UNIVERSITY OF GHANA

COLLEGE OF HEALTH SCIENCES

MATERNAL SERUM ANGIOPOIETIN AND VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) LEVELS IN PREECLAMPSIA AND PREGNANCY OUTCOMES

BY

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THIS DISSERTATION IS SUBMITTED TO THE UNIVERSITY OF GHANA, LEGON IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF MPHIL IN PHYSIOLOGY DEGREE

SEPTEMBER, 2021

DECLARATION

Candidate's Declaration

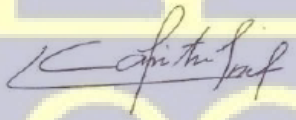
I hereby declare that this research work is the result of my own original research and that no part of it has been presented for another master's degree in this school or elsewhere.

Candidate's Signature:  Date: 30/05/2022

Name: Fidelis Bayor

Supervisor's Declaration

I hereby declare that the preparation and presentation of this research work has been supervised in accordance with the guidelines on supervision of project work laid by the College of Health Sciences, University of Ghana.

Main Supervisor's Signature:  Date: 30/05/2022

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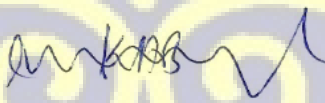
Co-supervisor's Signature:  Date: 30/05/2022

Name: Dr. Kwame Adu-Bonsaffoh

DEDICATION

I dedicate this research work whole-heartedly to my family and loved ones and a special dedication goes to my supervisors; Rev. Prof. Charles Antwi Boasiako and Dr. Kwame Adu-Bonsaffoh.

ACKNOWLEDGMENTS

My foremost thanks go to the Almighty God for His grace that made this work a reality.

My next thanks go to my able supervisors; Rev. Dr. Charles Antwi Boasiako and Dr. Kwame Adu-Bonsaffoh for their guidance and mentorship to conceptualize this work.

My sincere appreciation also goes to my family for the countless financial and moral support.

Finally, I thank you all who have contributed in every little way in cash and in kind to sustain the successful completion of work. Thanks once again, and May the good Lord keep you all blessed.

ABSTRACT

Introduction: Preeclampsia remains a major obstetric complication globally, accompanied by a significant burden of adverse maternal and perinatal outcomes especially among Sub-Saharan African (SSA) countries including Ghana. The high maternal and perinatal outcomes of preeclampsia may be due to the lack of consensus on the ideal clinical and therapeutic intervention which stems from the insufficient knowledge on the actual etiology and pathophysiological mechanisms that underline the disease progression.

Aim: To explore maternal serum levels of angiopoietins (1 and 2), Vascular endothelial growth factor (VEGF), and pregnancy outcomes in preeclampsia.

Methods: The study was a comparative cross-sectional study with cases and controls, conducted at the Obstetrics and Gynaecology Department of the Korle-Bu Teaching Hospital (KBTH). A systematic random sampling technique was used to recruit study participants consisting of women diagnosed with preeclampsia (cases), pregnant normotensives (control group 1), and non-pregnant normotensives (control group 2). A structured data extraction form was used to collect data on the demographic and clinical features of the study participants.

Results: A total of 263 women including 75 non-pregnant normotensives, 94 pregnant normotensives, and 94 with established diagnosis of preeclampsia (PE) were enrolled. The study revealed that maternal serum VEGF [4.71 (IQR: 3.65, 7.93) pg/ml], Ang-2 levels [1.25 (IQR: 0.90, 2.15) ng/ml] and VEGF/Ang-2 ratio [3.85 (IQR: 1.91, 11.38)] in the PE group were significantly lower than in the normotensives controls. Although elevated maternal serum Ang-1 levels were found, the difference between women with PE and normotensive controls did not reach statistical significance ($p>0.05$). VEGF levels were significantly lower in early-onset PE [(3.89 (2.87, 4.78)pg/ml;$p=0.014$] than in late-onset PE [5.23 (3.78, 16.97) pg/ml] while Ang-1/VEGF ratio was found to be higher in early-onset PE [20.62(15.76,

31.44)] than late-onset PE [17.55(6.22, 26.64)] (p=0.042). Also, after adjusting for maternal risk factors such as maternal age, parity, blood pressure, BMI, GA at recruitment, and GA at birth, maternal serum VEGF levels <62pg/ml was significantly associated with increased risk of preterm birth, poor Apgar scores <7 and low birth weight (LBW), while serum Ang-1 levels >84ng/ml and Ang-2 <17ng/ml were not associated with adverse pregnancy outcomes.

Conclusion: Lower maternal serum levels of VEGF, Ang-2 and VEGF/Ang-2 ratio were detected in PE compared to normotensive controls. VEGF levels were significantly reduced among early-onset PE with elevated Ang-1/VEGF ratio, than in late-onset PE and therefore may serve as good predictive biomarkers for the diagnosis of early-onset PE. Also, after adjusting for maternal risk factors, lower VEGF levels <62pg/ml was significantly associated with poor maternal and perianal outcomes but Ang-1 and Ang-2 did not show significant association.

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LIST OF ABBREVIATIONS

PE – preeclampsia

LMICs – low and middle-income countries

HELLP – hemolysis elevated liver enzymes and low platelets

HDP – hypertensive disorders of pregnancy

VEGF – vascular endothelial growth factor

PIGF – placental growth factor

sFlt-1 – soluble fms-like tyrosine kinase-1

sEng – soluble endoglin

Ang - angiopoietin

Ang-1 – angiopoietin 1

Ang-2 – angiopoietin 2

Tie-2 – angiopoietin receptor type 2

WHO – world health organization

eNOS – endothelial nitric oxide synthase

NO – nitric oxide

RAAS – renin angiotensin aldosterone system

ACOG – American college of Obstetricians and Gynaecologists

HLA – Human Leukocyte class 1 antigens

µL – microliter

KBTH – Korle Bu Teaching Hospital

Pg/ml – pictograms per millitre

BMI – body mass index

sBP – systolic blood pressure

dBP – diastolic blood pressure

MAP – mean arterial pressure

ELISA – enzyme-linked immunosorbent assay

PBS – phosphate-buffered saline

CHAPTER ONE

1.0 INTRODUCTION

1.1 Study Background

Preeclampsia (PE) is the most common hypertensive disorder in pregnancy associated with profoundly high adverse maternal and perinatal outcomes. It affects about 2 – 8% of all pregnant women and remains the principal cause of increased maternal and perinatal mortalities worldwide, especially among Low- and Middle-income countries (LMICs) (Joshi *et al.*, 2020; Portelli & Baron, 2018; Stocks, 2014; Tornyi *et al.*, 2021; Wang *et al.*, 2021). The prevalence of preeclampsia varies significantly from country to country depending on the economic and healthcare situation (Kharaghani *et al.*, 2016; Portelli & Baron, 2018).

Preeclampsia is associated with profoundly high maternal mortalities and the risk of short- and long-term health implications (Koual et al., 2013; Pankiewicz *et al.*, 2019; Pauli & Repke, 2015). In the United States and other developed countries, PE accounts for about 16% of all maternal mortalities (Johal *et al.*, 2014; Ton *et al.*, 2020). In Ghana, the overall maternal deaths primarily attributed to PE accounts for about 18% of maternal mortalities and represents the leading cause of maternal deaths in tertiary hospitals in the country (Adu-Bonsaffoh, 2012; Ghana Statistical Service, 2018).

Depending on the severity, patients with PE are at increased risk of cardiovascular and cerebral complications such as oliguria (below 500mls volume of urine in 24 hours), microvascular complications, cyanosis, and pulmonary edema (Gupte & Wagh, 2014; Portelli & Baron, 2018). Although the actual etiology of preeclampsia remains unclear, the major hypothesized pathophysiological mechanism is linked with the association between abnormal placentation and maternal systemic disease leading to systemic endothelial dysfunction. However, the specific mechanism by which this abnormal placentation leads to systemic

endothelial dysfunction has remained elusive in the area of clinical research and is still under investigation (Hariharan et al., 2016). Despite the limited knowledge on the actual etiology of PE, clinical research has demonstrated that an imbalance between antiangiogenic (e.g. sFlt-1 and sEng) and proangiogenic (e.g. VEGF and PIGF) factors expression play a crucial role in the pathogenesis of the disease. This imbalance ensuing from abnormal placentation due to multiple aetiologies leads to placental hypoperfusion and ischemia. This leads to increased expression of hypoxia-inducible transcription factors or vasoactive factors (e.g. sFlt-1 and sEng) which exceeds the placental expression of VEGF and thereby results in extensive vascular endothelial cell dysfunction (George & Granger, 2014)

In normal pregnancy, widespread angiogenesis favours adequate placental perfusion and fetal development. Fetal-derived extravillous cytotrophoblasts invade the uterine arteries, causes remodelling of uterine spiral arteries, and converts the high-resistance, low capacitance vascular system to low-resistance, high-capacitance uterine arteriolar system. This allows improved blood flow and adequate placental oxygenation and nutrition for the maintenance of pregnancy(George & Granger, 2014; Hariharan et al., 2016). In preeclampsia, the pathological changes of heterogonous origin in the placenta occur due to abnormal remodelling of the uterine spiral arteries. Thus, cytotrophoblast cells only invade the decidua segment of the uterus but fail to penetrate the myometrium. This leads to impaired conversion of the spiral arteries into large, tortuous high-capacitance vessels thereby resulting in impaired placental perfusion and ischemia(Hariharan et al., 2016; Marwa & Sahar, 2011).

The role of Angiopoietins (1 and 2) in the pathogenesis of PE has also been extensively described in clinical research especially for their role in vascular morphogenesis, remodelling and development of placental vasculature (Leinonen et al., 2010; Schneuer et al., 2014). However, the actions of these proangiogenic factors which are normally expressed by the placenta during the early stages of pregnancy differ significantly and are complimentary to

VEGF (Burton et al., 2019; Kappou et al., 2015). Angiopoietin 1 (Ang-1) appears to serve a protective role in normal pregnancy by promoting endothelial cell maturation, integrity, and stability, and the levels are normally elevated throughout gestation. It also reduces vascular permeability induced by VEGF. On the other hand, Angiopoietin 2 (Ang-2) causes endothelial cell destabilization and apoptosis in the absence of VEGF and its effects in normal pregnancy may be detrimental. However, in the presence of VEGF, Ang-2 renders endothelial cells accessible to VEGF (Burton et al., 2019; Kappou et al., 2015). In women with PE, elevated serum levels of Ang-1, as well as depressed Ang-2 levels, were demonstrated by other studies (Leinonen *et al.*, 2010; Schneuer et al., 2014). This study hypothesized that the depressed production of VEGF seen in women with PE removes the positive inhibition on tyrosine kinase receptor type-2 (Tie-2) resulting in a surge in serum Ang-1 concentration with a significant decline in serum Ang-2 levels. According to Apostolakis, Ang-1/ Ang-2 ratio showed a sensitivity of 47% and specificity of 87% in 25 to 28 weeks pregnant women who subsequently developed PE and can be used as a predictive biological marker for the diagnosis of late-onset of preeclampsia (Apostolakis *et al.*, 2009).

Although the role of Angiopoietins in PE has been described in other studies, findings on their relationship with VEGF and associated adverse maternal and perinatal outcomes have been conflicting and have not been adequately studied in clinical research especially among LMICs including Ghana(Phupong et al., 2021). This study explores the association between maternal levels of angiopoietins and VEGF and associated pregnancy and perinatal outcomes among Ghanaian women with preeclampsia.

1.2 Problem statement

Preeclampsia remains a major obstetric complication globally due to the significant burden of maternal mortalities and adverse pregnancy outcomes especially among Sub-Saharan African

(SSA) countries including Ghana (Adu-Bonsaffoh et al., 2017; Gathiram & Moodley, 2016; Joshi *et al.*, 2020, 2021). The high maternal and perinatal outcomes associated with the disease may be because no definitive decision regarding an ideal therapeutic intervention has been reached to optimize pregnancy outcomes.

Current management strategies of PE only depend on the timely delivery of the fetus and the placental (Burton et al., 2019; Li, *et al.*, 2018). The lack of consensus on the ideal clinical and therapeutic intervention stems from the insufficient knowledge on the actual etiology and mechanisms that underline the disease progression (Bell, 2010; Gathiram & Moodley, 2016; Uzan *et al.*, 2012) has made the determination of the prevalence and diagnosis of the disease extremely cumbersome if not impossible (Burton *et al.*, 2019).

Although clinical research has demonstrated that angiopoietins play a critical role in the pathogenesis of preeclampsia, much is focused on disease epidemiology. Studies on the levels of Ang 1 and 2 in preeclampsia and associated adverse maternal and perinatal outcomes have shown inconsistent results and have not been adequately exploited especially among LMICs (Han et al., 2012; Kappou *et al.*, 2015). These gaps in knowledge serve as major barriers to therapeutic interventions aimed at minimizing, preventing, and management of poor maternal and perinatal outcomes associated with preeclampsia in LMICs.

1.3 Justification

Hypertensive pregnancy disorders including preeclampsia have become a major global concern due to the accelerated adverse maternal and perinatal outcomes (Hutcheon *et al.*, 2011; Khowaja et al., 2015). The increased pregnancy complications associated with PE has imposed substantial ramifications on the global economy, welfare and social wellbeing of the community and families of pregnant woman and healthcare systems especially in LMICs whose poorly resourced healthcare systems are already overstretched (Khowaja *et al.*, 2015;

Von Dadelszen *et al.*, 2012). To determine the severity of the disease and its associated complications, several clinical indicators and biological markers including angiopoietins have been investigated but not applied clinically. Interventions which augment clinical management and thereby reduce adverse outcomes, as well as extensive and further studies are required.

This proposed study will provide data on the maternal levels of angiopoietins and associated pregnancy outcomes in women with PE as well as answer relevant questions on the biological mechanisms of angiopoietins in PE and their relationship with poor pregnancy outcomes. It will provide useful information for policy formulation and the development of more robust interventions for clinical practice aimed at reducing the burden of PE-related complications in Ghana.

1.4 Aims and Objectives

1.4.1 General Aim

To explore the relationship between serum levels of maternal angiopoietins, VEGF, and pregnancy outcomes in preeclampsia

1.4.2 Study objective

The aims of this study were;

1. to evaluate the serum levels of angiopoietins (1 and 2) and VEGF in preeclampsia
2. to compare the levels of serum Angiopoietins and VEGF in women with PE, non-pregnant and pregnant controls
3. to determine the association between maternal biomarkers and pregnancy outcomes in preeclampsia.

1.5 Conceptual framework

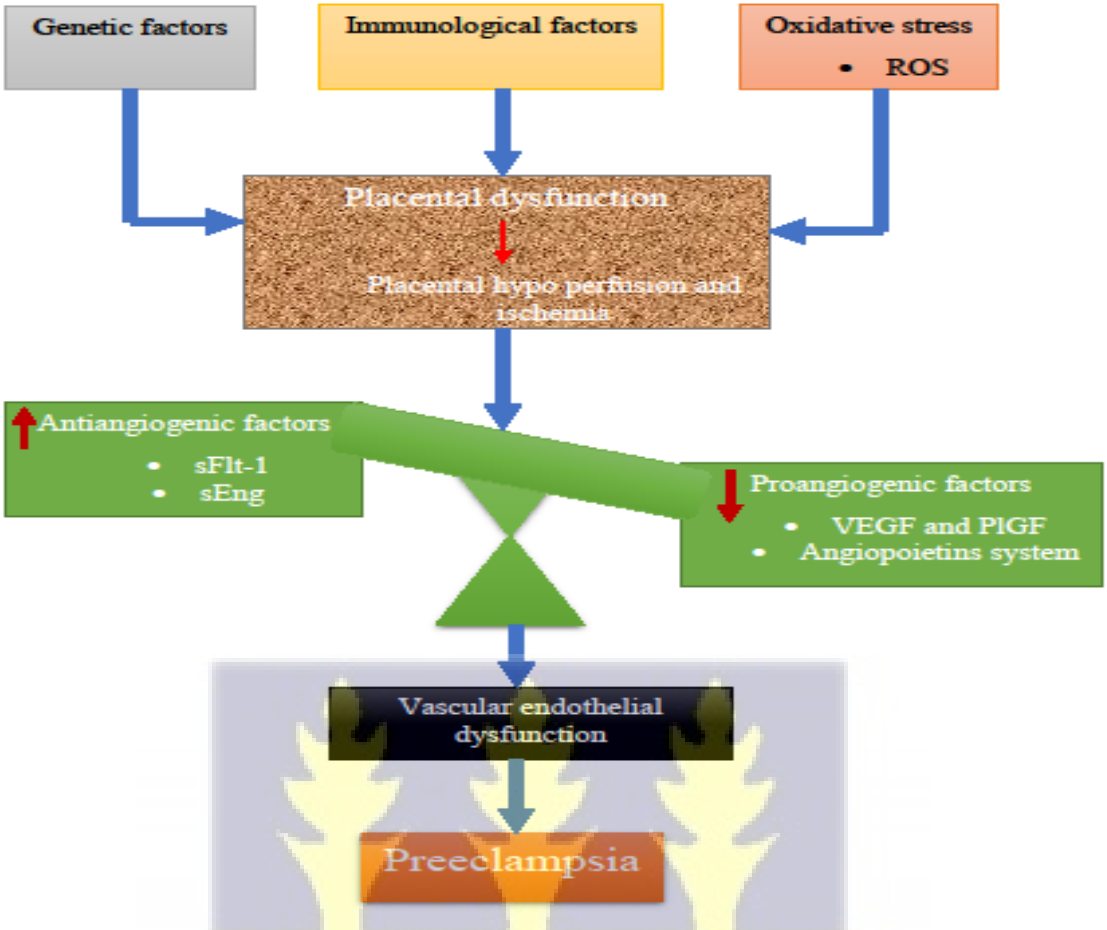


Figure 1: Conceptual framework

The cause of PE is multifactorial. The factors implicated in the biological pathway of PE have been presented in the conceptual framework (figure 1). The factors of interest in the framework include genetic factors and oxidative stress. These factors are associated with an imbalance between increased expression of antiangiogenic factors and decreased proangiogenic factors including a disruption in the angiopoietin system. Thereby results in placental dysfunction and systemic vascular endothelial dysfunction.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Preeclampsia

Preeclampsia (PE) is a heterogenous systemic group of diseases associated with pregnancy that affects many organ systems(Collier *et al.*, 2021). It presents with a novel onset of hypertension (blood pressure of ≥140/90 mmHg) taken on two conservative times, at least 4 hours apart, and proteinuria (elevated urine protein levels of ≥300mg/24hours) in a hitherto normotensive woman after 20 weeks of gestation (Collier *et al.*, 2021; Cruz *et al.*, 2020; Hariharan *et al.*, 2016; Portelli & Baron, 2018; Tornyi *et al.*, 2021).

PE is a common complication of pregnancy that is associated primarily with the placenta (Flint et al., 2019). The disorder occurs in the second trimester of pregnancy with varying degrees of adverse maternal and perinatal outcomes and poses a significant threat to the survival of both mother and the fetus (Han *et al.*, 2020; Redman & Sargent, 2005). According to Collier *et al.*, (2021), the disorder may be asymptomatic and may be undiagnosed until delivery while other pregnancies may manifest early onset of the disorder resulting in multiorgan dysfunction including vascular and neurological diseases (Collier *et al.*, 2021).

Preeclampsia is characterized by both maternal and perinatal complications: maternal complications include eclampsia, cerebral hemorrhage, acute renal failure, abruptio placentae; low platelets count (thrombocytopenia), hepatic dysfunction, risk of maternal death, pulmonary edema, and severe headache as well as an increased potential risk for metabolic and vascular consequences such as hypertension, acute coronary disease, and stroke. The perinatal complications include low births, intrauterine growth restriction, prematurity, intrauterine fetal demise, and early neonatal deaths (Collier et al., 2021; C. Han *et al.*, 2020; Hoedjes *et al.*, 2011).

The American College of Obstetricians and Gynaecologists (ACOG) criteria allow for classification of PE based on the onset of the disorder; early-onset (onset of the disorder before 34 weeks), or late-onset (after 34 weeks) (Adu-Bonsaffoh *et al.*, 2015; Han et al., 2020; Portelli & Baron, 2018) and the severity such as mild PE (elevated blood pressure of 140 – 160 mmHg/ 90 – 110 mmHg) to severe (elevated blood pressure of ≥160/110 mmHg, severe proteinuria (≥500mg/24 hours), hemolysis, elevated liver enzymes, and low platelet count (HELLP) syndrome (Hoedjes *et al.*, 2011; Marwa, S & Sahar, 2011). Although alpha-fetoprotein (AFP) and unconjugated estriol (UE3) were reported by Weitzner et al. to be associated with women with early onset of PE (Weitzner et al., 2020), both early and late-onset of PE share very similar aetiological features (Iacobelli *et al.*, 2017; Lisonkova & Joseph, 2013). Severe adverse maternal (e.g. placenta abruption, oligohydramnios, eclampsia, HELLP syndrome) and neonatal complications (e.g. small for gestational age, poor Apgar score <7 at 5 minutes, stillbirths and early neonatal deaths) are reportedly high and evident among women with early-onset of PE compared to those with late-onset. Additionally, Wadhwani et al., found that the late-onset of PE was more prevalent among primigravidas (Kınay *et al.*, 2015; Madazli *et al.*, 2014; Wadhwani *et al.*, 2020). Baker *et al.*, (2009) found that women with mild PE were reported to have high triglyceride levels and total cholesterol/high density-lipoprotein (HDL) ratio compared to normal pregnant subjects while severe PE was significantly associated with low levels of low-density lipoprotein (LDL) and low atherogenic profile compared to the control subjects. While depression and anxiety are common disorders of pregnancy, Abedian detected no significant difference between the severity of PE and depression and anxiety states among study subjects (Abedian *et al.*, 2015). On the other hand, other studies also proposed that significant adverse maternal outcomes including placenta abruption, maternal vitamin D deficiency, postpartum hemorrhage, posttraumatic stress disorder (PTSD), etc. and perinatal outcomes had a strong association

with severe PE compared to the mild PE (Bodnar *et al.*, 2014; Hoedjes *et al.*, 2011; Kongwattanakul *et al.*, 2018; Ton *et al.*, 2020).

Although the actual etiology or pathophysiological mechanism of PE is not yet known, several risk factors may contribute to its occurrence. These risk factors include extremes of maternal age (young or advanced maternal), poor antenatal care services (<8 antenatal visits), low level of formal education, primiparity, multiple pregnancies, hereditary, rural dwellers, single marital status, ethnicity (African and Hispanic American race, Latin Americans), multigravidity, polycystic ovarian syndrome, pyelonephritis or urinary tract infections, severe anemia, male fetus, the use of assisted reproductive technology and previous PE (Bilano et al., 2014; Li, *et al.*, 2018; Lisonkova & Joseph, 2013; Luealon & Phupong, 2010; Paré *et al.*, 2014). Additional risk factors include pre-existing medical disorders such as chronic hypertension, vascular and connective tissue disorders, diabetes, obesity, renal disease, and antiphospholipid antibody disorder (Bilano *et al.*, 2014; Luealon & Phupong, 2010; Paré *et al.*, 2014). Due to a lack of understanding of the aetiology and pathophysiology of PE, there is currently no definite consensus on effective prevention interventions and management strategies. The current clinical management of PE includes early detection, clinical monitoring, administration of hydralazine with labetalol, magnesium sulfate, aspirin, and delivery of the fetus and the placenta have shown promising results in reducing the disease progression and severe complications such as eclampsia (Amaral *et al.*, 2017; Dekker, 2014; English et al., 2015; Flint et al., 2019).

2.2 The burden of preeclampsia among Low- and Middle-income countries

Globally, PE with its associated increased risk of maternal and perinatal morbidity and mortality, affects 2 to 10% of all pregnancies (Young *et al.*, 2010). The incidence of PE varies by country, but it is a considerable burden and has a severe impact on countries with limited resources, such as low- and middle-income countries like Ghana (Noubiap *et al.*,

2019). Due to insufficient medical care in developing nations, PE is estimated to be prevalent (10%) in Asia and Africa, compared to 2 -5% reported in Europe and America (Kharaghani *et al.*, 2016; Portelli & Baron, 2018). When compared to Caucasians, the Chinese ethnic group had a lower rate of PE. This low rate of PE was partly linked to increased BMI or lifestyles that included periods of cohabitation with a spouse (Kharaghani *et al.*, 2016).

Poor maternal and perinatal outcomes associated with PE largely depend on the gestational age and the severity of the disease (Iacobelli *et al.*, 2017; Li, *et al.*, 2018a). Despite the progress in the prevention and clinical management, the burden of maternal and perinatal morbidity and mortality attributable to PE continue to rise among vulnerable communities especially in low- and middle-income countries with significant social, financial, and healthcare system implications (Firoz *et al.*, 2011; Khowaja *et al.*, 2015; Nicholson *et al.*, 2021).

According to the World Health Organisation (WHO), hypertensive disorders in pregnancy (HDP) including preeclampsia accounts for about 63,000 – 72,000 maternal mortalities annually, indicating a 10 – 15% of the overall maternal deaths directly associated with preeclampsia and eclampsia (Marwa & Sahar, 2011). The risk of adverse maternal and neonatal complications differs among different populations and from country to country. PE is associated with significant short-term and long-term adverse maternal and perinatal outcomes. The short-term adverse effects include: risk of prolonged hospital stay, cesarean sections, abruptio placentae, and eclampsia. Long-term complications include: risk of coronary artery disease, stroke, chronic hypertension, diabetes mellitus, and end-stage renal disease(Ton *et al.*, 2020). Incidence rate of severe and adverse complications associated with PE are higher in sub - Saharan countries than in developed countries (Meazaw *et al.*, 2020).

Similarly, the WHO estimated that PE is about seven-fold (2.8% of all live births) higher among developing countries in Asia and Sub-Saharan Africa compared to developed nations.

In Africa, significant variations in the prevalence of PE were detected from country to country. For instance, the incidence of PE varies from 2% to 16.7% in Nigeria compared to 1.8% to 7.1% variation in the incidence rates recorded from South Africa, Egypt, Tanzania, and Ethiopia (Osungbade & Ige, 2011). A meta-analysis and systematic review conducted in Africa indicated that the burden of HDP including PE was significantly high in Central and Western Africa (Noubiap et al., 2019). In Ghana, the overall maternal deaths are mainly attributed to PE and account for about 18% of maternal mortalities among tertiary hospitals in the country (Adu-Bonsaffoh, 2012; Ghana Statistical Service, 2018).

2.3 Embryology and physiology of the placenta during pregnancy

According to Herrick & Bordoni, placenta develops gradually after implantation during the first three months of gestation. This temporary but vital organ possesses the same genetic characteristics as a developing child and performs a variety of functions such as endocrine, immune, and physiological (Herrick & Bordoni, 2019). During the early stages of pregnancy, placentation occurs in a relatively hypoxic environment, which favours proper embryonic development. This low oxygen milieu appears to prevent trophoblast differentiation towards an invasive phenotype. Increased intervillous blood flow occurs around 10–12 weeks of gestation, exposing the trophoblast to higher oxygen tension (Po2) (Caniggia et al., 2000). To maintain a successful pregnancy, it therefore requires effective feto–maternal interface during early placentation (Herrick & Bordoni, 2019). Successful invasion of trophoblast cells deep into the decidua to the uterine spiral arteries is required for optimal placental perfusion (Caniggia et al., 2000; Staff et al., 2022).

Parenchyma, chorion, amnion, and the umbilical cord make up the placenta. The zygote's fetal structures separate the fetus from the endometrium. The amnion, chorion, yolk sac, and allantois are fetal tissues that develop from the chorionic sac. The decidua is the maternal part that comes from the endometrium. The decidua is divided into three sections: the decidua

basalis (deep at the implantation site), the decidua capsularis (which covers the implantation site), and the decidua parietalis (everything else) (Herrick & Bordoni, 2019). After fertilization, the fertilized ovum develops into a morula, which then takes in fluid and forms a blastocyst. The blastocyst then differentiates into the inner cell mass (embryo) and outer cell mass (trophoblast or fetal placenta). Following blastocyst implantation into the endometrium, the trophoblast differentiates into the syncytiotrophoblast, which secretes human chorionic gonadotrophic hormone (hCG) and the cytotrophoblast, which secretes enzymes that break down the bond between endometrial cells, allowing the syncytiotrophoblast to invade the endometrial wall. The chorion is made up of the syncytiotrophoblast, cytotrophoblast, and extraembryonic mesoderm and forms the placenta (Guttmacher et al., 2014; Herrick & Bordoni, 2019).

Trophoblast stem cells, also known as cytotrophoblast cells, are found in two types of chorionic villi: floating and anchoring villi. The vast majority of chorionic villi are floating villi, which are bathed in maternal blood and primarily perform gas and nutrient exchange for the developing embryo (Caniggia et al., 2000). Extravillous trophoblast cells invade the uterine wall up to the first third of the myometrium and its associated spiral arteries, disrupting the endothelium and smooth muscle layer and replacing the vascular wall. This causes the narrow calibre arteries to dilate and become distended uteroplacental arteries, increasing blood flow to the placenta and allowing the growing fetus to receive adequate oxygen and nutrients. The extravillous trophoblast cells' invasive activity is highest during the first trimester of pregnancy, peaking around 10–12 weeks and then declining. Pre-eclampsia is caused by insufficient invasion and often results in fetal intrauterine growth restriction, maternal hypertension, and proteinuria (Caniggia et al., 2000).

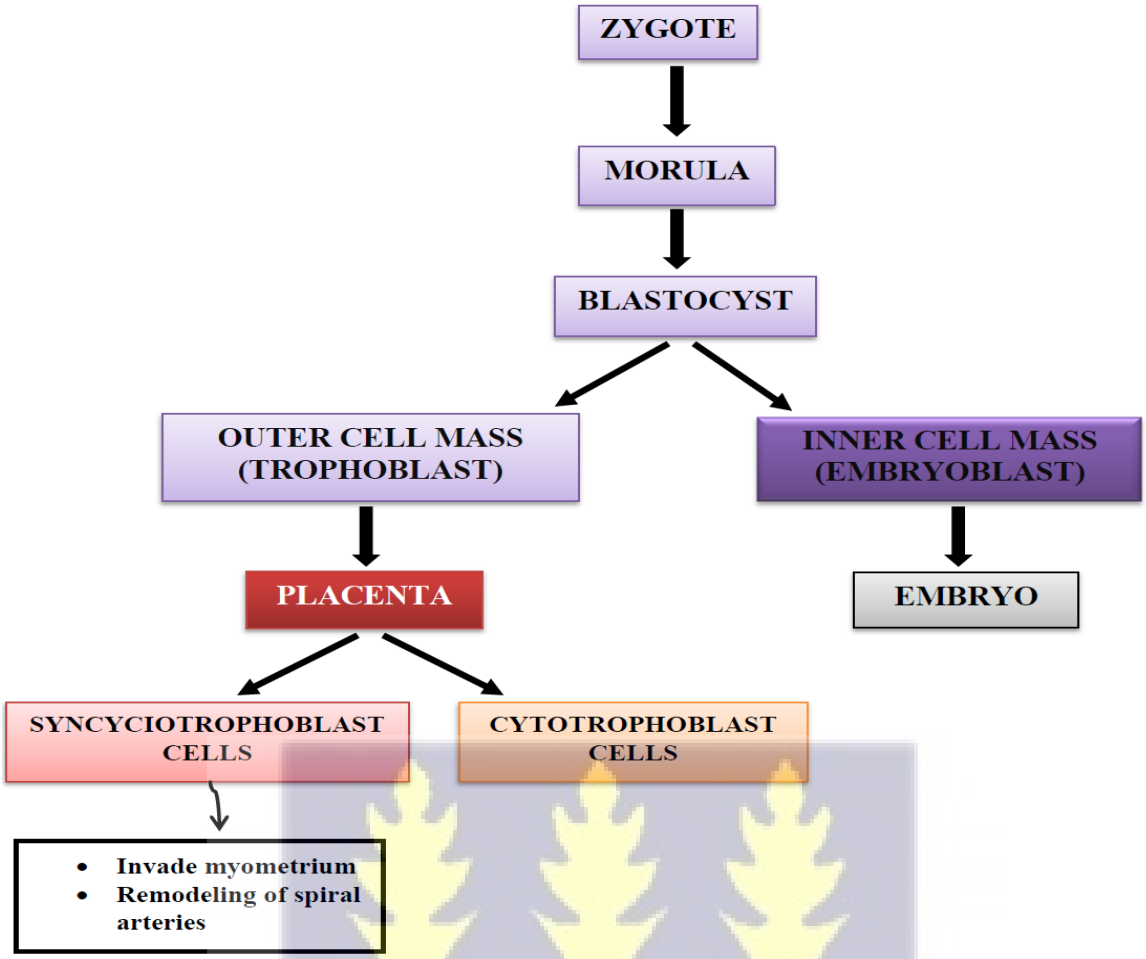


Figure 2: Embryology/development of the placenta during pregnancy

2.4 Pathophysiology of preeclampsia

PE is a heterogeneous disease of pregnancy which occurs after 20 weeks of gestation in women who were not hypertensive before the onset of the pregnancy. It manifests with a fresh onset of elevated blood pressure and urine proteins above the homeostatic threshold (George & Granger, 2014; Hutcheon *et al.*, 2011; Laresgoiti-Servitje, 2013; Rosser & Katz, 2013). Although there have been significant advances in clinical research, the etiology and pathophysiological mechanism of PE remains poorly understood. PE primarily is a multifaceted disorder of the placenta due to the abnormal functioning of the extravillous syncytiotrophoblasts leading to profound maternal inflammatory response and vascular endothelial dysfunction (Flint *et al.*, 2019; Hariharan *et al.*, 2016; Laresgoiti-Servitje, 2013;

Rosser & Katz, 2013). In addition to the placenta theory of the disorder, other theories including immunologic and genetic origins have been propounded to improve the understanding of the mechanistic pathway of the disease progression (Marwa & Sahar, 2011).

In normal pregnancy, the placenta largely serves as the maternal-fetal interface that guarantees adequate supply of oxygen and nutrients from the maternal circulation and elimination of waste products from the growing fetus throughout gestation (Al-Enazy *et al.*, 2017; Fu *et al.*, 2013; Ji *et al.*, 2013). It is a vascular-immunologic interface that allows adaptation to physiological changes of pregnancy, provides a harmonious coexistence whilst preventing rejection of the fetal allograft (Collier *et al.*, 2021; Ton *et al.*, 2020).

During normal placentation, physiologic differentiation of the trophoblast cell occurs through two fundamental pathways: (1) the villous pathway, characterized by the fusion of cytotrophoblast cells leading to the formation of multinucleated syncytiotrophoblast and (2) the extravillous pathway, where cytotrophoblast cells become an invasive phenotype characterized by invasion of the interstitial/endovascular extravillous trophoblasts of the decidua and a segment of the myometrium causing remodeling of the maternal vasculature. This differentiation is closely influenced by the interaction between oxygen tension, growth factors, hormones, transcription factors, and other signaling molecules (Ji *et al.*, 2013).

Widespread angiogenesis favors adequate placental perfusion and fetal development. Thus, invasion of the uterine arteries by fetal-derived extravillous cytotrophoblasts causes remodelling of uterine spiral arteries, converting the high-resistance, low capacitance vascular system to low-resistance, high-capacitance uterine arteriolar system. This allows improved blood flow and adequate placental oxygenation and nutrition for the maintenance of pregnancy (George & Granger, 2014; Hariharan *et al.*, 2016; Laresgoiti-Servitje, 2013).

In preeclampsia, pathological changes of heterogenous origin interfere with angiogenesis in the placenta due to failure of trophoblasts invasion and abnormal remodeling of the uterine

spiral arteries thus interfering with normal placental circulation and nutrition (Han *et al.*, 2020; Johal *et al.*, 2014). Thus, cytotrophoblast cells only invade the decidual segment of the uterus but fail to penetrate the myometrium. This leads to impaired conversion of the spiral arteries into large, tortuous high-capacitance vessels which results in vascular endothelial dysfunction impeding adequate placental perfusion with resultant placental ischemia (Hariharan *et al.*, 2016; Koppe *et al.*, 2014; Marwa & Sahar, 2011).

The poor placental perfusion and resultant ischemia cause a hypoxia-induced release of placental factors which lead to an imbalance between proangiogenic and antiangiogenic factors, increasing maternal oxidative stress, endothelial and immunological dysfunction (George & Granger, 2014; Han *et al.*, 2020; Hariharan *et al.*, 2016; Johal *et al.*, 2014). The ischemic placenta causes excessive expression of antiangiogenic factors such as soluble fms-like tyrosine kinase-1 (sFlt-1) and soluble endoglin (sEng) by the trophoblast cells into the maternal circulation. There is a correspondent decreased in proangiogenic factors such as vascular endothelial growth factor (VEGF) and placental growth factor (PlGF) which are necessary for vasculogenesis and maintenance of endothelial health. sFlt-1 blocks VEGF and PlFG from binding to their cognate receptors. This leads to impaired angiogenesis and decreased formation of vasodilators such as nitric oxide and prostacyclin. This results in vascular endothelial dysfunction characterised by increased synthesis of endothelin, reactive oxygen species (ROS) and enhanced vascular sensitivity to angiotensin II (Gilbert *et al.*, 2008; C. Han *et al.*, 2020; LaMarca, 2012; Mollie McDonnold & Gayle Olso, 2021).

The elevated maternal blood level of sFt-1 is manifested by severe hypertension, proteinuria, glomerular endotheliosis, increased risk of preterm birth, oligohydramnios, fetal growth restriction, and intrauterine foetal demise (Hariharan *et al.*, 2016; Mollie McDonnold & Gayle Olso, 2021). Also, in normal pregnancy endoglin (Eng), (a stress and proangiogenic factor) is derived from the syncytiotrophoblast cells and has a high affinity for transforming growth factor-β (TGF-β). In the presence of hypoxia, endoglin inhibits apoptosis in

endothelial cells. Eng also enhances the activity of endothelial nitric oxide synthase (eNOS) contributing to cardiovascular function. In PE, Eng is being antagonized by the antiangiogenic factor, soluble endoglin (sEng) release in response to oxidative stress or hypoxia (Flint *et al.*, 2019; Hariharan *et al.*, 2016).

While abnormal placentation and resultant ischemia remain the major hypothesized mechanism implicated in the pathogenesis of preeclampsia, the renin-angiotensin-aldosterone system (RAAS) also plays a fundamental role in its progression. In normal pregnancy, there is increased resistance to angiotensin II (Ang II) that aids maternal and placental vascular bed to remain low-resistance in the presence of low arterial pressure with correspondent improvement in blood flow. Normal pregnancy is associated with decreased uterine vascular contraction due to the increased expression of angiotensin receptor type 2 (AT_2) by the fetal kidney. Activation of AT_2 during pregnancy prevents cell growth, increases apoptosis, and promotes vasodilation and fetal tissue development (Xia, 2009). However, increased sensitivity to Ang II has been reported among pregnant women with preeclampsia although the specific mechanism that increases this sensitivity is unclear. It has been suggested that the Ang II type 1 receptor forms a heterodimer with bradykinin B2 which may increase the sensitivity to Ang II (Collier *et al.*, 2021; Laresgoiti-Servitje, 2013; Marwa, S & Sahar, 2011). Profound vasoconstriction ensues due to hypersensitivity of AT_1 to Ang II and thereby results in impaired blood flow.

Extravillous trophoblast cells may also produce a mixture of Human Leukocyte (HLA) class 1 antigens such as HLA-C, HLA-E, and HLA-G in the immunologic pathway. The expression of HLA class 1 antigens promotes the migration and activity of Natural Killer (NK) cells in the maternal decidua, resulting in dendritic cells proliferation. Dendritic cell proliferation leads to abnormal placentation and alteration in the maternal immune response to the fetal antigens(Marwa & Sahar, 2011). NK cells also increase the sensitivity to Ang II which has been reported among pregnant women with preeclampsia although the specific

mechanism that increases this sensitivity is unclear. It has however been suggested that the Ang II type 1 receptor forms a heterodimer with bradykinin B2 which may increase the sensitivity to Ang II(Collier *et al*., 2021; Laresgoiti-Servitje, 2013).

With regards to the genetic basis of PE, studies have shown that women who become pregnant for the first time with a family history of PE are at high risk. Again, a pregnant woman whose spouse's previous partner's pregnancy got complicated from PE is at potential risk of developing PE (Marwa & Sahar, 2011) although the inheritance pattern of the disorder still remain elusive.

The vascular endothelium performs several homeostatic activities, including vascular endothelial function, which are largely mediated by nitric oxide (NO). NO is an endothelium-derived relaxing factor primarily synthesized by a calcium-calmodulin dependent enzyme, endothelial nitric oxide synthase (eNOS), from the oxidation of L-arginine (Johal *et al.*, 2014). NO is a potent vasodilatory substance that acts by autocrine or paracrine signalling within the vascular endothelium in response to both mechanical and chemical stimuli (Johal *et al.*, 2014). It causes relaxation of vascular smooth muscles by binding and activating soluble guanylate cyclase receptor which catalyzes the production of the second messenger cyclic guanosine 3' 5' monophosphate (cGMP) (Johal *et al.*, 2014).

Endothelial (NOS) is known to modulate the activity of sFlt-1 by synthesizing nitric oxide (NO). However, deficient expression of eNOS has been detected in PE which increases oxidative stress by accelerating the severity of renal dysfunction. Deficiency in eNOS affects the biosynthesis of NO leading to impaired vasodilator effect on renal vasculature and renal dysfunction. Heme oxygenase (HO) is an enzyme responsible for converting heme into carbon dioxide (CO2), iron, and biliverdin. CO2 has a vasodilatory property on placental vasculature, allowing for adequate placental perfusion, and works synergistically with VEGF. HO potentiates the vasodilator effect of NO. Although the role of heme oxygenase (HO) in the progression of PE is still under investigation, other studies have demonstrated that this

enzyme contributes to fetal development and facilitates trophoblasts invasion of the uterine spiral arteries. In PE, the levels of HO were shown to be deficient contributing to oxidative stress (George & Granger, 2014; Hariharan *et al.*, 2016).

At the cellular level, HO prevents apoptosis by inhibiting reactive oxygen species (ROS) formation. Deficiency of HO will lead to accumulation of ROS thereby resulting in oxidative stress (George & Granger, 2014; Hariharan *et al.*, 2016).

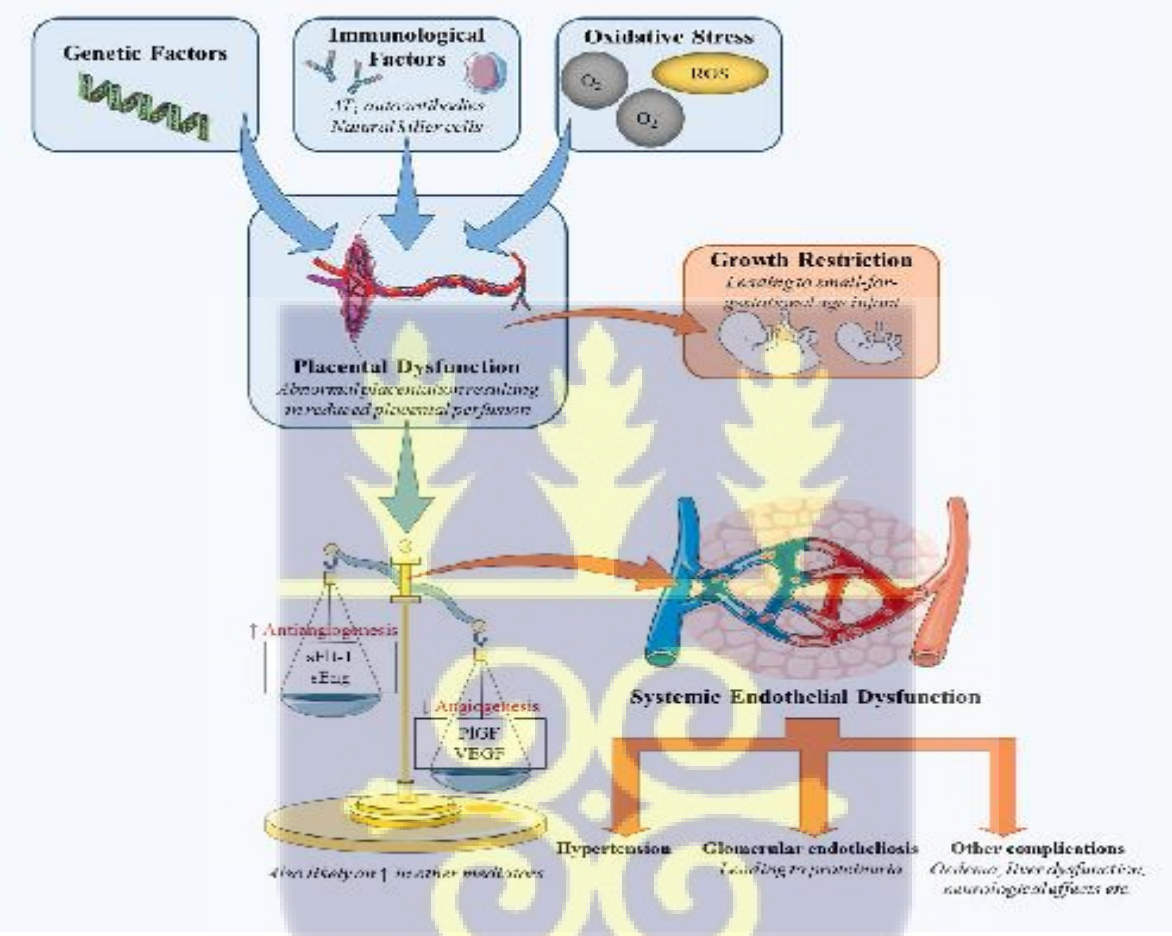


Figure 3a: Pathophysiological pathway of preeclampsia (Flint et al., 2019; Hariharan et al., 2016; Johal et al., 2014)

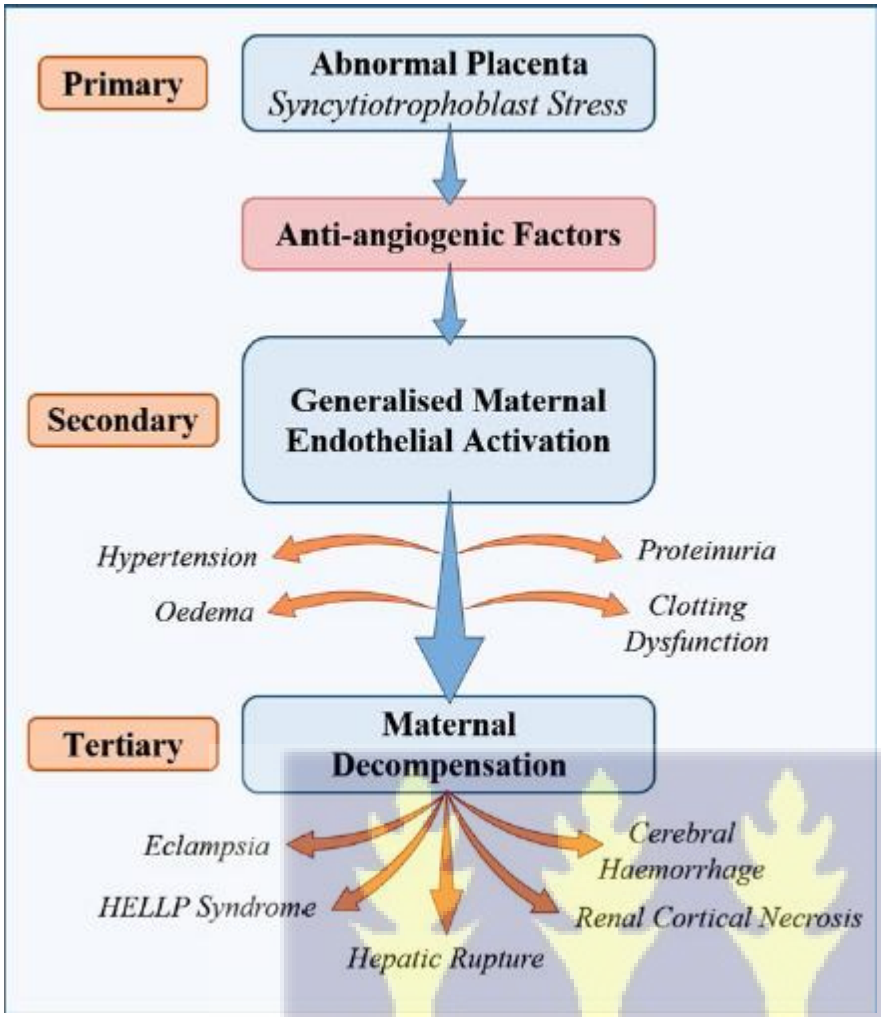


Figure 3b: Pathophysiological pathway of preeclampsia (Flint *et al.,* 2019; Hariharan et al., 2016; Johal *et al.*, 2014)

2.5 Soluble fms-like kinase-1 & VEGF in preeclampsia

In the last decade, despite the limited knowledge on the actual etiology of PE, clinical research has demonstrated that interplay between the expression of proangiogenic and antiangiogenic factors expression may play a crucial role in the pathogenesis of the disease. Evidence has shown that placental hypoperfusion and ischemia due to abnormal placentation accelerates the expression of hypoxia-inducible transcription factors (e.g. sFlt-1) which is responsible for the extensive vascular endothelial cell dysfunction in PE (George & Granger, 2014).

In PE, circulatory levels of sFlt-1 which are mainly expressed by placental trophoblast cells are shown to be significantly high 3 months before the onset of the symptoms. Further, sufficient evidence hypothesized that excessive expression of sFlt-1 primarily accounts for the abnormal placentation in preeclamptic women (Bergmann et al., 2010; George & Granger, 2014).

In normal pregnancy, proangiogenic factors such as VEGF and PIGF which are normally expressed in the invasive cytotrophoblast cell of the placenta inhibit sFlt-1. VEGF and PIGF are necessary for placental vasculogenesis and normal placentation in pregnancy. Perhaps, defective expression of VEGF and PIGF is due to the over-expression of sFlt-1 and inhibits their binding to their cognate receptors in PE (George, M E., & Granger, 2014; Laresgoiti-Servitje, 2013; Mollie McDonnold & Gayle Olso, 2021; Yi *et al.*, 2014).

According to George & Granger, both placental and plasma levels of sFlt-1 are considerably elevated in PE subjects, citing hypoxia and oxidative stress as the main triggers of sFlt-1 expression (George & Granger, 2014). Placental ischemia is also linked to excessive production of proinflammatory mediators such as Tumour Necrosis Factor- Alpha (TNF-α), Interleukins (IL-6, -8, 12, etc). TNF-α upregulation is another mechanism that appears to stimulate the release of sFlt-1 in the presence of hypoxia and ischemia in pregnant women who develop PE.

VEGF together with PIGF are potent proangiogenic factors that are necessary for maintaining vascular endothelial function in normal pregnancy (Guo *et al.*, 2021; Umapathy *et al.*, 2020). Findings of clinical research have proven that the levels of VEGF are abnormally low in PE than normal pregnant women. This assertion is concomitant with Kim *et al.*, (2012) who detected depressed levels of VEGF and visfatin in women with PE when compared to normal controls. According to Hong gene expression for VEGF was consistently low in women with PE as being reported by several other studies (Hong et al., 2014). As reported by

Andraweera, in Sri Lanka, VEGF gene polymorphisms were not associated with women with PE(Prabha H. Andraweera *et al*., 2013). Among Tunisian Arab women, Ben Ali Gannoun found a significant elevation of maternal VEGF and PIGF in women with early onset of PE (29-32 weeks of gestation) and (32-35weeks of gestation) compared to normal controls (Ben Ali Gannoun *et al.*, 2016). In another study, high VEGF levels were significantly associated with preterm delivery compared to term (Hong *et al.*, 2015).

According to Andraweera, VEGF correlates poorly with poor pregnancy outcomes including preterm delivery, small for gestational age, and can be used as a predictive biological marker for late-onset PE while elevated levels of sFlt-1 and PIGF were associated with severe adverse pregnancy outcomes and can be used as predictive markers for the development of PE with higher sensitivity and specificity (Andraweera *et al.*, 2012). However, other controversies regarding the expression of VEGF in PE exist. For instance, Tandon found that the levels of VEGF were significantly elevated in women with pregnancy-induced hypertension (PIH) (Tandon *et al.*, 2017). Similar contradictions were seen in other studies evaluating maternal levels of VEGF and endothelin-1 in preeclamptics and normal controls showed that VEGF levels were significantly higher in women with PE than the controls (Celik *et al.*, 2013). Ali also found that VEGF was significantly expressed in the placentas of women who developed PE (Ali *et al.*, 2019)

2.6 Angiopoietins in preeclampsia

Maintenance of a relatively stable balance between proangiogenic and antiangiogenic factors facilitates neovascularisation (formation of new vessels) which is necessary for normal placental growth and development throughout pregnancy (Kappou *et al*., 2015; Leijnse *et al*., 2018). In scientific research, four major ligands of the angiopoietin (Ang) system have been observed, thus, Ang (1 to 4). Ang-1 and Ang-2 have been implicated in the pathogenesis of PE (Kappou *et al.*, 2015; Leijnse *et al.*, 2018).

Angiopoietin 1 and 2 are proangiogenic factors expressed during early placenta development that play a crucial role in the growth of placental vasculature by binding to the same endothelial cell-specific tyrosine kinase receptor (Tie 2) (Sisay *et al.*, 2017) in the presence of VEGF (Schneuer *et al.*, 2014). These proangiogenic markers have antagonistic effects, in that; Ang-1 activates Tie 2 while Ang-2 inhibits Tie 2 in placental vasculature. Angiopoietins are said to promote placental growth and remodelling during placentation (Schneuer *et al.*, 2014). On the contrary, Schneier postulated that the Ang-1/Ang-2 ratio in the first trimester of pregnancy was associated with severe adverse pregnancy outcomes but poorer predictive outcomes values were found (Schneuer et al., 2014).

In normal pregnancy, Ang-2 acts as a natural antagonist of Ang-1 and is significantly elevated (Leinonen et al., 2010; Schneuer *et al.*, 2014). Stepan reported that serum levels of Ang-2, Ang-1/Ang-2 ratio were low with significantly increased Ang-2/sFlt-1 ratio at a gestational age between 25 and 28 weeks who later develop preeclampsia (Bolin *et al.*, 2009; Stepan *et al.*, 2009). Apostolakis also reported that Ang-1/ Ang-2 ratio showed a sensitivity of 47% and specificity of 87% in 25 to 28 weeks of gestation and can be used as a predictive biological marker for the diagnosis of late-onset of preeclampsia (Apostolakis *et al.*, 2009).

In contrast to Stepan, Han found that gene expression and plasma concentration of Ang-2 were significantly elevated in women with PE with evidence of positive correlations between them. He also added that maternal plasma concentration of Ang-2 recorded a 63% sensitivity rate with an 83% specificity rate of predicting severe PE (Han *et al.*, 2012). High Ang 1/Ang 2 ratios were also detected to be significantly associated with adverse pregnancy outcomes while maternal serum levels of Ang-1 and Ang-2 as markers for predicting adverse pregnancy outcomes (Schneuer *et al.*, 2014).

2.7 Relationship between Angiopoietins and VEGF in preeclampsia

During pregnancy, early placental expression of Ang-1 and Ang-2 are regulated by the presence of VEGF. For instance, VEGF promotes decoupling and downregulation of Tie-2 leading to diminution of active receptors on the cell surface. As a result, placental expression of Ang-1 is significantly high whereas declining levels of Ang-2 are normally observed in the early stages of pregnancy. However, these angiogenic markers have antagonistic effect on Tie-2. Binding of Ang-1 to the Tie-2 causes activation and promotion of capillary maturation while Ang-2 acts as a natural antagonist of Ang-1, destabilizing vessel growth thus accounting for the significant levels of Ang-2 (Leinonen *et al.*, 2010; Nadar *et al.*, 2005; Schneuer *et al.*, 2014). This destabilization favors vessel growth and enhanced vasculogenesis in the presence of VEGF. However, it is unclear how there is a shift from Ang-1 dominance to Ang-2 in later stages of pregnancy(Leinonen et al., 2010; Schneuer *et al.*, 2014).

In women with PE, elevated serum concentration of Ang-1 and low levels of Ang-2 have been reported by other studies (Leinonen *et al.*, 2010; Nadar *et al.*, 2005; Schneuer *et al.*, 2014). Ang-1 and Ang-2 regulate both vasculogenesis (De novo formation of vessels from endothelial progenitor cells) and angiogenesis (formation of new vessels from pre-existing ones) in the presence of VEGF-A. Additionally, Ang-1 is a weak mitogen involved in vascular endothelial cell maturation and stabilization while Ang-2 which is a natural antagonist to Ang-1, compliments the mitogenic activity of VEGF-A and its function is linearly related to the maternal concentration of VEGF(Geva *et al.*, 2002).

CHAPTER THREE

3.0 METHODOLOGY

3.1 Study Design

The study was a comparative cross-sectional study with cases and controls, comprising the following groups: 1) pregnant women with an established diagnosis of preeclampsia (cases), 2) pregnant normotensive women (control group 1), and 3) nonpregnant normotensive women (control group 2). A cross-sectional study is a form of observational study which allows the researcher to examine data at a particular time of a given population (DiPietro, 2010; Thompson & Panacek, 2007). It allows the record of information that is present in a population without manipulating the variables (Thompson & Panacek, 2007). Using a quantitative approach, this study design was chosen to find information regarding the association between serum concentrations of Angiopoietins and vascular endothelial growth factor (VEGF) in pregnant women with preeclampsia and their association with pregnancy outcomes.

3.2 Study Area

The study was conducted at the Department of Obstetrics and Gynaecology (O & G), Korle-Bu Teaching Hospital (KBTH), Accra, Ghana. As the largest tertiary healthcare institution in Ghana, the Department conducts approximately 11,000 deliveries annually. Being one of the affiliate departments of the University of Ghana Medical School, the Department is located within the KBTH. The department has a bed capacity of 240 for Obstetrics and 114 for Gynaecology. The department is made up of five units and each is headed by a senior consultant with other consultants, doctors, and midwives equally distributed among the units. The units are attached with respective clinics, theatres, and main wards. The department serves mainly as a referral centre for most healthcare facilities in the southern part of Ghana

with over 10 million people. It also serves as the primary training facility for medical and nursing students and postgraduate medical trainees of the Ghana College of Physicians and Surgeons and West African College of Surgeons.

3.3 Study population

Population refers to all individuals who have in common, certain characteristics that are of interest to the researcher. The study population was made up of Ghanaian citizens who met the study eligibility criteria as follows:

3.3.1 Inclusion criteria:

Participants with preeclampsia (cases):

1. Aged between 18 years and 42 years as at last birthday and without any medical condition or previous history of PE.
2. Established diagnosis of preeclampsia
3. Must have had an ultrasound scan done during the first trimester for confirmation and correct dating of pregnancy.

Pregnant normotensives (control group 1):

1. Aged between 18 years and 42 years as at last birthday.
2. Pregnant normotensives (blood pressure <140/90 mmHg after 20 weeks of gestation and negative urine protein results, in a previously normotensive woman).
3. Must have had an ultrasound scan done during the first trimester of pregnancy.

Non-pregnant normotensives (control group 2):

1. Aged between 18 years and 42 years as at last birthday
2. Non-pregnant normotensive (blood pressure <140/90 mmHg)

3.3.2 Exclusion Criteria for all study participants

1. Pregnant women with a history of underlying medical conditions such as long-standing hypertension, diabetes mellitus, smoking, kidney disease, thyroid dysfunction, urinary infection, inflammatory disorders, cardiac disease, and infectious diseases or women on any medication other than routine drugs (iron and folic acid).
2. Non-pregnant women with a history of underlying medical conditions such as hypertension, asthma, sickle cell disease, cardiac disease, and those on any hormonal medications such as contraceptives.
3. Pregnant women who decline to sign an informed consent and were unwilling to comply with the protocol guidelines
4. Not being in a steady state of health or sound mind

3.4 Ethical considerations

Throughout this study, procedures commensurate with ethical guidelines were followed. Ethical clearance and administrative approval were sought from the Ethical and Protocol Review Committee of the College of Health Sciences, University of Ghana (protocol number: CHS-Et/M.1 – 4.9/2021-2022). Further, all potential study participants were required to give their informed consent before their recruitment into the study. The potential risks and benefits of the study were elucidated to the participants individually. The participants were assured that their decision to either accept or decline participation would not in any way affect the normal management of their clinical conditions. Also, throughout the data collection and handling process, extreme respect was demonstrated for the rights and confidentiality of the study participants. All data related to the study were stored on a secure password-protected computer.

3.5 Sample Size Determination

The minimum sample size of this study for both cases and controls was determined using the formula:

$$\text{Sample size} = \frac{r+1}{r}\frac{SD^2(Z_\beta + Z_{\alpha/2})^2}{d^2}$$ (Charan & Biswas, 2013)

Where SD = Standard deviation = 2.21, taken from a previous study (Phupong *et al.*, 2021)

r = ratio of cases to control, thus, 1 for equal number of case and control

Zβ = Standard normal variate for power of 90% = 1.28

Zα/2 = Standard normal variate for level of significance at 95% = 1.96

d = Phupong et al. expected mean difference between cases and controls was 1.1

$$\text{Sample size} = \frac{1+1}{1}\frac{(2.21)^2(1.28+1.96)^2}{(1.1)^2} = 85$$

Therefore, a minimum of 85 cases and 85 controls were needed. After that, a 10% retention rate was added to the calculated sample size for the study to make a sample size of 94 participants for the cases and 94 for the controls.

3.6 Training of research assistants

Before the start of data collection, the research assistants involved in the data collection underwent a 3-day training course, on the overall project's procedures. The training course covered the administration of the different types of questionnaires, blood pressure measurements, and determination of urine proteins.

It also involved transport, laboratory processing and storage of blood samples.

Research assistants also received training on research ethics.

3.7 Study Recruitment

A screening log was completed by the principal investigator (PI) at the study site to provide documentation of all study participants that were reviewed for study eligibility and approached during admission to participate in the study.

The screening log included the following information:

1. Study identification number
2. Participant's name, age, gestational age
3. Participant's contacts – telephone.
4. Date of recruitment
5. Date of Study Consent
6. Participant's eligibility status for the study
7. Reasons for ineligibility for the study

3.8 Recruitment and sampling procedure

A systematic random sampling technique was used to recruit study participants. Registered pregnant normotensives and those with an established diagnosis of PE were recruited in their third trimesters that is, after 26 weeks of gestation at the antenatal clinic. Study participants were approached and assessed for eligibility to be included in the study as soon as the diagnosis of PE was established.

The same eligibility process outline above was used to recruit the non-pregnant normotensive controls from the gynecology clinic. To qualify for inclusion, all pregnant women must have done an ultrasound scan during the first half of their pregnancies for pregnancy confirmation and accurate pregnancy dating. The cases and controls were matched for age, parity, and gestational age.

For sampling of participants with preeclampsia, the sample frame consisted of all the preeclampsia record books registered at the antenatal clinics of the Obstetrics and Gynaecology Department. The first preeclampsia record book was selected at random. Before recruiting a participant, the aims and objectives of the study, the various procedures and tests to be used as well as the possible risks and benefits were explained. All relevant instructions for the study were discussed during the time of recruitment. Those willing to participate were then given their identification number and written informed consent was obtained.

3.9 Data collection tools and procedure

3.9.1 Sociodemographic and clinical characteristics

Using a standard structured data extraction form, the study participants were interviewed to obtain their demographic and clinical feature. Diastolic and systolic blood pressures were measured using the Mercury Sphygmomanometer blood pressure monitor with appropriate cuffs in the upright or supine position. Other relevant information such as blood pressure at booking (first antenatal care) was obtained from the participants' medical records. The non-pregnant normotensives had their blood pressures measured at the time of recruitment and were tested for proteinuria using dipsticks.

The American College of Obstetricians and Gynaecologists (ACOG) criteria adopted for the diagnosis of PE include blood pressure of ≥140/90 mmHg measured on two consecutive times, at least 4 hours apart and proteinuria (defined as ≥300 mg/24-hour urine protein or ≥1+ dipstick reading on a random urine sample) after 20th-week gestation in a previously normotensive woman(Adu-Bonsaffoh *et al.*, 2015; Hoedjes *et al.*, 2011). Early-onset PE was defined as PE that develops before 34 weeks of gestation, whereas late-onset PE develops at ≥34 weeks of gestation(Adu-Bonsaffoh *et al.*, 2015; Portelli & Baron, 2018). Severe preeclampsia was also defined according to ACOG criteria: as blood pressure of ≥160/110 mmHg, severe proteinuria (≥500mg/24 hours), haemolysis elevated liver enzymes and low

platelet count (HELLP) syndrome (defined by thrombocyte count <100*10^9/L, aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT) >30 U/L), convulsion, or fetal growth restriction (Hoedjes et al., 2011). The height and weight of study participants were recorded using their antenatal clinic (ANC) card. BMI in kg/m^2 was calculated for each study participant as body weight (kg) divided by the square of height (m). The WHO classification of BMI values was adopted: underweight as BMI < 18.5 kg/m^2; n ormal as BMI of ≥18.5 to <25kg/m^2; overweight at BMI of ≥25 to <30 kg/m^2; and obesity as BMI > 30kg/m^2.

3.9.2 Blood sampling

About 5mls of venous blood was obtained, after application of a tourniquet, from the antecubital fossa of each subject. In pregnant women with PE, the blood samples were obtained at the time of diagnosis before the start of any relevant medication used in the management of PE.

In the non-pregnant controls, the blood samples were obtained during the follicular phase of the menstrual cycle. The blood samples were collected into plain tubes. The blood samples were centrifuged at 250 rpm for 10 min within one hour of collection and the serum was aliquoted into cryo-tubes and stored in a fridge at −80 °C before the laboratory assays were performed. The sera were analyzed using Bio systems reagents in a semi-automated analyzer to determine levels of VEGF, Ang-1, and Ang-2.

3.10 Laboratory Analysis

3.10.1 Measurement of VEGF

For measurement of serum VEGF levels, a human VEGF enzyme-linked immunosorbent assay (ELISA) test kit (Quantikine, USA) was used. The assay was performed strictly according to the manufacturers' instructions. Recombinant human VEGF standard was diluted 2 times within a concentration range of 3000 pg/ml to 3100.25 pg/ml using 1.0 mL of

calibrator diluent RD6U. 100uL of standard, control, and serum samples were added to the wells and incubated for 2 hours at room temperature. After washing each plate four times, 200 µL of VEGF Conjugate (polyclonal antibody against VEGF conjugated to horseradish peroxidase) was added to each well and the plates were incubated for 2 hours at room temperature.

The plates were then washed 5 times and developed with 200 µL/well of 3,3′,5,5′-Tetramethylbenzidine TMB (4390A, Kem-En-Tec Diagnostics, USA) substrate for 25 min. The reaction was stopped using a sulphuric acid solution and optical densities read at 450 nm. The mean absorbance for each set of duplicate standard, control, and sample was then calculated, and the average zero standard optical density was subtracted to obtain the required values. The minimal detectable concentration of assay for VEGF as indicated in the manufacturer's manual was 62 pg/m. The Intra-assay and inter-assay coefficient of variation was 10% and the reported analytic sensitivity of the immunoassay was 62 pg/ml to 707 pg/mL for VEGF.

3.10.2 Measurement of Ang-1 and Ang-2

Measurements of serum Ang-1 and Ang-2 levels were done using the enzyme-linked immunosorbent assay (ELISA) test kits (Quantikine, USA). The assays were performed according to the recommendations of the manufacturer. All samples were double-checked and mean values for the individual sera were used for statistical analysis. As per the recommendation of the manufacturer's technical advice, interference with the measurement of each of the proteins did not occur in the presence of the Ang-1 and Ang-2 ELISA.

The ELISA plates were incubated overnight with 100 µL of diluted antibody for Ang-1 and Ang-2 in Phosphate buffered saline (PBS) (pH=7.2), followed by washing of plates three times (PBS pH 7.2and 5% Tween 20). 1% bovine serum albumin in PBS for 1 hour was used for the stabilization of any reaction. Recombinant proteins were serially diluted according to the manufacturer's guidelines. All samples were diluted 1:4 in reagent diluent. Samples that

produced higher OD than the standard were further diluted. The samples and standards were incubated for 1 hour at room temperature (on sker at 500rpm), followed by washing. Detection antibodies were added to the plates and were incubated for 1 hour.

After four times washing of the plates, streptavidin-HRP was added and incubated for 20 minutes. The substrate tetra methyl (TMB)/H202 was added and plates were incubated for 20 minutes at room temperature in the dark. 50 µL of stop solution (2N H2SO4) was then added to each well and OD was assessed at 450nm benzene. The minimal detectable concentration of the assays for Ang-1 and Ang-2, as indicated in the manufacturer's manual, were 62.5 and 17.0 pg/mL, respectively. The Intra-assay and inter-assay coefficient of variation was 10% and the reported analytic sensitivity of the immunoassay was 62.5 ng/ml to 84.0 ng/mL for Ang-1 and 17.0 ng/ml to 108.9 ng/mL for Ang-2.

3.11 Data Processing and Analysis

The data obtained in this study was entered into MS Excel 2010 and exported into SPSS version 22.0 for statistical analysis. Descriptive statistics (e.g. means, standard deviations frequencies, percentages, etc.) were used to summarize data on the demographic parameters (i.e. maternal age, gravidity, parity, and gestational age), clinical features (e.g. blood pressures, MAP and BMIs) and pregnancy outcomes of the participants. The parameters were compared between the normotensive controls and the PE subjects using Chi-square and one-way ANOVA and followed by a post hoc Tukey's analysis to determine the specific differences between the parameter in the subgroups with significant statistical differences.

Continuous variables such as serum levels of VEGF and angiopoietins were represented in median, interquartile ranges for nonparametric data followed by Kruskal-Wallis test to determine the specific differences between the PE subjects and normotensive controls. The maternal serum biomarkers of the participants with early- and late-onset PE, mild and severe were also compared using Mann-Whitney U test. A *P*-value of <0.05 was considered as sta-

tistically significant. The bivariate Pearson's correlation Coefficient and logistic regression analysis were used to determine the association between maternal biomarkers and pregnancy outcomes. Graphs and figures were also used to provide further illustration of the clinical parameters, laboratory tests, and pregnancy outcomes.

CHAPTER FOUR

4.0 RESULTS

4.1 Baseline characteristics of respondents

A total of 263 women were enrolled in this study including 75 non-pregnant normotensives, 94 pregnant normotensives, and 94 pregnant women with an established diagnosis of PE. The results presented in Table 1 showed that the average maternal age was 29.76±5.56 years for preeclamptics, 28.43±5.57 years for pregnant normotensives women, and 29.53±5.74 years for non-pregnant normotensives with no significant difference. Women with PE had significantly lower parity (0.77±1.23; p=0.004) compared to pregnant normotensives (1.32±1.34) but similar to the non-pregnant normotensives (0.83±1.08). The gravidity of women with PE (2.41±1.61; p=0.156) was similar to the pregnant and non-pregnant normotensive controls (2.83±1.71 and 2.44±1.54 respectively) was similar. Also, gestational age (GA) at recruitment was relatively the same for pregnant normotensives and pregnant women with PE (34.88±2.44 weeks and 34.31±2.74 weeks respectively) (p=0.131).

The blood pressures (BPs) at recruitment for the PE group were elevated (sBP= 164.97±19.46 mmHg and dBP= 105.94±10.82 mmHg) than the pregnant normotensives [mean sBP and dSP (111.71±11.40 mmHg and 69.64±8.50 mmHg) respectively] and non-pregnant normotensives [sBP and dSP (111.91±8.98 mmHg and 69.92±8.11 mmHg) respectively] (p<0.001). Also, mean arterial pressure (MAP) of women with PE (125.59±12.18 mmHg) was significantly elevated compared to the non-pregnant (83.90±6.66 mmHg) and pregnant (83.65±7.17 mmHg) controls (p<0.001). The results revealed that pregnant normotensives (27.33±8.72) and preeclamptics (29.45±6.26) had similar BMIs but were significantly overweight compared to the non-pregnant subjects (25.87±7.09).

Furthermore, pregnant women with PE had significantly worse pregnancy outcomes than the pregnant normotensives. For example, GA at birth was significantly lower among women with PE (36.20±1.71 weeks; $p<0.001$) compared to pregnant normotensives (38.91±1.28 weeks). Low birth weight (LBW) (2469.26±696.75 grams) also occurred among the PE group but not in pregnant normotensives (3237.23±507.63 grams) ($p<0.001$). Poor Apgar scores <7 were associated with women with PE [mean Apgar scores at 1st and 5th minutes (6.33±1.62 and 7.44±1.73) respectively] than the pregnant normotensives with mean Apgar scores of (7.20±1.06 and 8.47±1.07) respectively ($p<0.001$). Also, the risk of caesarean section delivery was higher in the PE subjects 68(72.3%) compared to the pregnant normotensives 21(22.3%) ($p<0.001$). Of the total number of preterm births recorded, 48 (96%) occurred in women with PE compared to only 2 (4%) in pregnant normotensive controls.

Table 1: General characteristics of women with and without preeclampsia and associated pregnancy outcome

Characteristic	Nonpregnant normotensive (n=75)	Pregnant normotensive (n=94)	Preeclampsia group (n=94)	P value
	Mean±SD	Mean±SD	Mean±SD	
Maternal Age (years)	29.53±5.74 [a]	28.43±5.57 [a]	29.76±5.56 [a]	0.228
Gradivity	2.44±1.54 [a]	2.83±1.71 [a]	2.41±1.61 [a]	0.156
Parity	0.83±1.08 [a]	1.32±1.34 [b]	0.77±1.23 [a]	0.004
GA at recruitment (weeks)	-	34.88±2.44	34.31±2.74	0.131
SBP at recruitment (mmHg)	111.91±8.98 [a]	111.71±11.40 [a]	164.97±19.46 [c]	<0.001
DBP at recruitment (mmHg)	69.92±8.11 [a]	69.64±8.50 [a]	105.94±10.82 [c]	<0.001
MAP (mmHg)	83.90±6.66 [a]	83.65±7.17 [a]	125.59±12.18 [c]	<0.001
BMI at Booking (kg/m²)	25.87±7.09 [a]	27.34±8.72 [b]	29.45±6.26 [b]	0.008
Pregnancy outcome				
GA at birth (weeks)		38.91±1.28	36.20±1.71	<0.001
Birth weight (grams)		3237.23±507.63	2469.26±696.75	<0.001
Apgar score at 1 min		7.20±1.06	6.33±1.62	<0.001
Apgar score at 5 min		8.47±1.07	7.44±1.73	<0.001
Mode of Delivery*				
SVD		76(74.5)	26(25.5)	<0.001
C/S		18(20.9)	68(79.1)	
Delivery*				
Preterm (n=50)		2(4.0)	48(96.0)	<0.001
Term (n=138)		92(66.7)	46(33.3)	

Statistically significant at p<0.05 on One-way ANOVA and chi-square; SD_Standard deviation; GA: gestational age; SBP: systolic blood pressure; DBP:diastolic blood pressure; MAP; mean arterial pressure; BMI: basal metabolic rate
[a]Non-pregnant nnormotensives
[b]Pregnant normotensives
[c]Preeclampsia group
*Results presented as number (percentage)

4.2 Determinants of pregnancy outcome associated with preeclampsia

The study's findings, as shown in Table 2, revealed that the rate of caesarean section was higher in late-onset PE 44/68 (64.7%) than the early-onset 24/68 (35.3%) although the association between the mode of delivery and the onset of the disease was not statistically significant (X^2=2.275). According to the data, pregnant women with early-onset PE delivered more females (38.8%) than males (22.2%) compared to late-onset PE subjects who delivered more males 35(77.8%) than females 30(61.2%) with no significant association found between sex of the baby and the disease onset (X^2=3.013). In the study, while poor Apgar score <7 at 1 minute was significantly associated with the onset of the disease (X^2=13.050), Apgar score <7 at 5 minutes was more prevalent in early-onset PE, 66.7%(8) than late-onset PE (4(33.3%)

(X2=8.271). Preterm birth and low birth weight were also found to be significantly associated with both early and late-onset of the disease (X^2=16.860) (table 2).

Assessment of pregnancy outcomes associated with mild and severe PE revealed that caesarean section 51(75.0%), Apgar scores 7 at 1 minute 31(73.8%) and 5 minutes 9(75.0%), preterm births 37(77.1%), and LBW 32(72.7%) were more common in those with severe PE compared to those with mild PE. However, no significant relationships between the disease severity and pregnancy outcome were found (X2=0.038). From the data, no significant association was found between the sex of babies and the severity of the disease (X^2=0.226). Furthermore, severity of the disease did not show significant relationship with the onset of the disease (X^2=1.185) although high prevalence of both early- 24(82.8%) and late-onset PE 47(72.3%) were found among the severe PE group compared to the mild PE (Table 2).

Table 2: Pregnancy outcome of preeclampsia

	Early-onset PE N (%)	Late-onset PE N (%)	X^2	Mild PE N(%)	Severe PE N(%)	X^2
Caesarean section n=68	24(35.3)	44(64.7)	2.275	17(25.0)	51(75.0)	0.038
Sex						
Male n=45	10(22.2)	35(77.8)	3.013	12(26.7)	33(73.3)	0.226
Female n=49	19(38.8)	30(61.2)		11(22.4)	38(77.6)	
Apgar at 1 min <7	21(50.0)	21(50.0)	13.050**	11(26.2)	31(73.8)	0.122
Apgar at 5 min <7	8(66.7)	4(33.3)	8.271**	3(25.0)	9(75.0)	0.002
Preterm delivery n=48	24(50.0)	24(50.0)	16.860**	11(22.9)	37(77.1)	0.128
LBW n=44	22(50.)	22(50.0)	14.218**	12(27.3)	32(72.7)	0.352
Onset of PE						
Early-	-	-	-	5(17.2)	24(82.8)	1.185
Late-	-	-		18(27.7)	47(72.3)	

***Pearson's Chi-square (X^2) significant at P<0.01; *P<0.05; PE_preeclampsia; LBW_low birth weight*

4.3 The maternal serum concentration of VEGF, Ang-1, and Ang-2

The study's findings revealed that maternal serum VEGF levels [4.71 (IQR: 3.65, 7.93) pg/ml] in the PE group were significantly lower than in the pregnant [83.52 (IQR: 78.64, 96.34) pg/ml] and non-pregnant (395.85 (IQR: 234.93, 625.06 pg/ml) normotensives (p 0.01). Although elevated maternal serum Ang-1 levels were found in the data, the difference

between women with PE [92.61 (80.92, 114.92) ng/ml] and normotensive [pregnant=99.26 (81.76, 113.12) ng/ml and non-pregnant=101.81 (84.65, 116.08) ng/ml] controls did not reach statistical significance (p>0.05). Serum Ang-2 levels were also significantly lower in women with PE [1.25 (IQR: 0.90, 2.15) ng/ml] than in pregnant normotensives [2.14 (IQR: 1.18, 5.73) ng/ml], but there was no significant difference in maternal serum Ang-2 levels between women with PE and non-pregnant controls [0.77 (IQR: 0.61, 1.04) ng/ml]. The data in Table 3 showed that Ang-1/Ang-2 ratio was higher in PE subjects [74.47 (IQR: 37.76, 107.33)] than in pregnant [45.98 (IQR: 16.11, 88.22)] normotensives, but significantly lower than the non-pregnant [144.41 (IQR: 84.81, 185.98) ng/ml] controls; (p0.01). The PE subjects had a lower VEGF/Ang-2 ratio [3.85 (IQR: 1.91, 11.38)] than the pregnant [39.59 (IQR: 14.12, 76.05)] and non-pregnant [458.66 (IQR: 244.37, 868.40)] controls (p<0.001) while Ang-1/VEGF ratio was also found to be significantly elevated in the PE group [24.50(9.44, 29.24);p<0.001] compared to the pregnant [1.15(0.94, 1.38)] and non-pregnant normotensive controls [0.37(0.16, .48)] (Table 3).

Table 3: Comparison between serum concentration of VEGF, Ang-1, and Ang-2 in preeclampsia and controls using Kruskal Wallis test followed by Posthoc Tukey's test

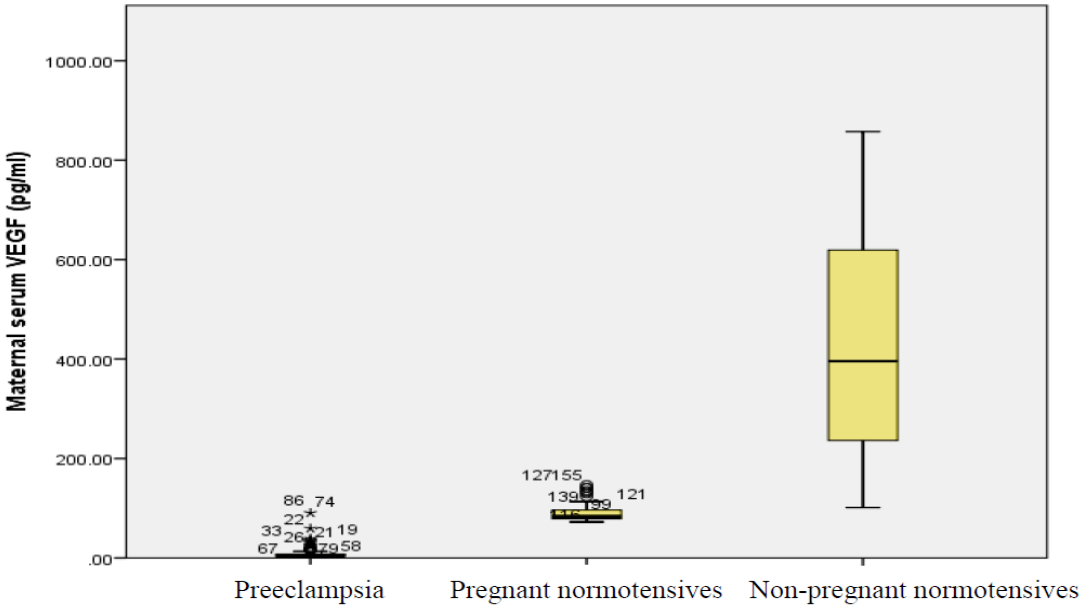
Maternal biomarkers	Pre-eclampsia	Pregnant normotensives	Non-pregnant normotensives	
	Median(IQR)	Median(IQR)	Median(IQR)	P value
VEGF (pg/ml)	4.71 (3.65, 7.93) [a]	83.52 (78.64, 96.34) [b]	395.85 (234.93, 625.06) [c]	<0.001
Ang-1(ng/ml)	92.61 (80.92, 114.92) [a]	99.26 (81.76, 113.12) [a]	101.81 (84.65, 116.08) [a]	0.749
Ang-2(ng/ml)	1.25 (0.90, 2.15) [a]	2.14 (1.18, 5.73) [b]	0.77 (0.61, 1.04) [a]	0.006
Ang-1/Ang-2	74.47 (37.76, 107.33) [a]	45.98 (16.11, 88.22) [b]	144.41 (84.81, 185.98) [c]	<0.001
VEGF/Ang-2	3.85 (1.91, 11.38) [a]	39.59 (14.12, 76.05) [b]	458.66 (244.37, 868.40) [c]	<0.001
Ang1/VEGF	24.50(9.44, 29.24) [a]	1.15(0.94, 1.38) [b]	0.37(0.16, .48) [b]	<0.001

Statistically significant at p<0.05 on kruskal wallis test; VEGF_vascular endothelial growth factor; Ang_angiopoietin; pg_picogram; IQR_Interquartile Range

[a]Pre-eclampsia

[b]Pregnant normotensives

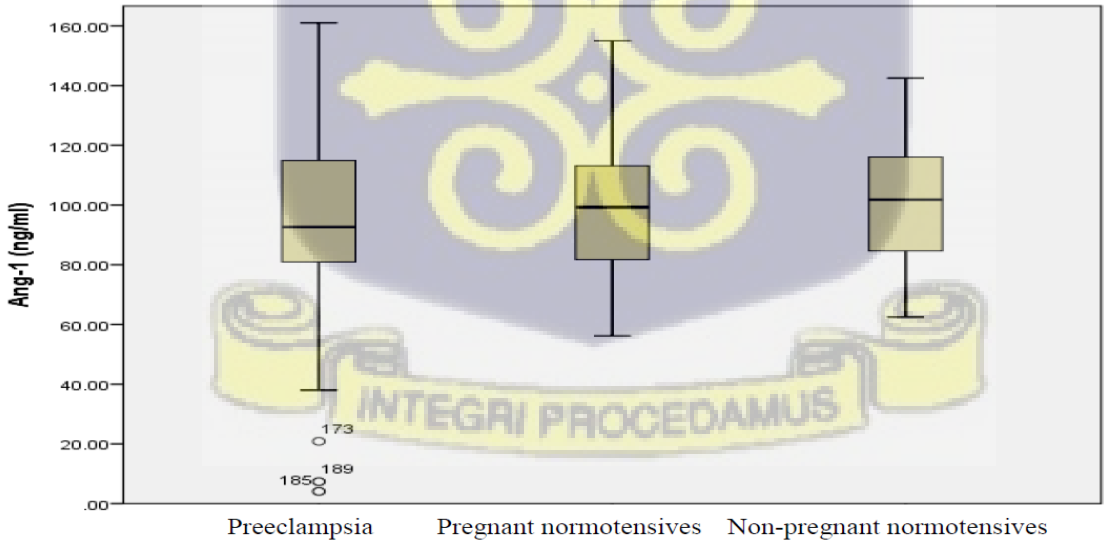
[c]Non-pregnant normotensives



p=0.001

Figure 4: Association between maternal serum levels of VEGF in preeclamptics and controls

Figure 3 shows that the maternal serum concentration of VEGF was significantly lower in preeclampsia compared to normotensive controls (p=0.001). For example, maternal VEGF levels were lower in the preeclamptic group compared to pregnant normotensives, whereas serum VEGF concentrations were significantly lower in the PE individuals compared to non-pregnant controls.



p=0.749

Figure 5: Association between maternal serum levels of Ang-1 in preeclamptics and controls

The data in figure 4 showed that maternal serum Ang-1 levels were significantly higher. Although maternal serum Ang-1 concentrations were higher in the PE compared to pregnant normotensives, no statistically significant difference was found. The same finding was made when comparing Ang-1 levels between PE subjects and non-pregnant normotensives (p=0.749).

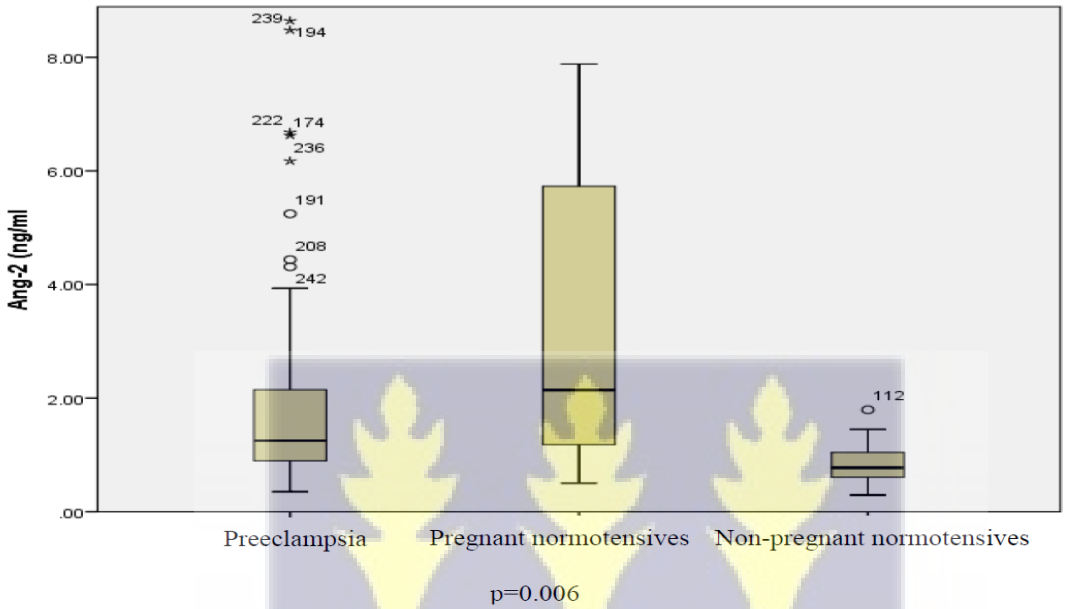


Figure 6: Association between maternal serum levels of Ang-2 in preeclamptics and controls

According to the data shown in the figure above, maternal serum levels of Ang-2 were found to be lower in women with PE compared to pregnant normotensives (p=0.006) and nearly equal to non-pregnant normotensives (Figure 5).

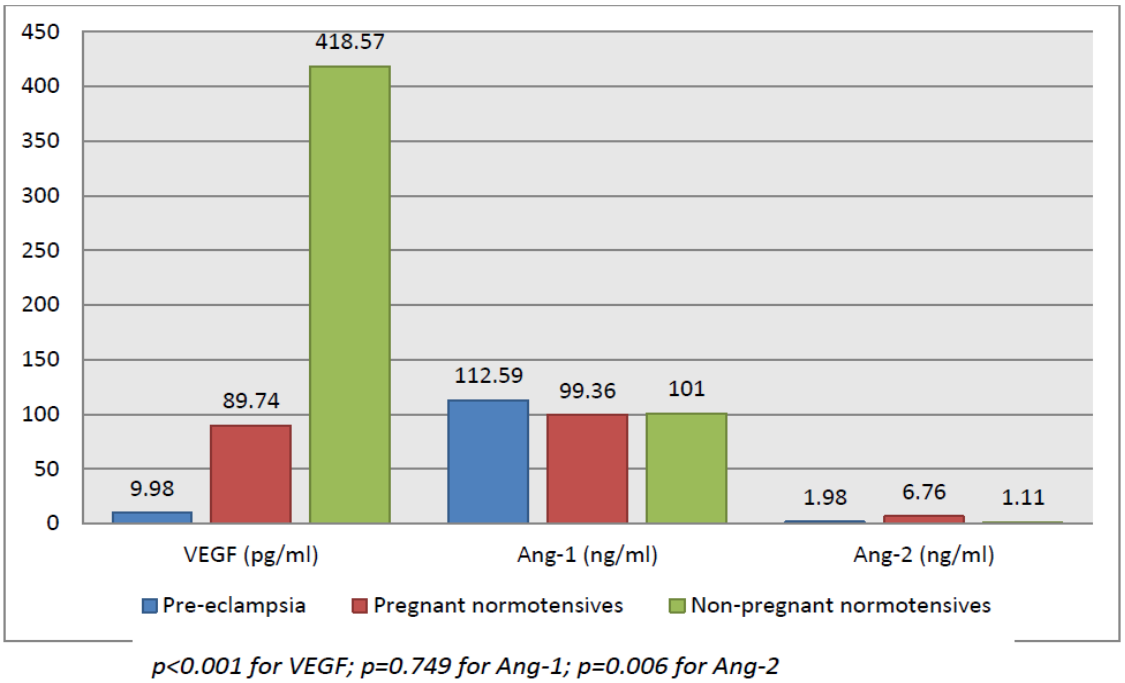


Figure 7: Relationship between serum VEGF, Ang-1, and Ang-2 in preeclampsia and normotensive controls

Figure 6 shows that serum VEGF concentrations in PE subjects were significantly lower (9.98 pg/ml) than in pregnant (89.74 pg/ml) and non-pregnant (418.57 pg/ml) normotensive controls (p0.001). Maternal serum Ang-1 levels in the PE (112.59 ng/ml) and normotensive groups (pregnant=99.36ng/ml and non-pregnant=101ng/ml) were not significantly different (p>0.05). Furthermore, serum Ang-2 levels in PE subjects (1.98 ng/ml) were similar to non-pregnant normotensives (1.11 ng/ml), but significantly lower than in pregnant normotensives (6.76ng/ml) (p=0.006).

4.4 Predicting the onset and severity of preeclampsia using maternal serum VEGF and angiopoietntins

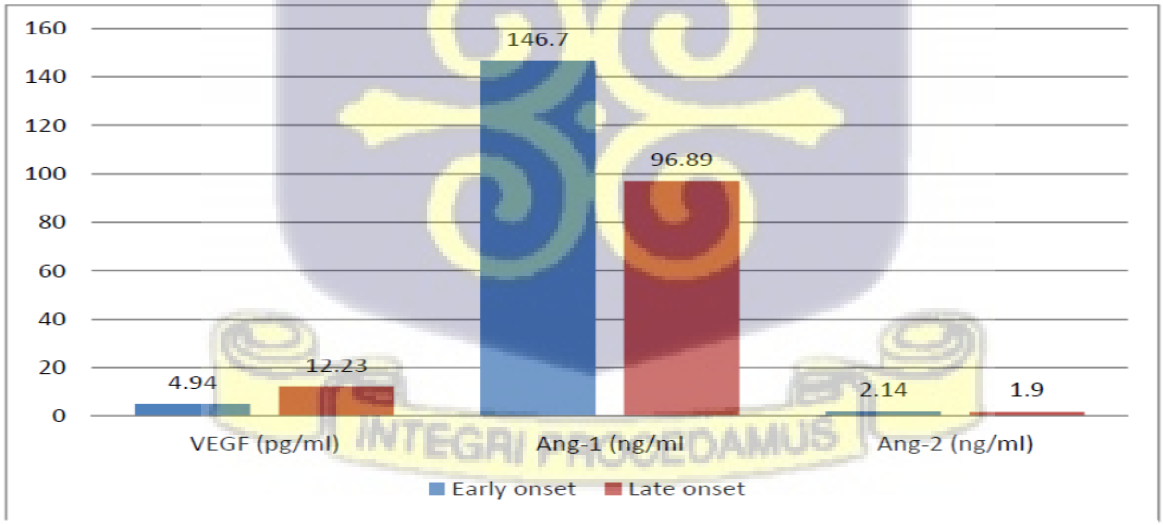
According to the data in Table 4, maternal serum VEGF levels were significantly lower in early-onset PE [(3.89 (2.87, 4.78)pg/ml;p=0.014] than in late-onset PE [5.23 (3.78, 16.97) pg/ml] and can be used as a predictive marker of early-onset PE. Early-onset PE serum levels of Ang-1 [94.04(64.47, 117.18) ng/ml] and Ang-2 [1.08(.83, 2.80) ng/ml] were found to be similar to late-onset PE [92.28(81.79, 114.92) ng/ml] and 1.26(.98, 1.94) ng/ml, respectively] (p>0.05) and may not be useful in predicting the onset of PE. Furthermore, no significant

differences in Ang-1/Ang-2 and Ang-2/VEGF ratios were observed between early-onset and late-onset PE [69.99(30.05, 128.70) and 2.77(1.31, 4.62) respectively] and late-onset PE [76.07(40.13, 107.33) and 4.33(2.31, 13.82) respectively] (p>0.05). The Ang-1/VEGF ratio was found to be higher in early-onset PE [20.62(15.76, 31.44)] than late-onset PE [17.55(6.22, 26.64)] (p=0.042) and may serve as a good marker in predicting early-onset PE. Furthermore, maternal serum levels of VEGF, Ang-1, Ang-2, Ang-1/Ang-2, Ang-2/VEGF, and Ang-1/VEGF ratios in mild PE did not differ significantly from those in severe PE (p>0.05) and cannot be used as good predictors of PE severity (Table 4).

Table 4: Maternal serum levels of VEGF, Ang-1, and Ang-2 in preeclampsia

Maternal biomarker	Preeclampsia					
	Early-onset	Late-onset		Mild	Severe	
	Median(IQR)	Median(IQR)	P value	Median(IQR)	Median(IQR)	P value
VEGF (pg/ml)	3.89(2.87, 4.78)	5.23(3.78, 16.97)	**0.014**	4.82(3.40, 13.18)	4.68(3.77, 7.93)	0.748
Ang-1(ng/ml)	94.04(64.47, 117.18)	92.28(81.79, 114.92)	0.238	85.58(57.20, 109.55)	94.04(81.79, 117.18)	0.451
Ang-2(ng/ml)	1.08(.83, 2.80)	1.26(.98, 1.94)	0.635	1.26(.96, 2.88)	1.25(.87, 2.04)	0.583
Ang-1/Ang-2	69.99(30.05, 128.70)	76.07(40.13, 107.33)	0.715	61.62(21.32, 113.84)	74.57(38.19, 107.33)	0.456
VEGF/Ang-2	2.77(1.31, 4.62)	4.33(2.31, 13.82)	0.117	3.59(1.35, 5.61)	3.86(1.91, 12.46)	0.381
Ang1/VEGF	20.62(15.76,31.44)	17.55(6.22,26.64)	**0.042**	16.49(9.86,30.01)	18.40(6.72,29.24)	0.550

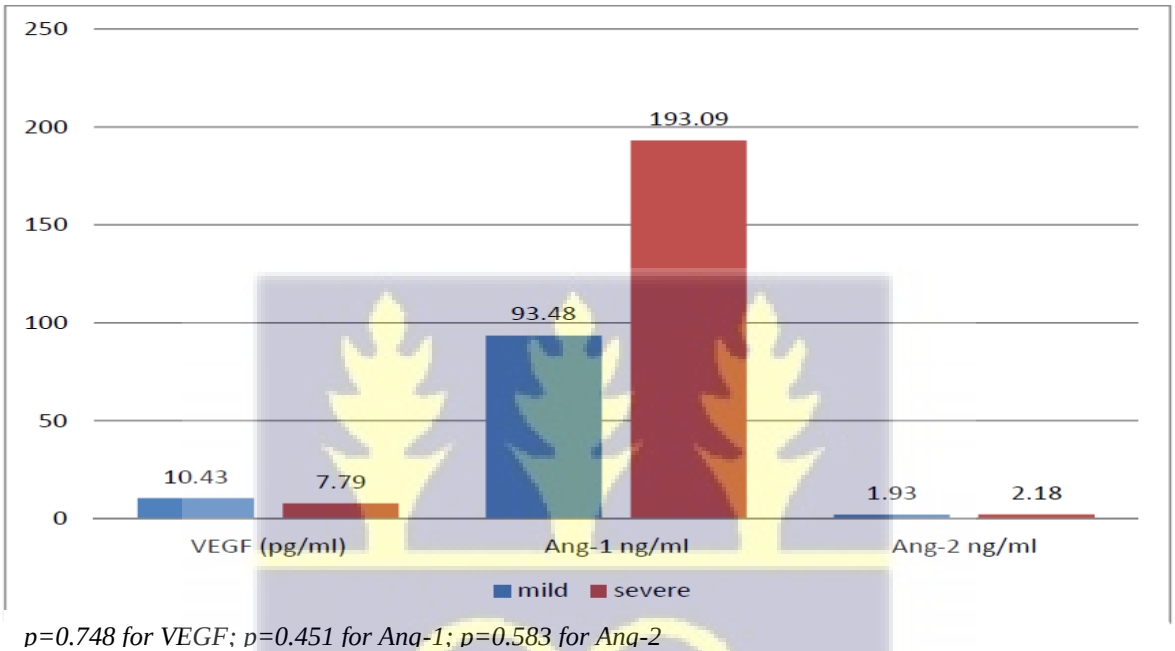
Statistically significant at p<0.05 on Mann-Whitney test; IQR_Interquartile Range; VEGF: vascular endothelial growth factor; Ang-1: angiopoietin 1; Ang-2: angiopoietin 2



p=0.014 for VEGF; p=0.238 for Ang-1 and p=0.635

Figure 8: Comparison of maternal serum levels of angiopoietins and VEGF between early-onset and late-onset of PE

According to the findings of this study illustrated in Figure 7, maternal serum VEGF levels were significantly lower in the early-onset PE (4.94 pg/ml) than in the late-onset PE (12.23 pg/ml) (p=0.014). Furthermore, although serum Ang-1 levels were higher in the early-onset group (146.7 ng/ml) than in the late-onset group (96.89 ng/ml), no significant difference was found (p=0.238). Again, maternal serum Ang-2 levels were similar in early-onset (2.14ng/ml) and late-onset (1.9ng/ml) of the disease (p=0.635)



p=0.748 for VEGF; p=0.451 for Ang-1; p=0.583 for Ang-2

Figure 9: Comparison of maternal serum levels of angiopoietins and VEGF between mild and severe preeclampsia

The data analysis shown in figure 8 revealed that there was no significant difference (p=0.748) in maternal serum VEGF levels between mild (10.43pg/ml) and severe PE (7.79pg/ml). Although serum Ang-1 levels in the severe PE were higher (193.09ng/ml) than in the mild PE (93.48ng/ml), this did not reach statistical significance (p=0.451). There was also no statistically significant difference (p=0.583) in serum Ang-2 concentrations between mild PE (1.93ng/ml) and severe PE (2.18ng/ml). According to the findings, there is no established relationship between the severity of the disease and its onset. This is because the data in Table 2 showed no correlation between the severity of the disease and the onset,

despite the fact that both early-onset and late-onset were found to be more common in severe PE compared to mild PE. Furthermore, as shown in Figures 7 and 8, there was no significant difference in maternal biomarker concentration.

4.5 Associations between maternal biomarkers and pregnancy outcome in preeclampsia

The association between maternal biomarkers and pregnancy outcome in PE was investigated using bivariate Pearson's correlation analysis. The study's findings revealed that maternal serum VEGF levels directly correlate with GA at birth (Pearson, r= 0.156; p<0.05), with highly significant correlations with Apgar scores at 1 minute, 5 minutes, and birth weight (Pearson, r=0.304, 0.330, and 0.536, respectively). Again, no correlation was found between maternal serum Ang-1 levels and GA at birth (Pearson, r=0.068), but were found to be inversely related to pregnancy outcomes such as Apgar score at 1 minute (Pearson, r=-0.145), Apgar score at 5 minutes (Pearson, r=-0.146), and birth weight (Pearson, r=-0.039). There was a significant positive correlation between serum Ang-2 levels and GA at birth (Pearson, r=0.180) and birth weight (Pearson, r=0.675; p0.01), but not with Apgar scores at 1 minute (Pearson, r=0.001) or 5 minutes (Pearson, r=0.057) (Table 5)

Table 5: Pearson's Correlation Coefficients Indicating the Association between Maternal Biomarkers and Pregnancy Outcomes

Pearson's Correlation	Variables	Maternal serum VEGF (pg/ml)	Maternal serum Ang-1 (pg/ml)	Maternal serum Ang-2 (pg/ml)
	GA at delivery	0.156*	0.068	0.180*
	Apgar Score at 1 min	0.304**	-0.145	0.001
	Apgar Score at 5 min	0.330**	-0.146	0.057
	Birth weight	0.536**	-0.039	0.675**

***Pearson correlation coefficient is highly significant at p < 0.01; *significant at p <0.05 (2-tailed)*

Using an analytic sensitivity of 62 pg/ml to 707 pg/mL for VEGF, the data in Table 6 show that after adjusting for maternal risk factors such as maternal age, parity, blood pressure, BMI, GA at recruitment, and GA at birth, maternal serum VEGF levels <62pg/ml was significantly associated with increased risk of preterm [aOR=3.881 (95%CI=0.002, 6.981); p<0.001], poor Apgar scores <7 at 1 minute [aOR=1.622(95%CI=0.301, 8.736); p<0.001] and 5 minutes [aOR=0.757(95%CI=0.036, 15.996)p=0.001] (p>0.05) and low birth weight (LBW) [aOR=0.096(95 percent CI=0.012, 0.740); p=0.025].

Table 6: Bivariate logistic regression model of VEGF levels <62pg/ml and Pregnancy outcome in PE

Outcome	Unadjusted Odd ratio(95%CI)	P value	Adjusted Odd ratio(95%CI)	P value
[a]Prematurity		0.000		<0.001
Yes	0.020(0.005,0.087)		3.881 (0.002, 6.981)	
No	Ref.		Ref.	
[b]Apgar score <7 at 1minute		0.000		<0.001
Yes	0.183(0.088,0.381)		1.622(0.301, 8.736)	
No	Ref.		Ref.	
[c]Apgar score <7 at 5minute		0.012		0.001
Yes	0.072(0.009,0.564)		0.757(0.036, 15.996)	
No	Ref.		Ref.	
[d]LBW		0.000		0.025
Yes	0.049(0.017,0.144)		0.096(0.012,0.740)	
No	Ref.		Ref.	

[a]Maternal age, parity, blood pressure, BMI, GA at diagnosis
[b]Maternal age, parity, blood pressure, BMI, GA at diagnosis, GA at birth
[c]Maternal age, parity, blood pressure, BMI, GA at diagnosis, GA at birth
[d]Maternal age, blood pressure, BMI, GA at diagnosis

With an analytic sensitivity of 62.5 ng/ml to 84.0 ng/mL for Ang-1, maternal serum Ang-1 levels >84ng/ml was not associated with adverse pregnancy outcomes such as preterm birth [aOR=1.003(95%CI=0.998, 1.008), poor Apgar scores <7 at 1 [0.989(95%CI=0.974, 1.003) and 5 minutes [aOR=0.991(95%CI=0.976, 1.007) and LBW [aOR=0.996(95%CI=0.990, 1.002) after adjusting for maternal risk factors (Table 7).

Table 7: Bivariate logistic regression model of serum Ang-1 >84ng/ml and Pregnancy outcome in PE

Outcome	Unadjusted Odd ratio(95%CI)	P value	Adjusted Odd ratio(95%CI)	P value
[a]Prematurity		0.495		0.286
Yes	1.004(0.992, 1.017)		1.003(0.998, 1.008)	
No	Ref.		Ref.	
[b]Apgar score <7 at 1minute		0.443		0.118
Yes	0.996(0.984, 1.007)		0.989(0.974, 1.003)	
No	Ref.		Ref.	
[c]Apgar score <7 at 5minute		0.236		0.264
Yes	0.996(0.989, 1.003)		0.991(0.976, 1.007)	
No	Ref.		Ref.	
[d]LBW		0.322		0.159
Yes	0.998(0.994, 1.002)		0.996(0.990, 1.002)	
No	Ref.		Ref.	

[a]Maternal age, parity, blood pressure, BMI, GA at diagnosis
[b]Maternal age, parity, blood pressure, BMI, GA at diagnosis, GA at birth
[c]Maternal age, parity, blood pressure, BMI, GA at diagnosis, GA at birth
[d]Maternal age, blood pressure, BMI, GA at diagnosis

Also, with an analytic sensitivity of 17.0 ng/ml to 108.9 ng/mL for Ang-2, maternal serum levels of Ang-2 <17ng/ml was not a good indicator for predicting adverse pregnancy outcome such as preterm birth [aOR=1.020(95%CI=0.000, 4.606)], poor Apgar scores <7 at 1 [aOR=0.995(95%CI=0.957, 1.035)] and 5 minutes [aOR=0.969(95%CI=0.922, 1.019)] and LBW [aOR=0.987(95%CI=0.911, 1.068)] (Table 8).

Table 8: Bivariate logistic regression model of serum Ang-2 <17ng/ml and Pregnancy outcome in PE

Outcome	Unadjusted Odd ratio(95%CI)	P value	Adjusted Odd ratio(95%CI)	P value
[a]Prematurity		0.125		p>0.05
Yes	1.145(.963, 1.360)		1.020(0.000, 4.606)	
No	Ref.		Ref.	
[b]Apgar score <7 at 1minute		0.463		0.815
Yes	1.018(0.971, 1.067)		0.995(0.957, 1.035)	
No	Ref.		Ref.	
[c]Apgar score <7 at 5minute		0.916		0.225
Yes	1.003(0.945, 1.065)		0.969(0.922, 1.019)	
No	Ref.		Ref.	
[d]LBW		0.269		0.743
Yes	1.081(0.941, 1.242)		0.987(0.911, 1.068)	
No	Ref.		Ref.	

[a]Maternal age, parity, blood pressure, BMI, GA at diagnosis
[b]Maternal age, parity, blood pressure, BMI, GA at diagnosis, GA at birth
[c]Maternal age, parity, blood pressure, BMI, GA at diagnosis, GA at birth
[d]Maternal age, blood pressure, BMI, GA at diagnosis

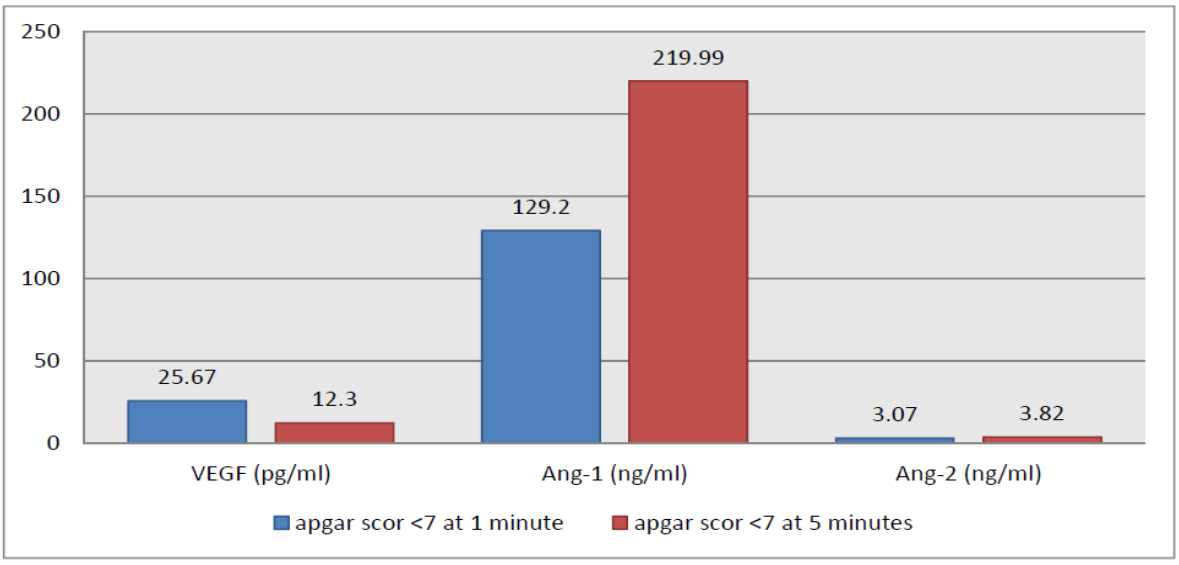


Figure 10: Association between biomarker levels and poor Apgar scores

According to the results of the study, low maternal serum VEGF levels were identified in low Apgar scores <7 at 1 minute (25.67 pg/ml) and 5 minutes (12.3 pg/ml). Furthermore, elevated serum levels of Ang-1 were observed in poor Apgar scores <7 at 1 minute (129.2 ng/ml) and 5 minutes (219.99 ng/ml), but depressed serum levels of Ang-2 were seen in Apgar scores <7 at 1 minute (3.07 ng/ml) and 5 minutes (3.82 ng/ml) (Figure 9).

CHAPTER FIVE

5.0 DISCUSSION

PE remains a serious maternal hypertension syndrome due to an angiogenic imbalance caused by a lack of pro-angiogenic factors in the blood and an excess of anti-angiogenic factors. As a result, maintaining a reasonably steady balance of proangiogenic and antiangiogenic factors promotes neovascularisation (formation of new vessels). This is required for optimal placental growth and development during pregnancy (Kappou *et al.*, 2015; Leijnse *et al.*, 2018). The study's goal was to look at the link between maternal serum angiopoietins, VEGF levels in preeclampsia, and pregnancy outcomes and how this can contribute to improved understanding of the pathogenesis of PE.

5.1 Baseline information and pregnancy outcomes of preeclampsia

Although the actual pathophysiological mechanism of PE is not yet known, several risk factors including extremes of maternal age, poor ANC services, low level of education, primiparity, multiple pregnancies, hereditary, rural dwellers, male fetus, maternal medical condition such as diabetes, obesity, and renal disease may contribute to its occurrence (Bilano et al., 2014). Regardless of maternal risk factors, multiparity is a strong protective factor and is associated with a lower incidence of preeclampsia; rather, nulliparous women have greater rates (Košir Pogačnik et al., 2020). This present study found that primiparous women (0.77±1.23; p=0.004) were at increased risk of PE compared to the pregnant normotensives (1.32±1.34) but similar to the non-pregnant normotensives (0.83±1.08). This study's findings also were comparable to those of a recent study conducted in Tokyo, Japan, which found that multiparity was related to a lower risk of preeclampsia (Maeda et al., 2021). Similar findings were reported by several other studies (Bilano *et al.*, 2014; Lisonkova & Joseph, 2013; Paré *et al.*, 2014).

Conventionally, normal physiological process of pregnancy is characterised by significant weight gain occurring in normal pregnancy, due to the increased maternal physiological changes and development of the gravid uterus. However, overweight and obesity may increase the risk of pregnancy complications including PE. This study revealed that the PE subjects were overweight (29.45±6.26) similar to the pregnant normotensives (27.33±8.72), but significantly higher than the non-pregnant controls (25.87±7.09). This study asserts that the normal physiological changes or growing nature of the uterus could account for the increased weight gain although the overweight detected in the study may also accelerate the development of pregnancy complications including PE. According to Poorolajal & Jenabi, (2016) overweight and obesity were significantly associated with PE and may be used to predict the risk of PE. Sohlberg et al., (2012) also reported that high BMI increases the risk of PE in all categories and severity of the disease whiles obesity increases the risk of PE about three folds (Roberts *et al.*, 2011). These findings were similar to those of this study.

The study demonstrated that although maternal age, gravidity, and GA at diagnosis of both study group and controls were similar, PE subjects were at increased risk of preterm delivery (36.20±1.71 weeks) with a 96% overall prevalence rate of preterm birth compared to the pregnant controls (38.91±1.28 weeks). As a common pregnancy complication of placental origin (Flint et al., 2019), PE is associated with varying degrees of adverse maternal and perinatal outcomes and poses a significant threat to the survival of both mother and the fetus (C. Han et al., 2020). For example, PE has been linked to perenatal problems such as low birth weight, intrauterine growth restriction, preterm births, intrauterine fetal demise, and early neonatal mortality, according to studies (Collier et al., 2021; C. Han *et al.*, 2020; Hoedjes *et al.*, 2011).

In the current study, significant adverse pregnancy outcomes such as LBW <2500 grams, poor Apgar scores <7 at 1minute (6.33±1.62), and preterm deliveries (98%) occurred in the PE group compared to the pregnant controls. Similar findings were reported by Miller *et al.*,

(2010) and also by Parra-Pingel *et al.*, (2017). In the study, PE subjects were at increased risk of preterm delivery (36.20±1.71 weeks) with a 96% overall prevalence rate compared to the pregnant controls (38.91±1.28 weeks). This is consistent with findings by Morisaki et al., (2017) who found that the risk of preterm delivery was mediated by an increased risk of PE. Similar findings were reported by Davies et al., (2016) in a population-based case-control study who found a significant positive correlation between PE and the risk of preterm delivery. Other studies emphasized that PE is a major risk factor for preterm delivery with increased maternal and perinatal complications (Pattanashetti *et al.*, 2013; Rolnik *et al.*, 2017).

According to Madazli et al., (2014) severe adverse maternal (e.g. placenta abruption, oligohydramnios, eclampsia, HELLP syndrome) and neonatal complications (e.g. small for gestational age, poor Apgar score <7 at 5 minutes, stillbirths and early neonatal deaths) were reportedly high and evident among women with early-onset of PE compared to those with late-onset. In contrast, findings of the present study discovered that the onset of the disease had no effect on mode of delivery or the gender of the baby, despite the fact that caesarean section and male gender were more common in late-onset PE [64.7% (68) and 77.8% (35) respectively] than in early-onset PE. Significant associations were found between the onset and adverse pregnancy outcomes such as low Apgar scores <7 at 1 and 5 minutes, preterm birth, and low birth weight despite the fact that the prevalence were the same in both early- and late-onset PE. This contradicts with findings of other studies indicating that severe adverse maternal and perinatal complications such as fetal growth restriction, poor Apgar scores, preterm births, and LBW were strongly associated with the early-onset PE compared to the late-onset (Shankar *et al.*, 2019; Stubert *et al.*, 2014; Wadhwani *et al.*, 2020).

Furthermore, the study revealed that pregnancy outcomes such as caesarean section 51(75.0%), Apgar scores <7 at 1 minute 31(73.8%) and 5 minutes 9(75.0%), preterm births 37(77.1%), and LBW 32(72.7%) were more common in those with severe PE compared to

those with mild PE. However, no significant relationships between the disease severity and pregnancy outcome were found. The study's finding collaborates with reports by Amorim et al., (2015) who found that severe PE is significantly associated with adverse pregnancy outcomes and also by other studies that significant adverse maternal and perinatal outcomes had a strong association with severe PE compared to the mild PE (Bodnar *et al.*, 2014; Hoedjes *et al.*, 2011; Kongwattanakul *et al.*, 2018; Ton *et al.*, 2020).

According to Staff & Redman, (2018), both early- and late-onset PE have significant clinical differences and are associated with adverse pregnancy outcomes. The early-onset for example, has been linked to poor placentation and foetal growth restriction, whereas maternal factors have been proposed to induce the late-onset condition, which does not have a placental influence. In the current study, severity of the disease did not show significant relationship with the onset of the disease (X^2=1.185) evidenced by high prevalence of both early- 24(82.8%) and late-onset PE 47(72.3%) occurring among the severe PE group compared to the mild PE. This suggests that the severe form of the disease may present with placental insufficiency and dysfunction, as a result, increase the risk of both early- and late-onset PE compared to the mild PE. This agrees with a study by Staff & Redman, (2018).

5.2 Maternal serum angiopoietins, VEGF levels in preeclampsia and pregnancy outcomes

Adequate uteroplacental perfusion is required for a normal pregnancy outcome, which is achieved by extravillous trophoblast (EVT) transformation of the uteroplacental vasculature following proliferation, migration, and invasion of these cells into the maternal decidua. The physiological role of pro-angiogenic factors such as VEGF and PIGF, which are primarily produced by the placenta's extracellular matrix, has a significant influence on uteroplacental vascular remodelling during normal pregnancy (Hoffmann et al., 2009; Mandala & Osol, 2012). According to Hoffmann et al., (2009) the circulating levels of placental endocrine

gland-driven vascular endothelial growth factor (EG-VEGF), which is thought to play a key role in trophoblast differentiation and angiogenesis during the first trimester of normal pregnancy, may be altered in PE (Zhou et al., 2010). Findings of the present study revealed that maternal serum VEGF levels [4.71 (IQR: 3.65, 7.93) pg/ml] and VEGF/Ang-2 ratio in the PE group were significantly lower than in the pregnant [83.52 (IQR: 78.64, 96.34) pg/ml] and non-pregnant (395.85 (IQR: 234.93, 625.06 pg/ml) normotensives ($p< 0.01$). This study's findings were consistent with those of Zhou et al., (2010), who discovered that serum VEGF levels in PE subjects were significantly lower when compared to those in the normal pregnant group, despite VEGF mRNA expression in PE placental tissue. The lower serum VEGF levels detected in the current study may have had an impact on the successful invasion and remodelling of the uteroplacental vasculature, resulting in impaired vasculogenesis, impaired placental perfusion, and poor pregnancy outcomes.

Furthermore, Ang-1 and Ang-2 are also pro-angiogenic factors, promoting endothelial health, vascular maturation, and stability. They cause angiogenesis within the placental vascular by binding to the same endothelial cell-specific tyrosine kinase receptor, and their activity is tightly regulated by the physiological action of VEGF (Burton et al., 2019; Kappou et al., 2015; Melincovici et al., 2018). Several studies proposed that Ang-1 and Ang-2 have been shown to have antagonistic properties during normal pregnancy. This is due to the fact that, whereas Ang-1 promotes vascular cell maturation, growth, and stabilization, Ang-2 promotes apoptosis and destabilization of vessel growth in the absence of VEGF (Leinonen *et al.*, 2010; Melincovici *et al.*, 2018; Nadar *et al.*, 2005; Schneuer *et al.*, 2014).

To buttress this point, unlike Ang-1, Ang-2 is a strong mitogen which activity appears to compliment the function of VEGF in normal pregnancy by rendering endothelial cells more accessible to VEGF (Burton *et al.*, 2019; Geva *et al.*, 2002; Melincovici *et al.*, 2018). Also, the function of Ang-2 is hypothesized to be linearly related to VEGF, as a result, Ang-2

concentrations in PE have been reported to be significantly depressed with elevated levels of Ang-1(Kamal & El-khayat, 2011; Nadar *et al.*, 2005) and this hypothesis agrees with the results of the current study which identified that maternal serum Ang-2 levels were significantly depressed in women with PE in comparison to the pregnant controls. In the study, although elevated maternal serum Ang-1 levels were found, the difference between women with PE and normotensive controls did not reach statistical significance ($p>0.05$). In contrast, Leinonen et al. reported that at 12 – 15 weeks of gestation maternal serum Ang-1 and Ang-2 were high, and elevated serum levels of Ang-2 occurred at 16- 20 weeks of gestation in women who subsequently developed PE. Ang-1/Ang-2 and Ang-1/VEGF ratios were also found to be higher in PE subjects than in pregnant normotensives controls (p0.01). This finding contradicts with Bolin et al., who found that serum Ang-1/Ang-2 ratio was significantly lower in pregnant women between 25 to 28 weeks of gestation who later developed PE.

Also, maternal serum VEGF levels were significantly lower in early-onset PE than in late-onset PE while Ang-1/VEGF ratio was higher in early-onset PE than late-onset PE and therefore may serve as good predictive markers in predicting early-onset PE. This finding differs significantly from findings by Cim et al., (2017) who found no significant difference in serum VEGF levels among the different subgroups of PE(Cim *et al.*, 2017) and also by Puttapitakpong & Phupong, (2016) who rather found serum Ang-2 to be associated with GA at 16 – 18 weeks and identifying it as a marker to predict early-onset of PE. Furthermore, maternal biomarkers in mild PE did not differ significantly from those in severe PE ($p>0.05$) and cannot be used as good predictors of PE severity but contradicts with Sala et al., report that serum VEGF levels were high in late-onset and severe forms of PE compared to the early-onset and mild form of the disease

5.3 Associations between maternal biomarkers and pregnancy outcomes in preeclampsia

According to Schneuer *et al.,* high Ang 1/Ang 2 ratios were significantly associated with adverse pregnancy outcomes while maternal serum levels of Ang-1 and Ang-2 can be used as markers for predicting adverse pregnancy outcomes. On the other hand, this study identified maternal serum concentration of VEGF and Ang-2 to be positively correlated with pregnancy outcomes such as GA, Apgar scores, and birth weight while maternal serum Ang-1 level was inversely correlated with pregnancy outcomes such as GA at delivery, Apgar scores <7 at 1 and 5 minutes and birth weight. Also, after adjusting for maternal risk factors such as maternal age, parity, blood pressure, BMI, GA at recruitment, and GA at birth, maternal serum VEGF levels <62pg/ml was significantly associated with increased risk of preterm, poor Apgar scores <7 and low birth weight (LBW), while serum Ang-1 levels >84ng/ml was not associated with adverse pregnancy outcomes. Also, serum levels of Ang-2 <17ng/ml did not show any significant associations with adverse pregnancy outcome. This affirms findings by Schneuer et al (2014) and Akolekar, et al (2009) that after adjusting for maternal and clinical risk factors, women with low Ang-2 levels and high Ang-1/Ang-2 ratio had increased risk of developing most adverse pregnancy outcomes.

5.4 Strengths

Major strengths of this study include; the adoption of the American College of Obstetricians and Gynaecologists criteria, a widely accepted criterion used for categorizing PE into early and late-onset as well as into mild and severe PE. This makes determination of the potential risk of pregnancy outcomes or complications related to PE much easier. This criterion also allows the prediction of which biomarkers must be of special interest to augment clinical intervention and management strategies to minimize the disease progression and reduce adverse pregnancy outcomes.

According to literature, Angiopoietins are pro-angiogenic factors that promote vasculogenesis and angiogenesis, however, they remained poorly researched and their association with pregnancy outcomes has not been clearly stated. This study has deeply exploited multiple biomarkers such as serum levels of Ang-1 and Ang-2 and their relationship with VEGF including how they predict adverse pregnancy outcomes which also represent a major strength in this study

5.5 Limitations

This study has limitations despite the strong background in describing the biological bases of maternal serum angiopoietins and VEGF in PE. Also, measurement of possible differences in haematological tests, lipid profiles, and liver function tests (LFTs), renal function tests which are essential clinical indicators for diagnosing PE and this serves as a limitation. The criterion used during the sampling procedure made participants' cooperation and consent to inclusion criteria extremely problematic. In Ghana, funding for scientific research projects is not readily available locally and is especially difficult to secure international funds. Due to this shortfall and the high cost of reagents, the research team could not secure research funding for this study. As a result, other relevant biomarkers including soluble fms-like tyrosine kinase-1 (sFlt-1), soluble endoglin (sEng), Nitric oxide, and Angiotensins, all of which are implicated in the pathogenic pathway of PE could not be tested.

Again, the time frame for this study was not enough to allow the recruitment of many more participants. Also, using a cross-sectional study design was another limitation. A longitudinal study design would have been better where pregnancy is studied from the first trimester through to the term.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

This study concluded that PE is associated with significant adverse maternal and perinatal outcomes such as caesarean sections, preterm births, poor Apgar scores and LBW with high risks occurring among those with severe PE compared to the mild PE. This poses a significant threat to the survival of both mother and the fetus. Low maternal serum levels of VEGF and Ang-2 and VEGF/Ang-2 ratio were observed in PE compared to the normotensive controls, while no significant differences in maternal serum levels of Ang-1 were observed between the PE group and the normotensive controls. Ang-1/Ang-2 and Ang-1/VEGF ratios were also found to be higher in PE subjects than in pregnant normotensives controls. Futhermore, maternal serum VEGF levels were significantly lower in early-onset PE than in late-onset PE while Ang-1/VEGF ratio was higher in early-onset PE than late-onset PE and therefore may serve as good predictive markers for the diagnosis of early-onset PE.

Maternal serum concentration of VEGF and Ang-2 had a positive correlation with pregnancy outcomes such as GA, Apgar scores, and birth weight while maternal serum Ang-1 level inversely correlated with pregnancy outcomes. After adjusting for maternal risk factors such as maternal age, parity, blood pressure, BMI, GA at recruitment, and GA at birth, maternal serum VEGF levels <62pg/ml was significantly associated with increased risk of preterm birth, poor Apgar scores <7 and low birth weight (LBW), while serum levels of Ang-1 >84ng/ml and Ang-2 <17ng/ml did not show any significant associations with adverse pregnancy outcome.

6.2 Recommendations

To expedite clinical management and reduce the progression of preeclampsia, it is recommended that;

1. Further investigation and evaluation of the biological bases of Angiopoietins and their relation with VEGF in a longitudinal study is required
2. Evaluation of the association between angiopoietins and antiangiogenic factors such as soluble fms-like tyrosine kinase-1 and soluble endoglin is required to enhance understanding of the biological pathway of preeclampsia
3. A longitudinal study on the maternal angiopoietins on PE should be conducted where pregnancy is studied from the first trimester to the term.
4. Further research should be focused on biological studies in Ghana to enhance an adequate understanding of the biological pathways of preeclampsia and to integrate this scientific knowledge into clinical practice.

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Appendix I

Ethical approval

UNIVERSITY OF GHANA
COLLEGE OF HEALTH SCIENCES
ETHICAL AND PROTOCOL REVIEW COMMITTEE

EPRC/SEPT/2021 **September 28, 2021**

Ref. No.:.....

Mr. Fidelis Bayor
Dept. of Physiology
University of Ghana Medical School
Korle-Bu.

ETHICAL CLEARANCE

Protocol Identification Number: ***CHS-Et/M.1–4.9/2021-2022***

FWA: 000185779 **IORG: 0005170** **IRB: 00006220**

The College of Health Sciences Ethical and Protocol Review Committee (EPRC) at its **September 24, 2021** full board meeting reviewed and approved your research protocol.

Title of Protocol: **"Maternal serum angiopoietins and vascular endothelial growth factor (VEGF) levels in preeclampsia and pregnancy outcomes"**

Principal Investigator: Mr. Fidelis Bayor

This approval requires that you submit six-monthly review report(s) of the study to the Committee and a final full review report to the EPRC at the completion of the study. The Committee may observe, or cause to be observed, procedures and records of the study before, during and after implementation.

Please note that any significant modification(s) to this project/study must be submitted to the Committee for review and approval before its implementation.

You are required to report all serious adverse events related to this study to the EPRC within seven (7) days verbally and fourteen (14) days in writing.

As part of the review process, it is the Committee's duty to review the ethical aspects of any manuscript that may be produced from this study. You will therefore be required to furnish the Committee with any manuscript for publication.

This ethical clearance is valid till September 28, 2022.

Please always quote the protocol identification number in all future correspondence in relation to this protocol.

Signed: .....
Professor Andrew Anthony Adjei
Chair, Ethical and Protocol Review Committee

cc: **Provost, CHS**
Dean, UGMS
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Appendix II

Study questionnaire

BACKGROUND INFORMATION:

1. Study ID Number…….
2. Hospital ID…… Maternity ward……
3. Initials:…… Tel.....
4. a. Date of Hospital Arrival……...
   b. Date of Hospital Discharge/Death……...
   c. Duration of hospital stay……..
5. Occupation…….
6. Marital Status: 0: married/cohabitation
   1: divorced/separated 2: Single 3: Other
7. Which tribe do you belong to? (ethnicity)
   1=Akan 2=Ga 3=Ewe 4=North 5=Other
8. Age in years…….....
9. Which religion do you belong to?
   (0: Christianity 1: Islam 2: Traditional 4: Others)…….
10. What is the level of formal education you have attained? (0: None 1: Primary 2: Junior High Secondary 3: Senior High Secondary 4: Tertiary)……

Obstetric History

11. Number of pregnancies (including current pregnancy)…….
12. Number of previous births (excluding current delivery)……
13. Have you lost a pregnancy before? Yes =1 No =2
14. If yes, how many (a) miscarriages (b) induced abortions (c) stillbirths
15. History of previous caesarean section:
    Yes =1 No =2
16. Previous history of hypertension in pregnancy
    Yes =1 No =2
17. Number of antenatal visits with current pregnancy.....

Maternal Outcome Indicators

18. a. Height (M) at booking …….
    b. Weight (Kg) at booking …….....
    c. BMI (Kg/m^2) at booking ……..……
19. a. Gestational age at booking in weeks……
    b. Blood Pressure at Booking……
20. a. Gestational age at diagnosis in weeks……..
    b. Blood Pressure at diagnosis……
21. Was the patient referred here?
    Yes =1 No =2

a. If yes, what was the reason for referring?

22. Medical history:
    a. Hypertension 1=Yes 2=No
    b. Diabetes 1=Yes 2=No
    c. Sickle Cell Disease 1=Yes 2=No
    d. HIV 1=Yes 2=No
    e. Malaria
    f. UTI
    g. Other……

Labour and Delivery

23. Weight (Kg) before delivery…….
24. Dexamethasone before delivery: 1=Yes 2=No
    a. Time to delivery after dexamathaxone……
25. Gestational age in weeks at delivery…….
    Date of delivery…….
26. Premature rupture of membranes:
    1=Yes 2=No
27. Spontaneous labour: 1=Yes 2=No
28. Induction of labour: 1=Yes 2=No
29. Augmentation of labour Yes =1 No =2
30. Mode of delivery: Vaginal=1. Caesarean=2

a. Estimated blood loss……

b. Use of prophylactic misoprostol Yes =1 No =2

31. Maternal Complications

a. Placental abruption (APH) Yes =1 No =2
b. Postpartum Haemorrhage Yes =1 No =2
c. Emergency Laparotomy Yes =1 No =2
d. Acute renal failure/dysfunction Yes=1 No=2
e. Intracranial haemorrhage/Coma/CVA: Yes =1 No=2
f. HELLP Syndrome: Yes =1 No =2
g. Admission to ICU (6h Recovery): Yes=1No=2
h. Eclampsia: Yes =1 No =2
i. Severe preeclampsia: Yes =1 No =2
j. Coagulation disorders (bedside clotting time >7 min): Yes =1 No =2
k. Puerperal sepsis: Yes =1 No =2
l. Re-laparotomy: Yes =1 No =2
m. Wound dehiscence:
n. Transfusion of blood products: Yes=1 No=2
1. If yes, how many units of whole blood?……..... or FFPs?……..
32. Haemoglobin within 1 week before delivery:……..
33. Haemoglobin 48 hours after delivery……
34. Maternal death Yes =1 No =2
a. If yes, what is the cause of death……...
35. **Hypertension in pregnancy**:……..

Perinatal Outcome Indicators

36. Sex 1. Male 2. Female
37. Birth weight (Kg)……
38. Length (CRL)……..
39. Placental weight (kg)……..……
40. APGAR at 1minute……
APGAR at 5minnutes……
41. Liquor state: meconium stained: Yes =1 No =2
42. a. NICU admission: Yes =1 No =2 b. If yes, what was the duration of stay?
43. Oxygen therapy given: 1=Yes 2=No
44. Resuscitation given: 1=Yes 2=No
45. Respiratory distress/asphyxia Yes =1 No =2
46. a. Stillbirth: Yes =1 No =2
b. If yes is it MSB (=1) FSB (=2)
47. Early neonatal death Yes =1 No =2
48. If perinatal death occurred, what was the main cause ?.....
49. IUGR Yes =1 No =2
50. Gestational age as assessed by paediatricians

Laboratory Tests

51. VEGF
52. Angiopoietins (Ang-1 and Ang-2)

THANK YOU

Date of Data Collection:……
Data Collector's Name:……