweza kuwasiliana na wafuatao:

Mtafiti mkuu, Dk. Chege Rahab Njeri

Idara ya Obstetrics na Gynecology, Chuo Kikuu cha Nairobi

Namba ya simu. 0723241102.

Au

Mwenyekiti,

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Barua pepe: uonknh_erc@uonbi.ac.ke

Idhini

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Sahihi ya Mshiriki _____ Tarehe _____

Saini ya Mtafiti / Msaidizi _____ Tarehe _____

Annex III: Study Questionnaire

Date _____ Serial number _____

SECTION A: socio-demographic and clinical characteristics

1. Age _____ years.

2. Gestation ~~———~~weeks

3. Marital status
    a) Single ☐
    b) Married ☐
    c) Separated ☐
    d) Divorced ☐

4. Educational level
    a) Tertiary☐
    b) Secondary ☐
    c) Primary ☐

5. Occupational level
    a) Self employed ☐
    b) Unemployed ☐

6. Parity
    a) Nulliparous ☐
    b) 1 ☐
    c) 2 ☐
    d) ≥3

7. History of previous prolonged bleeding after trauma.

a. Yes ☐

b. No ☐

History of bleeding after bleeding (for mothers who have previously given birth, for primigravidas, history of prolonged bleeding after trauma, heavy menses)

b. Yes ☐

b. No ☐

SECTION B:Features of Severity

8. Bp:
   a) At admission: mmHg _____

   b). after 4 hours:mmHg _____

Symptom(s)	YES	NO	Duration of onset in days
headache			
Visual disturbances			
Epigastric and or right upper quadrant pain			
Respiratory distress			

(pulmonary edema)			

9.

10. Liver enzymes:
    a) AST levels:Up to 40U/L
    b) ALT levels:Upto 35U/L

11. Creatinine levels: Up to 90umol/L

SECTION C:Hemostatic parameters

12. Platelet count

    a) >150,000/L☐
    b) 100,000-150,000/L☐
    c) 70,000-100,000/L☐
    d) <70,000/L☐

13. Prothrombin time: Normal between (11.8 to 15.1 seconds):

14. Activated partial thromboplastin time: (Normal between 24.3 to 35 seconds)

SECTION D: Maternal outcomes

15. Post-Partum Hemorrhage: Estimated blood loss after delivery: _____ mls.

16. Disseminated Intravascular Coagulopathy
    a) Yes ☐
    b) No ☐

17. Gestational age at delivery : _____ weeks

18. Mode of delivery

    a) Vaginal delivery ☐
    b) Assisted vaginal delivery ☐
    c) Caesarean section ☐

19. Duration of labor (for SVD)

    a) 1st stage _____ min.

    b) 2nd stage _____ min.

SECTION E: Fetal outcomes

20. Fetal Growth Restriction

    a) Yes ☐
    b) No ☐

21. Intra Uterine Fetal Death

    a) Yes ☐
    b) No ☐

DISSERTATION

PREVALENCE AND TYPES OF ABNORMAL COAGULATION PARAMETERS IN PRE-ECLAMPSIA WITH SEVERE FEATURES AT KENYATTA NATIONAL HOSPITAL, A CROSS SECTIONAL STUDY, 2020

DR- CHEGE RAHAB NJERI- H58/88236/2016.

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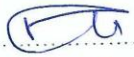
Sign: 

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