SERUM LEVELS OF ANGIOGENIC AND ANTIANGIOGENIC FACTORS IN GHANAIAN WOMEN DIAGNOSED WITH PRE-ECLAMPSIA AND ITS RELATION TO FETAL OUTCOMES

MASTER OF PHILOSPHY IN PHYSIOLOGY

By

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DECLARATION

I, **LIM ABLA KWAWUKUME**, author of this thesis do hereby declare that, with the exception of references to other people's work which has been duly cited, this work has entirely resulted from my personal original research under the supervision of Rev. Dr. Charles Antwi- Bosiako and Dr. Kwame Adu- Bonsaffoh and has not been presented for any other degree elsewhere.

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DEDICATION

This work is dedicated to my parents, Professor Yao and Dr. Mrs. Susu Kwawukume without whose mentorship and love this pursuit of knowledge would not have come to fruition

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LIST OF ABBREVIATIONS

ALT	Alanine aminotransferase
AST	Aspartate Aminotransferase
EDTA	Ethylene diamine tetracetic acid
dBP	Diastolic blood pressure
GA	Gestational age
MAP	Mean arterial pressure
NICU	Neonatal intensive care unit
PE	Preeclampsia
PLGF	Placental growth factor
sBP	Systolic blood pressure
sFLT-1	Soluble fms like tyrosine kinase receptor 1

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ABSTRACT

Background: Pregnancies complicated by pre-eclampsia are associated with a high rate of maternal and fetal morbidity and mortality. The only effective treatment of pre-eclampsia is premature delivery of the baby and the placenta which can result in neonatal complications. Antiangiogenic and angiogenic factors have been implicated in the pathophysiology of pre-eclampsia resulting in of placental ischemia with subsequent maternal endothelial cell dysfunction and fetal complications.

General Aim- The aim of the study was to determine the association between the serum levels of antiangiogenic factor, sFLT-1, angiogenic factor, PLGF and the fetal outcome in pre-eclampsia.

Methodology- This study was a case control study which included a total of 120 participants, comprised 60 pre-eclamptic women and 60 pregnant women with no complications. A volume of 6mls of blood was drawn from the antecubital fossa using a 10mls syringe from each participant. This was then divided into two 3mls each, one portion was poured into the plain bottle and the other half into an EDTA tube and labelled with indelible ink appropriately. Blood samples were drawn as follows: at presentation, during the active phase of labour and 24 hours after delivery for each participant. The human soluble fms- like tyrosine kinase 1 and placental growth factor ELISA kit were used to measure the antiangiogenic and angiogenic factors respectively. Full blood count blood, urea, electrolytes, creatinine and uric acid, ALT, AST and albumin were measured. Digital weighing scale was used to measure the weight of each baby and the weight of the placenta. The APGAR scores were calculated at 1 and 5 minutes after delivery. Neonatal admission into NICU and neonatal mortality were recorded. Questionnaires on demographics and knowledge of pre-eclampsia were analyzed. Statistical significance was set at a probability of 0.05

Results- The median serum level of sFLT-1 at diagnosis was significantly higher in women diagnosed with pre-eclampsia compared to controls 49622.6pg/ml (IQR:22256.7-122818.6) versus 14842pg/ml (IQR:6289.0-20607.6) p<0.001. Similarly, the median sFLT-1 level during delivery was 50634.6pg/ml(IQR:24171.1-118934.5) and 25046.6pg/ml (IQR:13570.5-40565.4) p=0.002 while the level 24 hours after delivery was 7521.7pg/ml (IQR: 1321.8-41049.5) and 2477.4pg/ml (IQR: 1038.0-7438.2) p= 0.021 in the case and control respectively.

The median serum level of PLGF in women diagnosed with pre-eclampsia was significantly lower than controls,78.7pg/ml (IQR: 46.9-188.7) versus 1016.7pg/ml (IQR:280.7-2177.2), p<0.001. Likewise, the median level of PLGF during delivery was 65.1pg/ml(IQR:41.7-105.3) in cases and 202.6pg/ml(IQR:84.8-418.6) in controls, p<0.001. The median level of PLGF 24 hours after delivery was marginally lower in the case 52.9pg/ml (IQR:20.0-114.3) as compared to the control 58.7pg/ml(IQR:30.75-127.1) p= 0.386.

The increased serum levels of the anti-angiogenic sFLT-1 and low serum level of angiogenic factor, PLGF was associated with NICU admissions, neonatal death and low birth weight. However, mothers who had still birth had a low sFLT-1 (p=0.403) and PLGF levels (p=0.736).

Conclusion- A high serum sFLT-1 and low PLGF levels in women diagnosed with pre-eclampsia is associated with greater NICU admissions, low birth weight and neonatal death. Furthermore, low sFLT-1 levels after high levels in utero regardless of the PLGF level is associated with still birth in women diagnosed with pre-eclampsia. Therefore, neonatal outcomes may improve if there is regular clinical monitoring of sFLT-1 and PLGF levels for women with pre-eclampsia.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Hypertensive disorders of pregnancy (HDP) are principal causes of maternal and neonatal morbidity and mortality in developing countries (Khan et al., 2006)(Ferrazzi et al., 2018)(Jeyabalan, 2013). Pre-eclampsia is a condition in pregnant women after 20 weeks of gestation, identified by an increase in systolic blood pressure of 140mmHg or diastolic pressure of 90mmHg or more followed by proteinuria of more than 300mg/L in urine/24h (Tannetta & Sargent, 2013)(Mol et al., 2016)(Spracklen et al., 2014)(Rodrigues Helmo et al., 2017). It is often also categorized as mild or severe and early (less than 34 weeks) or late (more than 34 weeks) to integrate the severity of the disease and management approach (Jeyabalan, 2013). Pre-eclampsia is one of the few complications whose impact is felt globally. It remains a major clinical challenge as it is known to affect about 2-8% of pregnant women worldwide (Duley, 2009). Some studies have attributed a genetic component to it and its said to have an estimated heredity of about 55% (Gray et al., 2017). It remains a disease of important public health concern because it needs prompt intervention. Whereas other causes of maternal mortalities can have interventions in place to prevent them, there is no effective intervention to prevent pre-eclampsia and the only known cure is delivery of the baby and placenta (Jeyabalan, 2013).

In the United States of America, 4% of pregnancies are affected by pre-eclampsia and it is the second leading cause of maternal mortality(Statement, 2018). In Ghana, it is one of the leading causes of maternal and neonatal mortality (Gumanga et al., 2011)(Adu-Bonsaffoh et al.,

2017)(Adu-Bonsaffoh et al., 2013). A recent study done in 2013 in one of the Ghana's tertiary hospitals put the prevalence of pre-eclampsia at 38% (Adu-Bonsaffoh et al., 2013). If left untreated, pre-eclampsia can lead to eclampsia. Maternal complications include renal failure, hemolysis, elevated liver enzymes, and thrombocytopenia (HELLP syndrome), liver failure, cerebral edema with seizures and ultimately death (Mutter & Karumanchi, 2008)(Afrasiabi et al., 2014) and these complications can be life threatening (Benton et al., 2016). The fetus is also not spared of the many complications of pre-eclampsia such as prematurity, intrauterine growth restriction, low APGAR scores, still birth and low birth weight.

Pre-eclampsia and cardiovascular diseases (CVD) share several risk factors, including insulin resistance, obesity, diabetes, and inflammation (Gloria, 2017)(Roberts et al., 2011). In spite of extensive research into the etiology of pre-eclampsia, the exact cause of the disease still bafflers many researchers and clinicians, as such it has been described as the disease of theories (Ahmed & Ramma, 2015a)(McLaughlin et al., 2015)(Pipkin & Rubin, 1994). Some authors proposed that maternal factors such as smoking may be the causative factor in women with pre-eclampsia (Janakiraman et al., 2009) while some experts are of the opinion that the deficiency of macro element such as calcium and magnesium may be the causative factor in pre-eclampsia (Kobina et al., 2014). This may be because altered calcium homeostasis as shown by increased calcium excretion is associated with high blood pressure (Sandhya Panjeta Gulia, Madhusudan Chaudhury, NaseemNoorunnisa3, C D Balakrishnan4, 2012).

Pre-eclampsia has also been proposed to be due to abnormal placentation causing placental factors to enter maternal circulation and cause endothelial dysfunction. This results in proteinuria and

hypertension which are the key features of the disease (Solomon & Seely, 2004). Angiogenic factors are factors known to aid in angiogenesis and are of extreme importance in normal pregnancy. However, when there is an imbalance between angiogenic factors and anti angiogenic factors, pre-eclampsia may result (Rodrigues Helmo et al., 2017).

The rise in the anti angiogenic factors is specific to pre-eclampsia (Ramma et al., 2012) with high maternal soluble fms-like tyrosine kinase-1 (sFLT-1 also known as sVEGFR) and low placenta growth factor (PLGF) known to play a role in the pathophysiology of pre-eclampsia (Ahmed & Ramma, 2015b). It has been seen that soluble fms- like tyrosine kinase 1 (sFLT-1), a circulating antiangiogenic protein is increased in the placenta of some women with pre-eclampsia while placenta growth factor (PLGF), an angiogenic protein may be decreased (Mol et al., 2016).

Levels of sFLT-1 and PIGF are not only related to the diagnosis of pre-eclampsia but may also predict pre-eclampsia related adverse outcomes within 2 weeks (Rana et al., 2012). Recent work has shown that the placenta of women with pre-eclampsia is abnormally developed and probably relatively hypoxic. Maternal endothelial dysfunction, possibly related to circulating factors elaborated from the abnormal placenta, is a key feature (Mutter & Karumanchi, 2008).

In spite of the major progress made during the millennium development goals era, major challenges remain in reducing maternal and infant mortality. Though globally, maternal mortality rates have declined, the rate in sub Saharan Africa with Ghana not being an exception is unacceptably high. Annual report from Komfo Anokye and Korle Bu Teaching Hospitals show that eclampsia results in the death of about 11 people every month at the Komfo Anokye teaching

hospital (*Eclampsia:Major Cause of Maternal Deaths*, 2014) and is the leading cause of hypertensive related deaths in the Korle Bu Teaching Hospital.

Neonatal outcome when inadequate has many socio cultural effects on the family and society as a whole. New and improved neonatal intensive care unit (NICU) facilities have an effect on improving survival. The global, social and economic burden of pre-eclampsia is immense and still the only effective therapy is delivery of the baby and placenta. Management of the patient with pre-eclampsia depends on the time of presentation (early or late) and on the severity of the disease (severe or mild). Delivering women with mild pre-eclampsia is earmarked at 37 completed weeks while controlling the blood pressure and monitoring the growth of the baby. In those who present with early onset pre-eclampsia (less than 34 weeks), the decision between delivery or other treatment depends on the severity of the pre-eclampsia (and its risks to both mother and neonate) versus the risk of the neonate being born prematurely. Delivery depends on how well the blood pressures are controlled, whether there is impaired renal and liver function tests, fetal distress and the Obstetrician's judgement.

The hypothesis that pre-eclampsia may be caused by an imbalance of circulating angiogenic factors suggests that these changes may precede the onset of clinical disease and may be useful in designing screening and/or diagnostic tests to identify patients at risk for pre-eclampsia. Accurately predicting the occurrence or absence of pre-eclampsia is important as severe presentations may result in the deterioration of both maternal and fetal conditions, leading to iatrogenic preterm birth. Such a test would be invaluable to clinicians who may offer close follow-up and therapeutic interventions early

in the course of the disease. However, whether the imbalance in angiogenic and antiangiogenic factors seen in pre-eclampsia results in the pathophysiology of the disease remains a mystery (15).

This study examined the relationship between sFLT-1and PLGF and the pathogenesis of pre-eclampsia and its relation to fetal outcome in a case control study on blood samples and clinical information from women in the Obstetric and Gynaecological department of the Korle Bu Teaching Hospital, Accra.

1.2 Problem statement

Pre-eclampsia occurs worldwide and it is a major contributor to maternal and perinatal morbidity and mortality. (Adu-Bonsaffoh et al., 2013). Despite knowing this, no effective pharmacological agents exist to treat pre-eclampsia and the only solution is the premature termination of the pregnancy (Ahmed & Ramma, 2015b). The main goal of expectant management of pre-eclampsia is to improve perinatal outcomes by delaying delivery until later in gestation. However, some neonates who are delivered early do not have adverse complications like anticipated whereas those who are delayed and delivered late rather end up with neonatal complications and even death.

The discovery of the role of angiogenesis-related factors, soluble fms-like tyrosine kinase-1 (sFLT-1) and placental growth factor (PLGF), in the underlying pathophysiology of placenta dysfunction resulting in pre-eclampsia has marked an important step for improving its early diagnostic and prognostic assessment (Herraiz et al., 2017). There is the need to investigate if these markers can be used to enable clinicians decide on when to objectively deliver women with pre-eclampsia. A

blood test which is able to detect fetal outcome with better accuracy would provide clinicians with a valuable screening tool that may reduce adverse neonatal outcomes.

1.3 Justification

In Ghana, though some work has been done on proangiogenic factors like VEGF, there is limited data on the contribution of PLGF and sFLT-1 in the pathophysiology of pre-eclampsia (Adu-Bonsaffoh et al., 2017). In addition, there is paucity of data on its association with fetal outcomes. Determining the levels of sFLT-1 and PLGF in women at risk for pre-eclampsia can help decide whom to hospitalize and whom to send home especially in our setting where we have limited bed space available at our tertiary facilities. It also helps to identify pregnancies at high risk for immediate delivery and fetal and maternal outcomes (Stepan et al., 2015).

Increased levels of uric acid have been shown to be a key clinical feature of pre-eclampsia with higher levels correlating with significant maternal and fetal morbidity and mortality. Perhaps performing a simple ELISA test to determine the antiangiogenic and angiogenic factor levels may be superior to serum uric acid in determining the severity of pre-eclampsia. If sFLT-1 and PLGF levels can be used to predict fetal outcome, it may be more beneficial than uric acid as performance of this test requires minimal expertise and less blood sample (Lam et al., 2005). Delivery of women who have pre-eclampsia is done at the discretion of the obstetrician. In so doing, the neonate may be delivered late when the disease is thought to be seemingly under control but suffer the many complications associated with pre-eclampsia. There should be a standard guideline in place to determine when to objectively deliver women with pre-eclampsia to prevent neonatal

complication. Such a guideline is not in existence thus this study sought to bridge this gap in knowledge.

1.4 Null Hypothesis

Elevated levels of sFLT-1 and low levels of PLGF at diagnosis does not predict adverse fetal outcomes

1.5 Aim

The aim of this study was to determine the association between the serum levels of sFLT-1, PLGF and their relationship to fetal outcome in Ghanaian women diagnosed with pre-eclampsia.

1.6 Specific Objectives

The specific objectives of the study were to:

i. Determine maternal serum levels of PLGF and sFLT-1 in women diagnosed with pre-eclampsia at the point of diagnosis, during labour and 24hours after delivery.
ii. Determine maternal levels of PLGF and sFLT-1 in uncomplicated pregnancy at the time of diagnosis, delivery and 24 hours after delivery.
iii. Determine changes that occur in the maternal serum levels of PLGF and sFLT-1 in women diagnosed with pre-eclampsia and uncomplicated pregnancies.
iv. Correlate the maternal serum levels of placenta growth factor and sFLT-1 to birth weight, placental weight, fetal death, NICU admission and APGAR score as a function of fetal outcome in women diagnosed with pre-eclampsia.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Introduction

Hypertensive disorders in pregnancy, specifically pre-eclampsia is a major public health concern for women and infants. Among the hypertensive disorders of pregnancy, pre-eclampsia represents a monumental burden to obstetric and neonatal outcome. A study conducted in Ghana in December 2013 showed that hypertension and its related disorders is the third leading cause of maternal mortality in pregnant women in Ghana (Der et al., 2013) and it contributes significantly to the the maternal mortality ratio (MMR) for Ghana which is quoted as 310 deaths per 100,000 live births (GSS et al., 2017)

Pre-eclampsia is a leading cause of maternal and perinatal morbidity and mortality, with an approximated 50,000–60,000 pre-eclampsia-related deaths per year worldwide (Susilo et al., 2015)(World Health Organization, 2005). In this chapter, we reviewed the definition of pre-eclampsia, the local impact of pre-eclampsia, the epidemiology of risk factors, some complications associated with the disease and specific antiangiogenic and angiogenic factors.

2.2 Definition of pre-eclampsia

The definition of pre-eclampsia has been recently revised to include atypical presentation of pre-eclampsia. According to the International Committee of the International Society for the Study of Hypertension in Pregnancy (ISSHP), pre-eclampsia is characterized by new onset hypertension after 20 weeks of gestation with one or more of the following;

1. Proteinuria of more than 300mg/l per day or urine dipstick of more than 2+ (1g/L).

2. Maternal organ dysfunction such as renal insufficiency, liver involvement, neurologic complications and haematological complications.

3. Utero placental dysfunction (Nakanishi et al., 2017)(Ferrazzi et al., 2018).

Clinically, because women can present with a spectrum of symptoms, pre-eclampsia can be categorized into mild or severe depending on the gravity of hypertension and proteinuria, or on the existence of other signs and symptoms of end-organ injury (Steiner et al., 2013).

It is also further subdivided into early onset disease (<34 weeks) and late onset disease (>34 weeks)

(Vaddamani et al., 2017)(Sonek et al., 2017). This classification is necessary because it has been suggested that the maternal and perinatal mortalities of early onset and late onset preeclampsia are different. Recent literature has stated that women diagnosed with early onset pre-eclampsia have more maternal and perinatal complications.(E. et al., 2018)

Eclampsia is defined as the development of convulsions and/or unexplained coma during pregnancy or postpartum in patients with features of pre-eclampsia (Smith, 1976). It is the worse form of pre-eclampsia and can cause irreparable destruction to the brain, liver and kidneys. When this condition is poorly managed it can lead to life threatening conditions like coma, cerebral damage, liver rupture, renal failure, cerebral haemorrhage, pulmonary oedema, cortical damage, cerebral edema cerebrovascular accident and ultimately untimely maternal death (Khatun et al., 1997).

2.3 Epidemiology of Preeclampsia

The epidemiology of pre- eclampsia spans globally and it is estimated that about 2- 8% of pregnant women worldwide are affected by this condition (Duley, 2009). It is a major public health concern

with its incidence much higher in developing countries than developed countries.(World Health Organization, 2005)

In Africa, Calabar state in Nigeria has an incidence of 1.2% (Kooffreh et al., 2014) while a study done in 2017 in Zimbabwe recorded an incidence of 1.3%(Ngwenya, 2017). The prevalence of preeclampsia is much higher in these developing countries ranging from 1.8% to 16.7%(Bilano et al., 2014) with Ghana recording a high prevalence of 38% in a study done by Adu- Bonsaffoh et al (ADU-BONSAFFOH et al., 2017).

2.4 Risk factors

The risk factors are diverse and no single test predicts risk or prognosis accurately(Tardif et al., 2018). Africans are at a higher risk for pre-eclampsia than Caucasians (Breathett et al., 2014)and this is also true when Africa Americans were compared to European Americans (Nakimuli et al., 2014)

There is a role for genetic predisposition to preeclampsia. Sisters of the affected women who have preeclampsia as well as daughters of women who have preeclampsia have an increased risk of developing the disease (Nakimuli et al., 2014).

Secondary analysis of WHO global survey done in 2014 showed that increased maternal age and low socio- economic factors are greatly associated with pre-eclampsia. The study also showed that significant risk factors for the development of preeclampsia were absence of antenatal care, high body mass index (BMI), nulliparity, chronic hypertension, gestational diabetes, cardiac or renal disease and urinary tract infection (Bilano et al., 2014).

Paternal factors also contribute to the risk of pre- eclampsia as a change in partner has been found to be significantly associated with the development of preeclampsia (Bilano et al., 2014).

2.4 Pathophysiology of pre- eclampsia

Pre-eclampsia has been described as a disease of theories as there are a lot of theories attributing to its cause. Several mechanisms have been described and common among them are the fact that preeclampsia results in increased levels of anti-angiogenic factors, oxidative stress and systemic inflammation.

2.4.1 Abnormal spiral artery remodeling in pre-eclampsia

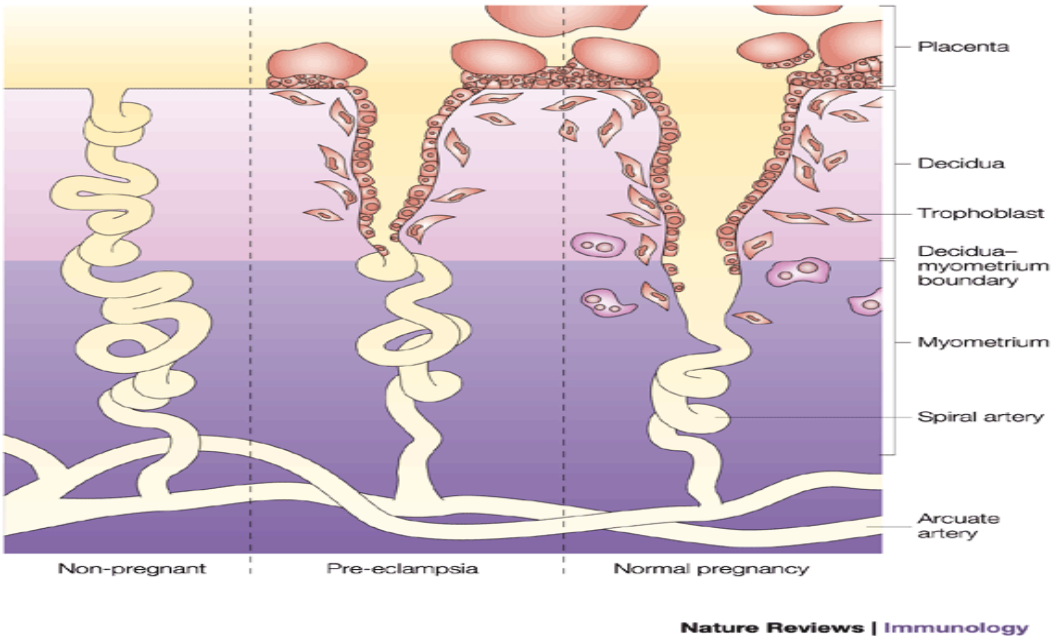

Figure 2. 1: Diagram showing spiral artery invasion in pre-eclampsia compared to normal pregnancy and non-pregnant women

The placenta has a paramount role and it is central in the development of this syndrome, thus alterations in placental functions may contribute to the pathogenesis of pre-eclampsia (Szpilbarg & Damiano, 2017). The proposed theory is that there is inadequacy of endovascular trophoblast invasion together with the absence of remodeling of the spiral arteries and this appear to be responsible for the non- performance of placental perfusion. The resulting ischemic placenta

releases cellular debris and anti- angiogenic factor into the maternal circulation**.** The placenta is known for its essential role in exchanging nutrients, oxygen and waste products between the mother and the developing fetus. In order to execute its role, the placenta must have low resistance vessels which can easily transport the nutrients oxygen and waste. Bonafide placental development involves the processes of vasculogenesis which is the formation of new blood vessels and angiogenesis which is the growth of the already formed blood vessels (Mutter & Karumanchi, 2008). Early in fetal development, the placenta is composed of primary villi which are columnar structures consisting of cytotrophoblast cells surrounded by a layer of syncytiotrophoblast. Mesenchymal cells later invade the villi forming secondary villi and give rise to placental blood vessels. Mesenchymal derived macrophages (Hofbauer cells) express VEGF and other angiogenic factors and are thought to initiate vasculogenesis (11).

However, the fact that preeclampsia stems from abnormal placentation has been disputed by Lyall *et al* who showed that these findings are not only in the disease preeclampsia, but in other conditions such as premature rupture of membrane and intrauterine growth restriction(Bilano et al., 2014).

2.4.2 Elevation in maternal systemic inflammation

Systemic inflammation has been proposed to be the cause of pre-eclampsia and is one of the proposed theories regarding pre-eclampsia. In human pregnancy, the elevation in pro-inflammatory status does not seem to precede the onset of pre-eclampsia (Bilano et al., 2014) and inflammation evidenced by increased IL-6 levels has been seen in normal pregnant women.(Kronborg et al., 2011)

2.4.3 Antiangiogenic and angiogenic factors in pre-eclampsia

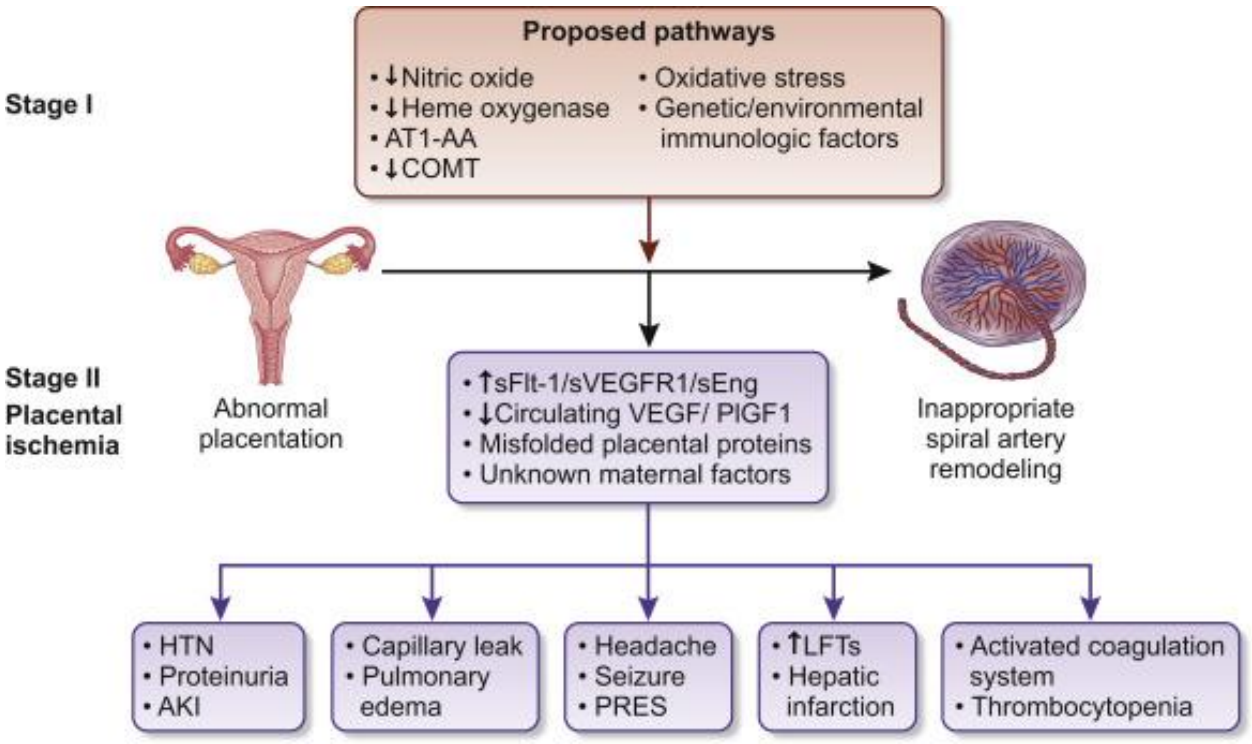

Figure 2. 2: Diagram showing the role of angiogenic and antiangiogenic factors in the pathophysiology of pre-eclampsia

Recent theory in the last decade suggests that the high levels of antiangiogenic factors antagonizes the beneficial effects of the angiogenic factors and result in pre- eclampsia. During normal pregnancy, the concentration of the anti-angiogenic factor sFLT-1 (soluble fms-like tyrosine kinase-1) is low thus the proangiogenic factors, VEGF (vascular endothelial growth factor) and PIGF (placental growth factor) can function reliably. This angiogenic balance is crucial for maintaining physiological anticoagulation and vasodilation of the maternal endothelium. In conditions of stress and low oxygen tension such as seen in pre-eclampsia, the placenta increases its production of sFLT-1 and this antagonizes VEGF and PLGF. The anti-angiogenic factor, sFLT-1 has been shown to be increased before the clinical onset of pre-eclampsia.(&NA;, 2007) This

sFLT-1 is able to form a heterodimer with cell surface VEGF receptors therefore acting as a competitive inhibitor by simply binding vascular endothelial growth factor A [VEGFA] and placental growth factor [PLGF]. This results in an imbalance of the factors therefore causing generalized endothelial dysfunction resulting in the symptoms of pre-eclampsia (Tardif et al., 2018)(Charnock-Jones, 2016).

2.4.4 The role of Vascular endothelial growth factor (VEGF) in pre- eclampsia

Angiogenesis is the formation of new capillaries by the endothelial cells from pre-existing blood vessels. In adults, it is triggered by low oxygen in ischemic or growing tissues, which release factors such as VEGF. The vascular endothelial growth factor-A (VEGF-A) and its receptor-2 (VEGFR-2) trigger most of the mechanisms in activating and regulating angiogenesis. The VEGF family of proteins includes five sub-types in vertebrates; VEGF-A, -B, -C, -D and placental growth factor (PIGF). These factors play a crucial role in the formation and maintenance of blood and lymphatic vasculatures through the activation of specific tyrosine kinases VEGF-receptors (VEGF-R) (Pauty et al., 2017).

VEGF has been implicated in the role of converting cytotrophoblasts from epithelial to endothelial cell type for proper invasion of maternal spiral arteries (Luttun & Carmeliet, 2003a). VEGF has been shown to play a role in decreasing vascular tone and blood pressure by arousing nitric oxide and other vasodilatory prostacylins in endothelial cells (Maynard et al., 2003). In recent antiangiogenic clinical trials, VEGF signaling inhibitors have resulted in hypertension and proteinuria (Maynard et al., 2003). Knockdown of VEGFR-1 in mice also leads to embryonic lethality

2.4.5 The role of Placental growth factor (PLGF) in pre- eclampsia

Placental growth factor is a member of the vascular endothelial growth factor (VEGF) family and is mainly expressed in the placenta, although it is also expressed at low levels in many other tissues, including the heart, lung, thyroid, liver, skeletal muscle and bone. There are four isoforms and of these four, PLGF 1 and 2 are the most abundant. PLGF can bind to VEGFR-1 (vascular endothelial growth factor-1 receptor-1) or FLT-1 (fms-related tyrosine kinase-1) and its soluble variant sFLT-1 (soluble fms-like tyrosine kinase-1). However it cannot bind to VEGFR-2 (vascular endothelial growth factor receptor-2), also known as KDR (kinase insert domain receptor) (Chau et al., 2017). PIGF is especially important for vasculogenesis during the embryonic period (Rana et al., 2012)(Rodrigues Helmo et al., 2017) and it's a key factor for the normal growth of new blood vessels as well as for the proliferation, survival, and fenestration of endothelial cells (Herraiz et al., 2017).

The main role of PLGF in tissues other than the placenta is angiogenesis in response to pathological ischaemia or injury. It was observed that knockout mice ($PLGF^{-/-}$) have impaired angiogenesis and arteriogenesis during pathological conditions such as heart, limb and ocular ischaemia however, physiological exercise induced ischaemia in skeletal and cardiac muscle does not stimulate PLGF production. Accordingly, PLGF expression is upregulated by hypoxia in non-trophoblast cells. In contrast, transcriptional activity of PLGF in trophoblast is suppressed by hypoxia and increased by a normoxic environment pointing to a specific regulatory mechanism and function in these cells. The placenta is known to produce PLGF thus circulating PLGF is prominently elevated in pregnancy. The function of PLGF in the placenta is likely to be in the promotion of development and maturation of the placental vascular system. This is supported by the fact that implantation sites of PLGF knockout mice show abnormal placental vasculature.

Concentrations of PLGF are low in the first trimester of an uncomplicated pregnancy and increases from week 11 to 12 onwards to a peak at week 30, after which it decreases (Chau et al., 2017).

2.4.6 The role of soluble fms like tyrosine kinase receptor (sFLT-1) in pre- eclampsia

Soluble fms like tyrosine kinase receptor (sFLT-1) is produced by syncytiotrophoblasts due to alternate splicing of the RNA messenger of the VEGFR-1 gene. It is similar to the normal VEGF receptor but has a single transmembrane and cytoplasmic membrane. The PLGF/VEGF can bind to this receptor but by so doing it inhibits its action (Rodrigues Helmo et al., 2017). It lacks the cytosolic domain thus its function is restricted to reducing the levels of free VEGF and PLGF available to signal via intact Flt1 and fetal liver kinase-1 (Luttun & Carmeliet, 2003a). It therefore acts as a potent antagonist and induces endothelial cell dysfunction (Masuyama et al., 2007);56). Placental dysfunction triggers an imbalance in the maternal circulation of angiogenesis regulatory factors characterized by elevated concentrations of antiangiogenic factors released by the dysfunctional hypoxic placenta. sFLT-1 is responsible for the maternal dysfunction, causing peripheral vasoconstriction in an attempt to raise maternal blood pressure. This is intended to increase the oxygenated maternal blood flow through the intervillous space, but eventually can lead to pre-eclampsia (Herraiz et al., 2017).

Placental sFLT-1 mRNA is known to be up-regulated in pre-eclampsia, leading to increased systemic levels of sFLT-1 that fall after delivery. Recent studies showed that the average serum level of sFLT-1 was almost five times higher in patients with severe pre-eclampsia than in normotensive pregnant women (Maynard et al., 2003).

Increased sFLT-1 levels reduce circulating VEGF and PLGF below a critical threshold required

for the maintenance of an established vasculature. This causes endothelial dysfunction, which may disrupt the blood-brain barrier and cause intracranial hypertension, result in edema in the liver, and affect glomerular function (Luttun & Carmeliet, 2003b).

2.5 Neonatal outcomes associated with pre-eclampsia

Pre-eclampsia's effect on the child is said to continue to childhood with an increased risk of obesity and blood pressure and these may also continue to adulthood (Herzog et al., 2017). Some common neonatal complications associated with pre-eclampsia are low appearance, pulse, grimace, activity and respiratory score common known as the (APGAR), increased need for neonatal intensive care unit (NICU) admission, intrauterine growth restriction and low birth weight. Most of these complications results from severe forms of pre-eclampsia. Apgar is a quick score that can be used to check the physiological maturity of the infant and is one of the determinant of NICU admission. (Susilo et al., 2015).

It has been shown that the ratio of the antiangiogenic to angiogenic factor (sFLT-1/PLGF) of 85 or greater can be strongly associated with adverse outcomes and suggests impending delivery within 2 weeks of presentation in women presenting with signs and/or symptoms of pre-eclampsia (Rana et al., 2012). A ratio of 38 or less is widely accepted for eliminating pre-eclampsia (Herraiz et al., 2017).

Adverse outcome is difficult to predict in patients who have pre-eclampsia as some people who have pre-eclampsia are able to carry pregnancy to near full term with no complications while others may develop complications like eclampsia without elevation in blood pressure and proteinuria (32).

Measurement of angiogenic and antiangiogenic factors

Angiogenic and antiangiogenic factors can be measured using either serum or serum. Patients with pre-eclampsia have low levels of antiangiogenic factors both in serum and serum. Serum levels are higher than serum probably because the clotting factors in the serum are also capable of releasing angiogenic and antiangogenic factors (Oggè et al., 2010).

CHAPTER THREE

3.0 METHODOLOGY

3.1 Introduction

This was a case control study done in the department of Obstetrics and Gynaecology, Korle-Bu Teaching Hospital. Each woman with pre-eclampsia (Case) was matched to 1 normotensive pregnant woman (Control). A total of 120 subjects were enrolled (60 pre-eclamptic and 60 normotensive pregnant women). The neonatal adverse outcomes were assessed. After data was collected, it was classified, analyzed and tabulated using SPSS version 20.

3.2 Study design

The study design was a case control study done between the months of September 2018 to February 2019 at the Obstetrics and Gynecological department of the Korle- Bu teaching hospital in Accra, Ghana. The patients diagnosed with pre-eclampsia were recruited and followed up to 24 hours after delivery to see the trend of the angiogenic and anti-angiogenic factors and the relationship of these factors to fetal complications.

3.3 Study site

The study was conducted at the Department of Obstetrics & Gynaecology of the Korle-Bu Teaching Hospital (KBTH) in the capital city of Ghana. Korle-Bu Teaching Hospital is currently the third largest Hospital in Africa and the leading national referral centre in Ghana. The hospital is the premier health facility in the country. It has a bed capacity of 2000 and is a training institution for the College of Health Sciences, Nursing school and Medical school of the University of Ghana.

The Obstetrics and Gynaecology department is one of the departments in the Korle Bu Teaching Hospital. It provides 240 beds for Obstetric cases and 114 beds for Gynaecological cases. The department is further divided into five units. A senior consultant heads each unit with other consultants and doctors equally distributed among the units. Each of the five obstetric units has a fixed antenatal clinic day, which is also the duty day for the unit. On each clinic day new patients are booked and old patients are followed up. Early pregnancy cases are managed at the gynaecology clinic and are transferred to the antenatal clinic at twenty weeks of gestation. The department manages an average of 130 patients diagnosed with pre-eclampsia in a month. Averagely, a total number of 3500 patients attend clinic in a month. The pregnant women who report to the clinic routinely have their blood pressure measured after which their urine is tested for glucose and ketones before they attended to by their doctor.

3.4 Study population

The target population was pregnant women who attended the antenatal clinic of the department of Obstetrics and Gynecology of the Korle Bu Teaching Hospital between the months of September 2018 and February 2019.

3.5 Sampling technique

The sampling technique employed was systematic sampling

3.5.1 Selection of subjects

In a study done between January and February 2013, it was reported that the average number of people who had pre-eclampsia and attended the antenatal clinic (ANC) in KBTH was 3 per day (ADU-BONSAFFOH et al., 2017). The maximum number of participants to be recruited in a day

in this study was one. Therefore, the number 3 was divided by 1, thus every 3rd attendant diagnosed with pre-eclampsia was selected to be part of the study until the sample size was obtained. The selection of the first patient was done by cutting 3 identical pieces of paper, and then numbering them 1- 3. They were then folded identically and put in a bowl and thoroughly mixed. A blindfolded assistant picked the first piece of paper with the number 2 written on it. The cases were therefore picked from the 2nd attendant diagnosed with pre-eclampsia and the rest at intervals of 3. For every case selected, a normotensive pregnant woman was gestationally matched and also selected.

3.6 Case definitions

American College of Obstetrician Gynecologists' (ACOG) criteria was used for the diagnosis of pre-eclampsia (Roberts James M, Druzin Maurice, August Phyllis A, Gaiser Robert R, Bakris George, Granger Joey P, Barton John R, Jeyabalan Aurun, Bernstein Ira M, 2013). Accordingly, systolic blood pressure above 140 mmHg and diastolic blood Pressure above 90 mmHg was measured in a sitting position at least twice after a 15 minutes' rest and in at least two consecutive measurements, six hours apart, and proteinuria of 300 mg or more in a 24-hour urine collection or ≥ 1+ dipstick on a random urine sample was considered as pre-eclampsia.

Severe isolated systolic hypertension was defined as systolic blood pressure of 160mmHg or more

Severe isolated diastolic hypertension was defined as diastolic blood pressure of 110mmHg or more

Mean arterial pressure was calculated by the formula 1/3(SBP-DBP) + DBP where SBP is systolic blood pressure and DBP is diastolic blood pressure

Severe both systolic and diastolic blood pressure was defined as a combination of both severe isolated systolic blood pressure and diastolic blood pressure.

Pre-eclampsia was sub-classified as either early onset pre-eclampsia (<34 weeks' gestation) or late onset pre-eclampsia (=> 34 weeks' gestation).

Low birth weight was defined as birth weight less than 2.5kg

The gestational age for the cases were between 22 weeks and 40 weeks while that for the controls were between 27 weeks and 40 weeks.

3.7 Inclusion criteria

The study included pregnant women between the ages of 18 and 45 years. The following were also included

- Normal pregnant women with no complication were gestationally matched to women with pre-eclampsia and recruited from the O&G department of the Korle Bu Teaching Hospital. (control group)
- Pregnant women with pre-eclampsia (cases) as diagnosed by an Obstetrician from the O&G department of the Korle -Bu Teaching Hospital

Where normal means pregnant women with no complications

3.8 Exclusion criteria

- Patients who had a history of chronic hypertension, diabetes mellitus, urinary infection, cardiovascular diseases, thyroid dysfunction, infectious disease, sickle cell disease, neoplastic conditions and any renal disease obtained from history, investigations and medical records were excluded from the study.
- Pregnant women who were on any medical treatment other than iron, folic acid and calcium supplements were also excluded from the study.
- Patients who were unable to give informed consent or unwilling to comply with the requirements of the protocol were excluded from the study.

3.9 Sample size calculation

Estimated minimum sample size of participants included in the study was determined by using a formula from the site **sourceforge.net**;

In this formula, using a 1:1 matched study design,

The minimum odds ratio to detect = 4

Percentage exposed among the controls = 20%

Power = 90%

Probability of exposure- discordant pair =50%

Alpha risk = 5%

The estimated sample size (number of pairs):

Number of exposure- discordant pairs= 25

Number of pairs= 50

Total sample size = 100

Thus the minimum sample size calculated was 100 which was comprised of 60 (sixty) cases and 60 (sixty) controls.

3.10 Methods and materials

3.10.1 Interview

The study population was interviewed by means of well-structured questionnaire (refer to appendix) upon recruitment to determine:

- Demographics
- Knowledge of pre-eclampsia
- Complication of pre-eclampsia

Maternal complications as diagnosed by the obstetrician and laboratory test results were obtained from the medical record book

3.10.2 Neonatal outcomes

APGAR- Appearance, pulse, grimace, activity and respiratory rate is a score used to measure the physical condition of the newborn infant. It is obtained by adding points. Each parameter has a minimum score of 0 and a maximum score of 2 making a total minimum and maximum score of 0 and 10. The score was calculated immediately at 1 and 5 minutes after birth.

Birth weight was measured using a digital scale. Scales were checked daily for reliability.

Placenta weight was measured using a digital scale.

Length of neonate was measured using an infantometer with the baby flat on its back and legs extended and measured from top of the head to the heel of one foot.

Chest circumference of neonate was measured around the chest to the nearest 1 mm at the level of the nipple line.

Head circumference of neonate was measured around the head to the nearest 1 mm using the occipital and frontal regions as marks.

Gestational age was calculated using ultrasound done during the first trimester.

Still birth was recorded as an APGAR score of 0 at one and five minutes.

Neonatal death was defined as any death within the first 28 days of life. It was obtained by telephone calls to the mother on day 29.

3.10.3 Procedures

3.10.3.1 Blood pressure measurement

Participants had their blood pressure measured upon presentation to the clinic to determine if they had pre-eclampsia. The blood pressure was taken using the Omron digital sphygmomanometer. Once the diagnosis was established and the patients consented, they were recruited into the study. The patients were allowed to rest for 10 minutes prior to taking the measurements. The left upper arm was flexed at the elbow and the cuff slided up the arm until the bottom edge was about ½ above the elbow crease and the artery mark directly over the brachial artery. The Start button was then pushed and blood pressure measurements recorded twice, one minute apart and the average blood pressure reading recorded.

3.10.3.2 Collection of Blood samples

A volume of 6mls of blood was drawn from the antecubital fossa using a 10mls syringe for all patients and controls. This was then divided such that 3mls was poured into the ethylene diamine

tetracetic acid (EDTA) vacutainer and the remaining 3mls poured into the serum for gel separator vacutainer and labelled with indelible ink. Rubber tourniquet was applied around the arm above the elbow for less than one minute and the antecubital fossa cleaned with 70% methylated spirit. Phlebotomy was performed and the desired amount of blood drawn. The blood was then stored for haematological and chemistry analysis. A second sample of blood was taken into the EDTA bottle during delivery and a third 24 hours after delivery.

The tubes were mixed gently to avoid hemolysis. Samples which were hemolyzed were rejected. After the blood was taken, it was allowed to clot by leaving it undisturbed at room temperature. The clot was removed by spinning the sample at a centrifugal speed of 1000xg for 10 minutes. The resulting supernatant (serum) obtained was carefully removed using a Pasteur pipette into clean Eppendorf tubes apportioned into 0.5ml aliquots and then packed in cryoboxes. Repeated freeze-thaw cycles was avoided and the serum obtained was stored in the freezer at the department of Physiology at -25 degrees and later transported to the Nogouchi laboratory on dry ice once all the samples were obtained.

3.10.3.3 Measurement of serum sFLT-1 and PLGF levels

The sFLT-1 and PLGF ELISA kits were used strictly following manufacturers protocol. The sandwich ELISA technique was used and both case and control were run together to avoid bias. The samples were measured in duplicate and the mean values of individual samples were used for statistical analysis. Analysis of sFLT-1 and PLGF was performed on the serum after all samples had been obtained using an automated biochemistry analyzer. Maternal serum levels of sFLT-1 and PLGF (with both levels measured in picograms per milliliter) were determined.

Serum was also used for the blood urea and creatinine test, liver function test and uric acid test.

The sodium EDTA anticoagulant blood was used for haematological parameters.

Haematological parameters were measured using auto-haematological analyzer. Measurements included haemoglobin levels, total WBC counts and platelet count. Blood, urea, electrolyte, creatinine, uric acid, AST, ALT and albumin were measured using Hitachi 917 automated analyzer (Roche Diagnostics, Indianapolis, IN). The sodium meta bisulphite method was used to detect sickle cell disease. Strict quality assurance procedures were followed.

3.10.3.4 Urine sample collection and reading

Fifteen (15mls) of mid- stream urine was collected into clean dry plastic containers by the participants and a fresh urine strip was dipped into it up to the test area, for not more than two seconds. Excess urine was removed by draining the strip along the brim of the container making sure the test area does not touch the container. This was done to prevent excess urine on the strip from reacting with chemicals between adjacent pads leading to incorrect results. The test result was read by holding the strip horizontally and comparing it with the colour chart on the strip container under good lighting condition. The urine sample was then discarded after the strip had been read and reported.

3.10.3.5 Dipstick testing for proteinuria

The presence of urine protein was determined using the dip stick qualitative method. The manufacturer's instructions were strictly adhered to. The principle of the assay is based on protein "error of indicators". Anion is the specific pH indicator attracted by a cation on the protein molecule and makes the indicator further ionized, which changes the colour from yellow to blue green.

Dipstick strip was impregnated with buffered tetrabromophenol blue. The strip was dipped in urine and a colour change was read after 1 minute. The colour of the dipstick changed from yellow to various shades of green depending on the amount of protein in the urine and was graded as follows;

- 1+ 0.3 gram per litre
- 2+ 1.0 gram per litre
- 3+ 3.0 gram per litre
- 4+ greater than 10g

3.10.4 Reagents for measuring antiangiogenic and angiogenic factors

Human vascular endothelial growth factor receptor 1 **(**also known as sFLT-1) and human placenta growth factor (PLGF) was purchased from R&D Systems in Minneapolis, Minnesota, USA.

3.10.5 Principle and procedure of measuring the levels of placenta growth factor (PLGF)

The test principle applied in this method was Sandwich enzyme immunoassay. The mouse anti-human capture antibody specific to Placenta Growth Factor (PLGF) provided in the kit was used to coat a NUNC maxisorp 96-well microtiter plate overnight at room temperature. Samples were added to the appropriate microtiter plate wells and were detected with a Biotinylated Goat Anti-Human PIGF detection antibody. Next, streptavidin conjugated to Horseradish Peroxidase (HRP) was added to each microplate well and incubated. After TMB substrate solution was added, only those wells that contained Placenta Growth Factor (PLGF), biotin-conjugated antibody and enzyme-conjugated streptavidin exhibited a change in color. The enzyme-substrate reaction was terminated by the addition of sulphuric acid solution and the color change measured spectrophotometrically at a wavelength of 450nm ± 10nm. The concentration of Placenta Growth

Factor (PLGF) in the sample was then determined by comparing the optimal density of the samples to the standard curve.

The procedure was as follows:

3.10.5.1 Plate preparation

1. The Capture Antibody was diluted to the working concentration in PBS without carrier protein. The 96-well microplate was immediately coated with 100µL per well of the diluted Capture Antibody. The plate was sealed and incubated overnight at room temperature.
2. Each well was aspirated and washed with Wash Buffer. The process was repeated two times for a total of three washes. Each well was washed by filling with Wash Buffer (400µL) using an auto washer. Complete removal of liquid at each step was essential for good performance. After the last wash, the remaining Wash Buffer was removed by aspiration or by inverting the plate and blotting it against clean paper towels.
3. The plates were blocked by adding 300µL of Reagent Diluent to each well and incubated at room temperature for a minimum of 1 hour.
4. The aspiration/wash was repeated as in step 2 and by so doing the plates were ready for sample addition.

3.10.5.2 Assay procedure

1. 100µL of sample or standards in Reagent Diluent, or any appropriate diluent was added per well. This was then covered with an adhesive strip and incubated for 2 hours at room temperature.
2. The aspiration/wash as in step 2 of Plate Preparation was repeated.

3. 100µL of the Detection Antibody diluted in Reagent Diluent was added to each well. This was covered with new adhesive strip and incubated for 2 hours at room temperature.
4. The aspiration/wash as in step 2 of Plate Preparation was repeated.
5. 100µL of the working dilution of Streptavidin-HRP was added to each well. The plates were covered and incubated for 20 minutes at room temperature. The plates were placed in an area avoiding direct light.
6. The aspiration/wash was repeated as in step 2.
7. 100µL of Substrate Solution was added to each well and incubated for 20 minutes at room temperature. The plates were placed in an area avoiding direct light.
8. 50µL of Stop Solution was added to each well. The plates were gently tapped to ensure thorough mixing.
9. The optical density of each well was determined immediately, using a microplate reader set to 450 nm.

3.10.6 Principle and procedure for measuring the vascular endothelial growth factor receptor 1 (VEGF R1/sFLT-1) assay

The test principle applied in this method was Sandwich enzyme immunoassay. The mouse anti-human capture antibody specific to Vascular Endothelial Growth Factor Receptor 1 (VEGF R1) provided in the kit was used to coat a NUNC maxisorp 96-well microtiter plate overnight at room temperature. Samples were added to the appropriate microtiter plate wells and were detected with a Biotinylated Goat Anti-Human VEGF R1 detection antibody. Next, streptavidin conjugated to Horseradish Peroxidase (HRP) was added to each microplate well and incubated. After TMB substrate solution was added, only those wells that contained Vascular Endothelial Growth Factor Receptor 1 (VEGF R1), biotin-conjugated antibody and enzyme-conjugated

streptavidin exhibited a change in color. The enzyme-substrate reaction was terminated by the addition of sulphuric acid solution and the color change measured spectrophotometrically at a wavelength of 450nm ± 10nm. The concentration of Vascular Endothelial Growth Factor Receptor 1 (VEGF R1) in the sample was then determined by comparing the optimal density of the samples to the standard curve.

The human soluble fms-like tyrosine kinase-1 (sFLT-1) ELISA (Enzyme-Linked Immunosorbent Assay) kit was used.

3.10.6.1 Plate preparation

1. The Capture Antibody was diluted to the working concentration in PBS without carrier protein. The 96-well microplate was immediately coated with 100µL per well of the diluted Capture Antibody. The plate was sealed and incubated overnight at room temperature.
2. Each well was aspirated and washed with Wash Buffer, repeating the process two times for a total of three washes. Each well was washed by filling with Wash Buffer (400µL) using an auto washer. The liquid was completely removed at each step for good performance. After the last wash, any remaining Wash Buffer was removed by aspirating or by inverting the plate and blotting it against clean paper towels.
3. The plates were blocked by adding 300µL of Reagent Diluent to each well and incubated at room temperature for a minimum of 1 hour.
4. The aspiration/wash as in step 2 was repeated and the plates, ready for sample addition.

3.10.6.2 Assay procedure

1. 100µL of sample or standards in Reagent Diluent, or any appropriate diluent was added per well. This was then covered with an adhesive strip and incubated for 2 hours at room temperature.
2. The aspiration/wash as in step 2 of Plate Preparation was repeated.
3. 100µL of the Detection Antibody diluted in Reagent Diluent was added to each well. This was covered with new adhesive strip and incubated for 2 hours at room temperature.
4. The aspiration/wash as in step 2 of Plate Preparation was repeated.
5. 100µL of the working dilution of Streptavidin-HRP was added to each well. The plates were covered and incubated for 20 minutes at room temperature. The plates were placed in an area avoiding direct light.
6. The aspiration/wash was repeated as in step 2.
7. 100µL of Substrate Solution was added to each well and incubated for 20 minutes at room temperature. The plates were placed in an area avoiding direct light.
8. 50µL of Stop Solution was added to each well. The plates were gently tapped to ensure thorough mixing.
9. The optical density of each well was determined immediately, using a microplate reader set to 450 nm.

3.10.7 Haematological analysis

The Sysmex 500i Haematological Analyser was used to measure the haematological parameters at a room temperature of 15- 30 degrees. The EDTA anticoagulant whole blood was mixed very well and the manufacturer's instruction for operating the haematology analyzer was strictly adhered to.

3.11 Data management and analysis

Data was entered into an excel spread sheet and analyzed using Statistical package for social sciences version 20 (SPSS). The analysis was done as follows;

3.11.1 Statistical analysis

3.11.1.1 Descriptive statistics

Continuous variables such as age, weight, height, body mass index, systolic blood pressure, diastolic blood pressure, serum sFLT-1, serum PLGF, number of antenatal visit, APGAR score at one and five minute, head circumference, chest circumference and total length of neonate were tested for homogeneity of variances using the Levene's test and for normality using the Shapiro Wilk test before analysis. The results were presented as mean +/- SD. All of the variables were normal except the sFLT-1 levels, the PLGF levels and placenta weight thus these were reported using the median value. The median serum levels of sFLT-1 and PLGF between the normotensive pregnant and pre-eclamptic women were also compared using non-parametric Mann-Whitney U test and results presented using box plot and line graph. The pre-eclamptic group was, further, sub-classified into early- and late-onset pre-eclampsia. The Wilcoxon test was used to compare significance between paired data such as the levels of sFLT-1 before and after delivery. Categorical data such as marital status, highest level of formal education, mode of delivery, cigarette use and knowledge of pre-eclampsia was expressed as proportions.

Graphical displays such as box plots and scatter graphs was used to compare sFLT-1 levels in cases and controls, bar graph was used to display the admission into the neonatal intensive care unit, proportion of cases who had low birth weight and those who had still birth.

Line graph was used to show the changes in sFLT-1 and PLGF levels from the first presentation through delivery to twenty- four hours after delivery. For dichotomous data such as severe systolic blood pressure and severe diastolic blood pressure, frequencies were calculated.

3.11.1.2 Inferential statistics

Hypotheses comparing measures of centrality was evaluated using the independent sample t-test. The paired sample t test was used to compare the levels of sFLT-1 and PLGF before delivery and 24 hours after delivery. The independent sample t test was used to compare means of some parameters such as birth weight, placenta weight, APGAR score at one and five minute and head and chest circumference between the cases and controls. Spearman's rank correlation and linear regression were used to assess association between variables such as birth weight and PLGF levels, placenta weight and PLGF levels, systolic blood pressure and sFLT-1 levels and birth weight and sFLT-1 levels. Scatter graphs showed the correlation between birth weight and PLGF and placenta weight and PLGF. Any probability (*p)* value less than α, where α is 0.05 was regarded as statistically significant.

3.12 Dissemination of results

The outcome of this study was presented to the Department of Physiology of the University of Ghana Medical School at a seminar, as part of the Master of Philosophy programme in Physiology. It was also presented to the Obstetrics and Gynaecology Unit of the Korle-Bu Teaching Hospital and the Noguchi Memorial Institute for Medical Research.

Additionally, the finished paper was presented as a thesis in partial fulfillment for the award of M.Phil. degree in Physiology. Copies of the completed work have been made available at the

Physiology Department of the University of Ghana Medical School, the Balme Library and the School of Graduate Studies of the University of Ghana. This would provide easy access of the materials to other interested researchers.

3.13 Ethical approval

The study has been approved by the Ethical and Protocol review committee of the College of Health Sciences, University of Ghana (protocol identification number: CHS-Et/M. B-P2.14/2017-2018). Informed consent was obtained for all enrolled women. Participants got all their laboratory tests done at no cost to them and this has been kept in their folders for future reference. Data was handled solely by the investigators with no access granted to any unauthorized person for any purpose without permission. Data will be securely kept for up to five years after publication.

3.14 Consent

Signed or thumb-printed informed consent was obtained from participants and their participation in the study was voluntary.

CHAPTER FOUR

4.0 RESULTS

4.1 Introduction

This chapter was grouped into three sub-sections- clinical characteristics, the neonatal outcomes and the antiangiogenic and angiogenic factors. The cases were first compared with the controls. Then, the cases were categorized into early and late onset pre-eclampsia and severe and mild pre-eclampsia and reported as such.

4.2 Clinical Characteristics of Preeclampsia and Controls

The minimum age was 18 years while the maximum age was 43 years for all participants. The mean ages of the cases and controls were (30.00 ± 6.1) years and (31.28 ±4.4) years respectively (p value > 0.05). The mean number of pregnancies including index pregnancy were 3 ± 2 for the case group and similarly 3 ± 2 for the control group. The mean weight and height for the cases were (74.78 ±18.5) kg and (1.62± 0.1) m while that for the control was (77.06 ±1.8) kg and (1.60 ± 0.1) m respectively. Both groups of women were overweight with no significant difference in their weights (p= 0.448) and heights (p=0.099) respectively.

4.3 Demographic Characteristics

The demographic characteristics were similar between the two groups indicating that participants in the two groups were comparably homogeneous. Of the cases, 53 (88.3%) were either cohabiting or formally married while 7 (11.7%) were single. A similar trend was noticed in the control where majority of them were either married or cohabiting (98.3%). The total number of women who had

a previous history of pre-eclampsia were 4 (2 in each group respectively). The mean number of antenatal visits for the cases were 6 ±2 while that for the controls were 5 ± 2. Knowledge on pre-eclampsia was generally low in both groups with a total of 8(6.7%) participants having prior knowledge of pre-eclampsia. Six out of the eight were from the control group. All participants who took part in this study denied smoking.

No maternal mortality was recorded in both groups. Of the sixty cases diagnosed with pre-eclampsia, 15 had maternal complications such as HELLP (haemolysis, elevated liver enzymes and low platelets) syndrome, placenta abruption, intrauterine fetal death and post-partum hemorrhage (PPH). Two women in the control group had PPH. There was a higher prevalence of caesarean section delivery (75%) among the cases while a higher prevalence of vaginal delivery among the control group (61.7%).

4.4 Maternal Clinical Characteristics

4.4.1 Maternal Clinical Characteristics in Preeclampsia and Control

Table 4. 1: Comparing clinical characteristics between pre-eclampsia and control

Variable	**Pre-eclampsia**	**Control**	**p value**
	Mean ± SD (N=60)	**Mean ± SD (N=60)**	
sBP diagnosis (mmHg)	171.3 ±17.4	109 ±11.3	<0.001*

dBP diagnosis (mmHg)	109.2 ±12.4	67.3 ±9.6	<0.001*
GA diagnosis (weeks)	34.7±3.9	33.4±3.7	0.062
MAP diagnosis	129.9 ±12.9	81.2 ±9.3	<0.001*
GA delivery (weeks)	35.0 ± 3.8	38.7 ±1.4	<0.001*

SD: standard deviation; sBP: systolic blood pressure, dBP: diastolic blood pressure, MAP: mean arterial pressure; GA: gestational age. * Significant at $p<0.05$

The mean systolic blood pressures at diagnosis for the case group and the blood pressures at recruitment for the control group were (171.3±17.4) mmHg and (109± 11.3) mmHg respectively. Similarly, the mean diastolic blood pressures at diagnosis for the case group and that for the control group at recruitment were (109.2 ±12.4) mmHg and (67.3 ±9.6) mmHg respectively. As shown on page 40 (table 4.1), there were significant differences between the systolic and diastolic blood pressures in both groups. The mean arterial pressures were (129.9 ±12.9) mmHg and (81.2 ±9.3) mmHg for both cases and controls respectively with a notable significance between the two groups ($p<0.001$)

4.4.2 Maternal Clinical Characteristics in early and late Onset Pre-eclampsia

Table 4. 2: Comparing maternal clinical characteristics in early and late onset pre-eclampsia

Variable	**Early onset PE**	**Late onset PE**	**p value**
	Mean ± SD	**Mean ± SD**	
sBP diagnosis (mmHg)	177.9 ±18.2	168.0 ±16.1	0.037*
dBP diagnosis (mmHg)	113.4 ±11.0	107.1 ±12.7	0.063
MAP (diagnosis)	134.9 ±11.7	127.4 ±12.8	0.032*
GA at diagnosis (weeks)	30.0 ±2.6	37.0 ±1.8	<0.001*
GA at delivery (weeks)	30.5 ±2.7	37.2 ±1.6	<0.001*

SD: standard deviation; sBP: systolic blood pressure, dBP: diastolic blood pressure, MAP: mean arterial pressure; GA: gestational age PE: pre-eclampsia. * Significant at p<0.05

Among the women diagnosed with pre-eclampsia, 20(33.3%) of them were less than 34 weeks' gestation (Early onset pre-eclampsia) while the remaining 40 (66.7%) were diagnosed at 34 weeks

or more (Late onset pre-eclampsia). When these two groups were compared, differences in the mean systolic blood pressures in early and late onset pre-eclampsia were significant (p=0.037). However, the differences in the mean diastolic blood pressures for both groups were not significant (p=0.063). The mean arterial pressures were significantly different between early and late onset pre-eclampsia. (p=0.032).

Women with early onset pre-eclampsia were diagnosed at (30.0 ±2.6) weeks which was lower than those with late onset pre-eclampsia who were diagnosed at (37.0 ±1.8) weeks (p<0.001). Similarly, those with early onset pre-eclampsia were delivered at an earlier age of (30.5 ±2.7) weeks while those with late onset were delivered at a gestational age of (37.2 ±1.6) weeks. (p<0.001). Refer to Table 4.2

4.4.3 Maternal clinical characteristics in severe and mild pre-eclampsia

Table 4. 3: Comparing maternal clinical characteristics in severe and mild pre-eclampsia

Variable	**Severe PE**	**Mild PE**	**p value**
	Mean ± SD	**Mean ± SD**	
sBP diagnosis (mmHg)	184.7 ±12.0	158.0 ±10.0	<0.001*
dBP diagnosis (mmHg)	118.1 ±8.0	100.3 ±9.2	<0.001*

MAP (diagnosis)	140.3± 7.6	119.5± 7.5	<0.001*
GA at diagnosis (weeks)	33.7±4.3	35.6±3.3	0.066
GA at delivery (weeks)	34.0± 4.1	35.9± 3.1	0.050*

SD: standard deviation; sBP: systolic blood pressure, dBP: diastolic blood pressure, MAP: mean arterial pressure; GA: gestational age. * Significant at p<0.05

Table 4.3 shows that there were significant differences between systolic blood pressure at diagnosis (184.7 ±12.0mmHg) in women with severe pre-eclampsia when compared to women with mild pre-eclampsia (158.0 ±10.0mmHg; p<0.001). Similarly, there were significant differences in the diastolic blood pressures between those with severe pre-eclampsia were (118.1 ±8.0) mmHg and (100.3 ±9.2) mmHg (p<0.001) respectively. The mean arterial pressures at diagnosis were (140.3±7.6) mmHg and (119.5± 7.5) mmHg in those with severe and mild pre-eclampsia respectively.

Those with severe pre-eclampsia were delivered at a gestational age of (34.0± 4.1) weeks in contrast to (35.9 ±3.1) weeks in those who had mild pre-eclampsia (p=0.051).

4.5 Haematological and biochemical parameters in pre-eclampsia

4.5.1 Haematological parameters in women diagnosed with early and late onset pre-eclampsia

Table 4. 4: Comparing haematological parameters in early and late onset pre-eclampsia

Variable	**Early onset PE**	**Late onset PE**	**p value**
	Mean ± SD	**Mean ± SD**	
WBC	11.1 ±3.1	10.0±3.1	0.202
Hb (g/dl)	11.1±1.9	10.2±1.8	0.069
Plt	256.4±92.5	267.7±83.6	0.636
Bedside clotting(min)	5.0±1.1	4.7±1.0	0.317

WBC- white blood cell, Hb- haemoglobin level, Plt- platelet level

The haematological parameters were compared between early and late onset pre-eclampsia. There was no significant difference in white blood cell (WBC) count between early and late onset pre-eclampsia. The haemoglobin levels were higher in those diagnosed with early and late onset pre-eclampsia respectively with a strong trend towards significance (11.1±1.9g/dl and 10.2±1.8 g/dl; p=0.069). By contrast, platelet count was lower in those with early onset pre-eclampsia with no statistical significance (p= 0.636).

4.5.2 Haematological parameters in women diagnosed with severe and mild pre-eclampsia

Table 4. 5: Comparing haematological parameters in severe and mild pre-eclampsia

Variable	**Severe PE**	**Mild PE**	**p value**
	Mean ± SD	**Mean ±SD**	
WBC	10.3 ±3.1	10.5 ±3.2	0.790
HB (g/dl)	10.8 ±1.9	10.2 ±1.9	0.192
Plt	243.9 ±80.2	284.0 ±88.4	0.071
Bedside clotting time	4.8 ±0.8	4.7 ±1.2	0.621

WBC- white blood cell, Hb- haemoglobin level, Plt- platelet level

Haematological parameters were compared between severe and mild pre-eclampsia. With the exception of platelet count which was lower in severe than mild pre-eclampsia (243.9 ±80.2 versus 284.0 ±88.4; p= 0.071), all other parameters did not show any statistical difference (p>0.05).

4.6 Biochemical parameters

4.6.1 Biochemical parameters in early and late onset pre-eclampsia

Table 4. 6: Comparing biochemical parameters in early and late onset pre-eclampsia

Variable	**Early onset PE**	**Late onset PE**	**p value**
	Mean ± SD	**Mean ± SD**	
Sodium (mmol/l)	130±8.2	134.7±7.9	0.036*
Potassium(mmol/l)	3.6±0.7	3.5±0.6	0.420
Chloride(mmol/l)	102.9±3.2	103.9±3.5	0.291
Urea(mmol/l)	2.7±1.3	2.6±1.3	0.650
Creatinine(umol/l)	60.4±34.0	54.2±17.7	0.487
Uric acid	288.2±108.5	293.6±55.9	0.800
AST(U/l)	57.3±75.4	45.7±51.4	0.492
ALT(U/l)	45.5±55.6	51.0±67.1	0.755
Albumin(g/l)	33.2±5.0	32.5±5.8	0.650

AST- aspartate transaminase, ALT- alanine transaminase, * Significant at $p<0.05$

The sodium levels were lower in early onset pre-eclampsia than late onset pre-eclampsia (130±8.2mmol/L and 134.7±7.9mmol/L; p=0.036). There were no statistical differences in potassium levels, chloride levels, urea levels, creatinine levels, uric acid levels, aspartate aminotransferase (AST) levels, alanine aminotransferase (ALT) levels and albumin levels between women who developed early onset pre-eclampsia and those who developed late onset pre-eclampsia.

4.6.2 Biochemical parameters in women diagnosed with severe and mild pre-eclampsia

Table 4. 7: Comparing biochemical parameters in severe and mild pre-eclampsia

Variable	**Severe PE**	**Mild PE**	**p value**
	Mean ± SD	**Mean ±SD**	
Sodium(mmol/l)	132.2 ±7.5	134.1 ±9.0	0.387
Potassium (mmol/l)	3.5 ±0.6	3.6 ±0.7	0.810
Chloride (mmol/l)	103.4 ±3.4	103.7 ±3.5	0.730
Urea (mmol/L)	2.7 ±1.4	2.6 ±1.2	0.781
Creatinine	61.0 ±26.8	51.3 ±19.7	0.130
Uric acid	277.8 ±93.4	306.2 ±52.7	0.159
AST (u/l)	40.7 ±59.8	59.3 ±60.4	0.243
ALT(u/l)	30.6 ±34.6	68.9 ±79.2	0.024*
Albumin (g/dL)	32.4 ±5.4	33.1 ±5.7	0.621

AST- aspartate transaminase, Alt- alanine transaminase * Significant at $p<0.05$

The biochemical markers were compared between severe and mild pre-eclampsia as shown on table 4.7. There was no statistical difference seen among the two groups except with regards to the Alanine transaminase where it was 30.6 ±34.6U/L and 68.9 ±79.2U/L in the severe and mild groups respectively.

4.7 Neonatal outcomes

4.7.1 Neonatal outcomes in women diagnosed with pre-eclampsia and control

Table 4. 8: Comparing neonatal outcomes between pre-eclampsia and control

Variable	**Pre-eclampsia Mean ± SD (N=60)**	**Control Mean ± SD (N=60)**	**p value**
Birth weight (kg)	2.3 ±0.9	3.3 ±0.5	<0.001*
APGAR at 1 minute	6.1 ±2.2	7.3 ±1.2	<0.001*
APGAR at 5 minutes	7.2 ±2.4	8.6 ±0.8	<0.001*
Placenta weight (g)	315.5 ±55.6	455.9 ±72.1	<0.001*

Head circumference (cm)	31.9 ±3.4	35.0 ±1.9	<0.001*
Chest circumference (cm)	29.3 ±4.0	34.0 ±2.0	<0.001*
Total length (cm)	46.2 ±6.1	51.0 ±3.0	<0.001*

SD: standard deviation; APGAR: appearance, pulse, grimace, activity; respiratory score; * Significant at p<0.05

The average birth weight for the case was (2.31 ±0.86) kg as opposed to (3.30± 0.48) kg for the control (p=0.000). The APGAR scores at one and five minutes were generally lower in the cases group at 6.1±2.2 and 7.2± 2.4 respectively with a strong statistical significance (p<0.000) Refer to Table 4.8. The APGAR scores at one and five minutes were 7.3 ±1.2 and 8.6 ±0.8 respectively in the control groups. The mean placenta weight in the case was (315.50± 55.58) g while that in the control was (455.83 ± 72.05) g; p<0.001). The mean head circumference, total length and chest value in the cases group were recorded at (31.85 ±3.41) cm, (46.20 ± 6.05) cm and (29.30 ± 3.95) cm respectively. That in the control group were recorded as (34.92 ± 1.89) cm, (50.61 ± 2.95) cm and (34.03 ± 2.02) cm respectively and there was statistical significance (p<0.001) for all parameters between the two groups.

4.7.2 Neonatal outcomes in early and late onset pre-eclampsia

Table 4. 9: Comparing neonatal outcomes in early and late onset pre-eclampsia

Variable	**Early onset PE**	**Late onset PE**	**p value**
	Mean ± SD	**Mean ± SD**	
Birth weight (kg)	1.5±0.6	2.7±0.6	<0.001*
APGAR at 1 min	4.8±2.8	6.7±1.5	0.011*
APGAR at 5 minutes	5.8±3.2	7.8±1.5	0.001*
Placenta weight (g)	270.0±56.0	326.9±51.0	0.065*
Head circumference (cm)	28.5±4.2	33.1±1.9	0.001*
Chest circumference (cm)	25.7±4.6	30.7±2.6	<0.001*
Total length (cm)	39.4±6.7	48.8±3.0	<0.001*

SD: standard deviation; APGAR: appearance, pulse, grimace, activity; respiratory score; PE: pre-eclampsia, *Significant at p<0.05

Mothers who had early onset pre-eclampsia had neonates with a birth weight of (1.5±0.6) kg as compared to (2.7±0.6) kg in those with late onset pre-eclampsia ($p<0.001$) as shown in Table 4.9 When the APGAR scores were compared between early and late onset pre-eclampsia, the scores at one minute were 4.8±2.8 and 6.7±1.5 ($p=0.011$) respectively while the scores after five minutes were 5.8±3.2 and 7.8±1.5 ($p=0.001$) respectively. Women diagnosed with early onset pre-eclampsia had smaller placentas (270.0±56.0) g than those who had late onset pre-eclampsia (326.9±51.0) g with a barely detectable statistical difference ($p=0.065$).

These anthropometric measurements were compared between the two groups. Those with early onset pre-eclampsia had a head circumference of (28.5±4.2) cm while those with late onset recorded a value of (33.1±1.9) cm ($p=0.001$). The chest circumference of the neonate was (25.7±4.6) cm and (30.7±2.6) cm respectively ($p=<0.001$) while the total length of the neonate was of (39.4±6.7) cm and (48.8± 3.0) cm respectively ($p<0.001$) in those with early and late onset pre-eclampsia respectively.

4.7.3 Neonatal outcomes in women diagnosed with severe and mild pre-eclampsia

Table 4. 10: Comparing neonatal outcomes in women diagnosed with severe and mild pre-eclampsia

Variable	**Severe PE**	**Mild PE**	**p value**
	Mean ± SD	**Mean ± SD**	
Birth weight (kg)	2.1 ±0.9	2.5 ±0.8	0.127
APGAR at 1 minute	6.5 ±1.6	5.8 ±2.6	0.256
APGAR at 5 minutes	7.6 ±1.7	6.7 ±2.8	0.184
Placenta weight (g)	314 ± 70.0	317±41.0	0.908
Head circumference (cm)	31.7±3.4	32.0±3.4	0.694
Chest circumference (cm)	28.9±4.3	29.7±3.6	0.453
Total length (cm)	45.3±6.6	47.1±5.4	0.295

SD: standard deviation; APGAR: appearance, pulse, grimace, activity; respiratory score;

Table 4.10 shows that those with severe pre-eclampsia had neonates who recorded a birth weight of (2.1 ±0.9) kg while those who had mild pre-eclampsia had neonates with an average birth weight of (2.5 ±0.8) kg with a significance of 0.120.

Women with severe pre-eclampsia had neonates who had APGAR scores of 6.5± 1.6 at one minute and 5.8± 2.6 at five minutes (p=0.2556) while those with mild pre-eclampsia had neonates who scored an APGAR of 7.6± 1.7 and 6.7± 2.8 (p=0.184) at one and five minutes respectively. The placenta weight was (314 ± 70.0) g in those who had severe pre-eclampsia and (317±41.0) g; p=0.908 with mild pre-eclampsia. The head circumference of the neonates born to mothers who had severe and mild pre-eclampsia were (31.7±3.4) cm and (32.0±3.4) cm (p=0.694) respectively. The chest circumference of neonates whose mothers had severe and mild pre-eclampsia were (28.9±4.3) cm and (29.7±3.6) cm respectively (p= 0.453) and the total length, (45.3±6.6) cm and (47.1±5.4) cm (p=0.295) respectively.

4.8 Serum levels of Angiogenic and Antiangiogenic factors

4.8.1 Median serum sFLT-1 levels before and after 24 hours of delivery in both case and control

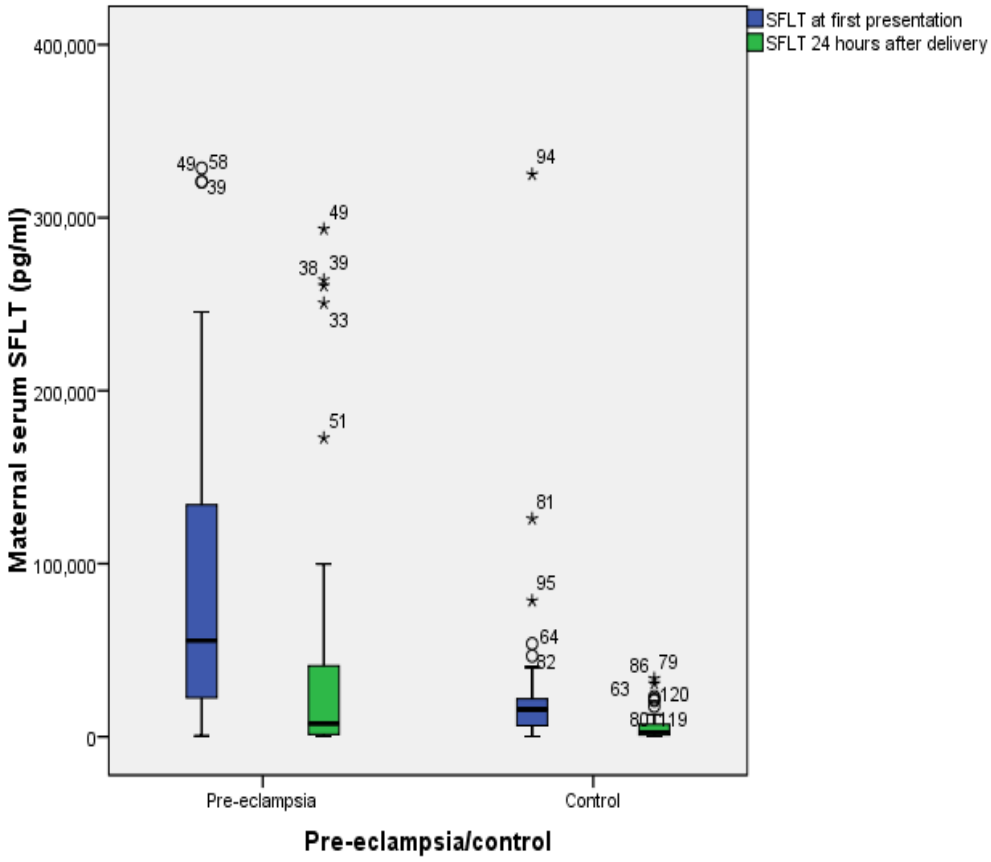

Figure 4. 1: Maternal median serum levels of sFLT-1 at diagnosis and 24 hours after delivery in women diagnosed with pre-eclampsia and controls

The above figure 4.3 shows a box plot representing the level of the serum sFLT-1 before and after delivery in both case and control groups. In the illustration, 133534.2pg/ml, 21556.6pg/ml

and 22870.0pg/ml, 6964.0pg/ml represent the 75th and 25th percentiles respectively in the case and control group.

The median level of sFLT-1 in women diagnosed with pre-eclampsia [49622.6pg/ml IQR: 22256.7- 122818.6)] was significantly higher than controls [14842.5pg/ml, IQR: 6289.0-20607.6)] $p<0.001$. Similarly, the sFLT-1 levels during delivery and 24 hours after delivery was significantly higher in the cases than control group. ($p<0.002$, $=0.021$). The median level of sFLT-1 in women before delivery [54109.3pg/ml, IQR:22870.0-133534.2)] was significantly higher than controls [15197.7pg/ml, (IQR:6964.0-21556.6)] $p<0.001$.

Among both case and control, there was a significant fall in the sFLT-1 levels when compared at first presentation and 24 hours after delivery, $p<0.001$

4.8.2 Median serum PLGF levels before and after 24 hours of delivery in both case and control

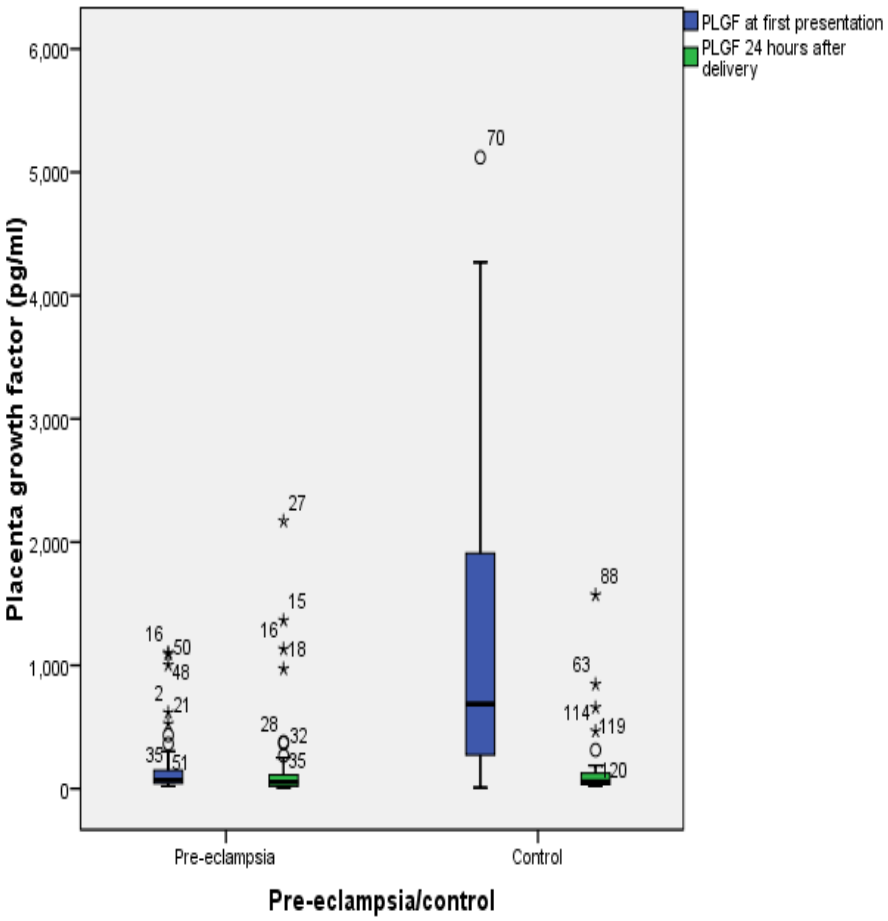

Figure 4. 2: Maternal median serum levels of PLGF at diagnosis and 24 hours after delivery in women diagnosed with pre-eclampsia and controls

The above box plot shown in figure 4.4 represents the level of PLGF before and after delivery in both cases and controls. The median serum level of PLGF at diagnosis and during delivery in the case group was [78.7pg/ml, IQR:46.9-188.7)] and [65.1pg/ml, IQR:41.7-105.3)] respectively while the level in the control group was [1016.7pg/ml, IQR:280.7-2177.2)] and [202.6pg/ml, IQR:

84.8-418.6)] with a clear strong statistical significance, p<0.001. The median PLGF level in the women diagnosed with pre-eclampsia before delivery [68.7pg/ml; IQR:44.5-161.0)] was significantly lower than in the control group [715.7pg/ml (IQR:280.7-1933.3)], p<0.001. However, the PLGF level in the case group 24 hours after delivery was [52.9pg/ml (IQR: 20.0-114.3)] and this was not statistically different from the control group [59.7pg/ml (IQR: 30.8-127.1)] p=0.386

There was a significant fall in the PLGF levels before and after delivery in both case and control p<0.001, =0.041

4.8. 3 The relationship between sFLT-1 and PLGF levels in case and control

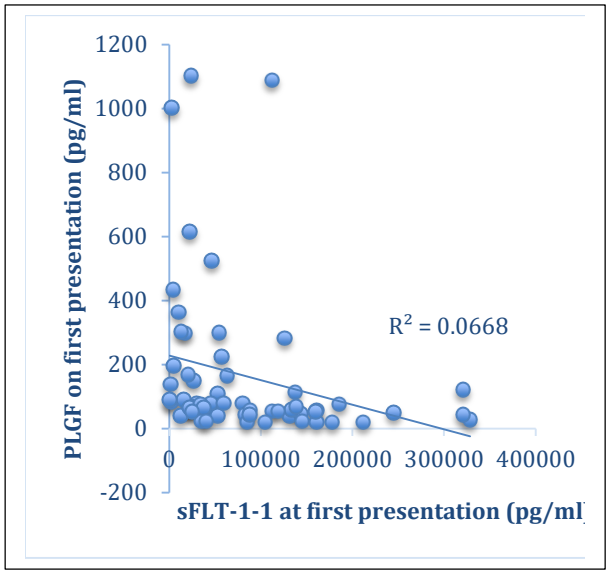

Figure 4. 4: Correlation between maternal serum levels of PLGF and sFLT-1 at first presentation in women with uncomplicated pregnancies

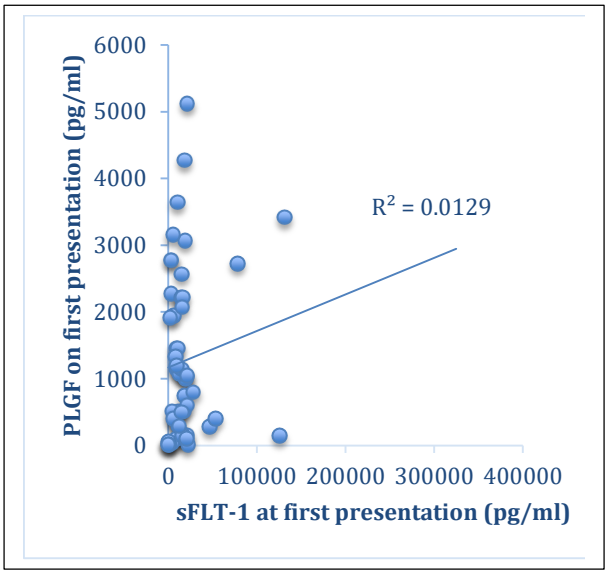

Figure 4. 3: Correlation between maternal serum levels of PLGF and sFLT-1 at first presentation in women diagnosed with pre-eclampsia

As illustrated above, a negative correlation ($R^2 = 0.0668$) was seen between PLGF and sFLT-1 in the women diagnosed with pre-eclampsia while a positive correlation ($R^2 = 0.0129$) was seen in the control group.

4.8.4 Maternal serum levels of sFLT-1 and PLGF in women diagnosed with early and late onset pre-eclampsia

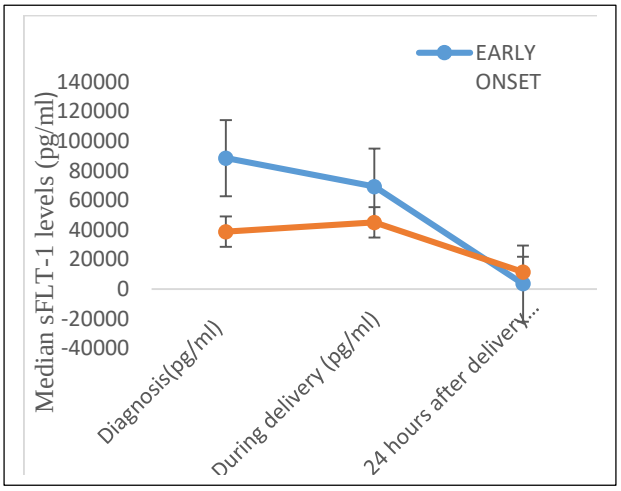

Figure 4. 6: Figure showing the median sFLT-1 levels during pregnancy in early and late onset pre-eclampsia in both case

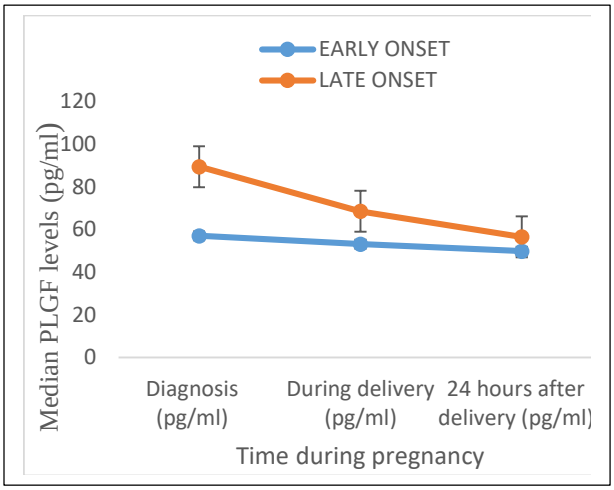

Figure 4. 5: Figure showing the median PLGF levels during pregnancy in early and late onset pre-eclampsia in both case and control

When the median serum sFLT-1 level at diagnosis was compared between early and late onset pre-eclampsia, the level was significantly higher in the early onset [88490.9pg/ml, IQR:29873.9-160392.4)] than the late onset group [(38731.4pg/ml, IQR:17570.4-82962)]; p=0.028pg/ml. The sFLT-1 levels in early and late onset preeclampsia during delivery and 24 hours after delivery were not statistically different; p=0.665 and p=0.518 respectively.

The median serum PLGF level at diagnosis in early and late onset pre-eclampsia were [57.0pg/ml (IQR:42.4-100.3)] and [89.4pg/ml (IQR:58.5-298.1)] respectively though this level did not reach statistical significance p=0.059. The median serum PLGF level during delivery was [53.1pg/ml

(IQR: 28.9-97.1)] in early as compared to late onset pre-eclampsia [68.5pg/ml; (IQR: 52.9- 114.7)] p=0.208. The serum PLGF level 24 hours after delivery was marginally lower in the early (49.8pg/ml (IQR: 20.0-127) than late onset group (56.5pg/ml (IQR: 20.0-111.3) p=0.617.

4.8.5 Serum levels of maternal sFLT-1 and PLGF levels in women diagnosed with severe and mild pre-eclampsia

Table 4. 11: Comparing median serum levels of maternal sFLT-1 and PLGF in women diagnosed with severe and mild pre-eclampsia

Variable	**Severe PE**	**Mild PE**	**p**
	Median	**Median**	**value**
sFLT-1 at diagnosis (pg/ml)	63308.4 (24373.3-131976.1)	45359.2 (IQR:21565.8-119377.1)	0.392
sFLT-1 at delivery (pg/ml)	75317.4(30185.4-163447.9)	35862.9(IQR:12599.0-90683.7)	0.024*
sFLT-1 24 hours after	6373.4 (IQR:1660.0-41471.9)	9940.8 (IQR:1260.9-38371.3)	0.738

delivery (pg/ml)			
PLGF at diagnosis (pg/ml)	68.9 (IQR: 41.0-165.1)	82.1(IQR:58.2-290.6)	0.208
PLGF at delivery (pg/ml)	61.6 (IQR:22.7-101.0)	72.0 (IQR:54.0-118.5)	0.088
PLGF 24 hours after delivery (pg/ml)	38.7 (IQR:20.0-109.5)	58.2 (IQR: 28.4-118.2	0.236

PLGF: placenta growth factor; sFLT-1: soluble fms like tyrosine kinase 1, * Significant at $p<0.05$

Median level of sFLT-1 at diagnosis and during delivery in those who had severe and mild pre-eclampsia was [63308.4pg/ml (IQR:24373.3-131976.1)], [75317.4pg/ml (30185.4-163447.9)] and [45359.2pg/ml (IQR:21565.8-119377.1)], [35862.9pg/ml (IQR:12599.0-90683.7)] respectively p=0.392, 0.024. The median sFLT-1 level 24 hours after delivery was [6373.4pg/ml (IQR:1660.0-41471.9)] and [9940.8pg/ml (IQR:1260.9-38371.3)]; p=0.738 in the severe and mild pre-eclampsia group respectively. The median level of PLGF at diagnosis and during delivery in women who had severe pre-eclampsia and mild pre-eclampsia was [68.9pg/ml (IQR: 41.0-165.1)], [61.6pg/ml (IQR:22.7-101.0)] and [82.1pg/ml, (IQR:58.2-290.6)] [72.0pg/ml (IQR:54.0-118.5)] respectively p=0.208, 0.088. However, the median serum PLGF level 24 hours after delivery in

the women diagnosed with severe and mild pre-eclampsia was [38.7pg/ml (IQR:20.0-109.5)] and [58.2pg/ml (IQR: 28.4-118.2; p=0.236)] respectively.

4.9 Neonatal outcomes

4.9.1 Serum sFLT-1 levels and PLGF in women diagnosed with pre-eclampsia and their neonatal outcomes

Table 4. 12: Maternal birth outcomes and their relationship with angiogenic and antiangiogenic markers

Variables	**Median sFLT-1**	**P value**	**Median PLGF**	**P value**
NICU admission		0.015*		0.004*
Yes	104693.8 (IQR:48447.3-161205.0)		51.7 (IQR: 34.5-79.3)	
No	38731.4(IQR:22256.7-112389.4)		79.1(IQR:57.0-298.1)	
Still birth		0.403		0.736
Yes	17570.4(IQR:8318.0-145258.0)		54.4(IQR: 30.3-366.0)	
No	54808.8 (IQR:24373-134053.5)		68.9 (IQR: 45.3-148.7)	
Early neonatal death		0.182		0.061
Yes	159694.6 (IQR: 131976-178021.8)		45.3(IQR:27.9-58.2)	
No	46331.4 (IQR:22388.5-112441.8)		75.7 (IQR:50.9-166.8)	
Low birth weight		0.104		0.032*
Yes	87838.0 (IQR:22520.3-159694.6)		55.2 (IQR:41.0-89.4)	
No	40578.5(IQR:23087.9-100440.2)		79.1(IQR:57.6-79.1)	

Significant at p≤0.05; SD –standard deviation; * Significant at p<0.05

The median sFLT-1 and PLGF levels in women who had their neonates admitted to NICU were 104693.8pg/ml (IQR:48447.3-161205.0) and 51.7pg/ml (IQR: 34.5-79.3) while the levels in women whose neonates were not admitted into the NICU were 38731.4pg/ml (IQR:22256.7-112389.4) and 79.1pg/ml (IQR:57.0-298.1) respectively (p=0.015, p=0.004). The median maternal serum levels of sFLT-1 in those who had still births were 17570.4pg/ml (IQR:8318.0-145258.0) and the levels in those who did not have still birth were 54808.8pg/ml (IQR:24373-134053.5); p=0.403 while the PLGF levels were 54.4pg/ml (IQR: 30.3-366.0) and 68.9pg/ml (IQR: 45.3-148.7) respectively in those who had still births and in those who did not have still births. (p= 0.736)

The median sFLT-1 levels in mothers who lost their babies were 159694.6pg/ml (IQR: 131976-178021.8) while the levels in those mothers who did not suffer neonatal death were 46331.4pg/ml (IQR:22388.5-112441.8), p= 0.182. The median serum PLGF levels in those who lost their babies were 45.3pg/ml (IQR:27.9-58.2) as compared to 75.7pg/ml (IQR:50.9-166.8) (p =0.061). The median sFLT-1 levels in women who had neonates with low birth weights versus those who did not have low birth weight were 87838.0pg/ml (IQR:22520.3-159694.6) and 40578.5pg/ml (IQR:23087.9-100440.2) respectively. The median PLGF levels in women who had neonates with low birth weight were 55.2pg/ml (IQR:23087.9-100440.2) while the levels were 79.1pg/ml (IQR:57.6-79.1) in those who did not have low birth weight (p=0.032).

4.9.2 Low birth weight in women with pre-eclampsia

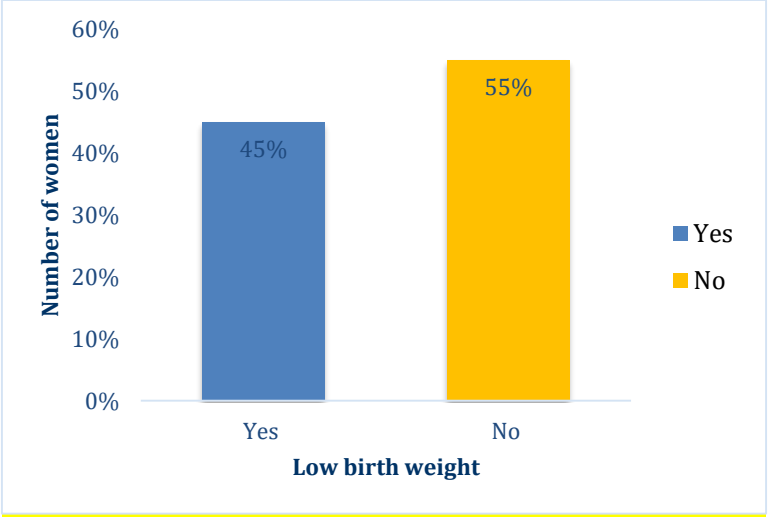

Figure 4. 7: Number of women whose neonates were classified as low birth weight versus normal birth weights

The figure 4.7 shows that among the women diagnosed with pre-eclampsia, 27 (45%) of them had neonates who had low birth weight.

4.9.3 The relationship between birth weight and serum levels of sFLT-1 and PLGF

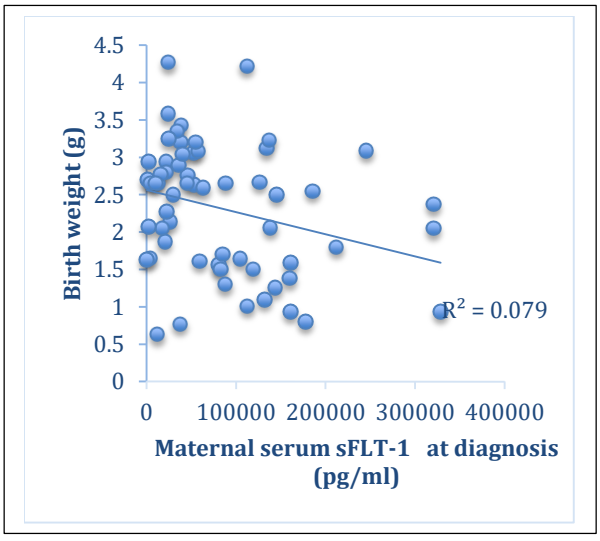

Figure 4. 8: Correlation between birth weight and maternal serum sFLT-1 in women diagnosed with pre-eclampsia

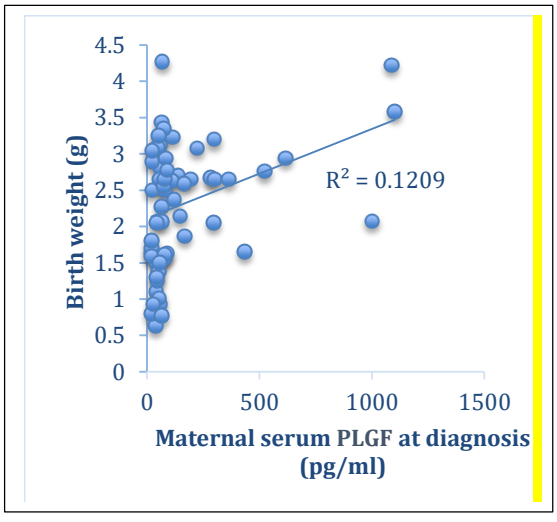

Figure 4. 9: Correlation between birth weight and maternal serum PLGF in women diagnosed with pre-eclampsia

As shown in Figure 4.8, there is an inverse relationship between birth weight and sFLT-1 at diagnosis (R=0.08) with a significance of p=0.029. The opposite trend was seen in Figure 4. 9 which shows that as the birth weight increases, PLGF levels increase. This showed a positive correlation (R=0.35) with a significance of 0.006. The PLGF explained 12.1% of the variation in birthweight.

4.9.4 NICU admissions in women with pre-eclampsia

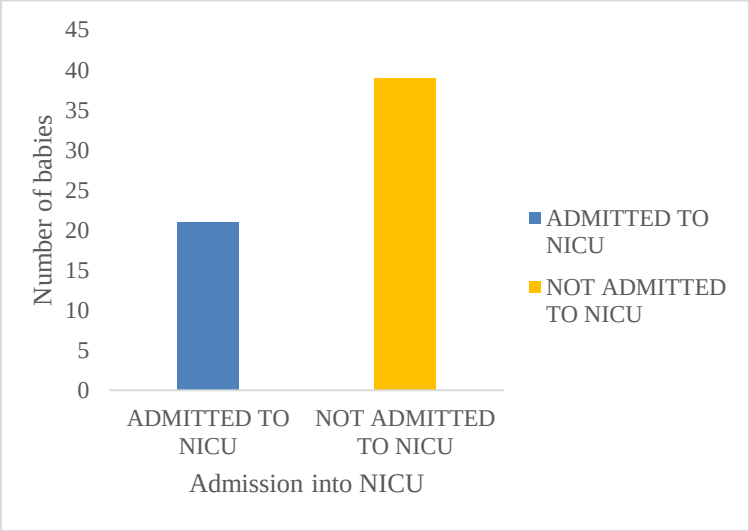

Figure 4. 10: Number of neonates admitted to NICU compared to those not admitted to NICU in women diagnosed with pre-eclampsia

None of the women in the control group had their neonates admitted into the neonatal intensive care unit. However, regarding the sixty deliveries done in the cases group, 21 of neonates were admitted into the neonatal intensive care unit. (Figure 4.10)

4.9.5 Neonatal death in women with pre-eclampsia

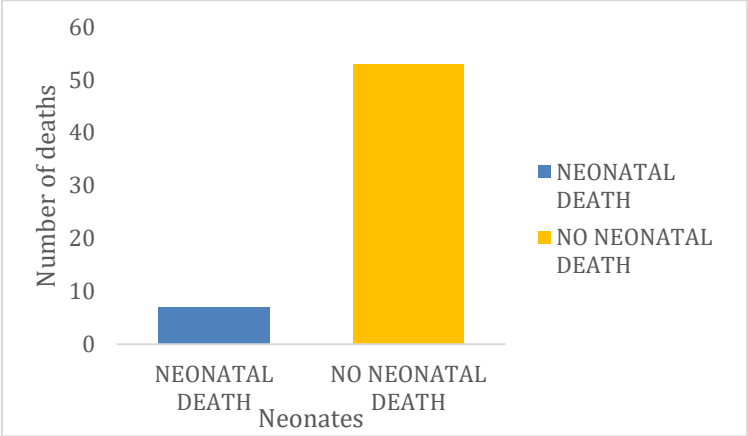

Figure 4. 11: Neonatal death versus no neonatal death in women diagnosed with pre-eclampsia

No neonatal death was recorded in the control group. However, among the women who had pre-eclampsia, 7 (11.7%) of them recorded early neonatal deaths as illustrated in Figure 4.11.

4.9.6 The relationship between placenta weight and serum PLGF and sFLT-1

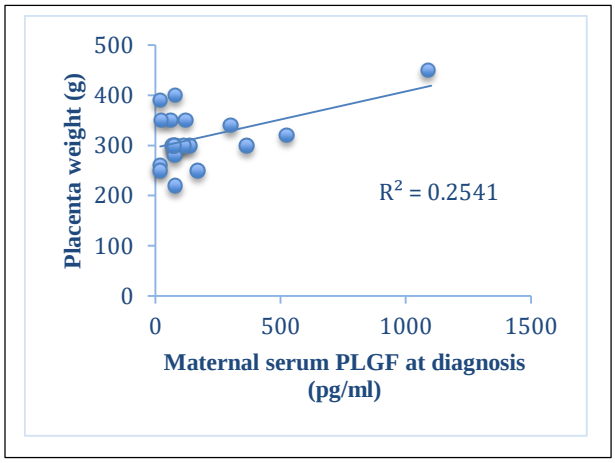

Figure 4. 13: Correlation between placenta weight and maternal serum PLGF levels in women diagnosed with pre-eclampsia

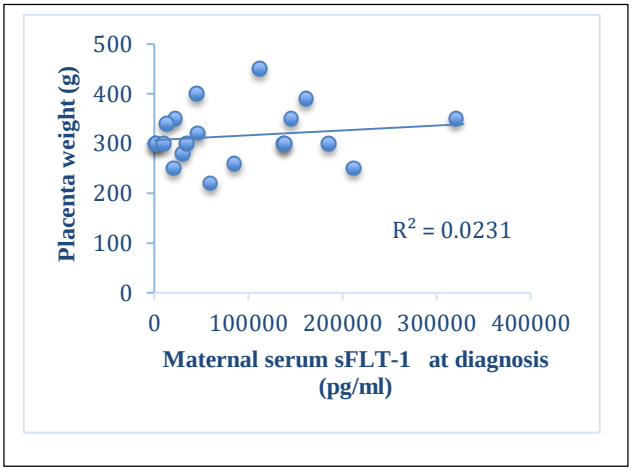

Figure 4. 12: Correlation between placenta weight and maternal serum sFLT-1 levels in women diagnosed with pre-eclampsia

A positive correlation (R=0.50) was noticeable between PLGF and placenta weight at a significance of 0.023. The variation in the placenta weight that was explained by the PLGF level was 25.4% as illustrated in figure 4.12

A weakly positive correlation (R=0.15) was noticeable between placenta weight and serum maternal sFLT-1 levels but this was not statistically significant (p=0.522)

CHAPTER FIVE

5.0 DISCUSSION AND CONCLUSION

5.1 Introduction

Neonatal mortality and morbidity is common following pregnancies associated with pre-eclampsia (Susilo et al., 2015). The focus of this research was to determine the association between antiangiogenic and angiogenic factors and neonatal outcome and to determine if these factors can be used to predict neonatal outcomes in Ghanaian pregnant women. To achieve this, pregnant women diagnosed with pre-eclampsia, who were between the ages of 18 and 43 years and who had no other medical condition were recruited as cases, and women who were in the same age interval, pregnant with no medical complications were recruited as controls. The antiangiogenic factor, sFLT-1 and angiogenic factor, PLGF were evaluated for both groups and the results of the cases were compared with controls. The findings have been categorized according to the specific objectives set for the study and discussed accordingly below.

5.1.2 General findings:

The maternal demographic characteristics of both women diagnosed with pre-eclampsia and controls were similar. Even though both case and control were overweight, the BMI of cases were higher than that of the controls. This is in disagreement with current literature which states that BMI of women diagnosed with pre-eclampsia as compared to control are obese (Markin & Medvyedyeva, 2018)(O'Brien TE, Ray JG, 2003). However, on further assessment of these articles, it was realized that the authors compared the BMI of these women before they were diagnosed with pre-eclampsia. This study assessed and compared the BMI between case and

control of women when they were diagnosed with pre-eclampsia. The women with pre-eclampsia may have had conditions such as hyperemesis gravidarum or poor appetite earlier in their pregnancy which resulted in a smaller weight and corresponding smaller BMI(Castillo & Phillippi, 2015). The mean arterial pressures were not surprisingly higher in the women diagnosed with pre-eclampsia as the definition of pre-eclampsia requires the blood pressure to be elevated(Ferrazzi et al., 2018). During the course of the research, none of the women admitted to smoking. This was expected because generally in Ghana, the prevalence of smoking is low(Owusu-Dabo et al., 2009).

5.2 Maternal serum levels of placenta growth factor (PLGF)

In this study, it was observed that the PLGF levels were generally lower in women diagnosed with pre-eclampsia compared to controls. This is consistent with studies which showed that women with pre-eclampsia have lower circulating maternal serum levels of PLGF(Habli et al., 2007) (Kasdaglis et al., 2010). This reveals the importance of PLGF in the maintenance of endothelial cell health during pregnancy(OHKUCHI et al., 2007)

When these levels were compared between early and late onset pre-eclampsia, the levels were lower in women with early onset pre-eclampsia (as seen in figure 4.6). This was not unexpected and agrees with previously published data which states that women with early onset pre-eclampsia have lower levels of PLGF and a more exaggerated disease than late onset pre-eclampsia(OHKUCHI et al., 2007). The reduction in serum PLGF levels may have been due to a combination of reduction of free PLGF, due to its binding with sFLT-1 (which is elevated in affected women) and a decreased expression of PLGF (Sandhya Panjeta Gulia, Madhusudan Chaudhury, NaseemNoorunnisa3, C D Balakrishnan4, 2012). In contrast to other studies which

showed increased or no change in PLGF levels in women diagnosed with pre-eclampsia, this study showed a clear reduction in PLGF levels in women diagnosed with pre-eclampsia.

Women with severe pre-eclampsia similarly, had lower levels of PLGF though this was not statistically significant. These findings suggest decisions should not be made based on the severity of the blood pressure alone, as this might not correlate well with the severity of injury and endothelial dysfunction in women with pre-eclampsia**.** Findings from this study showed that PLGF was positively correlated with placenta weight. This supports the hypothesis that PLGF is an indicator of abnormal placentation as shown by the significantly reduced placenta weights in patients with pre-eclampsia than controls.

5.3 Maternal serum levels of soluble flt-1 (sFLT-1) in pre-eclampsia

The serum levels of sFLT-1 level were higher in women diagnosed with pre-eclampsia than controls. When early onset pre-eclampsia was compared with late onset, the sFLT-1 at diagnosis, during delivery and 24 hours after delivery were all higher in early onset pre-eclampsia than late onset pre-eclampsia. This shows that patients who present with early onset pre-eclampsia usually have a more aggressive course (Schaarschmidt et al., 2013). Notably, the levels exceed those of the late-onset cases. As it has been shown both women with uncomplicated pregnancies and those with pre-eclampsia showed an increase in sFLT-1 with advancing gestational age however, the exceeding sFLT-1 levels in the early-onset group demonstrate that these patients present fast acceleration of the anti-angiogenic state.

When the levels were compared among those with severe and mild pre-eclampsia (table 4.11), those with severe pre-eclampsia had higher levels of sFLT-1 similar to those who had early onset pre-eclampsia. We can therefore infer from this that those who present with early onset pre-eclampsia usually have severe pre-eclampsia and corresponding higher levels of sFLT-1. This is consistent with the literature that patients with severe pre-eclampsia have a higher level of this antiangiogenic factor, sFLT-1(Maynard et al., 2003). The levels were not significantly higher in women who had severe pre-eclampsia except when we compared the levels at delivery (table 4.11) suggesting again that we should not base clinical disease severity only on systolic blood pressure readings**.**

There was a positive correlation between systolic blood pressure and sFLT-1 and the results showed that the sFLT-1 level explained about 6.3% of the variation in the systolic blood pressure. This finding builds on previously published work that suggested that excess placental production of sFLT-1 contributes to the hypertension (Maynard et al., 2003). It has been shown that sFLT-1 antagonizes VEGF. The VEGF is known to incite prostacyclin and nitric oxide production reducing the vascular tone and ultimately blood pressure. High sFLT-1 levels prevent this mechanism from occurring thus resulting in the high blood pressures that occur in women with pre-eclampsia (Maynard et al., 2003) Animals treated with sFLT-1 have significantly higher mean arterial pressures as compared with controls and this finding was not different in this study (Amraoui et al., 2014).

5.4 The changes that occur in the maternal serum levels of PLGF and sFLT-1

The antiangiogenic factor, sFLT-1 and angiogenic factor, PLGF were compared before and after delivery in both cases and control. The sFLT-1 levels in women who were diagnosed with pre-eclampsia was reduced to a level seven times lower, 24 hours after delivery. In the control group, the value also decreased to a level four times lower than before delivery. This is in comparison with other research works which suggested that the rapid and pronounced decline in sFLT-1 levels after delivery in pregnant women suggests that sFLT-1 levels has its origin almost entirely from the placenta (Saleh et al., 2018)(Maynard et al., 2003). This trend was different in the angiogenic factor PLGF. The PLGF levels in early onset pre-eclampsia were lower than late onset pre-eclampsia agreeing with the literature that states that early onset pre-eclampsia is usually more severe than late onset pre-eclampsia with corresponding maternal and perinatal complications (Benton et al., 2016).

The PLGF level in women who had pre-eclampsia decreased during delivery and decreased further 24 hours after delivery. When the levels were compared before and after delivery there was no significant difference in the levels indicating that the placenta prior to delivery was acting as though it was not present. The placenta was thus nonfunctional putting fetus at risk of complications as it was being deprived of nutrients and oxygen.

In the control group, the PLGF levels were high before delivery consistent with findings that showed that PLGF promotes development and maturation of normal placental vascular system (Sandhya Panjeta Gulia, Madhusudan Chaudhury, NaseemNoorunnisa3, C D Balakrishnan4, 2012). The levels of PLGF in the control group fell during delivery and more so after delivery. The gestational age at delivery was 38.7 ±1.4 weeks thus the fall in PLGF is expected as it usually falls around 37 weeks' gestation. After delivery, the placenta which is the organ that produces

most of the PLGF during pregnancy is removed, thus greater fall in the PLGF levels is expected. This fall in the PLGF levels agrees with the literature which states that levels are usually higher prior to delivery and reduced to eight times lower 24 hours after delivery. The rise and fall of the serum PLGF concurs that the levels corresponds with different stages in placental development (Sandhya Panjeta Gulia, Madhusudan Chaudhury, NaseemNoorunnisa3, C D Balakrishnan4, 2012). When PLGF levels were compared before and after delivery, the levels were lower during delivery in both early and late onset preeclampsia but increased after delivery in both groups. (refer to figure 4.6).

Comparing the PLGF levels between early and late onset pre-eclampsia, the levels before delivery were higher in late onset than early onset preeclampsia probably because the pathophysiology in late onset preeclampsia is not due to placenta factors but rather maternal factors such as hypertension and obesity (Benton et al., 2016). In severe and mild pre-eclampsia, the PLGF levels decreased gradually and were at their lowest 24 hours after delivery. PLGF levels were however lower in severe pre-eclampsia at all stages but this was not significant meaning pre-eclampsia should not be classified based on the severity of blood pressures alone as blood pressures may not reflect the true detrimental state of the woman.

5.5 Maternal serum levels of PLGF and sFLT-1 and perinatal outcomes in pre-eclampsia

There was a significant difference in birth weight of the neonates between the pre-eclamptic and normotensive pregnant women with a p-value less than 0.001. When this was further compared between early and late onset pre-eclampsia, those with early onset recorded a statistically significant smaller birth weight. The results are comparable with other studies which showed a

similar trend in the birth weights of early onset pre-eclampsia (Markin & Medvyedyeva, 2018)(Vaddamani et al., 2017). This confirms that low birth weight is common in women with pre-eclampsia and strategies to prevent low birth weight could arrest pre-eclampsia associated neonatal deaths.

From figure 4.6, it is evident that as sFLT-1 levels increased, birth weight reduced showing an inverse relationship between sFLT-1 and birthweight. The sFLT-1 level explained only 7.9% of this variation. Yet, when the birthweight was compared with PLGF as shown in figure 4.6 B, it showed a positive relationship. The change noticed in this study though not significant is confirmed in the literature (Ullah et al., 2016). This shows how these antiangiogenic and angiogenic factors can serve as a guide to the birth weight. If the sFLT-1 level can be controlled or prevented from increasing, the birth weight of the fetus can increase steadily and likewise if the PLGF level can be increased, the birth weight would also increase.

In this study, neonates were delivered at an average gestational age of 35 weeks in women with pre-eclampsia and 39 weeks in the controls. The mothers whose neonates survived and were admitted to NICU had the level of sFLT-1 almost three times higher than those who were not admitted while the PLGF level was about one and a half times lower in neonates who were admitted to NICU (Refer to table 4.12). A similar trend was seen in those who had early neonatal death and those who had low birth weight. In those who had still birth, it was seen that they had a lower value of sFLT-1, though not statistically significant when compared with those who delivered live neonates. This paradoxical result of low sFLT-1 level in mothers who had still birth might be suggesting that the initial high levels affected the fetus in utero and resulted in the death of the baby. Thus if the sFLT-1 levels were monitored and noticed to be declining, prompt delivery of the fetus should be done. Perhaps if they were delivered at an earlier gestational age and the

sFLT-1 had not caused detrimental effects, they could have been quickly admitted to NICU where they would have had a better chance of survival.

A study done by Habli et al about a decade ago showed that patients with pre-eclampsia who were delivered between 35 and 37 weeks gestation still had a higher rate of NICU admissions(Habli et al., 2007). This probably means that as the disease advances, the neonatal outcome worsens hence the need for early delivery and NICU admissions. Thus if Ghana, or for that matter developing countries can increase their NICU facilities and provide necessary medications like steroids which can be given before delivery or surfactant after delivery in anticipation of respiratory complications that may occur in the neonate, neonatal mortality can be reduced.

The PLGF level in women who had still birth was lower than those who did not have still birth as seen on table 4.14. This is in concordance with a study done in Canada, New Zealand and the United Kingdom which associated still birth to low PLGF levels (Benton et al., 2016). Comparing early and late onset pre-eclampsia, adverse birth outcomes were more common in women with early onset pre-eclampsia. This study showed that those with early onset pre-eclampsia had neonates with smaller birth weights and a lower APGAR score than those with late onset pre-eclampsia and this was statistically significant (Susilo et al., 2015).

A strong positive relationship was seen between PLGF and placenta weight. Clinicians can use this to their advantage with the view that if PLGF is getting lower, with/without other signs of pre-eclampsia, the obstetrician should deliver the baby quickly. Considering that the placenta is the main source of nutrients for the fetus, it may mean that the fetus might not be getting enough nutrients to grow resulting in intrauterine fetal growth restriction.

The PLGF which is known to regulate placenta growth was seen to be about ten times lower in those who had pre-eclampsia. Similarly, its level was significantly low in those who had neonatal death and in those mothers whose neonates were admitted to the intensive care unit. This is possibly because the placenta which is known to supply nutrients and oxygen to the fetus might be malfunctioning thus the fetus is already in danger in utero resulting in neonatal death. The low levels of PLGF also reflected the frequent admission to NICU.

Women with severe pre-eclampsia have been associated with neonates with poor outcomes. Research has showed that women with pre-eclampsia tend to have infants with low APGAR scores (Susilo et al., 2015). Paradoxically, when severe and mild pre-eclampsia were compared in this study, those with severe pre-eclampsia had a higher APGAR score than those with mild pre-eclampsia, though this was not statistically significant. Mothers of those with mild pre-eclampsia may have been overlooked because they were seemingly thought to have a milder condition and more concentration may have been given to those who had severe pre-eclampsia. Thus any complication such as prolonged labour or fetal distress could have occurred and this may have accounted for the low APGAR score in this study.

5.6 Conclusions

The study showed that women with pre-eclampsia are more likely to have neonates with stillbirths, early neonatal deaths and frequent admission into NICU. Women with pre-eclampsia have a high sFLT-1 and low PLGF level than those who had uncomplicated pregnancies. The study also revealed that in women who had still stillbirth, the sFLT-1 levels were paradoxically lower than

that in the controls because of the damage probably caused by high sFLT-1 levels in utero. The findings of this study indicate that pre-eclampsia could influence neonatal outcome significantly. Constant monitoring of maternal sFLT-1 -1 and PLGF levels, together with clinical signs and symptoms, may improve neonatal outcome

5.7 Novelty of the study

This is first study in Ghana relating the levels of angiogenic factors in pre-eclampsia to neonatal outcomes.

5.8 The limitations of the study

A semi-quantitative method was used to measure urine proteins instead of a more objective method might have influenced the diagnosis of preeclampsia.

RECOMMENDATION

It is recommended that, maternal health education to individual pregnant women on pre-eclampsia be increased, instead of mass education. Based on the findings reported in the current study, a larger longitudinal study is recommended in different tertiary facilities in Ghana to determine if the same trend in the antiangiogenic and angiogenic factors would be seen.

A urine albumin creatinine ratio should be done instead of the urine dipstick as it's a more objective method and gives a true reflection of the urine proteins produced in 24 hours.

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APPENDIX A: CONSENT FORM

CONSENT FORM

My name is Lim Abla Kwawukume and I'm conducting a research on pre-eclampsia, a pregnancy complication which is very common in Ghana. I am going to give you information and invite you to be part of this research. There may be some words that you do not understand. Please ask me to stop as we go through the information and I will take my time to explain. If you have questions later, you can ask them of me.

Pre-eclampsia is a serious complication that may occur in some pregnant women. It is a leading cause of maternal and fetal morbidity and mortality. The only known treatment however is delivery. In recent times, research has shown that some factors in the blood can predict this disease. The reason I am doing this research is to determine if the level of these factors in the blood when pregnant can determine the fetal outcome.

I am inviting all adults between 18-40 years to participate in this research. Your participation in this research is entirely voluntary. It's your choice whether to participate or not, all the services you receive at this hospital will continue and nothing will change. You may change your mind later and stop participating even if you agreed earlier.

The research will take place over 4 months. We will take about 3mls of blood three times and analyze the samples at no cost to you. The information that we collect from this research will be kept confidential. Any information about you will have a number on it instead of your name.

If you agree to participate in this research, please sign this form

I have read the foregoing information, or it has been read to me. I have had the opportunity to ask questions about it and any questions that I have asked have been answered to my satisfaction. I consent voluntarily to participate as a participant in this research.

Print Name of Participant_____

Signature of Participant _____

Date _____

Print name of witness_____ ***AND*** ***Thumb print of participant***

Signature of witness **_____**

Date _____

APPENDIX B: QUESTIONNAIRE

SERUM LEVELS OF ANTI ANGIOGENIC AND ANGIOGENIC FACTORS IN PRE-ECLAMPSIA AND THEIR RELATION TO FETAL OUTCOME

Demographics

1. Participant ID number …….
2. Hospital ID number……..
3. Initials……
4. Date of hospital admission *(day/month/year)*……..
5. Date of discharge *(day/month/year)*……..
6. Duration of stay in the hospital *(days)*……..
7. Age of participant *(years)*……..
8. Occupation……..
9. Marital status- 0. married/co-habitating 1. Single 2. Divorced/separated 3. Other
10. What is the highest level of formal education attained 0. Primary 1.JSS 2. SSS 3. Tertiary

Obstetric history

11. Number of pregnancies including index pregnancy……..
12. Previous history of pre-eclampsia 1. Yes 2. No
13. Number of antenatal visits with current pregnancy …….
14. Gestational age (*in weeks*)……

Maternal outcome indicators

15. Weight (kg) at booking-……
16. Height (m) at booking-……

17. BMI (Kg/m2)-…….

18. Gestational age at diagnosis (*in weeks*)……..

19. Systolic BP at time of recruitment…….

20. Diastolic BP at time of recruitment ……

21. Urine protein at booking – 0. Negative 1.= 1+, 2=2+, 3=3+, 4=4+, 5= not checked

22. Urine protein at diagnosis – 0. Negative 1= 1+, 2=2+, 3=3+, 4=4+, 5= not checked

23. Have you ever smoked cigarettes 1. Yes 2. No

24. Have you ever heard about pre-eclampsia 1. Yes 2. No

Labour and delivery

25. Gestational age at time of delivery *(weeks)*……..

26. Systolic blood pressure at delivery……

27. Diastolic blood pressure at delivery……..

28. Urine protein at delivery– 0. Negative 1.= 1+, 2=2+, 3=3+, 4=4+, 5= not checked

29. Mode of delivery- 1. Vaginal 2. Caesarean section 3. Assisted vaginal delivery

30. Estimated blood loss (*mls)*……..

Perinatal outcome

31. Sex- 1. Male 2. Female

32. Birth weight*(g)*-

33. Placental weight *(g*)-

34. APGAR at 1 minute-

35. APGAR at 5 minutes-

36. Was neonate admitted to NICU? 1. Yes 2. No

b. If yes, what was the duration of stay *(days*)…….

37. Still birth 1. Yes 2. No

b. If yes 1. Fresh still birth 2. Macerated

38. Early neonatal death 1. Yes 2. No

39. Intrauterine growth restriction 1. Yes 2. No

Laboratory tests

40. Full blood count

a. WBC-

b. Hb-

c. Plt-

41. Blood urea and electrolyte

a. Na-

b. K+

c. Cl-

d. Urea –

e. Creatinine-

f. Uric acid-

42. Liver function test

a. AST

b. ALT

c. Albumin

d. Total protein

43. SFLT at diagnosis……. At delivery…… 24hrs after delivery…….

44. PLGF at diagnosis……. At delivery…… 24hrs after delivery……

APPENDIX C: PERSONNEL OF STUDY TEAM

Principal Investigator	(100%)	Lim Abla Kwawukume
Clinical research coordinator	(40%)	Dr Bonsaffoh
	(30%)	Rev Dr Antwi Bosiako
	(30%)	Prof Gyan
Data collection personnel	(40%)	Lim Abla Kwawukume
	(40%)	Yaw Kusi Mensah
	(40%)	Patricia Kankam
Data analysis personnel	(30%)	Lim Abla Kwawukume
	(30%)	Edward Someah – Addae
	(20%)	Yaw kusi mensah
	(20%)	Dorotheah obiri
Lab technicians		Noguchi Memorial Institution
		Korle Bu Teaching Hospital