sFLT1/PlGF RATIO AS A PREDICTOR OF DEVELOPMENT OF PREECLAMPSIA

AND ADVERSE MATERNAL AND PERINATAL OUTCOMES IN WOMEN AT

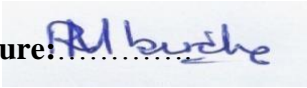**KENYATTA NATIONAL HOSPITAL, NAIROBI KENYA**

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DEPARTMENT OF OBSTETRICS AND GYNAECOLOGY

A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF MEDICINE IN OBSTETRICS AND GYNAECOLOGY, FULCULTY OFMEDICINE, UNIVERSITY OF NAIROBI.

2022

Declaration

I certify that this proposal is my original work. It has not been presented for the award of a degree in any other institution.

Signature:............................ **Date: 9th May, 2022**

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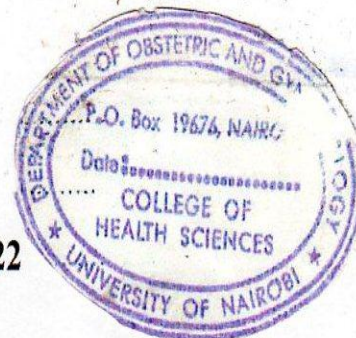
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I would also like to thank The Nairobi Hospital for allowing us to use their laboratory, Roche diagnostics for providing the kits at no cost and the patients who took part in the study.

Dedication

This dissertation is dedicated to my family: My father Mzungu Mwadai, my mother Dama Mlanda, my brother Dzombo Mzungu and sister Umazi Mzungu who would give anything to see me accomplish my dreams.

List of abbreviations

ANC- Antenatal clinic

BP- Blood pressure

ERC- Ethics Review Committee

FGR- Fetal growth restriction

FLT- Fms like tyrosine kinase

sFLT-1- Soluble fms like tyrosine kinase 1

HELLP- Syndrome of hemolysis elevated liver enzymes, low platelets

KNH- Kenyatta National Hospital

NICE- National Institute for Health and Care Excellence

NBU- New born unit

NH- Nairobi Hospital

NLR- Negative likelihood ratio

NPV- Negative predictive value

PE- Preeclampsia

PI- Principal Investigator

PlGF- Placental growth factor

PPH- Post-partum hemorrhage

PPV- Positive predictive value

SPSS- Statistical package for social sciences

VEGF- Vascular endothelial growth factor

WHO- World Health Organization

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Definition of terms

Chronic hypertension- Blood pressure above 140/90mmHg present before pregnancy or diagnosed before 20 weeks of pregnancy (1)

Gestational hypertension- BP above 140/90 mmHg diagnosed after 20 weeks of pregnancy with no proteinuria(1)

Preeclampsia-blood pressure of >140/90mmHg on 2 occasions at least 4 hours apart, after 20 weeks gestation in previously normotensive women AND any of: proteinuria dipstick 1+ or more, platelet count of <150,000/mL, elevated liver enzymes, new onset kidney function disturbance (creatinine of >90umol/L or raising serum creatinine), or CNS disturbances.(1)

Early onset preeclampsia- onset before 33 weeks + 6 days gestation(2)

Late onset preeclampsia- onset after 34 weeks and 0 days gestation(2)

High risk for preeclampsia- 1 major risk factor or 2/ more moderate risk factors(3)

Adverse neonatal outcomes- Admission to NBU

- Fetal growth restriction

- Intrauterine fetal demise

- Neonatal death within the first 24 hours

- Preterm birth- birth before 37 completed weeks (up to week 36+6 days)(4)

Sensitivity- proportion of true positives tests out of all with the disease

Specificity- proportion of true negatives out of all subjects who do not have a condition

Negative predictive value- proportion of true negatives out of all of all the negative tests

Positive predictive value- proportion of true positives out of all positive tests

Negative likelihood ratio- probability of not having the diease after a negative test

Positive likelihood ratio- probability of having the diease after a positive test result

Receiver operating characteristics curve- graphical plot of true positive rate (TPR) against the false positive rate

Adverse maternal outcomes- Acute kidney injury

- Admission to ICU

- Death

- Eclampsia

- HELLP syndrome

- PPH

- Preeclampsia

-Pulmonary edema

-Abruptio placenta

Abstract

Background: Preeclampsia affects 2-8% of pregnancies globally. In Kenya the prevalence is 5.6-6.5%. Preeclampsia/eclampsia causes 20% of direct maternal deaths in Kenyatta National Hospital (KNH). The diagnostic criteria for preeclampsia are proteinuria and elevated blood pressure beyond 20 weeks gestation. Maternal organ damage and adverse outcomes might happen before proteinuria becomes apparent. There is no single approved predictor of development of preeclampsia. Vascular endothelial growth factors; fms like tyrosine kinase: placental growth factor ratio(sFlt-1/PlGF), have shown promising results as predictors because they are elevated up to 1 month before development of preeclampsia.

Objective: To determine the utility of the sFlt-1/PlGF ratio as a predictor of the development of preeclampsia within 4 weeks and adverse outcomes in women at risk of preeclampsia in KNH.

Methodology: This was a prospective cohort study in which women between 24+0 to 36+6 weeks of gestation with risk factors for preeclampsia were enrolled, serum sFLT-1: PlGF measured, and then followed up to 24 hours post-partum to determine those who developed PE and adverse maternal and perinatal outcomes. It was conducted at Kenyatta National Hospital antenatal clinics. A sample of 50 women was obtained by consecutive sampling. A questionnaire was filled and data obtained was analysed using SPSS version 23. Demographic and clinical characteristics were summarized as median with interquartile range and frequencies with proportions. sFlt-1/PlGF ratio cut off of <38 was used to rule out preeclampsia within 1 week and >=38 to rule it in within 4 weeks and predict adverse maternal and perinatal outcomes. Sensitivity, specificity, negative and positive predictive values were calculated for the above outcomes. Best cut off to rule in preeclampsia within 4 weeks was determined using a receiver operating curve(ROC).

Results: 50 women with high risk for PE were recruited from the antenatal clinic between May and September 2020.The median sFlt-1/PlGF ratio at baseline for patients who developed preeclampsia within 4 weeks was higher at 24.2 (7.0-58.0) compared with those who did not 2.5 (1.3-3.7) though it was no statistically significant (P 0.005). sFlt-1/PlGF ratio <38 to rule out PE within 1 week had a sensitivity of 20.00%, specificity of 11.76% and a NPV of 60.00% (95% CI, 38.59%-78.17%). A ratio ≥38 to rule in preeclampsia within 4 weeks had a sensitivity of 53.85%, specificity of 93.02%. For predicting adverse perinatal outcomes, a ratio ≥38 had a sensitivity of 41.67%, specificity of 92.11% with PPV of 62.50% (31.76%-85.65%). A ratio of ≥38 for predicting adverse maternal outcomes had a sensitivity of 40.00%, specificity of 86.67% and PPV of 25.00% (8.28%-55.18%). Using an ROC curve, an sFlt-1/PlGF ratio cut off was set at 3.97 to rule in PE within 4 weeks. It had a sensitivity of 100% (75.29%-100%), a specificity of 74.42% (58.83%-86.48%), with an AUC 82.6% (CI, 68.6%-96.6%), PPV of 54.17% (41.51%-66.30%)

Conclusion: The sFlt-1/PlGF ratio cut off of 38 did not have very high NPV for ruling out and PPV for ruling in preeclampsia compared to other studies. A cut off of >3.97 to predict development of PE within 4 weeks and adverse maternal and perinatal outcomes has shown high sensitivity and PPV.

Recommendations: Lower cut off value for sFlt-1/PlGF ratio should be considered in our population to predict development of preeclampsia and adverse maternal and perinatal outcomes.

Chapter 1: Introduction

Background

The World Health Organization (WHO) places preeclampsia as the second commonest cause of direct obstetric maternal mortality causing 14% of mortality (5). In Kenya preeclampsia (PE)/eclampsia is the second commonest cause of direct maternal deaths accounting for 20% of deaths(6). It affects 2-8 in 100 pregnancies worldwide, and can be described as a multisystem disorder characterized by hypertension and proteinuria or maternal organ damage after 20 weeks of pregnancy (7).

Preeclampsia can be early or late onset. Early onset causes severe fetal growth restriction (FGR) requiring preterm delivery, increased risk of placental abruption, maternal complications and death. The pathogenesis of preeclampsia is not well known and has been thought to be caused by an imbalance in proangiogenic and antiangiogenic factors causing abnormal placentation and mal-perfusion(8). In preeclampsia, there is an increase of the circulating maternal serum antiangiogenic, soluble fms-like tyrosine kinase 1 (sFLT-1), and a decrease in the proangiogenic placental growth factor (PlGF) levels(9). The sFLT-1 antagonizes PlGF and the vascular endothelial growth factor (VEGF), causing endothelial damage and vasoconstriction which may lead to fetal growth restriction and hypertensive disease in pregnancy (10).

In search of predictors for preeclampsia, the sFLT-1 to PlGF ratio has been demonstrated to be raised in pregnant women up to 4 weeks before the onset of the disease and has been thought to be predictive of development of the syndrome(11).

The assessment tools that are in use currently e.g doppler studies are good predictors of fetal outomes but not maternal outcomes(12). Therefore there is need to develop assessment tools to

predict adverse maternal outcomes as well. The sFLT-1/PlGF ratio has been demonstrated to predict both fetal and maternal adverse outcomes (13).

Chapter 2: Literature review

Placental Growth Factor and Soluble fms-Like Tyrosine Kinase role in preeclampsia

Vascular endothelial growth factor (VGEF) is proangiogenic and supports trophoblastic growth during placentation. Placental growth factor is a member of the VEGF family. It is expressed mostly in the placenta but also in the liver, muscle, thyroid, bone, heart and lungs.(14) It competitively binds to Flt-1 which is a transmembrane receptor of VEGF, and sFlt-1. It however does not bind to VEGF receptor2. PlGF enhances VEGF by allowing it to bind to VEGF receptor2 which has a greater tyrosine kinase activity. sFlt-1 is a soluble isoform of Flt-1, which lacks the transmembrane domain but has a ligand binding domain capable of binding circulating VEGF and PlGF, preventing them from binding to transmembrane receptors therefore causing an antiangiogenic effect.(15) Placental growth factor is thought to promote growth and maturation of placental vasculature by influencing differentiation of natural killer cells which mediates trophoblastic invasion. Vascular endothelial growth factor plays a role in blood pressure (BP) regulation and maintenance of the glomerular filtration barrier integrity(16). Inhibitors of VEGF e.g. Bevacizumab have been demonstrated to cause high BP and proteinuria in patients with metastatic clear cell renal cancer who were enrolled in anti-vascular endothelial growth factor antibody trials(17). sFlt-1 administered to lab animals decreased VEGF and PlGF levels, and caused high BP, proteinuria, and glomerular endotheliosis, which are the same signs in PE(16).

sFlt-1 and PlGF in Plasma of normotensive Women vs preeclampsia

Expression of PlGF corresponds with different stages of placental development. It's level is low in the first trimester, but starts to go up from 11-12 weeks when there is spiral artery remodeling with the second wave of invasion at around 16-18 weeks, it peaks at 30 weeks and then decreases thereafter (18). PlGF concentrations in patients who developed PE followed a similar pattern but were significantly lower. The s-Flt-1 levels remain constant and start to rise towards the end of pregnancy at 33-36 weeks in normotensive women. In patients who developed PE, sFlt-1 levels were noted to start rising between 21-24 weeks of gestation which was weeks before development of clinical preeclampsia. (19)This results in a high sFlt-1/PlGF ratio in patients who develop preeclampsia. Low PlGF levels have also been noted in patients who got neonates who were small for gestation even if they subsequently did not develop PE. The rise in the ratio between sFlt-1 and PlGF has been demonstrated up to 5 weeks before preeclampsia becomes apparent (11).

PlGF versus sFLT:PlGF in the prediction of development of preeclampsia

The sFLT-1/PlGF ratio has shown high specificity for predicting PE. In a study by Stepan et al to compare PlGF and sFlt-1: PlGF to predict PE, specificity was higher with the ratio more so for PE developing after 34 weeks. Sensitivity was comparable (20).

SFLT-1: PlGF in preeclampsia development prediction

The PRediction of short-term Outcome in preGNant wOmen with Suspected preeclampsia Study (PROGNOSIS) a prospective, non-interventional, double-blind study conducted in 14 countries, set and validated a ratio, that would predict preeclampsia in singleton pregnancies 24 to 36 weeks 6 days with suspected preeclampsia.

A ratio of 38 had a NPV of 99.3% to rule out PE within 1 week and 94.7% to rule it in within 4 weeks. The PPV was 16.7% to rule out PE within 1 week at 38.6 to rule it in within 4 weeks.

For fetal outcomes, a ratio of < 38 had a NPV of 99.3% for adverse fetal outcomes within 1 week. A ratio of >38 had a PPV of 47.5% for development of adverse fetal outcomes within 4 weeks(21).

In a case-control study by Verlohren et al to determine gestational age specific cutoffs for the sFlt-1/PlGFas a diagnostic test for preeclampsia, 234 preeclamptic patients and a matched cohort of 468 patients with normal outcome were compared. The aim was to reach ≥95% sensitivity at the low cut off and ≥95% specificity at the high cut off. For early onset, the cutoffs at ≤33 and ≥85 resulted in a sensitivity/specificity of 95%/94% and 88%/99.5%, respectively.

For late onset, the cutoffs at ≤33 and ≥110 had a sensitivity/specificity of 89.6%/73.1% and 58.2%/95.5% for a diagnosis of preeclampsia. (22).

Gasser et al from Egypt in a case control study, looked at 45 cases and 45 controls between 24-34 weeks gestation. Those with PE/HELLP had an elevated sFlt-1/PlGF ratio compared with controls 34 weeks (590.1 vs 9.9, P .001). An sFlt-1/PlGF ratio cut off of >85 gave100% sensitivity and 100% specificity for development of preeclampsia(23).

sFlt-1: PlGF as a predictor of adverse maternal and fetal outcomes

In a prospective study by Rana S., Karumanchi SA. et al, 616 women with singleton gestation with a suspicion of preeclampsia, they evaluated the relationship of the sFLT-1: PlGF ratio to maternal and fetal adverse outcomes in 2 weeks(24). Adverse maternal outcomes were

hypertension plus any of: Platelet $\leq 100 \times 10^9$/L /uL, elevated AST or ALT, pulmonary edema, DIC, intracranial bleed, placenta abruption, eclampsia, acute kidney injury, or death. Adverse neonatal outcomes were iatrogenic delivery, umbilical artery Doppler indices that were abnormal, SGA, fetal and neonatal death. In those presenting at a gestational age <34 weeks, a cutoff of 85 had a specificity of 94.0% and sensitivity of 72.9% and, NPV of 87.3% and NLR of 0.29. PPV of 86.0% and positive likelihood ratio of 12.2 for adverse events(25).

The cutoff levels, optimal gestation for screening, single or multiple testing, and patients who would best benefit from such screening has not been agreed upon. Some countries like Germany have approved the test for the diagnosis of PE and in the UK as a rule-out in at risk women(26). NICE recommends PlGF to be used as a rule-out but not for diagnosis or rule-in in suspected preeclampsia between 20 to 34+6 weeks(27).

Conceptual framework

Independent variables

Risk factors for preeclampsia

Increased antiangiogenic factors
Decreased proangiogenic factors
High sFlt-1:PlGF ratio

Pregnant woman >20
weeks gestation

Endothelial damage
Placental insufficiency

Systemic changes, increased BP,
Proteinuria, liver changes,
reduced platelets

Maternal outcomes
Preeclampsia/eclampsia/HELLP
ICU admission
Acute kidney injury
Pulmonary oedema
PPH/Death

Dependent

variables

Perinatal outcome
Abruptio placenta
FGR, IUFD
Preterm birth
NBU admission
Neonatal death

Conceptual framework

Several risk factors are associated with increased preeclampsia risk. These risk factors include chronic hypertension, history of PE in a previous pregnancy, history of PE in a first degree relative, diabetes mellitus, renal disease, hypercoagulable states, such as antiphospholipid syndrome and advanced maternal age. Preeclampsia is thought to originate in the placenta, due to inadequate cytotrophoblast invasion causing widespread maternal endothelial dysfunction. Production of placental anti-angiogenic factors, soluble fms-related tyrosine kinase 1 has been shown to be increased in preeclampsia. These anti-angiogenic factors are released into the maternal circulation; causing disruption of the maternal endothelium and result in hypertension, proteinuria, and the other systemic manifestations of preeclampsia. Complications of preeclampsia include acute renal failure, placental abruption, seizures, pulmonary edema, acute liver injury, hemolysis, and/or thrombocytopenia and HELLP syndrome. Fetal complications include fetal growth restriction, preterm births and fetal death.

Statement of the problem

Placental vascular changes happen before clinical preeclampsia. Level of sFlt-1and PlGF are representative of these changes and are detectable before PE becomes apparent. Preeclampsia presents as elevated BP and proteinuria. Complications have been demonstrated to develop even before development of proteinuria and the level of proteinuria correlates poorly to outcomes.(28) There is no one reliable test that predicts development of preeclampsia and adverse outcomes.

National Institute for Health and Care Excellence (NICE) recommends the use of the triage PlGF test in combination with other diagnostic tests and clinical signs to rule out preeclampsia and risk of delivery within 2 weeks in women of gestational age between 20 to 34 weeks+ 6 days. A cut off of <12pg/ml had a sensitivity of 63%, specificity of 90%, PPV of 70% and NPV of 87% (27). This being a new concept, such recommendations do not exist in our local guidelines.

Doppler ultrasound parameters have been routinely used for fetal surveillance and to assess need for delivery during follow up of women with preeclampsia (29). Uterine artery Doppler has prognostic value for the fetal perinatal complications, but cannot predict maternal complications (12). The sFlt-1/PlGF ratio in combination with Doppler has demonstrated improvement in the sensitivity and specificity in predicting PE(30). The ratio and other screening tests could be useful tools in risk assessment in women at risk of PE.

A reliable predictor for women with suspected preeclampsia, those with rising BPs with no proteinuria, is needed to be able to identify those who are going to develop PE in the short term.

Study Justification

Management of PE requires close observation, recognition of signs and symptoms, diagnosis and optimizing the time of delivery to maximize maternal and fetal well-being. The ability to predict development of PE may decrease maternal and fetal adverse outcomes through closer monitoring and early referral for delivery at tertiary care centers. There is no local data on the use of vascular factors to predict PE or adverse maternal and perinatal adverse outcome.

A metanalysis by Agrawal et al reported that there was under reporting of studies that did not yield positive results(31). Studies that have been done using the ratio for prediction have used different cutoffs. Most studies don't report the length of time in which the test rules out or rules in the disease. Therefore more studies need to be carried out to validates the suggested sFLT-1/PlGF cut offs and determine the optimal time for screening in different patient groups.

Research Question

What is the utility of the sFLT1/PIGF ratio as a predictor of the development of preeclampsia and adverse maternal and neonatal outcomes in women at risk at KNH?

Broad Objectives

To determine the utility of the sFlt-1/PIGF ratio as a predictor of the development of preeclampsia within 4 weeks and adverse maternal and neonatal outcomes in women at risk in KNH.

Specific Objectives

In women at risk of preeclampsia, using the sFlt-1/PlGF ratio of 38 to predict development of:

1. Preeclampsia within 1 and 4 weeks

2. Adverse maternal outcomes (HELLP syndrome, AKI)

3. Adverse perinatal outcomes (FGR, Preterm birth, IUFD)

Chapter 3: Methodology

Study Design

This was a prospective cohort study in which women with one major, 2 or more moderate risk factors for preeclampsia were enrolled between the gestational age of 24+0 to 36+6 weeks of gestation and followed up to delivery and 24 hours postpartum and maternal and fetal outcomes determined.

Study Area

The study was conducted at the Kenyatta National Hospital antenatal clinics and wards as well as the records department. KNH is a national teaching and referral hospital that is located in Nairobi County 2 kilometers southwest of the central business district. The antenatal clinics run from Monday to Friday. It serves 3000-3500 clients per month, 1200-1500 being new clients. There are 3 ANC wards with 200 to 250 mothers at any one point. Some of the mothers are admitted through labor ward which on average serves 20 to 50 clients in 24 hours.

Study Population

Pregnant women of gestational age 24 weeks+0 days and above seen at the ANC KNH who had risk factors for PE.

Inclusion criteria

Pregnant women at 24 weeks + 0 days to 36 weeks + 6 days gestation at the first contact with at least one major or more than one moderate risk factors of preeclampsia(33).

Any one of:

- History of hypertensive disorder in prior pregnancy

- kidney disease

- autoimmune disease

- Diabetes mellitus/Gestational diabetes

- chronic hypertension

- Thrombophilia

Any 2/more of:

- Preeclampsia in first degree relatives

- In vitro fertilization

- Primigravida

- age <18 years or >40 years

- pregnancy interval > 10 years

- Multiple pregnancies.

Exclusion criteria

Women who were not able to give consent

Major congenital anomalies.

Sample Size Determination

In the PROGNOSIS Asia study, a prospective, multicenter, double-blind, observational study conducted at 25 sites by Xuming Bian et al a ratio of >38 for ruling in preeclampsia within 4 weeks had a sensitivity of 62.0% and 83.9% specificity,the prevalence of preeclampsia was 14.4%.(34) Therefore sample size will be calculated using Buderer's formula:

$$n = \frac{Z^2_{1-a/2} \times S(1 - S_p)}{L^2 \times (1 - Prevalence)}$$

Where,

n = Desired sample size

S_p = anticipated specificity set at 84%

$Z_{1-a/2}$ = value from standard normal distribution corresponding to desired confidence level (Z=1.96 for 95% CI)

L = absolute precision desired on either side (half − width of the confidence interval) of sensitivity which will be 0.12

$Prevalence$ = prevalence of the condition which is estimated at 14.4%

$$n = \frac{1.96^2 \times 0.84(1 - 0.84)}{0.12^2 \times (1 - 0.14)} = 41$$

Attrition rate expected = 30%, which translates to 12 patients, thereby a sample size of 53 patients was used.

Sampling Procedure

A consecutive sample of all women with risk factors and who met the inclusion criteria were selected until the desired sample size was reached.

Recruitment and Consent

In the antenatal clinics, women were screened for eligibility using a screening form by the triage nurse. When one met the inclusion criteria, they were directed to a room where the principal investigator (PI) was stationed. The routine ANC clinic was conducted then informed consent administered. If a patient consented, the questionnaire was filled by the PI and a blood sample drawn while observing protocols put in place for prevention of the spread COVID 19. Study participants were given a printout with the danger signs for preeclampsia and principal investigator's phone number and were directed to present to hospital for review if they experienced any symptoms of preeclampsia/eclampsia. The participants' phone numbers were taken for purposes of follow up, by the PI, and BP monitoring once the initial sFLT-1/PlGF ratio had been determined. Once the initial results were out, the participants were called and informed of the results.

Study Variables

objective	Exposure	outcomes	Sources of data
Development of preeclampsia	High sF1t-1/PIGF ratio.	Pre-eclampsia, Eclampsia, HELLP	Questionnaires Hospital file ANC card Referral notes
Maternal outcomes		Death, Eclampsia, HELLP, Acute kidney injury, ICU admission, Pulmonary edema, Postpartum hemorrhage, abruptio placenta	
Perinatal outcomes		Intrauterine death, fetal growth restriction, Neonatal death, , NBU admission, Preterm births (including iatrogenic), low birth weight	

Data collection

Data was collected using a questionnaire. (Appendix 5-Study questionnaire)

Study flow
May 2020

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│ Women at risk of preeclampsia,  │
│ 82                              │──────────┐
└─────────────────────────────────┘          │
                │                    ┌──────────────────────────────┐
                │                    │ Not meeting inclusion criteria│
                ▼                    │ 22                           │
┌─────────────────────────────────┐ └──────────────────────────────┘
│                                 │
│                                 │────────┐
└─────────────────────────────────┘        │
                │                   ┌──────────────────┐
                │                   │ Decline consent  │
                ▼                   │ 5                │
┌─────────────────────────────────┐└──────────────────┘
│ Women who gave consent          │
│ and were included 55,           │
└─────────────────────────────────┘──────┐
                │                 ┌──────────────────┐
                │                 │ Loss to follow   │
                ▼                 │ up,3             │
┌─────────────────────────────────┐└──────────────────┘
│ Analysed N=50                   │
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November 2020

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                ▼
┌─────────────────────────────┐
│ Preclampsia,15              │
│ No Preeclampsia, 35         │
└─────────────────────────────┘
        ╱               ╲
┌──────────────────┐  ┌──────────────────┐
│ Maternal outcomes│  │ Perinatal outcomes│
│ N=50             │  │ N=50             │
└──────────────────┘  └──────────────────┘
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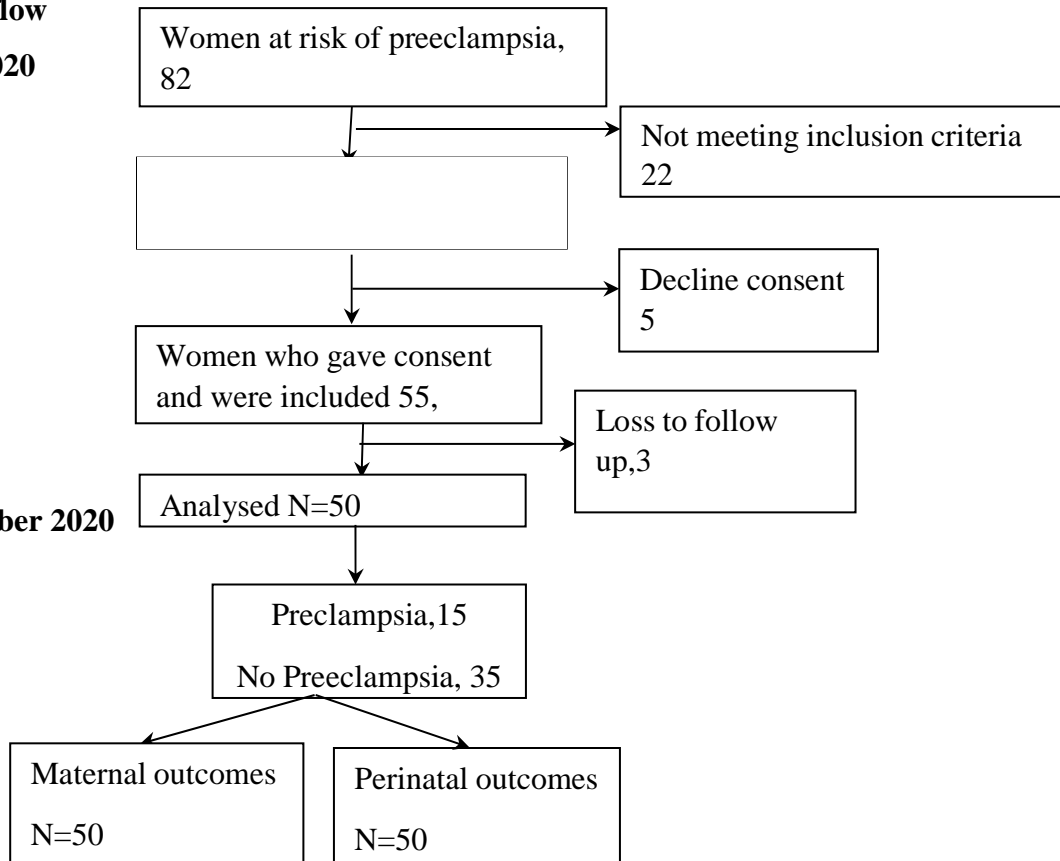
Study procedure

Women were screened for eligibility for inclusion into the study during the baseline visit (the first time they came into contact with the primary investigator).

Gestational age was calculated based on the last normal menstrual period for those with regular menses and sure of dates. Ultrasound dates done before 20 weeks gestation were used for those with irregular menses or not sure of dates. A combination of history e.g., 1st pregnancy test, 1st fundal height and quickening were used as estimates in those without ultrasounds and recorded at

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visit 1. Biodata, weight, height, medical history, routine pregnancy observations (RBS, urine dipstick proteinuria) were documented. A blood sample for sFLT-1/PlGF ratio was drawn. 6 participants with initial gestation of 24 weeks gave a second sample at 28 weeks.

The BP was measured with the patient seated with the back supported after resting for at least 3 minutes. BP cuff was placed at the level of the patient's midsternal level/4th intercostal space) and appropriately sized cuff using a digital blood pressure machine at the brachial artery (with the arm resting on a table at heart level).

Women were counselled on danger signs of preeclampsia and BP monitoring from a nearby clinic at home then followed up with weekly phone calls until birth to review BP readings and screen for any symptoms of preeclampsia. Those with any positive findings were advised to present to the hospital.

The preeclampsia status from every routine ANC visit was determined from the file: no preeclampsia, preeclampsia without severe features, preeclampsia with severe features, HELLP syndrome, superimposed preeclampsia on chronic hypertension and eclampsia. The fundal height was reviewed from the file to determine if there was FGR.

Collection of samples

Venous blood samples (=3 ml) were collected into plain tubes and at the end of each day transported in a cool box at room temperature to the Nairobi Hospital laboratory. The samples were used only for the purpose of this research and The Nairobi Hospital is responsible for safe disposal after the research came to an end.

Laboratory analysis and quality assurance

The analysis was done at the Nairobi Hospital Laboratory where the assay kits had been donated. The samples were centrifuged and analyzed on the same day. The Eclecsys PlGF, sFLT assay and cobas-e immunoassay analyzer from Roche diagnostics was used. The samples, calibrators and controls were at 20-25 °C prior to measurement using the manufacturer's instructions. Calibration was performed once per reagent lot using fresh reagent (i.e. not more than 24 hours since the reagent kit was registered on the analyzer). For quality control a PreciControl Multimarker was used. Controls for the various concentration ranges were run individually at least once every 24 hours when the test was in use.

Safety and monitoring of adverse events

Participants were required to report any adverse events to the principal investigator. The PI was responsible for the accurate documentation, investigation and follow up of any adverse events(e.g severe swelling and pain at the venipuncture site or death due to severe PE). The PI would report promptly to the KNH-UoN and TNH ERC all serious adverse events within 72 hours of their occurrence.(Appendix 4: Reporting adverse events)

Benefits from the Study

The study participants benefited directly from this study because they were closely monitored for development of any signs and symptoms of preeclampsia/eclampsia and immediate care instituted.

This study involved a black African population in Sub Saharan Africa, which has not been extensively studied.

Most studies evaluate sFlt-1/PlGF ratio in suspected preeclampsia, while this study evaluated women at high risk for PE but without any signs or symptoms for PE.

Study Limitations.

We were not able to provide patients with BP machines for home BP monitoring therefore most were not able to take daily BPs. Patients would get their weekly follow up BPs done at a nearby clinic and this might have delayed picking up an initial rise in BPs.

This study was not able to establish how often the test should be repeated to optimize pick up rate. It however serves as a baseline reference in the Kenyan population for studies in the future.

Loss to follow up due to the prospective nature, this was mitigated by making telephone follow ups. Loss to follow up was 5%. Those lost to follow up changed health facilities.

Ethical Consideration

Ethical clearance and approval were obtained from the Kenyatta National Hospital /University of Nairobi and the Nairobi Hospital Ethical Review Committee. Special authorization to collect data was obtained from the KNH research and programs department. Informed consent was also obtained from the women prior to administering the questionnaire by the Principal Investigator. All questionnaires had no identifying features of the women; they were maintained and stored in a secure place accessible to the Principal Investigator. Code numbers were used to identify the participants in a password-protected computer database.

Participants with sFlt-1/PlGF ratio >38(high risk for PE) were followed up with 2 telephone calls every week by the Principal Investigator to determine when they developed high BP.

Data Management and Statistical Analysis

Data was manually checked for completeness prior to entry and analysis with the use of Statistical Package for Social Sciences (SPSS) version 23. Demographic characteristics of the women were analyzed and summarized as medians and interquartile range after being subjected to a Shapiro Wilk test that showed skewedness. Clinical characteristics(hypertensive disorder in previous pregnancy, chronic hypertension, GDM/DM, medication)were summarized as frequencies and proportions. The Mood's median test was used to compare the levels of sFLT-1/PlGF in those who developed PE with those who did not develop PE. A P-value of <0.005 was considered to be statistically significant. To determine the predictive value of the sFlt-1/PIGF ratio, a cut off of <38 to rule out PE in 1 week and ≥ 38 to rule it in in 4 weeks was used, 2 by 2 tables were prepared for the different outcomes. Performance of the cutoff was determined by calculating sensitivity, specificity, negative and positive predictive value and area under the curve using ROC curves. An ROC curve was used to select the best cut-off ratio to predict development of preeclampsia within 4 weeks.

Chapter 4: Results

A total of 50 women with 1 major />1 moderate risk factors for PE were enrolled. 6 participants at 24 weeks gave a repeat sample at 28 weeks gestation. 56 samples were analysed for the preeclampsia outcome. 50 samples were analysed for the fetal and maternal adverse outcomes. 11 participants developed PE with severe features, 4 PE without severe features, 8 had pregnancy induced hypertension, 7 had chronic hypertension and 20 remained normotensive. A Fischer's exact test was used to compare baseline characteristics between those who developed PE and those who did not. There were no observed differences in the baseline characteristics like: age, parity, week of gestation at first contact and maternal weight. There were no differences in some clinical characteristics e.g. history of hypertensive disorder in prior pregnancy, history of DM and RBS, between those who developed PE and those who did not. The group that developed PE had significantly higher BP reading at the baseline visit of 140/90mmHg compared to those who did no, 128/79mmHg. There were more women with a history of chronic hypertension, multiple pregnancies, preterm births, fetal growth restriction and a lower birth weight in the PE group (Table 1, Table 2).

Table 1: Demographic and Clinical characteristics

	PE (n=15)	No PE (n=35)	p-value
Age (Median, IQR)	35.0 (32.5-38.0)	35.0 (29.0-37.0)	0.902
Occcupation (n, %)			
Employed	6 (40.0)	8 (22.9)	0.503
Self-employed	7 (46.7)	19 (54.3)	
Unemployed	2 (13.3)	8 (22.9)	
Weight, kg (Median,IQR)	82.0(79.5-87.5)	85.0(70.0-96.5)	0.758
Gravidity (n, %)			
Primigravida	3 (20.0)	4 (11.4)	0.415
Multigravida	12 (80.0)	31 (88.6)	
Week of gestation at baseline (Median, IQR)	31(28-35)	31(28-34)	0.902
BP a first visit (Median, IQR)			
Systolic	140 (135-150)	128 (113-137)	**0.001**
Diastolic	90 (88-96)	79 (74-90)	**0.003**
Proteinuria on follow up (n, %)			
1+	5 (35.7)	5 (83.3)	0.288
2+	5 (35.7)	1 (16.7)	
3+	3 (21.4)	0 (0.0)	
4+	1 (7.1)	0 (0.0)	

Table 2: Baseline clinical characteristics

	PE (n=15)	No PE (n=35)	p-value
Hypertensive disorder in previous pregnancy (n, %)	7 (46.7)	18 (51.4)	0.758
Chronic hypertension (n, %)	8 (53.3)	8 (22.9)	**0.049**
GDM/DM (n, %)	3 (20.0)	12 (34.3)	0.502
Interval since last pregnancy, years (Median, IQR)	4 (2-5)	2 (2-5)	0.384
Multiple pregnancy (n,%)	4 (26.7)	1 (2.9)	**0.024**
Medication at baseline visit (n,%)			
Aspirin	4 (26.7)	19 (54.3)	0.073
Iron/Folate	4 (26.7)	18 (51.4)	0.106
Anti-hypertensives	11 (73.3)	10 (28.6)	**0.003**

Table 3: Clinical outcomes

	PE (n=15)	No PE (n=35)	p-value
Maternal outcomes (n, %)			
AKI	1 (6.7)	0 (0.0)	0.300
HELLP	5 (33.3)	0 (0.0)	0.001
Neonatal outcomes			
Admission to NBU (n, %)	2 (13.3)	0 (0.0)	0.086
IUFD (n, %)	2 (13.3)	0 (0.0)	0.086
Preterm birth (n, %)	9 (60.0)	2 (5.7)	**<0.001**
Fetal growth restriction (n, %)	5 (33.3)	0 (0.0)	0.001
Birth weight (median, IQR)	2370 (2140-2635)	3300 (2945-3600)	**<0.001**

Prediction of preeclampsia

The median sFlt-1/PlGF ratio at baseline visit for patients who developed preeclampsia within one week was 45.5 (44.4-60.2) compared with those who did not 3.0 (1.6-7.6) which was not statistically significant. (**Table 4**,Figure 1)

The median sFlt-1/PlGF ratio at baseline visit for patients who developed preeclampsia within 4 weeks was 24.2 (7.0-58.0) which was significantly higher compared with those who did not 2.5 (1.3-3.7) (**Table 4**, Figure 2)

sFlt-1/PlGF <38 to rule out PE within 1 week had a specificity of 11.76% (4.44%-23.87%), sensitivity of 20.00%(0.51%-71.64%), NPV of 60.00% (95% CI, 38.59%-78.17%), NLR 6.80 (2.85-16.23)(Table 5)

An sFlt-1/PlGF ≥38 for ruling in PE within 4 weeks had a sensitivity of 53.85%(25.13%-80.78%), specificity of 93.02% (80.94%-98.54%, PPV 70.00% (41.23%-88.9%), PLR7.72 (2.32-25.67), NPV 86.96% (78.66%-92.34%), NLR0.50 (0.27-0.90),accuracy 83.93% (71.67-92.38%)(Table 5)
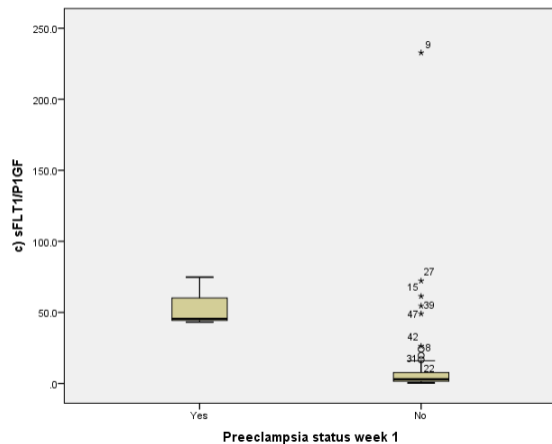
Figure 1: Median sFLT-1/PlGF ratio for participants with and without PE within 1 week
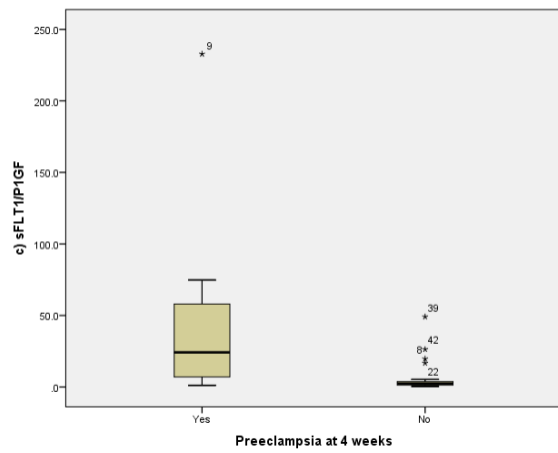
Figure 2: Median sFLT-1/PlGF ratio for development of preeclampsia

Table 4: Median sFlt-1/PlGF ratio for development of preeclampsia

	Preeclampsia	No Preeclampsia	p-value
Within 1 week(median, IQR)	45.5 (44.4-60.2)	3.0 (1.6-7.6)	0.074
Within 4 weeks (median, IQR)	24.2 (7.0-58.0)	2.5 (1.3-3.7)	0.005

Table 5: Predictive performance of sFlt-1/PlGF ratio

	TN/FN	TP/FP	NPV, % (95% CI)	PPV, % (95% CI)	Sensitivity, % (95% CI)	Specificity, % (95% CI)	NLR	PLR
sFlt1/PlGF<38, rule out within 1 weeks	6/4	1/45	60 (38.59%-78.17%)	2.17 (0.38%-11.40%)	20 (0.51%-71.64%)	11.76 4.44%-(23.87%)	60.00% (38.59%-78.17%)	0.23 (0.04-1.31)
sFlt-1/PlGF ≥38, rule in within 4 weeks	40/6	7/3	86.96 (78.66%-92.34%)	70 (41.23%-88.95%)	53.85 (25.13%-80.78%)	93.02 (80.94%-98.54%)	0.50 (0.27-0.90)	7.72 (2.32-25.67)

TN- True negative, FP- false positive, NPV- negative predictive value, PPV- positive predictive value, NLR- negative likelihood ratio, PLR- positive likelihood ratio

Prediction of adverse perinatal outcomes

50 samples were analysed. Adverse outcomes occurred in 12 of the participants between recruitment and delivery. Outcomes were tabulated as 1 for each participant even if more than 1 adverse outcome occurred in the same woman. Median sFlt-1/PlGF ratio for those who developed adverse outcomes was 20.1 (7.0 – 66.8), which was significantly higher than those who did not develop adverse outcomes 2.5 (1.4 – 3.8) (P 0.008)(Table 6).

An sFlt-1/PlGF ratio ≥38 to predict adverse fetal outcomes had a sensitivity of 41.67%(15.17%-98.34%), PPV of 62.50% (95% CI, 31.76%-85.65%), Positive Likelihood Ratio 5.28 (1.47-18.90) (Table 7)

Table 6: Median sFlt-1/PlGF for adverse outcomes

	Adverse	No adverse	p-value
Fetal (median, IQR)	20.1 (7.0 – 66.8)	2.5 (1.4 – 3.8)	**0.008**
Maternal (median, IQR)	24.2 (16.0 – 61.4)	2.7 (1.4 – 4.3)	**0.018**

Table7: sFlt-1/PlGF Ratio ≥38 to predict adverse perinatal outcome

	Yes	No	Total
≥38	5	3	8
<38	7	35	42
Total	12	38	50

Sensitivity 41.67% (15.17%-72.33%), Specificity 92.11% (78.62%-98.34%), Positive Likelihood Ratio 5.28 (1.47-18.90), Negative Likelihood Ratio 0.63 (0.39-1.03), Positive Predictive Value 62.50% (31.76%-85.65%), Negative Predictive Value 83.33% (75.44%-89.06%), Accuracy 80.00% (66.28%-89.97%)

Prediction of adverse maternal outcomes

50 samples were analysed. Adverse outcomes occurred in 5 participants between recruitment and delivery. Any participant with any adverse outcome was counted as 1. The median sFlt-1/PlGF ratio for women who developed adverse outcomes was 24.2 (16.0 – 61.4), which was significantly higher than those who did not develop adverse outcomes was 2.7 (1.4 – 4.3) (P 0.018) (Table 6). sFlt-1/PlGF ratio ≥38 for predicting adverse outcomes had sensitivity of 40.00% (5.27%-85.34), PPV 25.00%(8.28%-5.18%), PLR 3.00(0.81-11.08) (Table 7)

Table 7: sFlt-1/PlGF Ratio ≥38 to predict adverse maternal outcome

	Yes	No	Total
≥38	2	6	8
<38	3	39	42
Total	5	45	50

Sensitivity 40.00% (5.27%-85.34%), Specificity 86.67% (73.21%-94.95%), Positive Likelihood Ratio 3.00 (0.81-11.08), Negative Likelihood Ratio 0.69 (0.34-1.43), Positive Predictive Value 25.00% (8.28%-55.18%), Negative Predictive Value 92.86% (86.30%-96.41%), Accuracy 82.00% (68.56%-91.42%)

Determining a cut off for predicting development of preeclampsia within 4 weeks

Using an ROC curve for the data set that we had, an sFlt-1 /PlGF ratio of 3.97 was found to have the largest area under the curve (AUC) 82.6%(CI, 63.6-96.6%(Figure 3: ROC curve to determine best cut off to predict development of preeclampsia within 4 weeks)

It's perfomance in ruling in preeclampsia within 4 weeks is as follows: sensitivity 100% (75.29-100), Specificity 74.42% (58.83-86.48), PPV 54.17% (41.51-66.3) PLR 3.91(2.35-6.51) (**Table 8**)

Table 8: **sFlt-1/PlGF Ratio ≥3.97 to rule in preeclampsia within 4 weeks**

	Yes	No	Total
≥3.97	13	11	24
<3.97	0	32	32
Total	13	43	56

Sensitivity 100% (75.29%-100%), Specificity 74.42% (58.83%-86.48%), Positive Likelihood Ratio 3.91(2.35-6.61), Negative Likelihood Ratio 0.0, Positive Predictive Value 54.17%(41.51%-66.30%), Negative Predictive Value 100%, Accuracy 80.36% (67.57%-89.77%)

Table 9: sFlt-1/PlGF Ratio ≥3.97 to rule in adverse fetal outcome

	Yes	No	Total
≥3.97	10	29	31
<3.97	2	9	19
Total	12	38	50

Sensitivity 83.33% (51.59%-97.91%), Specificity 23.68% (11.44%-40.24%),Positive Likelihood Ratio 1.09(0.80-2.82),Negative Likelihood Ratio 0.70(0.18-2.82), Positive Predictive Value 25.64%(20.20%-31.960%), Negative Predictive Value 81.82%(52.90%,-94.75%), Accuracy 38.00% (24.65%-52.83%)
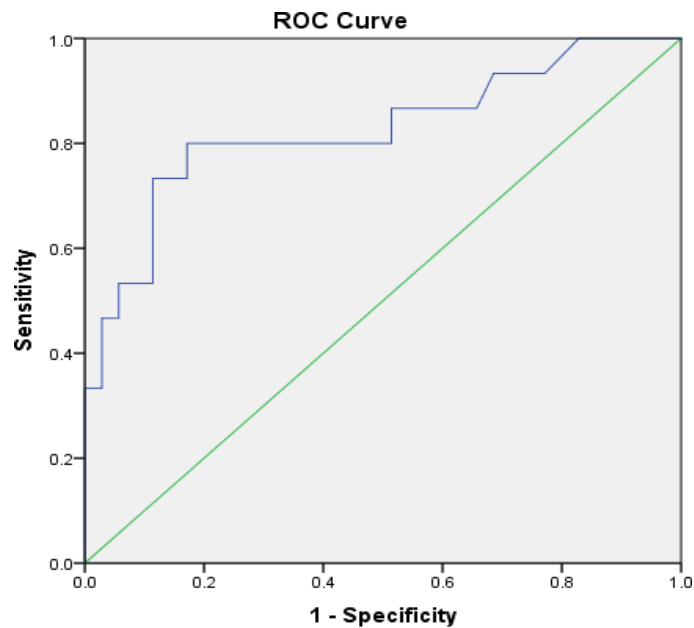
Figure 3: ROC curve to determine best cut off to predict development of preeclampsia within 4 weeks

Table 10: sFlt-1/PlGF Ratio ≥3.97 to rule in adverse maternal outcome

	Yes	No	Total
≥3.97	5	14	19
<3.97	0	31	31
Total	5	45	50

Sensitivity 100.00% (47.82%-100.00%), Specificity 68.89% (53.35%-81.83%), Positive Likelihood Ratio 3.21(2.08-4.96), Negative Likelihood Ratio 0.00, Positive Predictive Value 26.32%(18.78%-35.55%), Negative Predictive Value 100%, Accuracy 72.00% (57.51%-83.77%)

Chapter 5: Discussion

This study has demonstrated that the median levels of sFlt-1/PlGF ratio are higher in women who developed preeclampsia, maternal and fetal adverse outcomes for the first time in Kenya. This is in line with many other studies that have measured the ratio in normotensive and preeclamptic women. It was higher in women who developed preeclampsia at both 1 and 4 weeks, though it did not show statistical significance at 1 week, probably due to low patient numbers. sFlt-1/PlGF ratio of <38 had a lower sensitivity of 20.00% and NPV of 60% than in a study by Xuming et al where sensitivity was 76.5% with a NPV of 98.6% for ruling out preeclampsia within 1 week(34). This could be as a result of population differences, with the population we used having lower cut offs for the same. The cut off of <38 to rule out PE in 1 week might not be generalizable for all populations.

An sFlt-1/PlGF ratio of ≥38 to rule in PE within 4 weeks had a sensitivity of 53.85% and a PPV 70.00%, which was comparable to a study by Zeisler et al which had a sensitivity of 66.2%, PPVof 36.7%(35).

A cut off of ≥38 to predict adverse perinatal outcomes in this study had a sensitivity of 41.67%, specificity of 92.11%, PPV of 62.50%, in the PROGNOSIS Asia study which was evaluating adverse outcomes within 4 weeks the PPV was 53.5%. This study included adverse events that occurred anytime between recruitment and delivery and didn't restrict them to 4 weeks like in the PROGNOSIS Asia study(34).

The PPV for predicting maternal adverse outcomes with a cut off of ≥38 was 25% with a sensitivity of 40% and specificity of 86.67%. In a study by Ljijana et al which evaluated women with a confirmed diagnosis of PE a cut-off of 377·0 was found to have the best of sensitivity (75·0%) and specificity (92·3%) for predicting maternal complications(13).

An sFlt-1/PlGF cut off of >3.97 to predict PE within 4 weeks determined from an ROC had a sensitivity of 100%, specificity 74.42%, which compares to a cut off of 4 set in a study by Andersen et al that put sensitivity at 80%, specificity at 75.30% (36).

Chapter 6: Conclusion

The sFlt-1/PlGF ratio cut off of 38 did not have very high NPV for ruling out PE within 1 week and PPV for ruling it in or adverse maternal and neonatal outcomes within 4 weeks compared to other studies.

A cut off of >3.97 to predict development of PE within 4 weeks and adverse maternal and perinatal outcomes has shown high sensitivity and PPV.

Recommendations

Lower cut off value for sFlt-1/PlGF ratio should be considered in our population to predict development of preeclampsia and adverse maternal and perinatal outcomes. More prospective cohort studies in women at risk of PE in the African population are needed to establish a population-based cutoff and to conceptualize employment of biomarkers to the local situation .

This study did not set out to compare the predictive performance of the sFLT-1/PlGF ratio with the currently available modalities of screening for adverse outcomes. There is need for more studies to corroborate the biomarkers and ultrasound findings(Doppler,BPP and Biometrics) to improve diagnostic predictions and inform PE/ eclampsia management

Conflict of interest statement

The sFlt-1/PlGF assay kits used for this study were provided by Roche diagnostics. Roche diagnostics however was not be involved in the collection or entry of any data from this study. Roche diagnostics are not using this study as a way of marketing their test kits and are not giving any monetary incentives to the primary investigator.

The primary investigator has no conflict of interest to declare.

Time Frame

Activity	SEPT-DEC 2019	APRIL-MAY 2020	JUNE-OCTOBER 2020	NOVEMBER 2020	NOVEMBER 2020
Proposal development	■				
Ethical approval		■			
Data collection			■		
Data analysis				■	
Results					■

Budget

Item	Unit cost	Required	Total cost (Kshs.)
Printing paper	500	10	5000
Printing			10,000
Pens	20	50	1000
Flash disk	500	3	1,500
Research assistants			60,000
Airtime			4,000
Statisticians fee			40,000
Transport			50,000
SFLT-1/PlGF kits	10000	56	560,000
Laboratory technicians	3	70,000	210,000
Total			941,500

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Appendices

Appendix 1-Screening tool

Gestational age of 28 + 0 days to 36 + 6 days

Any one of:

- Hypertensive disease in a prior pregnancy

- chronic kidney disease

- autoimmune disease e.g., antiphospholipid syndrome or systemic lupus erythematosus

- Diabetes mellitus

- chronic hypertension

- Thrombophilia

Any 2/more of:

- History of preeclampsia in a first degree relative

- In vitro fertilization

- Primigravida

- age <18 years or >40 years

- pregnancy interval of more than 10 years

- BMI above 35 kg/m^2

- Multiple pregnancies.

Participant Information Sheet

Study title: The utility of the sFlt-1/PIGF ratio as a predictor of the development of preeclampsia and adverse outcomes in women at risk in KNH.

The Principal Investigator: DR. MBUCHE MZUNGU, a postgraduate student in the department of Obstetrics and Gynecology at the University of Nairobi. In this study. TEL: 0724768103.

Introduction

I would like to inform you about a study being conducted by the above listed 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) 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 KNH-UoN and Nairobi Hospital Ethics and Research Committee protocol No. _____

What is the study about?

The researcher listed above is interviewing individuals who are between 28 weeks to 34 weeks pregnant. The purpose of the interview is to find out if you are at high risk of developing high blood pressure (preeclampsia) in pregnancy. Participants in this research study will be asked questions about their age, previous history of high blood pressure in pregnancy, high blood sugar

and history of high blood pressure in first degree relatives. Participants will also have the choice to undergo test such as blood sugar, urine test and blood tests (sFLT-1: PlGF). There will be approximately 43 participants in this study 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 feel comfortable answering questions. The interview will last approximately 10 minutes. The interview will cover topics such as your medical history and counselling on danger signs for preeclampsia/eclampsia. In case you experience any of the danger signs(on the print out provided), you are required to present to KNH labour ward for review. After the interview has ended, we will take a blood sample of 3 milliliters. We will ask for a telephone number where we can contact you if necessary. Your contact information will be used only by people working for this study and will not be shared with others. The reasons why we may need to contact you include: informing you of the results and giving you a return date for a second blood sample to be drawn. You will also be provide with the principal investigators phone number to get in touch with whenever you make a visit to KNH.

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

Medical research has the potential to introduce psychological, social, emotional and physical risks. Effort 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 number to identify you in a password-protected computer database and will keep all of our paper records in a locked file cabinet. However, no system of protecting your confidentiality can be absolutely 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 you do not want to answer, you can skip them. You have the right to refuse the interview or any questions asked during the interview. 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 examinations/interviews. You may feel some discomfort when blood is drawn, and you may

have a small bruise or swelling from the site where the sample is drawn. In case of an injury, illness or complications related to this study, contact the study staff right away at the number provided at the end of this document. The study staff will treat you for minor conditions or refer you when necessary.

Other potential risks that may be related to the high risk for developing high blood pressure, and not related to the study; are your baby being small for age, premature separation and bleeding of the placenta before birth and demise of the fetus before birth.

Are there any benefits being in this study?

You may benefit by receiving knowledge on the development of high bold pressure in pregnancy and free blood testing for predictors of the same. We will refer you for more frequent clinic visit if you are at high risk for developing high bool pressure for a closer follow up. Also, the information you provide will help us better understand if high blood pressure in pregnancy can be predicted before it develops. This information is a contribution to science and development of guidelines in the diagnosis and management of high blood pressure in pregnancy.

Will being in this study cost you anything?

You will not incur any cost for the sFLT: PlGF blood test but will pay for all routine tests done in pregnancy and any other investigation, including scans, that your doctor orders.

Will you get refund for any money spent as part of this study?

Your transport to and from the hospital for the second visit will be reimbursed.

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 Ext. 44102 email uonknh_erc@uonbi.ac.ke. The Nairobi Hospital ERC tel; +254 202846045, fax +254 20 2728003. The study staff will pay you back for your charges to 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 any benefits.

Participant's statement

I have read this consent form or had the information read to me. I have had the chance to discuss this research study with a study counselor. I have had my 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 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 of the 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 have a blood sample drawn: 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 have willingly and freely given his/her consent.

Researcher's Name:_____**Date:** _____

Signature: _____

Role in the study_____

For more information contact Kenyatta National Hospital/University of Nairobi Ethics and Research committee, College of Health Sciences, P.O Box 19676 00202 Nairobi. Email uonknh-erc@uonbi.ac.ke

Fomu ya maelezo ya kiswahili.

Jina langu ni Dk. Mbuche Mzungu, daktari anayesomea taaluma ya daktari bigwa wa wanawake wajawazito katika hospitali ya kitaifa ya Kenyatta, chuo kikuu cha Nairobi.

Ningetaka kukujulishwa kwamba ninafanya utafiti kuhusu chembechembe za damu ambazo zinaweza kutabiri uwezekano na kupata shinikizo la damu katika ujauzito. Madhumuni ya maelezo haya ni kukusaidia kuamua iwapo ungetaka kuhusishwa katika utafiti huu. Jiskie huru kuuliza maswali kuhusu madhumuni ya utafiti huu, yanayotarajiwa kutoka kwako, madhara yoyote na manufaa, haki zako kama mhusika na jambo lingine lolote tatanishi. Baada ya kuridhika na majibu ya maswali yako, unaweza kuamua kushiriki au la. Utakapo kubali kushiriki, nitakusihi uweke sahihi kwenye karatasi hili. Kushiriki kwako ni kwa hiari, unaweza kutoka katika utafiti huu wakati wowote bila kutoa sababu na kutoka kwako hakutaathiri huduma utakazo pata katika hospitali hii ama nyingine ile.

Niendelee? NDIO/ HAPANA

Utafiti huu unafanyika kwa idhini ya hospitali ya Taifa ya Kenyatta- Chuo Kikuu cha Nairobi na Hospitali ya Nairobi Kamati ya maadali na utafiti protocol No_____

Kusudi

Mtafiti aliyetajwa hapo juu atahoji wanawake waja wazijo kuanzia wiki 28 hadi 34. Madhumuni ya mahojiano ni kujua kama uko katika hatari ya kupata shinikizo la damu kwenye uja uzito. Wahusika wataulizwa maswali kuhusu umri wao, nambari ya watoto walionao, historia ya ugonjwa wa shinikizo la damu na kadhalika. Wahusika watahitajika kutoa damu mara mbili. Kutakuwa na wahusika 88. Tunaomba idhini yako ya kuhusishwa katika utafiti huu.

Taratibu

Ukikubali kushiriki, mahojiano haya yatachukua dakika10. Maswali utakayo ulizwa yatakuwemo historia ya magonjwa yoyote. Utaelezwa kuhusu dalili za ugonjwa wa shinikizo la damu na kutakikana kufika katika chumba cha kujifungua cha KNH iwapo utaona dalili zozote. Utapewa nambari ya simu ya mtafiti mkuu na kutakikana kumpigia simu wakati wowote utakapo kuwa hospitali KNH. Maelezo yote utakayotoa yatahifadhiwa kwa siri na kutumiwa kwa madhumuni ya utafiti huu pekee.

Mililita 3 za damu zitolewa kwa ajili ya kipimo. Damu hiyo itapelekwa katika maabara ya Hospital ya Nairobi na viwango vya chembechembe za sFLT/PlGF kupimwa. Damu hiyo haitatumiwa kupima kitu kingine chochote.

Tutakuuliza nambari ya simu kwa madhumuni ya kukujilisha majibu na kupanga kliniki ya pili. Ukituachia nambari ya simu, hatutaitumia kwa madhumuni mengine ila kwa utafiti huu tu.

Madhara kwako kama mshiriki katika utafiti

Utafiti wa kisayansi unaweza kuleta madhara ya kisaikologia, kijamii, hisia na madhara ya kimwili. Hatari moja inayoweza kutokana na utafiti ni kupoteza faragha. Tutafanya yote tuwezayo kuweka maelezo yanayoweza kukutambulisha kwa siri. Tutatumia nambari kukutambulisha na kuweka maelezo hayo kwenye tarakilishi iliyokingwa kwa nywila. Hatutarajii ya kuwa kutakuwa na madhara yoyote makubwa isipokuwa maumivu na kuvimba mahali damu itakapotolewa. Iwapo utahisi kuwa utafiti huu unakudhuru kwa namna yoyote mweleze mtafiti mkuu kwenye nambari ya simu uliyopewa mwisho wa taarifa hii.

Faida kwako kama mshiriki katika utafiti

Tathmini bila malipo ya kiwango cha chembechembe za damu (sFLT-1/PlGF) zinazoweza kutabiri shinikizo la damu katika uja uzito.

Maelezo ya kina kuhusu uwezekano wa kupata shinikizo la damu kwenye uja uzito.

Malipo

Hutahitajika kulipia kipimo hiki cha sFLT-1/PlGF.

Utalipia vipimo vingine vyovyote vinavyofanywa kwa kawaida kwa mama wajawazito ambavyo daktari wako ataagiza ikiwemo uchunguzi wa picha.

Utaregeshewa tikiti utakayotumia kurudi mara ya pili kwa kipimo cha damu .

Maswali ya baadaye?

Ukiwa na maswali yoyote, unaweza kupiga simu ama kutuma ujumbe mfupi kwa namabri ya simu uliyopewa hapo chini. Kwa maelezo zaidi kuhusu haki zako kama mhusika katika utafiti

unaweza kuandika kwa mwandishi/ mwenyekiti, Kenyatta National Hospital-University of Nairobi Ethic and Research committee simu 2726300, barua pepe uonknh_erc@uonbi.ac.ke The Nairobi Hospital ERC simu; +254 202846045, fax +254 20 2728003.

Kujitoa

Ushiriki wako ni wa hiari na unaweza kukataa kushiriki ama kujitoa katika utafiti huu wakati wowote

Fomu ya saha(taarifa ya kusha).

Taarifa ya Mshiriki.

Nimesomamaelezo haya. Nimekuwa na fursa ya kujadili utafiti na mtafiti/ msaidizi. Madhara na faida zimeelezwa kwangu. Ninaelewa kwamba ushiriki wangu katika utafiti huu ni hiari na kwamba naweza kutoka wakati wowote. Ninaelewa kwamba jitihada zote zitafanywa kweka taarifa kuhusu utambulisho wangu binafsi.

Nimekubali kushiriki katika utafiti huu Ndio   Hapana

Nimekubali kutoa sampuli ya damu   Ndio   Hapana

Nimekubali kupeana nambari ya simu   Ndio   Hapana

Jina la mshirika_____

Sahihi_____**Tarehe**_____

Taarifa ya Mtafiti.

Nimeelezea kikamilifu maelezo muhimu ya utafiti huu kwa mshiriki aliyechaguliwa hapo juu na kuamini kuwa mshiriki ameelewa na ametoa idhini yake kwa hiari.

Jina la mtafiti_____**Tarehe**_____

Sahihi _____

Jukumu_____

Appendix 2: Consent form

I _____ do confirm that I have read/ been explained to the above study, understood the information presented to me and have had the opportunity to ask questions. I understand that my participation is voluntary and that I am free to withdraw from this study at any time without giving reason.

I agree to take part out of my own free will and no coercion or incentive has been offered.

Signature of participant_____   Date: _____

Signature of investigator_____   Date: _____

Appendix 3: Danger signs of preeclamsia/eclampsia

Are you experiencing any of the following? Present to KNH labour ward immediately and call Dr Mbuche Mzungu on 0724768103 once you get there;

Je unahisi dalili zozote zifuatazo? Tafadhali nenda katika chumba cha kujifungua cha KNH na umpigie simu Dk Mbuche Mzungu kwenye 0724768103 ukifika.

1. Loss of conciusnes/ kupoteza fahamu
2. Convulsions/ kufitika
3. Dizziness/ kizunguzungu
4. Blurring of vision/ kuto ona vizuri
5. Severe headache/Kuumwa na kichwa
6. Swelling of the laegs, hands and face/ kufura miguu, mikono na uso
7. Pain on the right upper abdomen?/Kuumwa na upande wa kulia wa juu wa tumbo
8. Heartburn? Kiungulia/ kuumwa kwenye chembe cha moyo
9. Nausea and vomiting/ kuchafukwa na roho na kutapika
10. Reduce urine volume/ kupungua kwa kiasi cha mkojo

Appendix 4: Reporting adverse events

Were any of these adverse events unexpected or more serious than expected?

☐ Yes ☐ No

1. If yes, did you send us an Adverse Event report? ☐ Yes ☐ No
2. Was the event attributable to a study procedure? ☐ Yes ☐ No

   If No, do not complete next AE section below

3. Was the event unexpected (not described in original application or consent form)

   ☐ Yes ☐ No

4. Was the event more serious than expected? ☐ Yes ☐ No

5. How was this event graded?

   - ☐ Mild (caused no limitation of usual activities)
   - ☐ Moderate (caused some limitations of usual activities)
   - ☐ Severe (caused inability to carry out usual activities)

7. Is this kind of adverse event described in the currently approved consent form?

   - ☐ Yes
   - ☐ No

   If not, will the event require changes in the consent form in research procedure?
      1. ☐ No
      2. ☐ Yes if yes attach a copy of the revised consent form with the changes highlighted.

8. Have you reported this event to the study sponsor? ☐ Not applicable ☐ Yes ☐ No

   if no, explain

9. Have you reported this event to the FDA ☐ Not applicable ☐ Yes ☐ No

   If no, explain

10. Have you reported this event to the NIH? ☐ Not applicable ☐ Yes ☐ No

    If no, explain

11. Has this kind of event happened before in connection with this study? ☐ Yes ☐ No

    If yes explain

12. Who is financially responsible for treatment of this adverse event?

    - ☐ Sponsor
    - ☐ Kenyan Health Department

- ☐ Others specify

13. What is the estimated cost of treatment?

14. Where was care provided?

15. Subject's study code number:

16. Subject's age:

17. Subject's gender:

18. Where did the event take place? (Name)

19. What time did the event start and when did it stop? date and time

20. Describe the Serious Adverse Event including a summary of all relevant clinical information.

21. Have you made any changes in study procedures to reduce the possibility that this adverse event will happen again?
    - ☐ Yes. Explain
    - ☐ No. Explain

Appendix 5-Study questionnaire

1) **Bio data**   Date/Tarehe:_____

    a) Serial NO: _____

    b) Age/ Umri:_____

    c) Occupation/Ajira:_____

    d) Marital status/Hali ya ndoa:_____

    e) Level of education:_____

    f) Religion/ Dini:_____

2) **Medical history**

    a) Height/ Kimo(cm):_____Weight/Uzito(kg):_____

    b) LNMP/hedhi ya mwisho:_____Parity:_____Gravidity:____

    c) Gestational age(weeks)_____Fundal height:_____

    d) Systolic BP(mmHg):_____Diastolic BP(mmHg):_____

    e) Urinalysis Protein:_____

    f) Random blood sugar(mmol/L):_____

    g) Do you have any of diseases listed below? Uko na ugonjwa wowote ufuatao?

        i) Diabetes mellitus/kisukari_____

        ii) Hypertension/ shinikizo la damu_____

        iii) Thrombophilia/ugonjwa wa damu kuganda_____

        iv) Kidney disease/ugonjwa wa figo_____If yes, please complete the following subsection

            (1) Urea level(mmol/L)_____

            (2) Creatinine level(mmol/L)_____

        v) History of hypertensive disorder in previous pregnancy/ ugonjwa wa shinikizo la damu kwenye ujauzito_____

        vi) History of preeclampsia in a first degree relative/ ugonjwa wa shinikizo la damu kwa jamaa wa karibu_____

        vii) Have you had assisted reproduction/ _____

        viii)   Is this your first pregnancy/ Je huu ni ujauzito wako wa kwanza_____

        ix) Interval since last pregnancy(month)/ mda tangu ujauzito wa mwisho_____

        x) Multiple pregnancy/ mapacha_____

h) Are you on any medication? / Unatumia dawa zozote?

    i) Aspirin

    ii) Iron-folic acid supplementation

    iii) Others:

i) Preeclampsia status, choose one

    i) No preeclampsia

    ii) Suspected preeclampsia

    iii) Preeclampsia without severe features

    iv) Preeclampsia with severe features

    v) Pregnancy induced hypertension

    vi) Chronic hypertension

    vii) Superimposed preeclampsia on chronic hypertension

3) 1 week visit

    a) sFLT (pg/ml)_____

    b) PlGF (pg/ml)_____

    c) sFLT1/PlGF_____

4) 4 weeks visit   Date:_____

    a) Gestational age(weeks)_____Fundal height:

    b) Systolic BP(mmHg):_____Diastolic BP(mmHg):_____

    c) Urinalysis Proteinuria:_____

    d) sFLT-1:_____PlGF:_____sFLT1/PlGF_____

    e) Preeclampsia status, choose one

        i) No preeclampsia

        ii) Suspected preeclampsia

        iii) Preeclampsia without severe features

        iv) Preeclampsia with severe features

        v) Pregnancy induced hypertension

        vi) Chronic hypertension

        vii) Superimposed preeclampsia on chronic hypertension

5) Scheduled/unscheduled visits with positive findings   Date:_____

    a) Interval since 1st visit: : _____

xxx

b) Systolic BP:_____Diastolic BP:_____

c) Gestational age:_____Fundal height:_____

d) Urinalysis   Protein: _____

e) Urea(mmol/L):_____Creatinine(mmol/L)_____

f) ALT:_____AST:_____ALP:_____

g) HB:_____PLT:_____

h) Preeclampsia status, choose one

   i) Suspected preeclampsia

   ii) Preeclampsia without severe features

   iii) Preeclampsia with severe features

   iv) Pregnancy induced hypertension

   v) Chronic hypertension

   vi) Superimposed preeclampsia on chronic hypertension

6) Delivery   **Date:**_____

   a) Systolic BP:_____Diastolic BP:_____

   b) Gestational age:_____

   c) Urinalysis   Protein:_____

   d) Perinatal outcomes

      i) Live birth:

      ii) IUFD

      iii) APGAR score at 5 minutes

      iv) Abruption placenta

      v) Neonatal deaths

      vi) Preterm birth- birth before 37 completed weeks(up to week 36+6

                days)

      vii) Admission to NBU

      viii)   Birth weight

      ix) Fetal growth restriction

e) Maternal outcome

    i) Death

    ii) Admission to ICU

    iii) HELLP

    iv) Eclampsia

    v) Post-partum hemorrhage

    vi) Pulmonary edema

    vii) Acute kidney injury

7) 24 hours postpartum

    a) Systolic BP_____Diastolic BP_____

    b) Urinalysis   Protein:_____Glucose:_____Nitrites:_____Blood:_____

    c) Preeclampsia status

        i) No preeclampsia

        ii) Suspected preeclampsia

        iii) Preeclampsia without severe features

        iv) Preeclampsia with severe features

        v) Pregnancy induced hypertension

        vi) Chronic hypertension

        vii) Superimposed preeclampsia on chronic hypertension

Appendix 6: ERC Approval

THE NAIROBI HOSPITAL

Our Ref. TNH/ADMIN/CEO/12/05/20

12 May 2020

Dr. Rebecca Mbuche Mzungu
Department of Obstetrics and Gynecology
University of Nairobi

Dear Dr Mzungu,

RE: sFLT1/PIGF RATIO AS A PREDICTOR OF DEVELOPMENT PREECLAPSIA, ADVERSE MATERNAL AND PERINATAL OUTCOMES IN WOMEN AT RISK AT A TERTIARY HOSPITAL IN KENYA

Reference is made to your request to carry out the above study at The Nairobi Hospital. We are pleased to advise that approval has been granted.

In line with the Research Projects Policy, you will be required to submit quarterly update reports of the study to the Committee. You are also required to submit a copy of the final findings for the Committee's records.

Do note that information/data collected and potential findings shall not be in conflict with the Hospital's Confidentiality Clause which states that "You will not without consent of the Association disclose any of its secrets or other confidential matters to anyone who is not authorized to receive them".

Please note that this approval is valid for the period May 2020 to May 2021, if an extension is required, a fresh application should be done before proceeding with the study.

You will also be required to seek for a research permit from the National Commission for Science, Technology and Innovation (NACOSTI).

Yours sincerely,
FOR: THE NAIROBI HOSPITAL

Dr. Allan Pamba
CHIEF EXECUTIVE OFFICER

C.c. Chairman - Bioethics & Research Committee
Director, Medical Services and Research
Director, Nursing Services

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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

3rd April   2020

Ref: KNH-ERC/A/121

Dr. Rebecca Mbuche Mzungu
Reg. No H58/86981/2016
Dept.of Obstetrics and Gynaecology
School of Medicine
College of Health Sciences
University of Nairobi

Dear Dr. Mzungu

RESEARCH PROPOSAL –sFLT1/PIFG RATIO AS A PREDICTOR OF DEVELOPMENT OF PREECLAMPSIA AND ADVERSE MATERNAL AND PERINATAL OUTCOMES IN WOMEN AT RISK AT A TERTIARY HOSPITAL IN KENYA (P679/11/2019)

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 3rd April 2020 – 2nd April 2021.
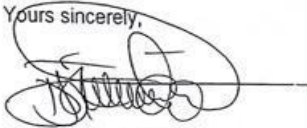
This approval is subject to compliance with the following requirements:

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

xxxiv

For more details consult the KNH- UoN ERC websitehttp://www.erc.uonbi.ac.ke

Yours sincerely,

PROF. M. L. CHINDIA
SECRETARY, KNH-UoN ERC

c.c.   The Principal, College of Health Sciences, UoN
       The Director, CS, KNH
       The Chairperson, KNH- UoN ERC
       The Assistant Director, Health Information, KNH
       The Dean, School of Medicine, UoN
       The Chair, Dept. of Obstetrics and Gynaecology, UoN
       Supervisors: Prof. Zahida Qureshi (UoN), Prof. Omondi Ogutu (UoN),
              Dr. Francis Kiigu, The Nairobi Hospital

Protect to discover