ASSOCIATION BETWEEN PRE-ECLAMPSIA AND PATTERNS OF EARLY PREGNANCY BLEEDING SEEN AMONGST POSTNATAL WOMEN TREATED AT THE KENYATTA NATIONAL HOSPITAL, NAIROBI, KENYA. A CASE CONTROL STUDY

Principal investigator:

H58/80924/2015

Dr Emmanuel Sinei,

A RESEARCH THESIS SUBMITTED IN PARTIAL FULFILMENT FOR MASTER'S DEGREE IN OBSTETRICS AND GYNAECOLOGY AT THE SCHOOL OF MEDICINE, UNIVERSITY OF NAIROBI

2021

DECLARATION

This is to declare that this thesis is my original work and has not been undertaken and presented for a degree in any other university. It was carried out with the guidance of my supervisors, and reference to work done by others has been indicated.

Dr Emmanuel Kiprono Sinei Date 3rd November 2021

SUPERVISORS' APPROVAL

This proposal has been submitted with the approval of my supervisors.

Professor Omondi Ogutu

MBChB; M.Med.(Obs/Gyn); PGDRM; FCOG(ECSA)

Chair, Department of Obstetrics and Gynaecology,

University of Nairobi.

Signed **Date 2nd November 2021**

Dr Diana Ondieki

MBChB; M.Med.(Obs/Gyn)

Consultant Obstetrician and Gynaecologist, Lecturer, Department of Obstetrics and Gynaecology,

University of Nairobi.

Signed.…… Date.…02/11/2

Dr Maureen Owiti

MBChB; M.Med.(Obs/Gyn)

Consultant Obstetrician and Gynaecologist, Kenyatta National Hospital.

Signed **3/11/2021**

CERTIFICATE OF AUTHENTICITY

This is to certify that this dissertation is the original work of **Dr. Emanuel Kiprono Sinei** a Master of Medicine student in Obstetrics and Gynaecology, registration number, **H58/80924/2015,** University of Nairobi. The research was carried out in the Department of Obstetrics and Gynaecology, School of Medicine, College of Health Sciences under the guidance and supervision of Professor Omondi Ogutu, Dr. Diana Ondieki and Dr. Maureen Owiti. It has not been presented in any other university for the award of a degree.

DEPARTMENT OF OBSTETRIC AND GYNAECOLOGY
P.O. Box 19676, NAIROBI
Date: 5/11/2021
COLLEGE OF HEALTH SCIENCES
UNIVERSITY OF NAIROBI

Signature: ..... **Date:** 5/11/2021

Professor Eunice Cheserem, MBChB, MMed (Obs/Gyn), PGDRM

Associate Professor, Faculty of Health Sciences,

Consultant Obstetrician and Gyanecologist, Kenyatta national Hosptal,

Chairperson, Department of Obstetrics and Gynaecology

University of Nairobi

LIST OF ABBREVIATIONS

ACOG- The American College of Obstetricians and Gynaecologists

PE- Pre-eclampsia

IUGR- Intra-uterine growth restriction

SGA- Small for gestational age

BJOG- British Journal of Obstetrics and Gynaecology

PIH- Pregnancy-induced Hypertension

EPVB- Early pregnancy vaginal bleeding

KNH- Kenyatta National Hospital

UON- The University of Nairobi

CME- Continuous medical education

IUD- Intra-uterine contraceptive device

D&C- Dilation and curettage

TOP- Termination of Pregnancy

DEFINITION OF TERMS

Preeclampsia: Defined using the ACOG definition(1) as systolic blood pressure greater than or equal to 140mmHg or diastolic blood pressure greater than or equal to 90mmHg on at least two occasions at least 4 hours apart after 20 weeks of gestation in a previously normotensive woman with proteinuria of equal to or greater than 0.3 grams in a 24 hour specimen or dipstick result of greater than or equal to 1+ OR new onset of any multisystem complication of pre-eclampsia.

Early pregnancy: The first twenty weeks of gestation.

LIST OF TABLES

Table 1: Frequency distribution of maternal characteristics based on presence or absence of pre-eclampsia among postnatal patients with singleton pregnancies treated at the Kenyatta National Hospital 18

Table 2: Frequency distribution of bleeding patterns based on presence or absence of pre-eclampsia among post-natal mothers with singleton pregnancies treated at the Kenyatta National Hospital 19

Table 3: Unadjusted and adjusted associations between maternal characteristics and pre-eclampsia among postnatal women with singleton pregnancies treated at the Kenyatta National Hospital 20

Table 4: Association between bleeding patterns and pre-eclampsia among postnatal mothers with singleton pregnancies treated at the Kenyatta national Hospital 21

Table 5: Unadjusted and adjusted association between maternal characteristics and bleeding in early pregnancy among postnatal mothers treated at the Kenyatta National Hospital 22

LIST OF FIGURES

Figure 1: Schematic representation of hypothesized pathway between maternal baseline characteristics, through vaginal bleeding to pre-eclampsia 9

Figure 2: Schematic representation of study procedures from recruitment to analysis 13

Figure 3: Schematic representation of study procedures from recruitment to analysis 17

CONTENTS

ABSTRACT v

CHAPTER ONE: INTRODUCTION 1

Background and Literature Review **1**

Study Justification **7**

Research Question: **8**

Study Objectives: **8**

Conceptual framework **9**

CHAPTER TWO: METHODOLOGY 10

Study Design **10**

Study site and description **10**

Sample size determination **11**

Study procedures **12**

CHAPER THREE: RESULTS 17

CHAPTER FOUR: DISCUSSION 23

Conclusion: **24**

Recommendations: **24**

REFERENCES 26

APPENDICES 29

Appendix 1: Participant Information and Consent Form **29**

Appendix 2: Study Questionnaire **33**

Appendix 3: Study Timelines **35**

Appendix 4: Budget **35**

Appendix 5: KNH/UoN ERC APPROVAL **36**

ABSTRACT

Background: Vaginal bleeding in early pregnancy is a common event estimated to occur in up to 40% of all pregnancies. The leading cause of such bleeding is miscarriage,affecting 16-25% of pregnancies. Suchbleeding is thought to occur due to abnormal functioningor development of the placenta and has been associated with an increased incidence of various adverse pregnancy outcomes among those pregnancies that proceed beyond 20 weeks of gestation.The outcome of interest for our study is preeclampsia. Pre-eclampsia is a rare condition estimated to complicate about 5-10% of pregnancies globally. The onset of pre-eclampsia cannot be reliably predicted,and this remains an area of ongoing research. The results of observational studies have been conflicting on the association between vaginal bleeding in early pregnancy and pre-eclampsia.

Study Objective: To study the association between vaginal bleeding in early pregnancy and the risk ofsubsequent pre-eclampsia.

Methods: We conducted our study at the Kenyatta National Hospital in Nairobi, Kenya. Our study was an unmatched case control study. Our sampling frame was all post-natal mothers within the first 72 hours after delivery treated at KNH. We stratified study participants into cases (post-natal mothers with pre-eclampsia in pregnancy) and controls (postnatal mothers without pre-eclampsia in pregnancy) and compared them with regards to history of per vaginal bleeding during the first 20 weeks of gestation. Study participants were identified from records in the post-natal wards. After obtaining informed consent from eligible participants, the information of interest was obtained using a structured study questionnaire administered by trained research assistants with verification of information given from patient case notes. The bleeding characteristics assessed were any bleeding in the first 20 weeks of pregnancy, maximal intensity of bleeding, duration of bleeding and number of episodes of bleeding. Descriptive statistics and regression analyses were used for data analysis using SPSS version 26 software.

Results: The study comprised 304 participants. 152 cases and 152 controls. Bleeding in early pregnancy was significantly associated with increased risk of subsequent pre-eclampsia (OR 2.110, 95% CI 1.184-3.763, p value 0.011).

Conclusion: Vaginal bleeding in early pregnancy is a common event and is associated with pre-eclampsia. The results of studies on this association are mixed. Further studies, especially in African populations whose risks have been found to vary from those of Indo-European populations, would shed further light on this issue.

Study significance: Few studies have been done globally looking at the association between early pregnancy bleeding and pre-eclampsia. No such study had been done previously on an African population though epidemiological data has shown racial disparities in risk of pre-eclampsia, with Africans having higher risk. Our study is the first of its kind carried out in a pre-dominantly African population and sheds light on early pregnancy bleeding patterns and their associated risk for pre-eclampsia. Our findings will inform future research on this topic in Africa.

Key words:

Pre-eclampsia
Early pregnancy
Threatened miscarriage

CHAPTER ONE: INTRODUCTION

Backgroundand Literature Review

Vaginal bleeding is common in pregnancy. Nicolaides, Poon and Kypros (2)estimated it to occur in 20 to 40 percent of pregnancies. The main sources of non-traumatic bleeding in early pregnancy as reported by Saraswat, Bhattacharya and Maheshwari (3)are ectopic pregnancy, miscarriage, implantation bleeding, trophoblastic disease, bleeding due to cervical, vaginal or uterine pathology including polyps and bleeding due to inflammation or infection of the genital tract.

Bleeding related to miscarriage is the most common non-traumatic cause of first trimester bleeding with a prevalence of between 16 and 25 percent reported in several observational studies(4,5,6,3,7,8). Any bleeding in pregnancy causes much anxiety to the mother, her family and to health care workers. An understanding of the possible outcomes of ongoing pregnancies complicated by early vaginal bleeding is important for reassurance of the mother, planning antenatal care and considering relevant interventions.

Weiss, Malone and Vivader (7) estimated the incidence of spontaneous abortion after first trimester bleeding to be 50% without sonographic evaluation for fetal viability. For those cases in which a viable fetus is noted on ultrasound examination, they reported a 95 to 98% chance of such pregnancies continuing beyond 20 weeks of pregnancy.

It has been hypothesized in several studies that vaginal bleeding in early pregnancy indicates an underlying placental dysfunction (9,4,6,7) which may manifest later in pregnancy as complications such as gestational hypertension/preeclampsia (9,4,10,11,12,7), preterm delivery, preterm premature rupture of membranes, intra-uterine growth restriction, placental abruption and increased caesarean rates among others.

Hasanet al (6)carried out a prospective cohort study between 2000 and 2009 looking at the patterns and predictors of vaginal bleeding in early pregnancy in the United States. They reported that 75.6% of all bleeding episodesamong study participants were described as spotting and most of them, 70.7%, were painless. The factors predictive of bleeding from this study were maternal age between 28 and 34 years, nulliparity, long or short cycle length, history of prior

miscarriage, history of induced abortion and infections in early pregnancy. Of note, BMI, ethnicity/race, marital status, poverty level, active or passive smoking, alcohol intake, caffeine intake were not predictors.This study demonstrated that bleeding in early pregnancy is a common event.

The peak incidence of bleeding at 6 to 7 weeks gestation was significant for our studyas this represents the period of luteo-placental shift when progesterone production begins to shift from the corpus luteum to the placenta. This supports the hypothesis that this bleeding occurs as a result of either defective development or functioning of the placenta.

Several studies have demonstrated that in normal pregnancies, optimal invasion of the endothelial walls of maternal spiral arteries is necessary for normal placentation (13,14). This leads to conversion of maternal spiral arteries into flaccid, utero-placental blood vessels(15,16). Miscarriage and preeclampsia manifest at different stages of pregnancy yet both have been shownto be associated with defective trophoblast invasion. (9,12). Piijenborg, Anthony and Davey (12) demonstrated this in their study that involved histological examination of placental bed tissue samples obtained from patients undergoing caesarean section delivery. Hustin et al (17) demonstrated histologically, suboptimal trophoblastic invasion in almost two thirds of early pregnancy loss tissue samples. Burton and Jauniaux (9) hypothesized that soon after implantation, migrating trophoblastic cells initially occlude maternal spiral arteries thereby limiting maternal blood flow into the placenta. This was consistent with the findings of Hempstock et al. (18)who showed that the early embryodevelops in a low oxygen environment. They further hypothesized that this was in order to protect differentiating embryonic cells from the harmful effects of free oxygen radicals produced during normal metabolic processes.

Watson et al (19) reported that at around the 10th week of gestation, the cellular plugs begin to dissipate, initially from the periphery withmaternal intra-placental circulation becoming fully established at about 12 weeks of gestation. This is marked by a significant rise in oxygen tension.These findings are supported by findings of previous studies (20,19,21,22,23,24) that carried out oxygen measurements in early placental tissues. Watson et al reported that partial pressure of oxygen in intraplacental tissues at 7-10 weeks is <20mmHg. They hypothesized that these conditions favour cellular differentiation and blastulation. This contrasted with oxygen partial pressures at 11-14 weeks whichrose to >50mmHg. They found that this rise was

associated with increased markers of oxidative stress (19) and hypothesized that this process is important for normal angiogenesis and cytotrophoblast proliferation, migration and fusion necessary at this stage of development.

Burton and Jauniaux (9) concluded that when trophoblast invasion is severely impaired, the plugging of maternal arteries is incomplete leading to premature establishment of maternal villous circulation. They further hypothesized that the resultant oxidative stress leads to widespread syncitiotrophoblastic damage and this is thought to be a likely contributory factor to miscarriage. This assertion is supported by findings from previous studies (25,26).

Hensley et al (27) reported that miscarriage represents the extreme end of a spectrum between it and normal placental development. They theorized that between these two conditions would be different degrees of trophoblastic invasion some of which would be compatible with ongoing pregnancy.They hypothesized that structural damage, apoptosis, necrosis and activation of stress response molecule signaling associated with the resultant oxidative stress would have effects later in pregnancy, contributing toendothelial dysfunction seen with preeclampsia.

Burton and Jauniaux (9) reported that as a result of these changes, placental perfusion would be impaired, depending on the degree of initial insult. They found thateven in normal pregnancy, oxygen concentrations fluctuate within the intervillous spaces in later pregnancy as a result of external compression of maternal arteries, intrinsic contraction of the same or redistribution of maternal blood flow for any reason including exercise and postural changes.This would result in transient episodes of hypoxia in the placenta and fetus as they constantly extract oxygen from the intervillous circulation. They hypothesized that these intermittent episodes of hypoxia lead to oxidative stress in the placenta through an ischemia-reperfusion phenomenon and added that the process would beexaggerated in placentas already exhibiting reduced perfusion due to impaired trophoblastic invasion.

Hafiner, Metzenbauer and Hofinger (28) hypothesized that the two forms of preeclampsia, namely early onset and late onset pre-eclampsia also represent a spectrum of oxidative stress induced by varying degrees of impairment of trophoblast invasion. They reported that in early onset preeclampsia, associated with FGR, placental volume is demonstrably reduced from as early as the 12^{th} week. They hypothesized that in late onset preeclampsia, the placental insult is less severe and the maternal vascular changes less extensive. Mayhew et al reported that in the

case of late onset pre-eclampsia, placental volume remains normal and the condition is not associated with growth restriction.(29)

As supported by the above literature, transition of maternal to placental circulation is a carefully coordinated process aimed at preventing overwhelming oxidative stress to the placenta. Trophoblast invasion, if incomplete, worsens intermittent perfusion of the inter-villous space seen in later pregnancy thus predisposing the mother to preeclampsia. From this, it can be hypothesized that early pregnancy losses and preeclampsia form a spectrum of conditions characterized by placental oxidative stress strongly linked to the depth of cytotrophoblast invasion of maternal spiral arteries.

A few observational studies have been done to evaluate the outcomes of pregnancies complicated by early pregnancy bleeding. Most studies were retrospective cohort studies where the exposure was bleeding in early pregnancy and several outcomes were reported on. We include in our review those studies that mention preeclampsia as an outcome of interest.

Sadat H. and Soghra Y, 2013(30) conducted a cohort study in India to assess the outcomes of pregnancies complicated by bleeding in the first trimester. The case arm constituted 236 gravida 1 and gravida 2 patients with the control group being made up of 944 gravida 1 and 2 patients with no history of bleeding in the first trimester. They found a significant association between bleeding in the first trimester and gestational hypertension (RR- 5.0) but no significant association between this and preeclampsia (RR- 1.7). This study did not differentiate between different bleeding patterns. Maternal characteristics and exposures were also not analyzed. The association found between vaginal bleeding in the first trimester and gestational hypertension is also significant for our study as this supports the hypothesis put forward by Burton G. and Jauniaux E.(9) that gestational hypertension and preeclampsia fall under the spectrum of conditions resulting from placental oxidative stress.

Weiss J., Malone F., Vivader J et al, 2003(7) conducted a large retrospective multicenter database study in New York to determine if patients with bleeding in the first trimester were at increased risk for poor pregnancy outcomes. 16,506 records with complete antenatal, birth and pediatric outcomes were reviewed. The samples were divided into three groups; 1-no bleeding 14,160 patients, 2-light bleeding (spotting only) 2094 patients and 3- heavy bleeding (like menses) 252 patients. They found that patients with vaginal spotting were more likely to have

preeclampsia compared to controls (OR 1.4, 95% CI 1.1-1.8). They found no significant difference in the incidence of preeclampsia or gestational hypertension between controls and those with heavy PV bleeding.

Funderburk J., Guthrie D. and Meldrum D. 1980 (31) carried out a retrospective cohort analysis of data of 25, 387 consecutive deliveries in Los Angeles between April 1963 and June 1964 to assess pregnancy outcome in deliveries complicated by bleeding in the first or second trimester. The control arm consisted of 25,118 patients while the case arm consisted of 259 patients who were further divided into two groups; those with heavy bleeding (more than one teaspoon in volume, recurrent, lasting more than one week or requiring treatment) and those with light bleeding (one episode of spotting lasting not more than one week). They found no significant association between bleeding in early pregnancy and the incidence of preeclampsia. In this study, light bleeding was not evaluated due to a small sample size.

Dadkhah F., Kashanian M. and Eliasi G. 2010 (5) conducted a prospective cohort study in Iran to evaluate the pregnancy outcome in pregnancies with threatened abortion. They defined threatened abortion as any bleeding in the first half of pregnancy. They compared 500 women with history of vaginal bleeding in the first half of pregnancy to 500 women with no history of vaginal bleeding. They reported no significant association between vaginal bleeding and preeclampsia. They did however report that 6.2% of those with bleeding in the first trimester developed pre-eclampsia while 4.6 % of those without bleeding in the first trimester developed pre-eclampsia. This represents a relative risk of 1.3.

Chhabra S., Tickoo C. and Kalra P. 2014 (4) conducted a prospective cohort study in India between 2009 and 2010 to determine perinatal outcomes among singleton pregnancies complicated by bleeding in the first 20 weeks of pregnancy. 7,040 patients were included in the study. 1020 had presented with bleeding in the first half of pregnancy. Of these, 507 had bleeding before 10 weeks while 513 had bleeding between the 10^{th} and 20^{th} weeks of gestation. Among those whose pregnancies that continued beyond 28 weeks, they found a significant association between bleeding in early pregnancy and pregnancy induced hypertension and pre-eclampsia compared to controls. No difference was found between the two case groups. No distinction was made between gestational hypertension and pre-eclampsia in this study. They also did not analyze different patterns of bleeding or maternal characteristics.

Wijesiriwardana A., Bhattacharya S., Shetty A. et al conducted a retrospective cohort study in Aberdeen, Scotland in 2006 to assess pregnancy outcomes in women with threatened miscarriage in the first trimester. Data from 39,260 pregnancies between 1974 and 2006 was obtained from records. Cases were defined as primigravidae with history of vaginal bleeding who delivered after 24 weeks of gestation (7627) while controls were primigravidae without vaginal bleeding who delivered within the same period (31633).They found no difference in incidence of pre-eclampsia between the two groups.

Oppenraij V., Jauniaux E., Christiansen O., et al 2009 (10) conducted a systematic literature review to evaluate the impact of early pregnancy events and complications as predictors of adverse obstetric outcomes. Literature search was done from MEDLINE and Cochrane databases for studies published between 1980 and 2008. Several early pregnancies events and their associated perinatal complications were reviewed. Among those early events with clinically relevant (OR>2.0) associations, threatened abortion was associated with PIH and preeclampsia.

In a systematic review of literature published in 2009 in the BJOG, Saraswat L., Bhattacharya S., Maheshwari A., et al (3) explored the effects of threatened miscarriage on maternal and perinatal outcomes. Preeclampsia and pregnancy induced hypertension were among the main outcome measures for the study. A total of 14 studies were included in the meta-analysis. Of these, 6 reported on preeclampsia/eclampsia and PIH. They concluded that the incidence of PIH, preeclampsia/eclampsia were not significantly altered by bleeding in the first trimester; OR 0.99, 95% CI 0.84-1.17.

Smits L., North R., Kenny L. et al (32)conducted a prospective cohort study looking at the association between different patterns of bleeding in early pregnancy and pre-eclampsia. While they reported no increased risk for pre-eclampsia among those who reported bleeding as compared to controls, they found that certain bleeding patterns were more likely to be associated with pre-eclampsia compared to others.

Apart from the studies by Weiss et al (7) and Smits et al (32), no distinction was made between different bleeding patterns in reported results. Maternal baseline characteristics were also not reportedthough these likely differed between women with and without bleeding and could have influenced the study outcomes. There was also no standard definition for the degree of intensity of bleeding in the different studies.

Study Justification

The aetiology of preeclampsia is poorly understood. Current consensus is that its aetiology is multi-factorial and the with the result being placental dysfunction. The multifactorial nature of its aetiology is the subject of much research globally looking at ways to predict its onset using risk stratification models based of maternal risk factors and clinical biomarkers(2). The use of risk scoring models and biomarkers in the prediction of pre-eclampsia was not recommended until recently.

The International Federation of Gynaecology and Obstetrics in May 2019 adopted the position that all pregnant women should be screened for pre-eclampsia using the first trimester combined test that comprises maternal risk factors, mean arterial pressure, uterine artery pulsatility index and placental growth factor as a one-step procedure at $11\text{-}13^{+6}$ weeks of gestation. A free online risk calculator designed by the Fetal Medicine Foundation is available for this purpose.(33)

Early pregnancy vaginal bleeding is an easily identifiable condition and could potentially be a cost-effective tool for identifying those at increased risk of developing PE. Few studies have been conducted globally looking at the association between early pregnancy bleeding and preeclampsia. The results of these studies have been conflicting. These studies have been carried out on predominantly Indo-European populations. We did not come across any such studies done on a predominantly African population though black race is a recognized independent risk factor for hypertensive diseases in pregnancy(2,1,34).

Comparability of previous studies has been hampered by not making distinction between different bleeding patterns during analysis, the lack of a standard definition of the different patterns and not including subjects' baseline characteristics that could be potential confounders.

One previous study by Weiss J., Malone F., Vivader J. et al (7)found that specific bleeding patterns, not early pregnancy bleeding as a whole, were associated with an increased risk of pre-eclampsia.

Our study is the first to assess early bleeding in pregnancy and its associated risk of preeclampsia in an African population and sheds light on specific bleeding characteristics that may be associated with preeclampsia while considering the effect of known confounding factors.

Theoretically, a positive association between the two conditions could inform changes in current practice such as initiation of therapies aimed at preventing or reducing the severity of preeclampsia in patients who present with vaginal bleeding in early pregnancy. Our findings could inform future research using more robust study design and larger sample sizes to further study this issue.

Null Hypothesis

There is noassociation between vaginal bleeding in early pregnancy and the risk of developing preeclampsia later in pregnancy.

Research Question:

Is vaginal bleeding in early pregnancy associated with an increased risk of subsequent pre-eclampsia?

Study Objectives:

Broad objective:

To study the association between vaginal bleeding in early pregnancy and subsequent preeclampsiaamong patients seen at the Kenyatta National Hospital.

Specific objectives:

1. To describe the early pregnancybleeding patterns seen among postnatal patients treated at the Kenyatta national Hospital
2. To determinethe association between different early pregnancy bleeding patternsand preeclampsia

Conceptual framework

This study is based on the work done by Burton and Jauniaux(9) that suggests that threatened abortion and preeclampsia represent a single spectrum of illnesses resulting from defective placental development. It follows that if these conditions do indeed represent a single spectrum of illness, then those patients diagnosed with either of them would share similar characteristics and risk factors.

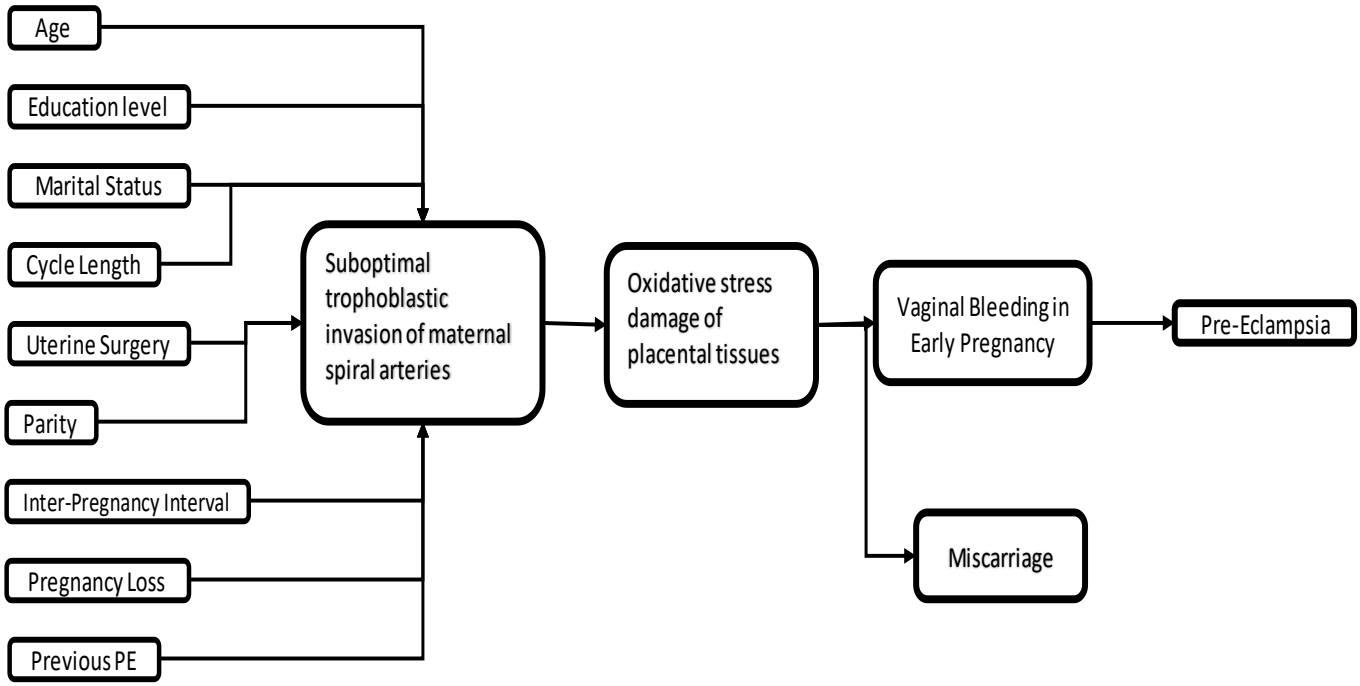


Figure 1: Schematic representation of hypothesized pathway between maternal baseline characteristics, through vaginal bleeding to pre-eclampsia

CHAPTER TWO: METHODOLOGY

Study Design

The study was an unmatched case control study. This design was suitable for our study because our outcome of interest, pre-eclampsia, is a rare condition with an estimated incidence of 6-9% of all pregnancies as described by Abalos et al(35). A case control design in this case allowed us to test our hypothesis using a relatively smaller sample size and over a shorter duration. This design was also appropriate considering available resources in terms of personnel and finances.

Study site and description

Our study was conducted at the Kenyatta National Hospital located in Nairobi, Kenya. The KNH is the oldest and largest public hospital in the country, having been founded in 1901. It is a tertiary, referral hospital that also serves as the teaching hospital for the University of Nairobi College of Health Sciences.

KNH provides care for both walk-in and referral patients in a variety of Medical Specialty Units including the Reproductive Health Unit. Patients come from all over the country and from neighbouring countries.

The RH Unit at Kenyatta comprises a labour ward with two attached operating theatres, three maternity wards in which both antenatal and postnatal patients are admitted, two gynaecology wards, one for acute gynaecological conditions and the other for cold gynaecological cases and a Reproductive health outpatient Clinic.

About 1700 deliveries are conducted each month in the RH Unit. Postnatal patients are all admitted in one of the postnatal units for post-delivery care before being discharged home. Our study shall be carried out in the post-natal wards. The postnatal wards were suitable for our study as our sample frame consisted of post-natal clients who were all admitted here before being allowed home.

Study Population

Thestudy population waspostnatal motherswho had singleton pregnancies receiving post-deliverycare at the Kenyatta national Hospital within the first 72 hours after delivery between 1st July 2019 and 30th September 2019.

In this study, cases were post-natal patients with singleton pregnancies within 72 hours of delivery who were diagnosed with pre-eclampsia during pregnancy. The control arm consisted of post-natal patients with singleton pregnancies within 72 hours of delivery who were not diagnosed with pre-eclampsia during pregnancy. We obtained this information from patient case notes.

Blood pressure measurements are routinely taken from all patients during ante-natal, intra-partum and post-partum monitoring and readings recorded in their case notes.

Inclusion and Exclusion Criteria

We included mothers who had singleton pregnancies and who gave informed consent to participate in our study. We excluded mothers who reported a history of physical trauma in early pregnancy, those who attempted to terminate their pregnancies, those with a diagnosis of chronic hypertension, renal disease, diabetes, connective tissue disorder or bleeding disorder. We also excluded patients without antenatal booklets or records of antenatal follow up.

Sample size determination

Sample size was calculated using the difference in proportions Fleiss JL formula (StatCalc epi-info^{Tm}) as outlined below. Findings from a study done by Smits J., North R., Kenny L. et al(32)were used in sample size determination. The following assumptions were considered during the calculation:

$$n = \left(\frac{r+1}{r}\right)\frac{(\bar{p})(1-\bar{p})(Z_{\beta} + Z_{\alpha/2})^2}{(p_1 - p_2)^2}$$

n = sample size per arm

r = ratio of unexposed to exposed, 1:1 in this case

P_1= proportion of cases with exposure = 40%

P_2= proportion of controls with exposure 25%

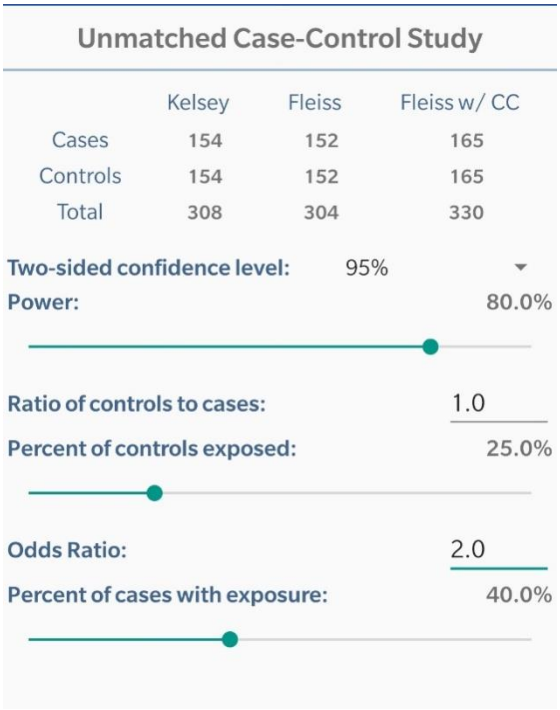
Ṕ =measure of variability, taken as 40+25/2 = 32.5

Z_{β}=Value corresponding to the power of the study, in this case 80% = 0.84

Zα = Value corresponding to the normal standard deviate at 95% C.I in this case = 1.96, with 0.05 level of significance

P_1- P_2 = effect size (difference in proportions) = 40 – 25= 15

Applying the Fleiss formula in StatCalc (epiinfo^{TM}) gives a sample size of 152 participants in each arm as below



Two-sided confidence level: 95%

Power: 80.0%

Ratio of controls to cases: 1.0

% of controls exposed: 25%

Odds Ratio: 2.0

The sample size using this formula comes to a total of **304**:

Cases – 152

Controls – 152

Study procedures

Sampling Procedure

Each maternity ward maintains a daily record of admissions. This record contains patient names, unique in-patient registration number and diagnosis. On each day of data collection, we generated a list of post-natal admissions in all three wards for the preceding 24 hours. We then performed an initial stratification of the patients into cases, those diagnosed with pre-eclampsia during pregnancy, and controls; those not diagnosed with pre-eclampsia during pregnancy. This information was obtained from patient case notes maintained in the wards. We then conducted sequential sampling from each stratum and recruited them into our study.

Study Enrolment Procedure

Patients in the sample lists were then approached individually in the wards. We provided them with an overview of our study and allowed them to ask any questions they had. Those who

agreed to participate were then interviewed privately in a side room in the wards. We then evaluated them for suitability in our study based on our inclusion and exclusion criteria. We obtained written, informed consent from those who were eligible in our study before the information of interest was collected.

The figure below shows the study flow:

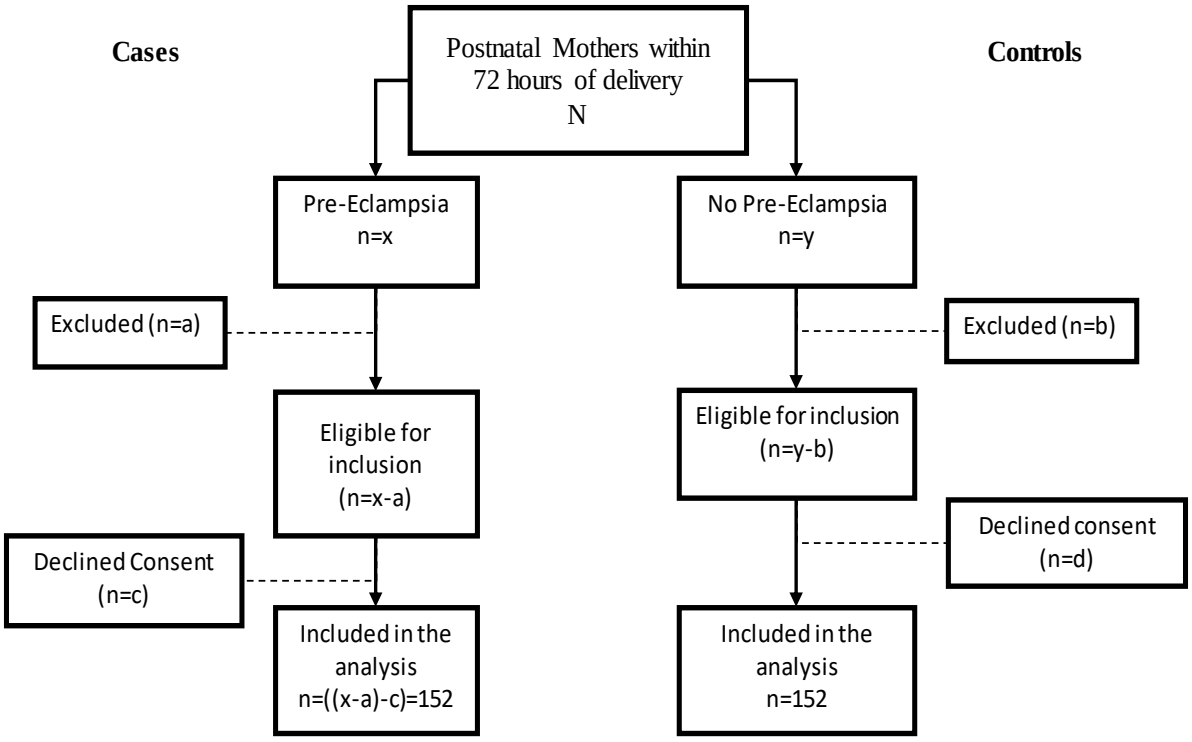


Figure 2: Schematic representation of study procedures from recruitment to analysis

Data Collection and Quality Assurance Procedures

We administered a structured study questionnaire to each eligible subject after enrollment in our study. This was done by trained research assistants. The process involved a single short interview, not lasting more than 15 minutes and was conducted privately in the ward side rooms.

Research assistants were recruited to assist in data collection and trained on sampling procedure, consent and data collection procedures. A pre-test of the study questionnaire was carried out and

necessary corrections made to reduce bias and prevent misinterpretations or ambiguity. Verification of information from available patient records was carried out where possible.

During digitization and data entry, we used code lists to minimize manual data entry. We also ensured data variable names were labelled in detail to avoid any confusion. We provided the data entry clerks with accompanying notes and documentation about the data provided.

We performed double entry of the data and double-checked coding of variables and verified data completeness. We performed interval random sampling of data entered and compared with original data to ensure quality.

Retention of the primary data collection tool, the study questionnaires, will be done for 3 years after the conclusion of the study.

Our study questionnaire is found in appendix 2.

StudyVariables

Our main study outcome was pre-eclampsia. We defined pre-eclampsia using the ACOG definition as systolic blood pressure greater than or equal to 140mmHg or diastolic blood pressure greater than or equal to 90mmHg on at least two occasions at least 4 hours apart after 20 weeks of gestation in a previously normotensive woman with proteinuria of equal to or greater than 0.3 grams in a 24 hour specimen or dipstick result of greater than or equal to 1+ OR new onset of any multisystem complication of pre-eclampsia. This information was obtained from a review of case notes in the patient ante-natal booklets and in-patient files. We grouped our study participants into cases and controls based on the presence(cases) or absence(controls) of pre-eclampsia.

Our main exposure variable was vaginal bleeding in early pregnancy. The ACOG defines early pregnancy as the first 20 weeks of pregnancy. We obtained this information duringface-to-face interviews with the study participants where we inquired as to whether they had any such bleeding. We also obtained this information from case notes from their ante-natal booklets where available. A description of the number of episodes, maximal bleeding intensity and maximal duration of bleeding was obtained from the participants at the same time.

Any bleeding that occurred 2 or more days from the last instance of bleeding was defined as a new episode. Duration of bleeding in days was also described by the participants. We included the longest bleeding duration for each patient with more than one episode of bleeding in our study. Bleeding intensity was described by each patient. We categorized bleeding intensity into spotting, light and heavy bleeding. Spotting was defined as bleeding that only resulted in staining of the inner garments. Light bleeding was defined as more than spotting but less than the participant's usual menses while heavy bleeding was defined as similar to or more than the participant's usual menses.The definition of bleeding patterns as set above madeour study comparable to the studies done by Weiss et al(7) and Smits et al. (32)

Other predictor variables included in our study were maternal age in years, marital status, highest attained level of education, usual cycle length in days, gravidity, inter-pregnancy interval, history of previous pregnancy loss, history of previous pre-term delivery and history of previous pre-eclampsia.

We defined gravidity as the total number of times the study participant had been pregnant, regardless of the pregnancy outcome. Conception date was calculated as LMP plus two weeks or derived from gestational age from early pregnancy scans where available. This allowed us to calculate the inter-pregnancy interval duration. We defined pregnancy loss as either an abortion, death of an embryo or fetus before 20 weeks of gestation or stillbirth, death of a fetus after 24 weeks of gestation. Pre-term delivery was defined as any birth before 37 completed weeks of gestation. Pre-eclampsia was defined using the ACOG definition earlier described. This information was obtained from participant interviews and verified from patient case notes.

Bias

Potential sources of bias in our study include recall and selection bias.

We attempted to reduce recall bias by interviewing our study participants soon after delivery. This was also reduced by the nature of our main exposure variable itself. As stated by Weiss J, Malone F. and Vivader, (7) "Any bleeding in early pregnancy is a distressing event not soon forgotten by the patient and any description of such events is subjective."

Selection bias is reduced in our study because our study population consists of women receiving postnatal care. In our study, we expect similar admission rates between cases and controls because they are all admitted for immediate post-natal care.

Ethical Considerations

We ensured confidentiality and security of information obtained from study participants by not disclosing any identifying personal information.Only the principal investigator had access to study materials. Participantshad the right to withdraw from the study at any point and were assured of continued scheduled care in the postnatal period even if they chose not to participate.

Departmental approval was sought before the research proposal was presented for ethical review by the KNH/UONERC.The study commenced once all corrections were made and an approval letter from the KNH/UON ERCobtained, Ref. No. KNH-ERC/A/260.

Data Management and Statistical Analysis

We used descriptive statistics to give a general description of our study sample. Means, frequencies and proportions were used for this. Chi square tests and independent T tests used to evaluate statistical significance of frequency distribution differences between the case and control groups.

We conducted singlelogistic regression analyses to evaluate the association between each of our study independent variables and pre-eclampsia. Unadjusted association estimates were thereby determined. We also conducted multiplelogistic regression analyses to evaluate association between maternal characteristics and pre-eclampsia while controlling for the effects of all the independent variables in our study thereby arriving at adjusted association estimates.

We conducted univariate analyses for each bleeding characteristic and compared them with no bleeding as the reference group with regards to association with preeclampsia. Odds ratios were determined, and Chi square test was used to determine statistical significance.

Our data analysis was performed using SPSS version 26 software.

CHAPER THREE: RESULTS

351 post-natal mothers admitted in the postnatal wards between 1st July 2019 and 30th September 2019 were recruited into our study. 190 had pre-eclampsia during pregnancy while 161 did not. A total of 47 were excluded, 9 from the control arm and 38 from the case arm. In most cases due to having one or more of our exclusion criteria. The total study population comprised 304 women. 152 cases and 152 controls.Study participant numbers at each stage of the study are detailed below:

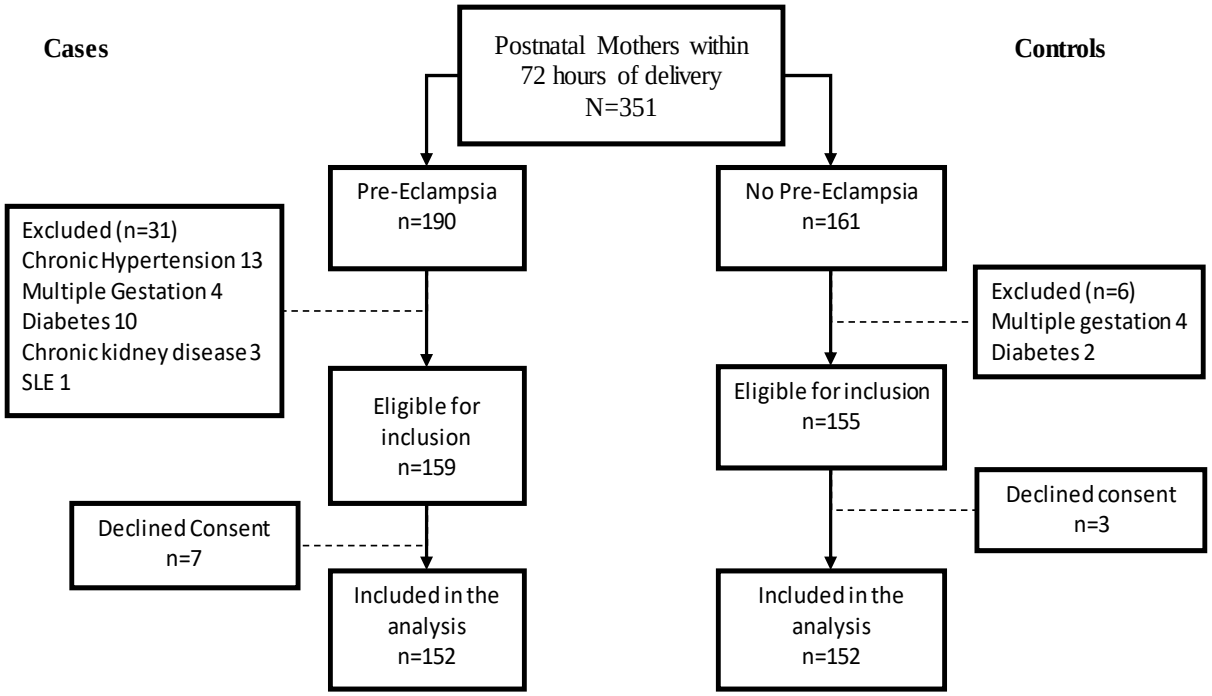


Figure 3: Schematic representation of study procedures from recruitment to analysis

The mean age at recruitment was 29 years. 85% of our study participants were married. 55% reported a usual menstrual cycle length of between 28 and 35 days. 40% reported a cycle length of less than 28 days. 22% reported history of prior uterine surgery. 29% were primigravidae. 35% of multiparas reported an inter-pregnancy interval of greater than 5 years. 27% had a history of pregnancy loss. 17% reported having had a previous pre-term delivery while 28% reported a history of pre-eclampsia in a previous pregnancy.

Frequency distributions of maternal characteristics in relation to presence or absence of preeclampsia are depicted in Table 1. We found statistically significant differences between

cases and controls with regard to PV bleeding in early pregnancy (p = 0.01), highest attained level of education (p=0.041), cycle length (p=0.000), previous pre-term delivery (p=0.035) and previous pre-eclampsia (p=0.000).

Table 1: Frequency distribution of maternal characteristics based on presence or absence of pre-eclampsia among postnatal patients with singleton pregnancies treated at the Kenyatta National Hospital

Maternal characteristic	Cases (n=152)	Controls (n=152)	p value*
Bleeding			0.01
Yes	40(26.3)	22(14.5)	
No	112(73.7)	130(85.5)	
Maternal age (y)	29.9±6.3	28.6±6.4	0.089
Maternal Age (yrs)			0.285
<25	34(22.4)	45(29.6)	
25-29	38(25)	43(28.3)	
30-34	49(32.2)	37(24.3)	
>35	31(20.4)	27(17.8)	
Marital status			0.198
Single	25(16.4)	18(11.8)	
Married	127(83.6)	132(86.8)	
Separated/Divorced	0(0)	2(1.3)	
Education			0.041
Primary	28(18.4)	38(25)	
Secondary	83(54.6)	61(40.1)	
Tertiary	41(27)	53(34.9)	
Cycle length (d)			0.000
<28 days	79(52.3)	43(28.3)	
28-35 days	64(42.4)	104(68.4)	
>35 days	8(5.3)	5(3.3)	
Gravidity			0.209
1	50(32.9)	40(26.3)	
>1	102(67.1)	112(73.7)	
IP interval (y)	5.02±2.9	5.09±3.5	0.865
IP interval (y)			0.287
<5	64(68.1)	62(60.8)	
5 or more	30(31.9)	40(39.2)	
Pregnancy Loss			0.788
Yes	29(28.4)	30(26.8)	
No	73(71.6)	82(73.2)	
Preterm delivery			0.035
Yes	24(23.5)	14(12.5)	
No	78(76.5)	98(87.5)	
Previous Pre-eclampsia			0.000
Yes	46(45.1)	14(12.5)	
No	56(54.9)	98(87.5)	

Data presented as n (%) or mean±SD
IP= inter-pregnancy
*Chi square or independent t test

62 study participants had a history of PV bleeding during the first 20 weeks of pregnancy, making up 20% of the total study population. Among those who bled, light bleeding was the most common intensity reported at 51%. 95% had bleeding of between 1 and 4 days duration while 41% reported only a single episode of bleeding. The frequency of bleeding among controls was 14% while among cases this was 26%. Frequency distribution of bleeding patterns among those participants who reported bleeding in early pregnancy are depicted in table 2 below. We found significant difference between cases and controls with regards to bleeding intensity (p=0.018).

Table 2: Frequency distribution of bleeding patterns based on presence or absence of pre-eclampsia among post-natal mothers with singleton pregnancies treated at the Kenyatta National Hospital

Bleeding pattern	Cases (n=39)	Controls(n=22)	p value*
Episodes			0.282
One	14(35.9)	11(50)	
More than one	25(64.1)	11(50)	
Maximal duration			0.182
1-4 days	36(92.3)	22(100)	
5 or more days	3(77)	0(0)	
Maximal intensity			0.018
Spotting	10(25.6)	13(59.1)	
Light	25(64.1)	6(27.3)	
Heavy	4(10.3)	3(13.6)	

Data presented as n (%)
*Chi square test

The association between maternal characteristics and pre-eclampsia are depicted in Table 3. Vaginal bleeding in early pregnancy was significantly associated with increased risk of subsequent pre-eclampsia in our study (ORa 3.85, p value 0.002). We found significant associations between pre-eclampsia and maternal age, education level, cycle length and history of pre-eclampsia in a previous pregnancy.

Compared to controls, we found that bleeding of more than one episode (OR 2.64, p value 0.01), of 1 to 4 days duration (OR 1.9, p value 0.03) and of light intensity (OR 4.84, p value 0.000) were associated with increased likelihood of pre-eclampsia. This is depicted in Table 4.

Table 3: Unadjusted and adjusted associations between maternal characteristics and pre-eclampsia among postnatal women with singleton pregnancies treated at the Kenyatta National Hospital

Variable	Unadjusted OR (95% CI)	p value	Adjusted OR (95% CI)	p value
Bleeding				
No	Reference		Reference	
Yes	2.11(1.18-3.76)	0.01	3.85(1.62-9.16)	0.002
Maternal age (y)	1.03(1.00-1.07)	0.09	1.06(1.00-1.13)	0.04
Marital Status		0.55		0.17
Single	Reference		Reference	
Married	0.69(0.36-1.33)	0.27	0.29(0.08-1.05)	0.06
Education level		0.04		0.05
Primary	Reference		Reference	
Secondary	0.95(0.50-1.80)	0.88	2.23(0.96-5.20)	0.06
Tertiary	1.76(1.04-2.97)	0.04	0.89(0.34-2.37)	0.82
Cycle length		0.00		0.03
<28	Reference		Reference	
28-35	0.34(0.21-0.54)	0.000	0.39(0.19-0.81)	0.01
>35	0.87(0.27-2.83)	0.82	1.03(0.23-4.96)	0.97
Gravidity		0.47		0.47
1	Reference		Reference	
>1	0.73(0.44-1.20)	0.21	0.73(0.44-1.20)	0.21
IP Interval	0.94(0.91-1.08)	0.89	0.92(0.83-1.03)	0.15
Pregnancy Loss				
No	Reference		Reference	
Yes	1.09(0.60-1.98)	0.79	0.67(0.31-1.48)	0.32
Preterm delivery				
No	Reference		Reference	
Yes	2.15(1.05-4.44)	0.04	1.23(0.49-3.06)	0.66
Previous PE				
No	Reference		Reference	
Yes	5.75(2.91-11.38)	0.000	7.14(3.07-16.61)	0.000

Table 4: Association between bleeding patterns and pre-eclampsia among postnatal mothers with singleton pregnancies treated at the Kenyatta national Hospital

	PE			
Bleeding pattern	Yes	No	Crude OR (95% CI)	p value
Any Bleeding				
No	112	130	Reference	
Yes	40	22	2.11(1.18-3.76)	0.01
Number of episodes				
None	112	130	Reference	
One	14	11	1.47(0.64-3.38)	0.36
More than one	25	11	2.64(1.24-5.60)	0.01
Maximal duration				
None	112	130	Reference	
1-4 days	36	22	1.90(1.06-3.42)	0.03
5 or more	3	0	8.12(0.41-158.90)	1.38
Maximal intensity				
None	112	130	Reference	
Spotting	10	13	0.89(0.38-2.11)	0.80
Light	25	6	4.84(1.92-12.21)	0.000
Heavy	4	3	1.55(0.34-7.06)	0.57

The association between maternal characteristics and bleeding in early pregnancy is depicted in table 5 below. We found no significant associations between any of the maternal characteristic variables in our study and vaginal bleeding in early pregnancy.

Table 5: Unadjusted and adjusted association between maternal characteristics and bleeding in early pregnancy among postnatal mothers treated at the Kenyatta National Hospital

Characteristic	Unadjusted OR (95%CI)	p value	Adjusted OR (95% CI)	p value
Age	0.98(0.94-1.02)	0.33	1.00(0.94-1.07)	0.95
Marital Status		0.12		0.54
Single	Reference		Reference	
Married	0.47(0.23-0.96)	0.04	0.52(0.16-1.66)	0.27
Education level		0.53		0.74
Primary	Reference		Reference	
Secondary	0.82(0.41-1.64)	0.58	0.88(0.38-2.08)	0.78
Tertiary	0.64(0.29-1.40)	0.26	0.67(0.24-1.89)	0.45
Cycle length in days				0.12
<28	Reference		Reference	
28-35	1.99(1.07-3.71)	0.03	2.43(1.02-5.78)	0.04
>35	2.75(0.76-9.92)	0.12	1.31(0.22-7.64)	0.77
Uterine surgery				
No	Reference		Reference	
Yes	0.81(0.42-1.54)	0.51	0.82(0.35-1.92)	0.65
Parity				0.48
1	Reference		Reference	
>1	1.04(0.56-1.91)	0.91	1.04(0.56-1.91)	0.91
IP interval	1.02(0.91-1.13)	0.79	1.01(0.89-1.14)	0.87
Pregnancy Loss				
No	Reference		Reference	
Yes	0.89(0.43-1.84)	0.74	1.08(0.47-2.48)	0.85
Preterm delivery				
No	Reference		Reference	
Yes	0.8(0.35-1.84)	0.60	1.32(0.52-3.38)	0.56
Previous PE				
No	Reference		Reference	
Yes	1.67(0.75-3.72)	0.21	0.68(0.27-1.71)	0.41

CHAPTER FOUR: DISCUSSION

We have conducted the first study on the association between early pregnancy bleeding and pre-eclampsia in a predominantly African population. Our results show that patients diagnosed with pre-eclampsia are twice as likely to report a history of bleeding during early pregnancy than those not diagnosed with pre-eclampsia.

Our findings support the pathogenic theory postulated by Jauniaux et al(9)that suggests that vaginal bleeding is a marker of placental oxidative damage secondary to suboptimal trophoblastic endothelial invasion of maternal spiral arteries leading to placental dysfunction and heralds pre-eclampsia in those pregnancies that proceed beyond 20 weeks of gestation.

Further analysis shows that there is variation in association with pre-eclampsia between different bleeding patterns. Our results show that those with more than one episode of bleeding, with bleeding of 1 to 4 days duration or bleeding of light intensity have a higher likelihood of developing subsequent pre-eclampsia. Such patterns may represent more significant placental tissue damage and dysfunction. The low reported incidence of heavy bleeding among our study participants is in keeping with previous studies. Heavy bleeding likely represents severe placental tissue damage and affected pregnancies are more likely to end in miscarriage than to proceed beyond 20 weeks of gestation(7).

The international federation of gynaecology and obstetrics currently recommends universal pre-eclampsia risk screening during the first trimester of pregnancy using a combined test that comprises maternal risk factors and serum biomarkers and physical measurements. Vaginal bleeding in early pregnancy is not a widely recognized risk factor for pre-eclampsia and is not included in this risk stratification model. This is in large part due to conflicting results from previous studies (32,7).

As stated earlier, most previous studies have been conducted in predominantly Indo-European populations. Given that epidemiological evidence has shown that there are racial differences in risk for pre-eclampsia, with Africans having higher risk(1), it is possible that there could also be differences in the prognostic value of risk factors between races.

Our findings are relevant in that they show a link between bleeding in early pregnancy and pre-eclampsia. This should prompt further studies on the prognostic value and utility of early pregnancy bleeding and its patterns in early pregnancy risk assessment for pre-eclampsia.

The strengths and limitations of our study deserve mention. Ours is the first study looking at the association between bleeding in early pregnancy and risk for pre-eclampsia in a predominantly African population. In our study, we evaluated pregnancies that progressed beyond 20 weeks of gestation and so excluded pregnancies that may have concluded before the earliest onset of pre-eclampsia. We also evaluated respondents of different gravidity and therefore could evaluate gravidity as a risk factor for both bleeding in early pregnancy and pre-eclampsia. We were also able to evaluate different bleeding patterns unlike several previous studies.

One of the main limitations was that we relied heavily on patient recall describing bleeding patterns. As Weiss et al (7)noted in their study, "…vaginal bleeding during pregnancy is a distressing event that any affected mother is not likely to forget". Of note, such subjective description is a feature in any study about vaginal bleeding in pregnancy, whether prospective of retrospective, as noted by Hasan et al(6). We attempted to improve recall by conducting our interviews within 72 hours of delivery. Finally, analysis of low frequency predictors was hampered by our small sample size. We did not include these in our analysis. Lack of, or incomplete documentation meant that on several instances, we were unable to accurately verify gestation at which bleeding occurred and therefore did not include this in our analysis.

Conclusion: Early pregnancy bleeding is a common event and is associated with increased likelihood of subsequent pre-eclampsia.Evaluation of vaginal bleeding and its patterns may have prognostic value in early pregnancy risk assessment for pre-eclampsia.

Generalizability: Our findings can be applied to women with singleton pregnancies who report bleeding during the first 20 weeks of pregnancy without any of the chronic conditions included in our study exclusion criteria as earlier stated.

Recommendations: We recommend future studies done locally, and of prospective design, enabling the investigators to evaluate timing of bleeding with greater accuracy, baseline risk factors such as BMI, renal and liver function at ANC booking and other pregnancy outcomes including miscarriage. Larger sample sizes would allow for better analysis of low frequency

predictors such as smoking and alcohol consumption during pregnancy. Histopathological studies of the placentas of women with history of vaginal bleeding in pregnancy may further elucidate this issue, providing information on possible structural abnormalities that may explain associated outcomes. We also recommend prospective studies on the utility of biomarkers and physical parameters in prediction of pre-eclampsia in our setting. The effect of use of therapies aimed at reducing incidence, risk or severity of pre-eclampsia in populations of women who present with bleeding in early pregnancy is another avenue for future research.

Conflict of interest declaration: There was no funding for our study. We have no conflict of interest to declare.

REFERENCES

1. Myatt L, Giftorn R, Roberts J. First trimester prediction of pre-eclampsia in nulliparous women. American journal of obstetrics and Gynaecology. 2012; 119(6): p. 1234.

2. Nicolaides L, Poon C, Kypros H. Early prediction of preeclampsia. Obstetrics and Gynaecology International. 2014 July; 2014(297397).

3. Saraswat L, Bhattacharya S, Maheshwari A. Maternal and Perinatal outcome in women with threatened miscarriage in the first trimester: a systematic review. British Journal of Obstetrics and Gynaecology. 2010 February; 117(3): p. 245-57.

4. Chhabra S, Tickoo C, Kalra P. Perinatal outcomes in cases with bleeding during first and early second trimester. International Journal of Medical, Health, Biomedical, Bioengineering and Pharmaceutical Engineering. 2014; 8(1).

5. Dadkhah F, Kashanian M, Eliasi G. A comparison between pregnancy outcome in women both with and without threatened abortion. Journal of early human development. 2010; 86: p. 193-96.

6. Hasan R, Baird D, Henning A. Patterns and predictors of vaginal bleeding in first trimester of pregnancy. Annals of epidemiology. 2010; 20(7): p. 524-31.

7. Weiss J, Malone F, Vivader J. Threatened Abortion: A risk factor for poor pregnancy outcome. American journal of Obstetrics and Gynaecology. 2004; 190: p. 745-50.

8. Wijesiriwardana A, Bhattacharya S, Shetty A. Obstetric outcome in women with threatened miscarriage in the first trimester. Obstetrics and gynaecology. 2006 March; 107(3): p. 557-62.

9. Burton G, Jauniaux E. Placental oxidative stress: From miscarriage to pre-eclampsia. Journal of the society for gynaecological investigation. 2004; 11: p. 342-52.

10. Oppenraaij V, Jauniaux E, Christiansen O. Predicting adverse obstetric outcome after early pregnancy events and complications: a review. Human reproductive update. 2009 July; 15(4): p. 409-421.

11. Petriglia G, Palaia I, Musella A. Threatened abortion and late pregnancy complications: A case control study. Minerva Ginecol. 2015 December; 67(6): p. 491-7.

12. Piijnenborg R, Anthony J, Davey D. Placental bed spiral arteries in hypertensive disorders of pregnancy. british Journal of Obstetrics and Gynaecology. 1991; 98: p. 648-55.

13. Carter A. When is maternal placental circulation established in man? Placenta. 1997;(18): p. 83-87.

14. Kliman H. The story of decidualization, menstruation and trophoblast invasion. American journal of Pathology. 2000;(157): p. 1759-68.

15. Hamilton W, Boyd J. Development of the human placenta in the first three months of pregnancy. journal of Anatomy. 1960;(94): p. 297-328.

16. Ramsey E, Donner M. Anatomy, Physiology, Radiology clinical aspects, atlas and textbook Stuttgart: Georg Thieme; 1980.

17. Hustin J, Jauniaux E, Schapps J. Histological study of the materno-embryonic interface in spontaneous abortion. Placenta. 1990;(11): p. 477-86.

18. Hempstock J, Jauniaux E, Greenwold N, Burton J. The contibution of plcental oxidative stress to early pregnancy loss. Human Pathology. 2003;(34): p. 1265-75.

19. Jauniaux E, Watson E, Bao Y, Skepper J, Burton G. Onset of maternal arterial bloodflow and placental oxidative stress. American Journal of Pathology. 2000;(157): p. 2111-22.

20. Rodesch F, Simon P, Donner C, Jauniaux E. Oxygen measurements in endometrial and trophoblastic tissuesduring early pregnancy. Journal of Obstetrics and Gynaecology. 1992;(80): p. 283-285.

21. Hustin J, Schaaps J. Echographic and anatomic studies of the maternotrophoblatic border during the first trimester of pregnancy. American journal of Obstetrica and gynaecology. 1987;(157): p. 162-68.

22. Jauniaux E, Jurkovic D, campbell S. In vivo investigations of anatomy and physiology of early human placental circulations. Ultrasound in obstetrics and gynaecology. 1991; 1: p. 435-445.

23. Jaffe R, Woods J. Colour doppler imaging and in vivo assessment of the anatomy and physiology of the early uteroplacental circulation. Fertility and Sterility. 1993;(60): p. 293-297.

24. Burton G, Jauniaux E, Watson A. Maternal arterial connections to the placental intervillous space during the first trimester of human pregnancy; The boyd collection revisited. American journal of Obstetrics and Gynaecology. 1999;(181): p. 718-724.

25. Jauniaux E, Zaidi J, Jurkovic D, Campbell S, Hustin j. Comparison of colour doppler features and pathological findings in complicated early pregnancy. Human reproduction. 1994;(82): p. 1582-88.

26. Schwarzler P, Holden D, Nielsen S, Hahlin M, Sladkevicius P, Bourne T. the conservative management of firstv trimester miscarriages and the use of colour doppler sonography for patient selection. Human Reproduction. 1994;(14): p. 1341-45.

27. Hensley K, Robinson K, Gabbita S, Salman S, Floyd R. Reactive oxygen species, cell signalling and cell injury. Free Radical Biology Medicine. 2000;(28): p. 1456-62.

28. Hafiner E, Metzenbauer M, Hofinger D. Placental growth from the first to the second trimester of pregnancy in SGA pregnancies and preeclamptic pregnancies compared to noral fetuses. Placenta. 2003;(24): p. 336-42.

29. Mayhew T, Ohadike C, Baker P, Crocker J, Mitchel C, Ong S. Stereological investigation of placental morphology in pregnancies complicate by preeclampsia with and without intrauterine growth restriction. Placenta. 2003;(24): p. 219-26.

30. Sadat H, Soghra Y. Late pregnancy outcomes in women with vaginal bleeding in the 1st trimester. Journal of Obstetrics and Gynaecology Societies of India. 2013; 63: p. 311-15.

31. Funderburk S, Guthrie D, Meldrum D. Outcome of pregnancies complicated by early vaginal bleeding. British Journal of Obstetrics and Gynaecology. 1980; 87: p. 100-105.

32. Smits L, North R, Kenny L. Patterns of vaginal bleeding during the first 20 weeks of pregnancy and risk of pre-eclampsia. Acta Obstetricia et Gynecologica Scandinavia. 2012 November; 91(11): p. 1331-8.

33. Poon LC, Shennan A, Kihara AB, Nicolaides KH. FIGO: A pragmatic guide for first trimester screening and prevention of Pre-eclampsia. international Journal of Gynaecology and Obstetrics. 2019 May; 145(S1): p. 1-33.

34. Norwitz E, Bellussi F. Early pregnancy prediction of preeclampsia. uptodate.com. 2019 June.

35. Abalos E, Cuesta C, Grosso A, Chou D, Say L. Global and regional estimates of preeclampsia and eclampsia. European journal of Obstetrics and Gynaecology. 2013 September; 170(1).

36. Cunningham F, Leveno K, Bloom S, Dashe J, Hoffman B, Casey B, et al., editors. Williams Obstetrics. In. New York: McGraw Hill; 2018. p. 709-753.

APPENDICES

Appendix 1:Participant Information and Consent Form

Title of the study: **VAGINAL BLEEDING IN EARLY PREGNANCY AND RISK OF PRE-ECLAMPSIA AMONG WOMEN TREATED AT THE KENYATTA NATIONAL HOSPITAL IN NAIROBI, KENYA**

Principal Investigator/Institutional affiliation: Dr Emmanuel Sinei, Master's Student in the University of Nairobi, Department of Obstetrics and Gynaecology

Introduction:

I would like to tell you about a study being carried out by the above researcher. The purpose of this consent form is to give you the information you will need to help you decide whether or not to be a participant in the study. Feel free to ask any questions about the purpose of the research, what happens if you participate in the study, the possible risks and benefits, your rights as a volunteer and anything else about the research or this form that is not clear. When we have answered all your questions to your satisfaction, you may decide to be in the study or not. This process is called 'informed consent'. Once you understand and agree to be in the study, I will request you to sign your name on this form. You should understand the general principles which apply to all participants in a medical research. I) Your decision to participate is entirely voluntary II) You may withdraw from the study at any time without necessarily giving a reason for your withdrawal III) Refusal to participate in the research will not affect the services you are entitled to in this health facility or other facilities. We will give you a copy of this form for your records.

May I continue? YES/NO

This study has approval by the Kenyatta National Hospital/University of Nairobi Ethic and Research Committee Protocol No……

What is this study about?

The researcher listed above is interviewing patients who have recently given birth in our hospital. The purpose of the interview is to find out if there is an association between bleeding

during the first 20 weeks of pregnancy and pre-eclampsia later in pregnancy. Participants will be asked questions about their marital status and education level, previous gynaecological and obstetric history, drinking and smoking habits and any history of bleeding in their just concluded pregnancy. There will be approximately 300 participants in the study, al randomly chosen. We are asking for your consent to consider participating in this study.

What will happen if you decide to be in this research study?

If you agree to participate in this study, the following things will happen:

You will be interviewed by a trained interviewer in a private area where you will feel comfortable answering questions. The interview will last approximately 15 minutes. The interview will cover topics such as, your previous gynaecological history, history of any previous pregnancies, environmental exposures such as smoking or alcohol use during your pregnancy and any history of bleeding in your just ended pregnancy.

Are there any risks, harms or discomforts associated with this study?

Medical research has the potential to introduce psychological, social, emotional and physical risks. Efforts should always be put in place to minimize the risks. One potential risk of being in the study is loss of privacy. We will keep everything you tell us as confidential as possible. We will use a code to identify you in a password-protected computer database and will keep all of your paper records in a locked file cabinet**.** However, no system of protecting your confidentiality can be completely secure, so it is still possible that someone could find out you were in this study and could find out information about you.

Also, answering questions in the interview may be uncomfortable for you. If there are any questions that may be uncomfortable for you, you may skip them. You have the right to refuse the interview or any questions asked in the interview.

It may be embarrassing for you to have to discuss certain information from your past gynaecological or obstetric history. We will do everything we can to ensure that this is done in private. Furthermore, all study staff and interviewers are professionals with special training in these interviews. Also, recalling certain distressing events in previous history may be stressful.The study staff will provide initial counselling in such an event and refer you appropriately when necessary.

Are there any benefits being in this study?

There are no direct benefits to you as an individual, monetary or otherwise, but your participation will help in increasing the understanding of preeclampsia and may inform changes in policy and guidelines in patient management**.**

Will being in this study cost you anything?

No. There will be no monetary costs for you for participating in this study.

What if you have questions in future?

If you have further questions or concerns about participating in this study, please call or send a text message to the study staff at the number provided at the bottom of this page.

For more information about your rights as a research participant you may contact the Secretary/Chairperson, Kenyatta National Hospital-University of Nairobi Ethics and Research Committee Telephone No 2726300 extension 44102 or email uonknh_erc@uonbi.ac.ke

The study staff will pay you back for charges incurred when calling these numbers if the call is for study related communication.

What are your other choices?

Your decision to participate in research is voluntary. You are free to decline participation in the study and you can withdraw from the study at any time without injustice or loss of benefits.

CONSENT FORM (STATEMENT OF CONSENT)

Participant's statement

I have read this consent form or had the information read to me. I have had the chance to discuss this study with a study counselor. I have had the questions answered in a language that I understand. The risks and benefits have been explained to me. I understand that my participation in this study is voluntary and that I may choose to withdraw at any time. I freely agree to participate in this research study.

I understand that all efforts will be made to keep information regarding my personal identity confidential.

By signing this consent form, I have not given up any legal rights that I have as a participant in a research study.

I agree to participate in this research study: Yes No

I agree to provide contact information for follow up: Yes No

Participant Printed Name:_____

Participant Signature/thumb Stamp:_____ Date:_____

Researcher's Statement:

I, the undersigned, have fully explained the relevant details of this research study to the participant named above and believe that the participant has understood and has willingly and freely given her consent.

Researcher's name:_____ Date:_____

Signature:_____

Role in the study:_____

For more information contact Dr Emmanuel Sinei at Tel:0720798828

Witness Printed name:_____ Contact:_____

Signature/Thumb Stamp:_____ Date:_____

Appendix 2: Study Questionnaire

Serial Number:

PART 1: BIODATA

1. Age in years…….
2. What is your marital status?

A. Single( )
B. Married ( )
C. Separated/Divorced ( )
D. Other ( )

3. What is your highest attained level of education?

A. Primary()
B. Secondary ( )
C. Tertiary ( )

PART 2: Gynaecological History

4. What is your usual menstrual cycle length in days?
5. Have you undergone uterine surgery in the past? Yes ( ) No ( )

If yes, what procedure was done?

A. Dilation and curettage ( )
B. Myomectomy ( )
C. Caesarean section ( )

6. Were you treated for any reproductive tract infection or notice abnormal vaginal discharge during the first 20 weeks of pregnancy?

Yes ( ) No ( )

PART 3: Obstetric History

7. What was your gravidity?

   Primigravida ( ) Multigravida ( )

8. What was the duration between your last delivery and the pregnancy under study? (Para 1+)

……. Months……. Years

9. Have you had preterm deliveries in a past pregnancy? Yes ( ) No ( )
10. Have you had a miscarriage or stillbirth in a previous pregnancy? Yes ( ) No ( )
11. Have you been diagnosed with preeclampsia in a previous pregnancy? Yes ( ) No ( )
12. Is there a history of preeclampsia in your family? Yes ( ) No ( )

PART 4: Maternal Exposures

13. Were you exposed to cigarette smoking during the first 20 weeks of your current pregnancy?

Yes ( )No ( )

14. If so, was it either:
    A. Active ( )
    B. Passive ( )
15. Did you consume alcohol during the first 20 weeks of your pregnancy? Yes ( ) No ( )

PART 5: Pregnancy History

16. Did you experience any episode(s) of vaginal bleeding during the first 20 weeks of your pregnancy? Yes ( ) No ( )
17. If so,

A. How many episodes of bleeding did you experience? (Any bleeding occurring at least 2 days after last event is considered a new episode)
    I. One ( )
    II. More than one ( )
B. What was the average duration of each episode?
    I. 1-4 days ( )
    II. 5 or more days ( )
C. What was the maximal intensity of bleeding in any episode?
    I. Spotting ( )
    II. Light ( )
    III. Heavy ( )
D. Did you report these symptoms to a medical care provider? Yes ( ) No ( )
E. Were you diagnosed with preeclampsia during your pregnancy? Yes ( ) No (

Appendix 3: Study Timelines

ACTIVITY	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV
ERC Process								
Research Assistants Training								
Questionnaire design and pre-test								
Data collection and entry								
Data analysis and Presentation								
Final Dissertation writing								

Appendix 4: Budget

Item description	Unit cost (Kshs)	Number of Units	TOTAL (Kshs)
Stationery, printing, photocopying and binding	15000	1	15000
Remuneration of research assistants (consenting and questionnaires)	100	300	30000
Statistician (data analysis)	25000	1	25000
TOTAL			**70000**

Appendix 5: KNH/UoN ERC APPROVAL

UNIVERSITY OF NAIROBI
COLLEGE OF HEALTH SCIENCES
P O BOX 19676 Code 00202
Telegrams: varsity
Tel:(254-020) 2726300 Ext 44355

KNH-UON ERC
Email: uonknh_erc@uonbi.ac.ke
Website: http://www.erc.uonbi.ac.ke
Facebook: https://www.facebook.com/uonknh.erc
Twitter: @UONKNH_ERC https://twitter.com/UONKNH_ERC

KENYATTA NATIONAL HOSPITAL
P O BOX 20723 Code 00202
Tel: 726300-9
Fax: 725272
Telegrams: MEDSUP, Nairobi

Ref: KNH-ERC/A/260

2nd July, 2019

Dr. Emmanuel Sinei
Reg. No.H58/80924/2015
Dept.of Obstetrics and Gynaecology
School of Medicine
College of Health Sciences
University of Nairobi

KENYATTA NATIONAL HOSPITAL APPROVED 02 JUL 2019 KNH/UoN-ERC P.O. BOX 20723 - 00202 NAIROBI

Dear Dr. Sinei

RESEARCH PROPOSAL: VAGINAL BLEEDING IN EARLY PREGNANCY AND RISK OF PRE-ECLAMPSIA AMONG WOMEN TREATED AT THE KENYATTA NATIONAL HOSPITAL IN NAIROBI, KENYA – A CASE CONTROL STUDY (P259/04/2019)

This is to inform you that the KNH- UoN Ethics & Research Committee (KNH- UoN ERC) has reviewed and approved your above research proposal. The approval period is 2nd July 2019 – 1st July 2020.

This approval is subject to compliance with the following requirements:

a. Only approved documents (informed consents, study instruments, advertising materials etc) will be used.
b. All changes (amendments, deviations, violations etc.) are submitted for review and approval by KNH-UoN ERC before implementation.
c. Death and life threatening problems and serious adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the KNH-UoN ERC within 72 hours of notification.
d. Any changes, anticipated or otherwise that may increase the risks or affect safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH- UoN ERC within 72 hours.
e. Clearance for export of biological specimens must be obtained from KNH- UoN ERC for each batch of shipment.
f. Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. (*Attach a comprehensive progress report to support the renewal*).
g. Submission of an *executive summary* report within 90 days upon completion of the study. This information will form part of the data base that will be consulted in future when processing related research studies so as to minimize chances of study duplication and/ or plagiarism.

Protect to discover