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LEVELS OF SELECTED BIOMARKERS IN PERIPHERAL BLOOD OF HEMOGLOBINOPATHY PATIENTS DIAGNOSED WITH PREECLAMPSIA, KORLE BU TEACHING HOSPITAL, ACCRA,2019

BY

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Declaration

I, Jessica Asante, do hereby declare that except for cited references and literature from studies carried out by others which have been duly acknowledged, this dissertation is the result of my research work done under supervision and has neither been presented nor submitted elsewhere in part or whole for another degree.

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Dedication

Firstly, to the faithful and dependable father, God Almighty, who gave me hope when all hope was lost and strength when I almost gave up.

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Abstract

Background: Preeclampsia is a multifactorial progressive disease that is characterized by a persistently high blood pressure above 140/90mmHg and proteinuria 2+ and above. It is known to cause 2-8% of pregnancy complications worldwide and its prevalence in Ghana is between 2-3%. Women with preexisting conditions such as abnormal haemoglobin genotypes are at a higher risk of developing preeclampsia during pregnancy. The shared pathophysiology between hemoglobinopathies and preeclampsia could make early detection of the condition difficult. More so, due to the unknown aetiology of the disease, the only way to solve it is to deliver the placenta. This may lead to obstetric and perinatal complications and also death depending on the severity of the disease. Biomarkers can be used to understand the pathogenesis and better diagnose the disease. This study determined selected circulating biomarkers in women with hemoglobinopathies diagnosed with preeclampsia.

Method: This was a cross-sectional study of women between the ages of 18-45 years that was delivered in the Obstetrics and Gynecology Department in KBTH, Accra. The study population was 108. It included hemoglobinopathy patients diagnosed with Preeclampsia and those without preeclampsia and normal haemoglobin patients diagnosed with preeclampsia and those without preeclampsia. Out of the several markers reviewed 4 potential markers were explored, N-Terminal Prohormone-Brain Natriuretic Protein (NTpro-BNP), Pregnancy Associated Plasma Protein -A (PAPP-A), Neuropilin-1(NEU-1) and Plasminogen Activator Inhibitor-1 (PAI-1). Gel electrophoresis was run to confirm the haemoglobin genotypes of the participants. A sandwich ELISA was run to determine the levels of the biomarkers in the study participants. A histogram was used to show the frequency of the genotypes. Fisher's exact test was done to determine an association. Spearman correlation test was done to assess correlation. A multivariate model was done to assess if the biomarkers can predict preeclampsia.

Results: From gel electrophoresis, it was found that the majority 54.6% (49/108) of the patients had normal hemoglobinopathy genotypes. The confirmation tests reclassified 0.08% of the participants with initial HbAA genotypes to different haemoglobin genotypes and 2.8% of participants with initial HbSS genotypes were also reclassified to different genotypes. Preeclampsia complicated pregnancy patients had the highest mean age of study participants was 31±4.5years. Among the women with PE and HbP, the majority (78.9%) of them delivered by caesarean section as well as those with HbP only and PE only. Among the women with HbP, mean serum levels of NTpro-BNP were higher in the preeclamptic group (40.76±21.6pg/ml, p=0.9309) compared to the non-preeclamptic group. Serum levels of PAI-1 were extremely high among the HbP and PE women (135.18±157.1pg/ml, p= 0.0105). The mean serum levels of PAPP-A, across the comparison groups, the women with HbP diagnosed with PE had a low mean serum level of PAPP-A (0.86±0.7ng/ml, p=0.4563). The highest median serum level of Neuropilin-1 was 1918pg/ml which ranges from 78.5pg/ml to 3134pg/ml in women with PE complicated pregnancy. There was a significant difference between the DBP of mothers at their first prenatal visit and preeclampsia diagnosis though the correlation was weak (r=0.1993, p=0.0456; r=0.4036, p=0.0080). In the multivariate model, it was found that none of the markers showed significance in predicting preeclampsia in pregnant women with hemoglobinopathy.

Conclusion: In conclusion, misclassifications of patients' sickling status were detected. The confirmation tests reclassified some participants of HbAA genotype and others of HbSS. There were variations in the concentrations of the selected biomarkers measured in pregnant women with hemoglobinopathies diagnosed with PE. This variation was only significant in the serum levels of PAI-1. All biomarkers investigated did not show any promising ability in predicting preeclampsia in women with HbP and PE and women without HbP and PE.

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List of Abbreviations

ACOG	American College of Obstetricians and Gynaecologists
Ang	Angiopoietin
ANOVA	Analysis of Variance
BMI	Body Mass Index
BP	Blood Pressure
DBP	Diastolic Blood Pressure
EDTA	Ethylenediaminetetraacetic Acid
ELISA	Enzyme-Linked Immunosorbent Assay
HbP	Hemoglobinopathy
Hb	Normal Hemoglobin
HDP	Hypertensive disorders in pregnancy
HELLP	Haemolysis, Elevated Liver Enzymes and Low Platelet Count
ISSHP	International Society for the Study of Hypertension in Pregnancy
KBTH	Korle-Bu Teaching Hospital
MDGs	Millennium Development Goals
Neuro-1	Neuropilin-1
NICU	Neonatal Intensive Care Unit
NMIMR	Noguchi Memorial Institute for Medical Research
NTpro-BNP	N-Terminal Prohormone Brain Natriuretic Peptide
PAI-1	Plasminogen Activator Inhibitor 1
PAPP-A	Pregnancy Associated Plasma Protein-A
PCR	Polymerase Chain Reaction
PE	Preeclampsia/Preeclamptic

PlGF	Placental Growth Factor
RNA	Ribonucleic acid
SBP	Systolic Blood Pressure
SDGs	Sustainable Development Goals
sFLT-1	Soluble fms-like tyrosine kinase-1
sVEGFR-1	Soluble Vascular Endothelial Growth Factor Receptor-1
UNDP	United Nations Development Programme
VEGF	Vascular endothelial growth factor
WHO	World Health Organization

CHAPTER ONE

1.1 Background

Hemoglobinopathies can be defined as a group of inherited disorders in which there is abnormal production or structure of the haemoglobin molecule (Laboratories, 2015). The most common inherited genetic diseases are hemoglobinopathies (HbP). Sickle Cell Disease is a term used to describe a group of genetic diseases that have a predominance of HbS. This is the most common hemoglobinopathy found in Africa including Ghana.

The thalassemia syndrome mostly results from globin chain imbalance, either in α-globin chain production or β-globin chain production which leads to ineffective red blood cell production.

Globally, about 5-7% of the world's population are carriers of hemoglobinopathies. In children below 5 years, 3.5% of the death that occurs is due to haemoglobin disorders with more than 330,000 infants born yearly with haemoglobin disorders. (Fattoum, 2009; Huntsman, 1976; Shrestha et al., 2020). Over 70% of pregnant women in the world's population carry a significant variant of haemoglobin (Bernadette & Matthew, 2008). More so, a study by Lippi & Mattiuzzi, (2020) reported a prevalence of 1.08% and 0.042% for sickle cell disorders and other hemoglobinopathies worldwide respectively.

Although the African population makes up 10% of the global population, over 70% of the total hemoglobinopathies recorded worldwide were recorded in Africa. The highest rate (10.7%) of conception in Africa supersedes that of the globe (2.73%)(Fattoum, 2009)

In Ghana, the prevalence of β- thalassemia carriers, sickle cell disease carriers and prevalence of Hb C carriers are 1.5%, 13.3% and 10.5% respectively(Acquah et al., 2020).

Among all the hemoglobinopathies, the homozygous form of haemoglobin S (HbSS) is known cause more severe and fatal complications compared to the other hemoglobinopathies. It is commonly called sickle cell anaemia (Aparecida et al., 2016). The presence of hemoglobinopathies can lead to pregnancy complications such as hypertensive disorders.

Hypertensive disorders of pregnancy (HDP) are a major cause of direct maternal morbidity and mortality(Fondjo et al.,2019). HDP is characterized by the onset of high blood pressure accompanied by proteinuria in some conditions(Fondjo et al., 2019). Chronic hypertension, preeclampsia, eclampsia, gestational hypertension and superimposed preeclampsia are a group of conditions that make up hypertensive disorders. HDPs can be grouped as pre-existing and pregnancy-induced hypertensive disorders(Singh et al., 2014). Studies and other reports have demonstrated that among hypertensive disorders, preeclampsia is a prominent cause of maternal and perinatal morbidity and mortality(Fondjo et al., 2019)

Preeclampsia (PE) is a hypertensive pregnancy-associated multisystem disorder responsible for maternal and neonatal morbidity and deaths globally. It is characterized by the development of high systolic blood pressure of 140mmHg and a diastolic pressure of 90mmHg or more with the development of protein in urine 0.3g/l or more 24 hours apart after 20 weeks of gestation (Adu-bonsaffoh, 2020; Atakul, 2019; Levine et al., 2004; Poon & Nicolaides, 2014).

Although the aetiology of PE is still unknown, there have been different existing theories of preeclampsia postulated as the underlying causes of preeclampsia. However, two main stages are thought to occur. During the first stage, there is an abnormal spiral artery modification leading to placenta hypoxia whereas the second stage involves the release of placental blood-related factors into maternal circulation and the abnormal expression of angiogenic, pro-inflammatory, and antiangiogenic factors(Fondjo et al., 2019; Steegers et al., 2010)

PE can be classified as early-onset pre-eclampsia and late-onset pre-eclampsia. Early-onset pre-eclampsia occurs before 34 weeks of gestation whereas late-onset pre-eclampsia occurs after 34 weeks of gestation. The probability that complications such as fetal growth restrictions, prematurity and maternofetal mortality could occur in the onset of pre-eclampsia is associated increasingly with early onset of pre-eclampsia than late-onset preeclampsia. Untreated preeclampsia can easily manifest as eclampsia which is the manifestation of seizures coupled with all known symptoms of pre-eclampsia (Fondjo et al., 2019; Madazli et al., 2014).

Clinical manifestations of preeclampsia include cardinal symptoms such as a consistent increase in blood pressure(hypertension), headache, oedema, blurry eyes and abdominal pains. In some cases, major complications such as end-organ damage, paralysis and even death could occur, if management fails(Fondjo et al., 2019)

Globally, 2-8% of pregnancy-related complication is caused by pre-eclampsia. It accounts for over 63000 deaths annually. The prevalence of pre-eclampsia is high in developing countries. In Africa, the prevalence of pre-eclampsia is 10% of all pregnancies which supersedes the prevalence estimates (2-8%) globally (Mol et al., 2016; Steegers et al., 2010). In Ghana, a prevalence of 7.5% in a study conducted in a tertiary hospital has been reported (Adu-Bonsaffoh et al., 2014, 2017). More so, a hospital-based study conducted in 2015 also showed that out of all deliveries made between five years, 2.5% of the deliveries were complicated by PE(Otu-Nyarko S., Quansah A.G., Sackey S., 2015).

Clinical risk factors of PE that are mostly used in predicting PE include age, obesity, gestational hypertension, gestational diabetes, genetics, family history, parity and multifetal gestation, pre-existing medical conditions such as chronic hypertension, infectious diseases (Hepatitis B, Human Immunodeficiency Virus, etc.) and hemoglobinopathies (sickle cell disorders and thalassemia syndromes)(Boafor et al., 2016). These risk factors if not predicted early may

worsen the maternal and perinatal outcome of PE. (Prophet et al., 2018; Sitepu & Rachmadsyah, 2019; Vieira et al., 2017)

Studies by Prophet and colleagues (2018) have shown that PE and SCD have shared the pathophysiology of pathologic dysfunction of the endothelium and trophoblast invasion during fetal development. In pregnant women with SCD or hemoglobinopathy, there is an increased risk of obstetrical complications as well as sickle-cell complications or mortality. Comparing the management of pregnant women with SCD in developing countries such as Ghana, and Nigeria and developed countries such as Germany and the USA, there is an improvement in management in developed countries, therefore, giving SCD pregnant women a better outcome.

Effective management of PE is based on early diagnosis and treatment of clinical symptoms. Although PE is known to have an association with a multifactorial effect and immunological balance, its aetiology is still not well understood (Mutter & Karumanchi, 2008). More so, proposed studies on biomarkers for preeclampsia are raised to a higher extent in normal pregnancy and preeclampsia without considering those at higher risk.

This study aimed at identifying selected biomarkers that play a key role in the pathogenesis of PE and hemoglobinopathies among high-risk groups. Findings will be an added advantage in early detection and emerging therapeutic strategies. This study determined serum levels of selected biomarkers and their correlation with preeclampsia among women with hemoglobinopathies.

1.2 Problem Statement

Maternal and obstetrical complications could occur during pregnancy and lead to fatal outcomes such as mortality of both mother and child. Maternal mortality is still a public health issue and one of the major causes is hypertensive disorders with pre-eclampsia being its lead cause. The rate of maternal mortality has been reduced from 451 deaths/100,000 live birth in

2007 to 310 deaths/ 100,000 live birth in 2017 globally (GSS et al., 2017). Although the reduction is significant, the set target by the current sustainable development goal to reduce maternal mortality to 75 deaths/100,000 live birth is still not achieved therefore there is the need to introduce interventions to reduce it. (World Health Organization, 2016)

Pre-eclampsia is a leading cause of pregnancy complications that may lead to mortality, particularly in women with underlying medical conditions such as hemoglobinopathies. Women with hemoglobinopathy diagnosed with pre-eclampsia are known to be at an increased risk of morbidity and mortality. Hemoglobinopathies are the most common monogenic autosomal and recessive diseases inherited by birth. Women with abnormal haemoglobin have an increased risk of maternal and obstetrical complications leading to maternofetal outcomes. Although novel medical management of such patients during pregnancy is yielding results there is still an elevated rate of obstetrical complications. Evidence has shown that there is shared pathophysiology among hemoglobinopathies such as SCD and pre-eclampsia (Prophet et al., 2018). It is therefore expected that women with both conditions will have an increased risk of mortality.

Early detection is the main challenge of pre-eclampsia diagnosis and treatment. A simple and easy way of detecting PE at a very early stage will reduce morbidity and mortality. A group of biomarkers that is indicative of pre-eclampsia in pregnant women with hemoglobinopathies can be useful in pre-eclampsia diagnosis, therapeutic and prognosis.

1.3 Justification

Although extensive studies have been conducted to understand the aetiology of preeclampsia a specific mechanism is yet to be outlined. Understanding the pathogenesis and pathophysiology of preeclampsia will influence the effective management of PE in pregnant

women especially those with hemoglobinopathies such as SCD. Several protein biomarkers have been explored with few showing promising associations with PE.

Early detection and management of patients with both conditions will need an effective tool such as a biomarker that can easily be used in the early diagnosis of pre-eclampsia. Currently, extensive studies on serum levels of potential biomarkers with a focus on high-risk populations such as patients with hemoglobinopathies are unavailable. Hence this study is investigated to explore some potential biomarkers in pre-eclamptic patients with hemoglobinopathy.

1.4 Conceptual Framework

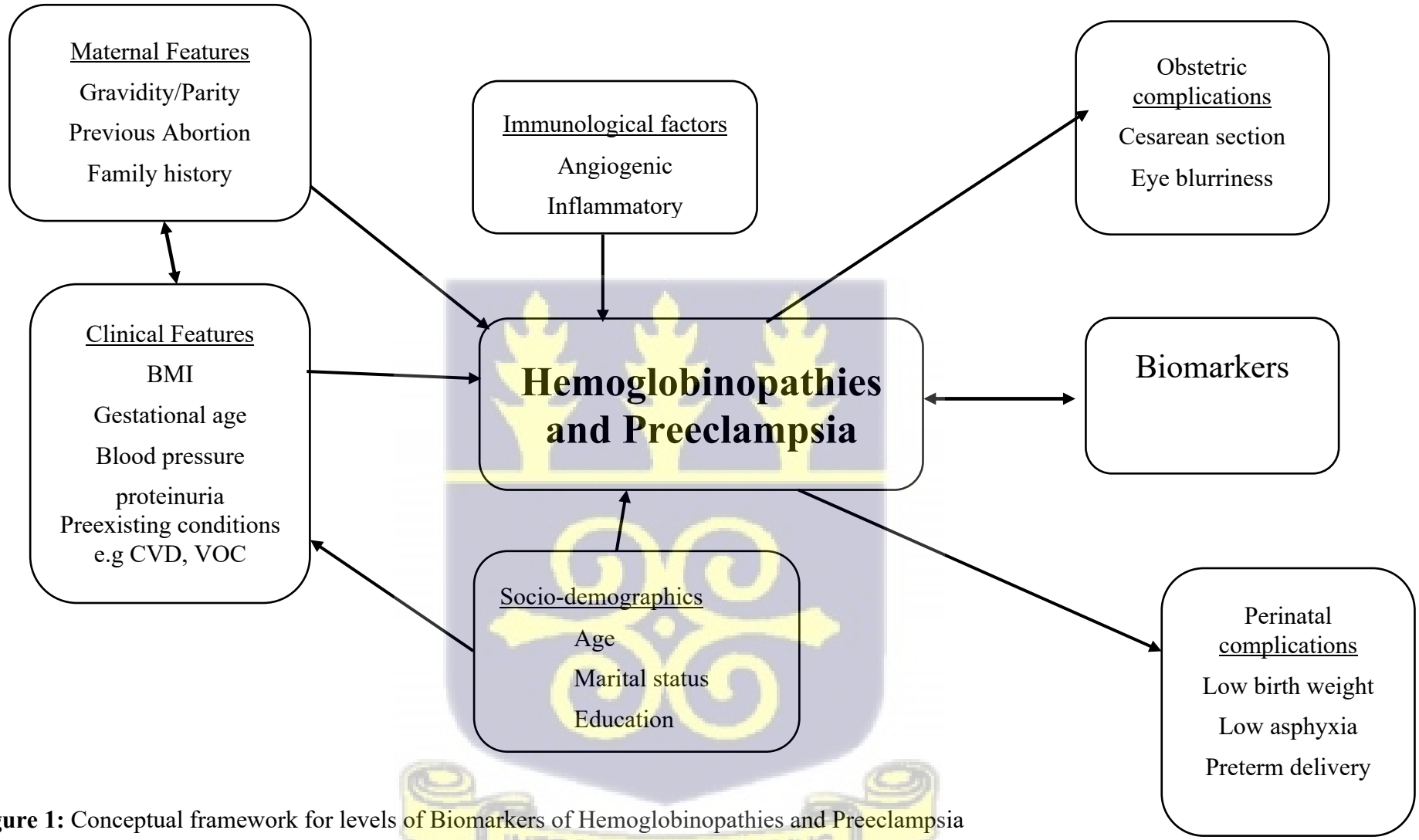

Figure 1: Conceptual framework for levels of Biomarkers of Hemoglobinopathies and Preeclampsia

1.4.1 Narrative of Conceptual Framework

Hemoglobinopathies and preeclampsia are two conditions that are known to share pathogenesis which causes endothelial dysfunction.

Hemoglobinopathies are genetic disorders that bring about abnormal haemoglobin in humans. Preeclampsia is a multi-system disorder caused by high blood pressure and proteins in urine. A woman with a hemoglobinopathy gene is known to be at high risk of getting preeclampsia. This is known to be associated with sociodemographic factors, maternal factors, clinical factors immunological factors and other risk factors such as preexisting conditions which include diabetes, sickle cell crisis and cardiovascular diseases. These factors if present is known to cause adverse maternal and perinatal outcome (Wilson et al., 2012a).

Sociodemographic factors such as maternal age, education, and marital status have been shown to have some association with pre-eclampsia and hemoglobinopathies. Advanced maternal age is associated with pre-eclampsia. Women who are >35 years are at a greater risk. The mother and child are highly exposed to adverse pregnancy outcomes such as birth complications, preterm birth and low apgar score (Tyas et al., 2020). Women who are more educated have shown a better knowledge of Preeclampsia (Fondjo et al., 2019)

Clinical features such as blood pressure and proteinuria are the immediate clinical symptoms that are manifested during preeclampsia. A woman with persistent blood pressure 140/90mmHg and above with proteinuria 2+ is diagnosed as pre-eclamptic (Sitepu & Rachmadsyah, 2019). The severity of pre-eclampsia is dependent on the increased level of blood pressure and proteinuria.

Other risk factors such as diabetes, sickle cell crisis and others are known to increase mortality in preeclamptic sickle cell women.

Immunological factors such as angiogenic and inflammatory factors play a role in the pathogenesis of these conditions and have shown a promising future as biomarkers (Obiri,2019).

Preeclampsia and Hemoglobinopathies are characterized by poor placentation in pregnant women and can lead to maternal and perinatal complications such as eye blurriness, chronic hypertension, low APGAR score, inter-uterine growth restriction, and maternofetal death.

Biomarkers are one of the technological tools used to study the pathogenesis of a disease and also for therapeutic purposes. A study of potential biomarkers in this area will help in the early diagnosis of preeclampsia in women with hemoglobinopathies.

1.5 Research Questions

1. What are the serum levels of NTpro-BNP, PAPP-A, Neuropilin-1 and PAI-1 in pre-eclampsia patients with hemoglobinopathy and those without preeclampsia?
2. What are the serum levels of NTpro-BNP, PAPP-A, Neuropilin-1 and PAI-1 in pre-eclamptic patients with normal pregnancy and those without preeclampsia?
3. What is the relationship between the serum levels of NTpro-BNP, PAPP-A, Neuropilin-1 and PAI-1 and the background characteristics?
4. Will the serum levels of NTpro-BNP, PAPP-A, Neuropilin-1 and PAI-1 make them good predictors of preeclampsia in pregnant women with hemoglobinopathy?

1.6. Objective of Study

1.6.1 General Objective

The general objective of the study was to determine the serum levels of NTpro-BNP, PAPP-A, Neuropilin-1 and PAI-1 in peripheral blood of hemoglobinopathy patients, Korle-Bu Teaching Hospital, Accra, 2019.

1.6.2 Specific Objectives

The specific objectives of the study were:

1 To confirm the haemoglobin genotypes of study participants.

2 To determine the serum levels of NTpro-BNP, PAPP-A, Neuropilin-1 and PAI-1in hemoglobinopathy patients with preeclampsia.

3 To determine the serum levels of NTpro-BNP, PAPP-A, Neuropilin-1 and PAI-1 in participants with normal haemoglobin without preeclampsia.

4 To determine the association of these selected circulating biomarkers and the background characteristics of the participants.

CHAPTER TWO

2.0 Literature Review

2.1 Maternal Mortality, Epidemiology, Causes and its economic impact

In most developing countries, pregnancy, as well as childbirth-related complications, are known to be the leading cause of disability and mortality among women of reproductive age(Patel D.A., Burnett N.M., 2003). The death of a woman during childbirth may pose a threat to the survival of her entire family. Also, the development of the newborn baby who may have survived, as well as the development of other young children in the family, is endangered by the death of the mother. Many women provide some support for the family by either working outside the home or taking major responsibility for household duties and child care. The increased levels of maternal mortality in many developing countries are very strong evidence of the neglect of the health needs of women despite the important roles they play in society (Graham et al., 2008).

Maternal mortality according to (World Health Organization, 1992) refers to the demise of a woman in her reproductive age whilst pregnant or within 42 days of delivery or cessation of pregnancy, from pregnancy-related causes or its management, excluding the deaths from incidental or accidental causes. Maternal deaths may either be direct or indirect based on their causes. In 2010, an estimated 287 000 maternal deaths occurred globally, mostly in low-income and middle-income countries(WHO, UNICEF, 1999).

It was estimated in 2008 that about 99% of maternal mortality transpired mostly in the developing countries of Sub-Saharan Africa and South Asia (Khan et al.,2006; UNICEF, 2019). In 2008, the maternal mortality rate in Ghana was estimated to be 451/100,000 live

births(GSS et al., 2017). While this is lower compared to its neighbours, Ghana's rate exceeds the global averages and is higher than the acceptable threshold.

Understanding the causes of deaths is a requirement for further advances in the reduction of maternal deaths which will aid effective policy and health programme decisions (World Health Organization, 1992). It is known from previous studies that the collection of routine and complete information about the causes of maternal death has not been possible due to inadequacies of data collection as well as the absence of vital registration systems in most countries. According to (Khan et al., 2006), it was reported the leading causes of maternal mortality in developing countries are haemorrhage and hypertensive disorders. A recent study by the Global Burden of Disease (GBD) provided estimates of maternal causes of death for the main direct causes as part of the analysis of all causes of death(Lozano et al., 2012).

The main causes of maternal deaths are essentially the same globally and they include postpartum haemorrhage, puerperal infections and hypertensive disorders of pregnancy, obstructed labour, and unsafe abortion. Globally, differences in behavioural as well as biological factors are known to increase the risk of a woman developing complications which may be life-threatening. Some interrelated factors may affect maternal mortality such as social status and position of women, economic resources and infrastructure of the country. Availability and accessibility of skills, materials, and facilities for adequate care are also some factors that affect maternal mortality. The World Health Organization has estimated that at least 15% of all pregnant women require rapid and skilled obstetric care; otherwise, they may end up suffering long-term morbidities(WHO, 1996). Even so, the needs of the woman in labour sometimes go unnoticed or unrecognized. If the needed help is not available, Childbirth may end up in the death or impaired health of the mother, the child or both of them.

Maternal deaths may occur past the 42-day window from the time of termination. The 10th revision of the International Classification of Statistical Diseases (ICD-10) then includes a

category of late maternal death as the demise of a woman from either direct or indirect obstetric causes more than 42 days but less than a year after termination of pregnancy (Graham et al., 2008). W.H.O estimates that the greatest proportion of maternal deaths are due to direct causes, including haemorrhage (25%), sepsis (15%), abortion (13%), hypertensive disorders of pregnancy (12%), and obstructed labour (8%). Indirect maternal deaths, on the other hand, are those caused by diseases such as heart diseases, iron deficiency, and malaria, amongst others that may have existed before pregnancy, but due to physiologic effects of pregnancy are aggravated and they make up about 20% of maternal deaths are due to indirect causes(WHO, 1996).

2.2 Hypertensive Disorders in Pregnancy

Hypertension is known to be the most common medical complication faced by pregnant women, complicating about 2-3% of pregnancies(Singh et al., 2014). Hypertensive disorders during pregnancy have been grouped into four, recommended by the National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy. Chronic hypertension, preeclampsia-eclampsia, preeclampsia superimposed on chronic hypertension, and gestational hypertension which is the transient hypertension of pregnancy or chronic hypertension identified in the latter half of pregnancy (Barton et al., 2001; Melamed et al., 2014).

2.2.1 Chronic hypertension

Chronic hypertension refers to high blood pressure that pre-exists before pregnancy that persists by the 12-week postpartum check-up after diagnosis within the first 20 weeks of pregnancy. Two types of severity are known: the mild type which is blood pressure up to 179mmHg systolic/109 mm Hg and the severe type which is blood pressure ≥ 180mmHg systolic or 110mmHg diastolic(Morgan et al., 2016; Nzelu et al., 2020; Podymow & August,

2017). Approximately 1-2% of pregnancies are complicated by chronic hypertension and its prevalence has been increasing due to late birthing (Nzelu et al., 2020). There is an increased risk of prematurity, intrauterine death, placental abruption, and caesarean delivery associated with this condition and the rate of complications is directly associated with the severity and duration of elevated blood pressures (Morgan et al., 2016). Approximately, first trimester patients with severe hypertension have a 50% increased risk of developing superimposed preeclampsia (Mégevand et al., 2019; Podymow & August, 2017; Report of the American College of Obstetricians and Gynaecologists' Task Force on Hypertension in Pregnancy, 2013).

2.2.2 Gestational hypertension

Gestational hypertension, also known as pregnancy-induced hypertension (PIH) or transient hypertension is the incipience of hypertension after 20 weeks of gestation. The determination of this type of hypertension is dependent on the patient having increased blood pressure of systolic ≥ 140mmHg or diastolic ≥ 90mmHg, previously normal blood pressures, no protein in the urine and no manifestations of preeclampsia(Barton et al., 2001; Melamed et al., 2014; Sadlecki et al., 2016; Sibai, 2003). Gestational hypertension is determined retrospectively when the patient does not develop preeclampsia and if blood pressure returns to normal by the 12-week postpartum visit. More so, prenatal results are worsened in women who progress to severe gestational hypertension based on the degree of blood pressure eminence than women with mild preeclampsia and hence require management is not different from those with severe preeclampsia (Adu-Bonsaffoh et al., 2017; Buchbinder et al., 2002; Catov et al., 2007; Endeshaw & Berhan, 2015).

2.2.3 Preeclampsia and Eclampsia

Preeclampsia is a multiorgan disease of unknown aetiology and is characterized by the development of hypertension and proteinuria after 20 weeks of gestation(Ahmad & Ahmed, 2004; Catov et al., 2007; Grotegut, 2016; Sitepu & Rachmadsyah, 2019). Eclampsia refers to the development of convulsions preceding pre-eclampsia. It occurs in patients with minimally heightened blood pressure and no proteinuria unexpectedly. The exact cause of eclampsia is not known but cerebral ischemia and oedema have been inferred to be its cause. The occurrence of an eclamptic seizure can be antepartum (53%), intrapartum (19%), or postpartum (28%)(Mahran et al., 2017; Mattar & Sibai, 2000).

2.3 Preeclampsia, Pathogenesis and Management

Preeclampsia is one of the main causes of maternal mortality, resulting in about 50,000–60,000 deaths annually globally(Report of the American College of Obstetricians and Gynaecologists' Task Force on Hypertension in Pregnancy, 2013). It is also associated with an increased risk of the mother and her child developing cardiovascular complications as well as diabetes mellitus later in life (O'Tierney-Ginn & Lash, 2014). Furthermore, preeclampsia is a multi-systemic syndrome which involves both genetic and environmental factors in its pathogenesis and pathophysiology and the only known treatment is delivery of the foetus and placenta (Fox et al., 2019; Kim, 2013).

There are subtypes of preeclampsia, which are based on the time of onset or recognition of the disease. The subtypes are divided into two main types, i.e., early- and late-onset preeclampsia(Staff et al., 2013). The late-onset preeclampsia comprises the majority (> 80%) of pre-eclamptic patients(Iacobelli et al., 2017; Madazli et al., 2014; Park et al., 2014). In the early-onset type, the clinical signs appear before 33 gestational weeks, while in the late-onset

type they occur at and after 34 weeks. However, it is the early-onset type that is responsible for most of the high maternal and foetal mortality rates(von Dadelszen et al., 2003).

Incomplete transformation of the spiral arteries is the main pathological feature of early-onset preeclampsia which results in a reduced amount of blood flow (hypoperfusion) to the placenta and reduced nutrient supply to the foetus leading to signs of foetal growth restriction, FGR (von Dadelszen et al., 2003). On the other hand, in the late-onset type, the spiral arteries are slightly altered in diameter with no signs of foetal growth restriction (Huppertz, 2008). This may be due to the reason that early-onset preeclampsia is related to placental hypoperfusion, whereas in the late-onset type there is no change or a shallow modification of the spiral arteries, leading to hyperperfusion of the placenta in some instances(Catov et al., 2007; Madazli et al., 2014; Yan, Zhong; Methodius, Tuuli; Anthony, 2011).

In a normal pregnancy, the extracellular fluid increases by 30-50% and plasma also increase by 30-40% due to a decrease in systemic vascular resistance and an increase in cardiac output (Kim, 2013; O'Tierney-Ginn & Lash, 2014). The decrease in systemic vascular resistance is thought to be a result of the presence of nitric oxide (Buhimschi et al., 1998; Darkwa et al., 2018; Lind et al., 2017). In early-onset preeclampsia, there is a decrease in plasma volume which occurs at 14 -17 gestational weeks, before the clinical onset of the disorder (Obiri, 2019; Steegers et al., 2010; von Dadelszen et al., 2003). The pathogenic process of preeclampsia starts during the first trimester before clinical signs are apparent. It is therefore difficult to identify early biomarkers. Generally, it is thought that the lack of adequate placental development is the root cause of early-onset preeclampsia because the only known treatment of the disorder is the delivery of the foetus and placenta.

The most popular theory of the pathogenesis of preeclampsia is that it is mostly immunologic. During a normal pregnancy, foetal syncytial trophoblasts invade and remodel maternal arteries

which causes them to dilate into huge, floppy vessels. The remodelling allows for accommodation of the vast, elevated maternal circulation required for adequate placental perfusion. In preeclamptic pregnancies, however, this remodelling is prevented due to placenta development which causes improper invasion of the maternal blood vessels which leads to intrauterine growth restriction and other foetal manifestations of the disorder. It has been studied that incomplete placentation is a result of maternal immunologic intolerance of foreign fetal genes(Fondjo et al., 2019; LaMarca, 2013; Lyall et al., 2013; Obiri, 2019). This theory is supported by evidence that, there is a high risk of pre-eclampsia in the first pregnancy which gradually decreases with the increase in the time a woman has lived with the father before becoming pregnant. The risk is also high in multiparous women impregnated by a new partner. There are other theories of the pathogenesis of preeclampsia that includes angiogenic factors that are increased soluble fms-like tyrosine kinase-1(sFlt-1), decreased placental growth factor levels (Cui et al., 2018; Levine et al., 2004) cardiovascular maladaptation and vasoconstriction, genetic predisposition (maternal, paternal, thrombophilia)(Levine et al., 2015; Mignini et al., 2006) immunologic intolerance between fetoplacental and maternal tissue, vascular endothelial damage or dysfunction (Wilson et al., 2012b). Several factors can be associated with preeclampsia and they include antiphospholipid antibody syndrome, chronic hypertension, chronic renal disease, elevated body mass index, maternal age older than 40 years, multiple gestations, pregestational diabetes mellitus and preeclampsia a past pregnancy especially one that was severe or before 32 weeks of gestation. The prevention of this condition through routine supplementation with calcium, magnesium, omega-3 fatty acids or antioxidant vitamins is inefficacious(Darkwa et al., 2017a). The risk of developing preeclampsia in high-risk women and those with low dietary calcium intakes is however reduced by calcium supplementation. (Darkwa et al., 2017b; Hofmeyr et al., 2014).

2.4 Risk Factors of Preeclampsia

Currently, several risk factors are known to play a key role in diagnosing PE or identifying the risk of PE during the early stage of pregnancy. Risk factors of PE could be classified as sociodemographic, maternal factors, immunological factors and clinical factors.

Sociodemographic factors associated with the risk of PE include age, education, marital status and others. Advanced maternal age (> 35 years) has been shown as one of the key risk factors for developing PE during pregnancy. Studies by Lamminpää et al., (2012) and Tyas et al., (2020) have shown that women above the age of 35 years developed PE with a higher risk of poor maternal outcomes compared to those in their reproductive age. More so, a study by Linda et al (2020) has also exhibited that women with a higher level of education have improved knowledge of PE. This means that women with low education levels have a higher risk of PE.

Maternal factors such as gravidity, parity, family history, obesity and others have been shown to have a relationship with the risk of developing PE. Nulliparous women are known to have a higher risk of PE and this has been proven by some studies (Fox et al., 2019; Mahran et al., 2017; Myers et al., 2011). More so, the presence of pre-existing medical conditions such as diabetes mellitus, sickle cell disease, autoimmune diseases, history of poor pregnancy outcomes also is risk factors that increase the risk of developing PE (Adu-bonsaffoh, 2020; Gathiram & Moodley, 2016; Luewan et al., 2018; Uzan et al., 2011). Immunologic and angiogenic factors IL 10 and increased levels of soluble fms-like tyrosine kinase-1(sFlt-1), as well as decreased levels of placental growth factor, also play a critical role as risk factors in increasing the development of PE in pregnancy (Cui et al., 2018; Levine et al., 2004).

These risk factors have the potential of increasing the perinatal and maternal outcomes in preeclampsia cases. High-risk complications from preeclampsia may lead to obstetric outcomes such as haemolysis elevated liver enzymes low platelets count (HELLP) syndrome,

eclampsia, acute renal failure, postpartum haemorrhage and mortality (Iacobelli et al., 2017; Mahran et al., 2017). Furtherly, stillbirth, low birth weight, Asphyxia, intrauterine growth restriction, preterm birth and to the extreme, neonatal death are some fetal and neonatal outcomes associated with the complications from preeclampsia (Oteng-Ntim et al., 2015Gathiram & Moodley, 2016).

2.5 Haemoglobin and Haemoglobinopathies in

2.5.1 Haemoglobin

The blood is made of unique, variant corpuscles which are the erythrocytes, the white blood cells and the blood platelets. In the red blood cells (RBCs) or erythrocytes is a tetrameric protein called the haemoglobin (Hb) whose primary function is to carry oxygen to the tissues, from the lungs (Ahmed Mostafa H., Ghatge Mohini S., 2017; Kato et al., 2018)

It comprises cofactor haem, which is linked to different combinations of globin subunits; with each carrying a molecule of oxygen(Kato et al., 2018) As afore-stated, Hb is expressed by both immature RBCs (reticulocytes) and mature RBCs (erythrocytes)(Kato et al., 2018)

Multiple genes encode several different globin proteins, and the variant combinations of their tetrameric structure result in multiple types of Hb, which are usually expressed at the embryonic, fetal and adult stages of life(Kato et al., 2018).

2.5.2 Variant forms of the haemoglobin

Haemoglobin may be present in variant forms depending on the diverse stages of development and mutations (Bain, 2017)(Keohane *et al.,* 2019). There are six human globin genes, located on two different chromosomes that encode the various forms of haemoglobin. α and ζ are found on chromosome 16 and β,γ, δ and ε are on chromosome 11(Bain, 2017) (Keohane *et al.,* 2019).

2.5.2.1 Hemoglobin A(HbA)

Haemoglobin A (HbA) is also known as adult haemoglobin and is produced six months after birth (Bain, 2017)(Keohane *et al.,* 2019). It consists of two alpha-globin genes (HbA1 and HbA2) and two beta-globin genes (HBB). Hence the subunit of Hb A is designated as $\alpha_2\beta_2$ (Bain, 2017).

2.5.2.2 Hemoglobin F (HbF)

Haemoglobin F (HbF) is also known as fetal haemoglobin since it is produced during embryonic life due to the cessation of the production of ζ and ε globin genes, leaving only α and γ chains; $\alpha_2\gamma_2$ (Bain, 2017). Genetic mutations can however cause subunits' changes and alterations in the amino acid sequence of the haemoglobin.

2.5.2.3 Hemoglobin S (Hb S)

Haemoglobin S (Hb S) is an example of such genetically mutated haemoglobin. It has the same structural subunit as the normal haemoglobin, Hb A; $\alpha_2\beta_2$. However, there has been a single nucleotide substitution which is a change of adenine for uracil at the sixth codon of the beta-globin gene. This causes the conversion of glutamic acid to valine(Bain, 2017; Eaton, 2020)

RBCs containing Hb S are referred to as sickle cells due to the formation of a "sickle" or crescent shape due to low oxygen tension and hydration levels in the intracellular cells (Bain, 2017).

2.5.2.4 Hemoglobin C (HBC)

Haemoglobin C (HbC), is found in about 28% of West African individuals and about 3% of Americans of African descent. As with Hb S, there is also a single nucleotide substitution leading to a change in amino acid sequence. However, with Hb C, guanine is substituted for adenine, resulting in the change of glutamic acid for lysine on the same sixth codon of the beta-

globin gene(Kohne, 2011; Laboratories, 2015; Shrestha et al., 2020). Polymerization of Hb S is observed during low oxygen tension, but that of Hb C is seen under high oxygen tension(Eaton, 2020).

2.5.2.5 Hemoglobin E (HbE)

There is also haemoglobin E, whose mutation is similar to that of Hb C. Nonetheless, its amino acid substitution of glutamic acid for lysine occurs at the twenty-sixth position of the beta-globin gene. (Bain, 2017).

Haemoglobinopathies

Haemoglobinopathies refer to genetic or hereditary disorders of the haemoglobin (Farmakis et al., 2017; Kohne, 2011). These firstly originated from the Mediterranean area and the greater parts of Africa and Asia. Hemoglobinopathies are now recorded in every part of the world. The major transporting factors responsible for this spread are migration and marriage (Kohne, 2011). According to (Kohne, 2011), these disorders can be categorized into two broad spectrums: thalassemia syndromes and variants of the haemoglobin structure.

When there is a defect in the synthesis of the haemoglobin, it is characterized by thalassemia syndromes. Here, the structure of the haemoglobin is normal however, the syntheses of the genes are where the defects lie (Kohne, 2011). In the abnormal structure variants, the alpha (α) and beta (β) globin genes are synthesized but mutations in protein synthesis led to a change in their structures(Kohne, 2011). There can, however, be mixed occurrences, where there are co-morbidities of the thalassemias and abnormal Hb structure variants (Kohne, 2011).

2.6 Sickle Cell Disease.

Sickle cell disease (SCD) refers to a plethora of inherited disorders such as sickle cell anaemia (SCA) HbS/ β-thalassemia and HbSC that is characterized by mutations in the β-globin gene

(HBB) (Kato et al., 2018; Kohne, 2011). It encompasses all manifestations of the level of the abnormal haemoglobin, S (HbS) (Grosse et al., 2011; Kohne, 2011). Individuals who are homozygous for the defective β- alleles have sickle cell disease while others, who are heterozygous for the defective β- allele carry the trait, but do not have the disease (Kato *et al*., 2018).

The term 'Sickle Cell Disease' includes different genotypes of homozygous HbS sickle cell anaemia (SS) and the double heterozygote states of sickle haemoglobin C disease (SC), sickle beta plus thalassemia (Sβ+Thal), sickle beta zero thalassemias (Sβ0thal), sickle cell anaemia with alpha thalassemia (SS αthal), and sickle cell anaemia with increased fetal haemoglobin (SS+F) (Grosse et al., 2011; Kato et al., 2018; Serjeant, 2001).

Sickle Cell Anaemia, the most severe form of SCD is strictly triggered by a homozygous mutation (haemoglobin S) and it presents as chronic anaemia. Until the 1970s, there was poor management of patients with sickle cell anaemia, and there was high maternal and foetal mortality associated with pregnancy.

Sickle Cell Disease has its origin in sub-Saharan Africa and the Middle East (Grosse et al., 2011; Kato et al., 2018; Stuart & Nagel, 2004) making it the most prevalent genetic disorder in individuals living in these regions. Sickle Cell Disease is now increasing as a result of population migration with increasing numbers of affected individuals in Europe and the USA (Serjeant, 2001). The disease is recognized as the most frequent inherited hemoglobinopathy with approximately 300,000 infants born globally yearly, mostly in countries such as Nigeria, India and the Democratic Republic of Congo (Wilson et al., 2012b). In recent times, screening techniques for newborns and preventive measures including vaccination and antibiotic prophylaxis from birth as well as the survival of patients have improved with a great decrease in maternal and neonatal mortality rates too (Green–top Guideline, 2011; Rajab et al., 2006).

About 300 000 children with Sickle Cell Disease are born each year(Angastiniotis et al., 1995; Grotegut, 2016; Kato et al., 2018) and two-thirds of these births are from Africa (Diallo & Tchernia, 2002; Grosse et al., 2011). In the UK, it is estimated that there are about 12,000-15,000 affected individuals and over 300 infants born with Sickle Cell Disease each year who are diagnosed as part of the neonatal screening programme (Streetly et al., 2009). The disease is associated with both maternal and foetal complications and is also associated with an increased incidence of premature labour, foetal growth restriction and acute painful crises during pregnancy(Elenga et al., 2016; Howard et al., 1995; Rajab et al., 2006; J. A. Smith et al., 1996). Some studies have described an increase in spontaneous miscarriage, antenatal hospitalisation, maternal mortality, delivery by caesarean section, infection, thromboembolic events and antepartum haemorrhage in individuals with the disease (Howard et al., 1995; Oteng-Ntim et al., 2015; Serjeant et al., 2004)

In the pathophysiology of HbSS, there is a single nucleotide substitution in the sixth codon of both alleles of HBB leading to an amino acid substitution of glutamic acid to valine (β^S). In a hypoxic condition or deoxygenation, HbS in RBCs polymerize and assume a 'sickled' cell or crescent shape of the RBCs. This leads to vaso-occlusive crises which are predominant in sickle cell diseases (SCDs)(Kato et al., 2018).

In individuals with HbAS, only one allele of HBB has the amino acid substitution of glutamic acid to valine (β^S) while the other allele is normal (Kato et al., 2018). They are thus referred to as carriers.

In individuals with HbSC, there is an amino-acid mutation of glutamic acid to valine in one β-allele and another mutation of glutamic acid to lysine in the other β-allele (Kato et al., 2018).

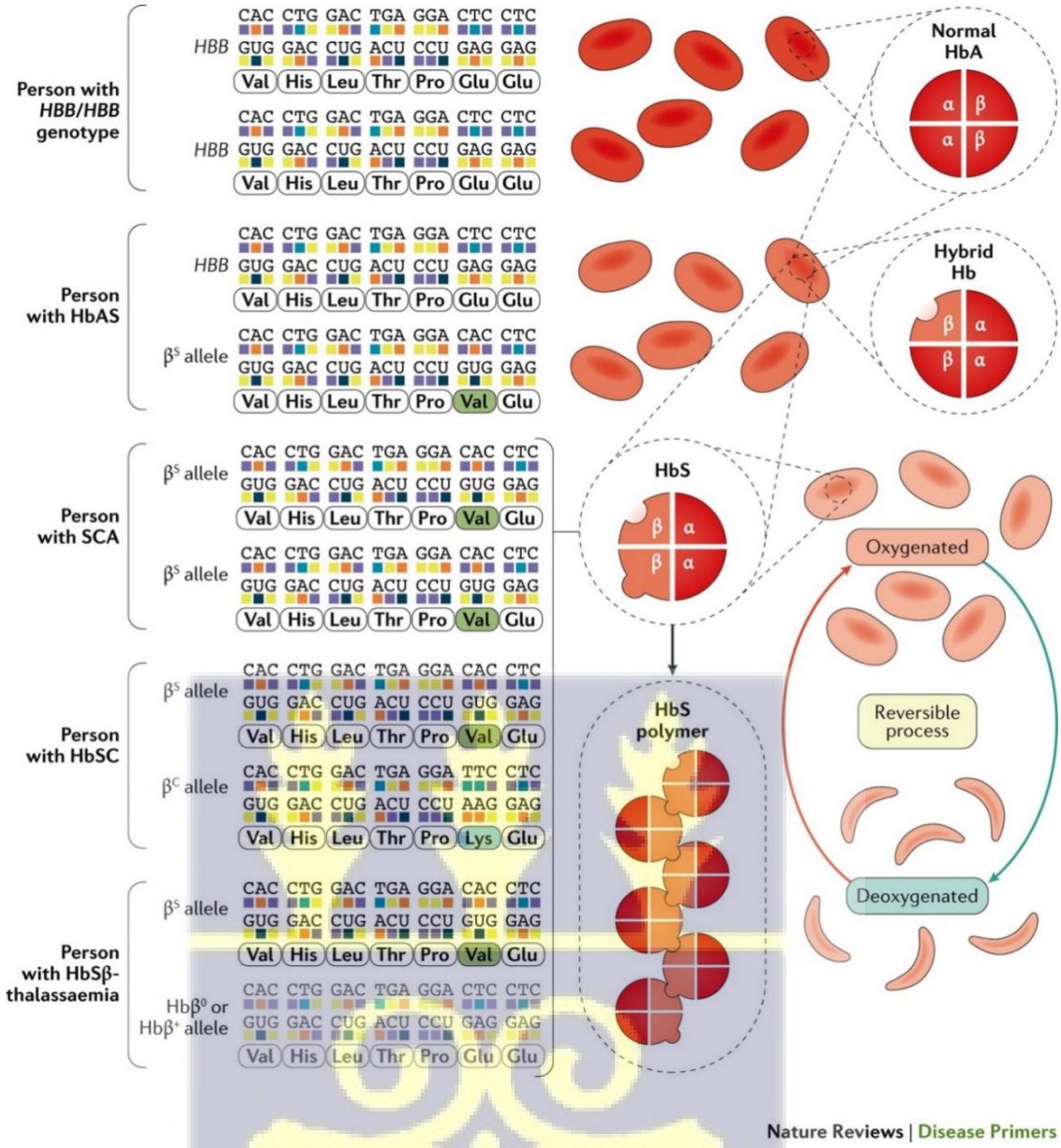

Figure 1: *Alterations in the β-globin gene of the haemoglobin* (Kato et al., 2018)

2.7 Hemoglobinopathies and Pregnancy

Pregnancy in sickle cell disease can be complicated as both the mother and child are at a high risk of serious outcomes. The physiological changes of pregnancy such as more metabolic demand increased blood viscosity and hypercoagulability become higher in patients with sickle cell disease, therefore, leading to increased occurrence of complications like a vaso-occlusive crisis, acute chest syndrome, osteonecrosis, hepatic necrosis, leg ulcers, and thromboembolic events. More so, vaso-occlusion occurring in the placenta leads to villous fibrosis, necrosis, and infarction, resulting in impaired uteroplacental circulation, which then causes chronic foetal hypoxia and other adverse foetal consequences (Hassell, 2005; Kuo & Caughey, 2016; Villers et al., 2008).

Sickle Cell Disease patients have delayed physical and sexual development (S., Orah; M.D., Platt; Roseenstock & M.S.P.H; Espeland, 1993). These are outcomes of factors like poor nutrition, repetitive infections, blood transfusions, painful crisis, and frequent hospital admissions (Jesus et al., 2018). Menarche onset is often delayed in women with Sickle Cell Disease and this is associated with the HbSS phenotype compared to the HbSC phenotype (Carvalho et al., 2017). Women within this situation have risk factors that may affect their ability to conceive, such as chronic inflammation, oxidative stress, transfusion-related hemochromatosis, and ovarian sickling which leads to ischemia and reperfusion injury to the ovaries.

Pregnancy in sickle cell disease is in conjunction with an elevated risk of obstetric complications like pre-eclampsia and eclampsia therefore their incidence is greater in sickle cell patients compared to non-sickle cell patients ((Boafor et al., 2016). Gestational diabetes is also found to be high but not as significant (Rogers & Molokie, 2010). Microvascular damage and decreased uteroplacental circulation in these mothers cause a very high risk of spontaneous

abortions and stillbirths. Other risk factors may include poor general health of the mother and drug abuse like tobacco, alcohol and narcotics(Hassell, 2005; Rogers & Molokie, 2010). Pregnancy worsens the pre-existing anaemia in women with Sickle Cell, leading to a higher incidence of severe anaemia and increased requirement for blood transfusions. There is also a higher rate of caesarean deliveries in these patients(Hassell, 2005; Jain et al., 2019).

The incidence of sickle cell disease-related complications like Acute Coronary Syndrome (ACS) is increased during pregnancy. Splenic functions become defective in such patients due to auto-splenectomy. Also, the disease, superimposed with the immune-compromised state of pregnancy leads to an increased risk of infections like pneumonia, pyelonephritis, UTIs, and postpartum infections, among others. Due to several obstetric and non-obstetric complications, maternal mortality is relatively higher in women with Sickle Cell Disease as compared to the general population. (Elenga et al., 2016)

Routine blood investigations like complete blood count, HIV, HBs Ag, and HCV should be done together with urine examination should be performed during prenatal visits, most importantly the first. The mother should understand the importance of the antenatal visit and it is recommended to visit an obstetrician every other week during the first two trimesters(Hassell, 2005; Rogers & Molokie, 2010; Ware et al., 2017). Blood pressure and urinalysis are also recommended to be performed at each consultation and midstream urine purposely for culture monthly as these women are prone to preeclampsia and increased risk of urinary tract infections (Piel et al., 2013). Mothers should also be explained to avoid precipitating factors of sickle cell crises like exposure to extreme temperatures, dehydration, and overexertion.

2.8 Hemoglobinopathies and Preeclampsia

Recent reports have demonstrated that sickle cell disease has a role to play in the pathophysiology of preeclampsia and eclampsia (Prophet et al., 2018, Jain et al., 2019). Thus, the physiological adaptations that the system of the primigravida and multigravida undergo, may overburden cells and organs that may have sustained injuries from the complications of sickle cell disease and thus may increase the rate of occurrence of preeclampsia or exacerbate its condition (Jain et al., 2019).

Endothelial dysfunction is one of the main pathophysiologies of preeclampsia. Sickle cell disease is also associated with endothelial dysfunction. Sickle cell disease (SCD) is characterized predominantly by intravascular hemolysis which occurs at high rates, in pregnant women resulting in the release of several amounts of damage-associated molecular patterns (DAMPs) into circulation (Prophet et al., 2018). Consequently, several inflammatory pathways are activated including the activation of the endothelial cells and injury (Addai et al., 1992; Prophet et al., 2018; Wilson et al., 2012a).

Sickle cell disease leads to the abnormal structure of the red blood cells (RBCs) due to the presence of Hb S. During hypoxic conditions, the RBCs 'sickle' and thus do not live their life span in the blood. Consequently, anaemia due to sickle cell is resulted (Bain, 2017).

The abnormal RBCs in sickle cell anaemia activate the endothelial cells, leucocytes and platelets, promoting the expression of cell adhesion molecules (VCAM-1 and ICAM-1 and E-selectin) and pro-inflammatory cytokines including TNF-α and IL-6(Teixeira et al., 2017). Protracted activation of the endothelial cells contributes to the formation of clusters which interact with the walls of the blood vessel and result in vascular occlusion crises (Teixeira et al., 2017).

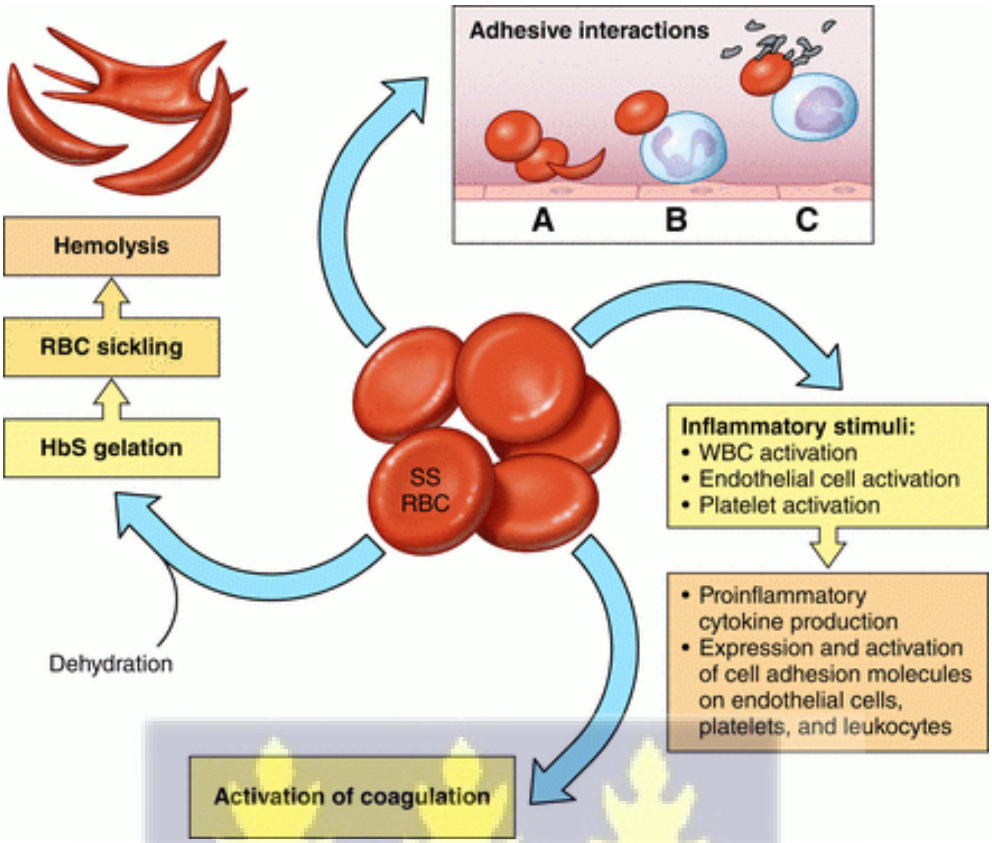

Figure 2: Mechanisms involved in endothelial dysfunction due to abnormal RBCs (http://omedicine.org/medicine-and-surgery/internal-medicine/blood-diseases-blood-tranfusion/sickle-disease-management-in-sub-saharan-africa/).

The bioavailability of nitric oxide (NO), (a potent vasodilator, maintaining vascular tone) is reduced. This is because, the rapid destruction of the abnormal RBCs leads to the availability of free Hb which react with the NO(Teixeira et al., 2017). Again, the chronic haemolysis facilitates the expression of arginase-I over-rides arginase-II and then catalyses the conversion of L-arginine to L-ornithine instead of L-citrulline and thus nitric oxide is not produced (Teixeira et al., 2017). According to (Teixeira et al., 2017), vascular stenosis and smooth muscle proliferation are also activated. Again, excessive production of reactive oxygen species induces oxidative stress and which affects the stability of NO production (Teixeira et al., 2017).

The vaso-occlusion crises, reduced bioavailability of NO, vascular stenosis, and production of pro-inflammatory cytokines arrive in the dysfunction of the endothelium (Teixeira et al., 2017).

The aforementioned may occur in the placenta leading to villous fibrosis, and infarction, which leads to an impairment in the uteroplacental blood flow and consequently, chronic hypoxia of the foetus which eventually ends in or exacerbates the condition of preeclampsia (Jain et al., 2019).

2.9 Impact of Biomarkers in Predicting Preeclampsia, Hemoglobinopathy or Both

Hulka and colleagues have defined biological markers as "cellular biochemical or molecular alterations that are measurable in biological media such as human tissues, cells or fluids"(Hulka, 1991). This definition over the years has evolved to include biological characteristics that can be objectively quantified and evaluated as an indicator of normal human biological processes, pathogenic processes or pharmacological responses to a therapeutic intervention(Mayeux, 2004; Nikuei et al., 2017). Biological markers can serve as tools and technologies that enable one to understand the prediction, cause, diagnosis, progression, regression or outcome of treatment of a disease(Mayeux, 2004). To study human diseases, biomarkers are used by an epidemiologist, physicists, and scientists. In the interim, biomarkers have been used in the diagnosis and management of cardiovascular diseases infections immunological and genetic disorders and cancers(Chen et al., 2001).

Extensive studies on biomarkers have shown reproducibility, specificity, sensitivity, and accuracy are criteria for useful biomarkers (Biomarkers Definitions Working Group Bethesda, 2001). Biomarkers can be classified into five groups based on their application in different disease stages. Firstly, it can serve as an antecedent to help identify developing diseases. Secondly, as a screening biomarker, it is used to screen for the presence of subclinical diseases. Furtherly, as a diagnostic tool, it is used to recognize diseases. The staging biomarkers are used

to categorize the severity of the disease. In predicting future disease occurrence, it serves as a prognostic tool(Biomarkers Definitions Working Group Bethesda, 2001).

Biomarkers are beneficial in several ways they contribute to clinical knowledge about pharmacology and also give the basics for the designing of a clinical trial. Biomarkers have been used to understand the pathogenesis and the prediction of several diseases(Nikuei et al., 2017).

In preeclampsia, it has been used to generate the two-stage theory in understanding the pathogenesis of preeclampsia to an extent. Although the pathogenesis is still not fully understood, biomarkers have been explored in the area of diagnosis and prediction of preeclampsia(Sitepu & Rachmadsyah, 2019).

Several biomarkers have been explored concerning predicting preeclampsia including angiogenic factors and inflammatory factors. Some of these biomarkers which have shown promising future include the family vascular endothelial growth factors (VEGF), the placental growth factors (PlGF), soluble fms-like tyrosine kinase 1(sFlt-1), N-Terminal Prohormone-Brain Natriuretic Protein (NTpro-BNP), Pregnancy Associated Plasma Protein -A (PAPP-A), Neuropilin-1(NEU-1) and Plasminogen Activator Inhibitor-1 (PAI-1). These biomarkers may serve as pro-angiogenic and anti-angiogenic factors that play a crucial role in the pathogenesis, diagnosis and treatment of preeclampsia(Luewan et al., 2018; Sadlecki et al., 2016; Try et al., 2014; Udenze et al., 2017).

CHAPTER THREE

3.0 METHODS

3.1 Study Design

The study was cross-sectional. The study population comprised all pregnant women between the ages of 18 years to 45 years of gestational age 20 weeks and above who delivered at the Department of Obstetrics and Gynaecology, Korle-Bu Teaching Hospital. Demographic and clinical data were obtained from the patient's hospital folder and antenatal book using a study questionnaire. The target population of the study were pregnant women with hemoglobinopathies diagnosed with PE. Other groups such as (1) pregnant women with hemoglobinopathies but not diagnosed with PE. (2) pregnant women with normal haemoglobin diagnosed with PE and (3) pregnant women with normal haemoglobin not diagnosed with PE were also recruited for the study. Recruitment into the study was based on participants voluntarily consenting to be part of the study. Laboratory samples were obtained before delivery and processed for the serum and transported to the designated laboratory. The study was carried out between July 2019 and March 2020.

3.2 Study Site

The study was conducted in the Department of Obstetrics and Gynaecology (OG), Korle-Bu Teaching Hospital (KBTH) one of the leading national referral centres in Ghana. The OG Department of KBTH is the largest in the hospital with approximately 12,000 deliveries. Located in the KBTH is the National Sickle Cell Referral Centre which manages pregnant women with other hemoglobinopathies to provide specialist obstetric care for pregnant women with SCD. Approximately 200 pregnant women assess the clinic with 1500 antenatal visits (Obiri, 2019).

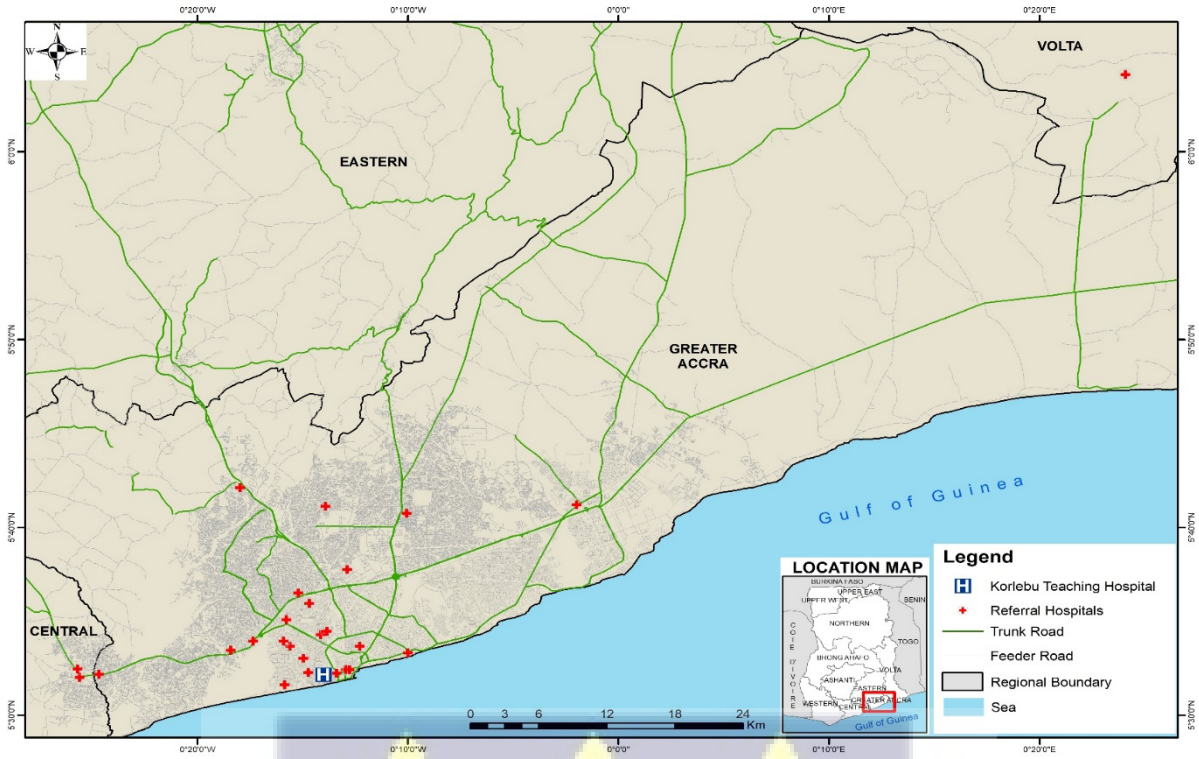

Figure 3: A map showing the geographical location of the study site

Source:(Obiri, 2019)

3.7 Selection of Biomarkers

In selecting the biomarkers, the biomarkers were investigated to identify their function and importance in pregnancy. The biomarkers selected were Neuropilin-1 (Neuro-1), N-Terminal-pro Brain Natriuretic Peptide (NTpro-BNP), Human Pregnancy Associated Plasma Protein-A(PAPP-A) and Plasminogen Activator Inhibitor 1(PAI-1). This was based on their pathologic effects, their implication and significance in PE, hemoglobinopathies (SCD) or both.

3.3 Variables

3.3.1 Dependent Variables

The dependent variable is pregnant women with hemoglobinopathy diagnosed patient with PE. The secondary dependent variables included (1) pregnant women with hemoglobinopathy but not diagnosed with PE. (2) pregnant women with normal haemoglobin diagnosed with PE and (3) pregnant women with normal haemoglobin not diagnosed with PE

3.3.2 Independent Variables

The independent variables measured include sociodemographics like age and body mass index. It also included maternal features such as parity, gravidity, sickling status, history of abortion, blood group and sickling genotype. Perinatal outcomes such as Apgar, birth weight, stillbirth, NICU admission and serum levels of biomarkers (Table 1).

Table 1: Study Variables and scale of measurement

Dependent Variables			
Variables	**Indicators**	**Scale of Measurement**	**Data Collection Technique and Tool**
Pregnant women with hemoglobinopathy diagnosed patient with PE	Presence of haemoglobin genotypes SS, AS, AC, and SC and presence of Protein in urine and blood pressure exceeding 140/90mmHg	Categorical/Binary	Records Review/questionnaire Laboratory Results
Pregnant women with hemoglobinopathy but not diagnosed with PE	Presence of haemoglobin genotypes SS, AS, AC, and SC and presence of Protein in urine and blood pressure not exceeding 140/90mmHg	Categorical/Binary	Records Review /questionnaire Laboratory Results
Pregnant women with normal haemoglobin diagnosed with PE	Presence of haemoglobin genotypes AA and presence of Protein in	Categorical/Binary	Records Review/questionnaire Laboratory Results

	urine and blood pressure exceeding 140/90mmHg		
Pregnant women with normal haemoglobin not diagnosed with PE	Presence of haemoglobin genotypes AA and presence of no Protein in urine and blood pressure not exceeding 140/90mmHg	Categorical/Binary	Records Review /questionnaire Laboratory Results
Independent Variables			
Sociodemographic	a. Age b. Body mass index	a. Continuous b. Continuous	Records Review /questionnaire
Maternal Factors	a. Parity b. Gravidity c. Sickling status d. History of abortion e. Blood group f. Haemoglobin genotype	a. Continuous b. Continuous c. Categorical/Binary d. Categorical/Binary e. Categorical f. Categorical	Records Review/ questionnaire Laboratory Results
Maternal Birth Outcome	a. Mother alive b. Presence of Birth Complications	a. Categorical b. Categorical	Records review/ questionnaire
Perinatal Outcome	a. Baby alive b. Apgar score at 1 minute and 5 minutes c. Sex d. Stillbirth	a. Categorical/Binary b. Categorical/Binary c. Categorical/Binary d. Categorical/Binary	Records review/ questionnaire
Serum level of Biomarkers	a. NTpro-BNP b. PAPP-A c. Neuropilin-1 d. PAI-1	a. Continuous b. Continuous c. Continuous d. Continuous	Laboratory Results

3. 4 Study Population

3.4.1 Inclusion Criteria

All pregnant women between 18 – 45 years of gestational age ≥ 20 weeks at the study site who have clearly understood the study and voluntarily decide to join after signing informed consent were recruited. Specifically;

(1) Pregnant women with haemoglobin genotypes (SS, SC, AC, AS, CC) and preeclampsia
(2) Pregnant women with haemoglobin genotypes (SS, SC, AC, AS, CC) without preeclampsia
(3) Pregnant women with normal haemoglobin genotypes (AA) with preeclampsia
(4) Pregnant women with normal haemoglobin genotypes (AA) without preeclampsia

All Hemoglobin genotypes were confirmed by isoelectric focusing (IEF) electrophoresis

3.4.2 Exclusion Criteria

Participants who were unable to give informed consent or unwilling to comply with the recruitment of the protocol were excluded from the study.

Participants with comorbid conditions including diabetes and malignancies were excluded

Participants who met the inclusion criteria but were critically ill were excluded from the study based on physicians' consent.

3.8 Sample Size Determination

The Cochran formula was used to determine the sample size was calculated from Cochran's formula $n = z^2p\ (1\text{-}p) / e^2$

Where N = the minimum sample size, Z = standard score for the 95% confidence limit = 1.96

P = the known prevalence of 12 % for hemoglobinopathies in Greater Accra (Oppong, 2017), e = the allowable error margin=10%, and the minimum number of study participants that were recruited for the study was 41. 10% of the calculated sample was added to the sample size to cater for the non-response of participants and the total sample size was 46 participants. A sample size of 108 was attained after sample collection.

3.6 Sampling Method

A consecutive purposive sampling method was used for selecting all eligible pregnant women willing to participate in the study. The point of contact with participants was at delivery in the labour ward when the pregnant women were sent there to deliver. In the labour ward, the study was introduced to a pregnant woman by the research assistant. The informed consent and processes involved were explained to her and when she consents, she was recruited into the study.

3.5 Recruitment of Participants

Pregnant women who present for delivery at the obstetrics and Gynaecology Department of KBTH were screened by a rotating team of trained research assistants.

Clinical Preeclampsia: A pregnant woman who presents with a history of sustained hypertensive (blood pressure ≥140mmHg systolic or ≥ 90mmHg diastolic at least 4 hours apart) with proteinuria (≥300mg of total protein measured in a 24-h urine collection or two readings of 1+ or higher on urinalysis) ≥ 20 weeks of gestation(Acquah et al., 2020) as diagnosed by an obstetrician.

Non-Preeclamptic Pregnancy: A pregnant woman at ≥ 20 weeks of gestation with a history of sustained blood pressure < 140mmHg systolic or < 90mmHg diastolic without proteinuria at the time of enrolment as confirmed by a clinician/midwife and medical records.

Hemoglobinopathy Pregnancy: A pregnant woman diagnosed with an abnormal haemoglobin genotype (SS, SC, AC, AS, CC) confirmed by electrophoresis, at ≥ 20 weeks of gestation with a history of sustained blood pressure < 140mmHg systolic or < 90mmHg diastolic without proteinuria at the time of enrolment as confirmed by a clinician/midwife and medical records

Normal Hemoglobin Pregnancy: A pregnant woman with haemoglobin genotype (AA) at ≥ 20 weeks of gestation with a history of sustained blood pressure < 140mmHg systolic or < 90mmHg diastolic without proteinuria at the time of enrolment as confirmed by a clinician/midwife and medical records

3.9 Sample Collection and Processing

For the biomarker screening, 5ml of peripheral blood was taken from the participant before delivery for the enzyme-linked immunosorbent assay (ELISA). The blood was transferred from a 5ml syringe used to withdraw the blood into a serum separator tube (SST) and was transported in a cooling chest to the designated laboratory (lab). At the lab, the sample was allowed to coagulate at room temperature for an hour. The blood was centrifuged at 1500rcf for 20 minutes. After centrifugation, the serum was aliquoted into well-labelled cryovials with the patient's ID. The aliquot serum was placed in a sock and stored in a Liquid nitrogen gas (LN_2) tank. Sample in LN_2 tank is transported to Noguchi Memorial Institute for medical research for permanent storage in a -80°C.

To confirm the haemoglobin genotypes of the participants, two blood spots were made on labelled filter paper dried and placed into a Ziploc bag with silica gel to serve as a desiccant.

3.10 Laboratory Investigation

3.10.1 Confirmation of Haemoglobin genotypes of Participants by Gel Electrophoresis

3.10.1.1 Sample Preparation

In sample preparation, two portions of the blotted paper were punched at a diameter of 3.2mm each into a microplate well. The microplate was placed on a shaker for an effective elution after the addition of 50µl haemoglobin electron solution.

3.10.1.2 Isoelectric Focusing (IEF) Gel Preparation

Firstly, the gel electrophoresis instrument to be used was set up. A complete agarose IEF gel was used for the electrophoresis. Before loading the samples, the IEF gel was allowed to attain room temperature. The IEF electrophoresis unit was prepared by cleaning the cooling plate surface carefully with water-drenched tissue paper. It was ensured that the cooling tube from the water bath was connected to the plate. 2ml distilled water was pipetted unto the centre of the cooling plate. The gel was then opened and turned flipped. and the right side was cleaned with gauze and labelled for easy identification. The gel was placed on the cooling plate using the parabola technique to ensure that no bubbles springs were present between the gel and the cooling plate. The sample application template was placed on the surface of the gel.

5µl of the resulting mixture of each sample was loaded into the respective wells as indicated in the tray. 5µl of controls were also introduced in the respective wells. The controls were

Two wicks soaked with anode solution were placed at the right and left edges of the gel and a wick soaked with cathode solution was placed in the centre of the gel. The electrode cables were placed on the gel with two cathode ends and an anode end in the middle of the gel.

The circuit was closed and the gel was run at 1200V, 200mA, and 26W for the electrophoresis unit. The electrophoretic migration was processed for an hour and a half with intermittent cleaning of the electrode cable.

3.10.1.3 Fixation Washing and Drying of Haemoglobin Genotyping Electrophoresis Gel

For fixing, the gel was soaked in 12% trichloroacetic acid solution for 8 minutes and was placed on the rocking shaker. The gel was soaked in 400ml deionised water for 12 minutes in a washing tray placed on a rocking shaker. After 5 cycles of washing the gel was placed in an oven to dry. Once dried the gel was tagged for identification together with the respective schedule and result sheet. The band's size was used to determine the genotypes present.

3.10.2 Measuring the serum levels of selected biomarkers (Neuro-1, PAPP-A, NTpro-BNP, PAI-1) in serum using Sandwich Enzyme-Linked Immunosorbent Assay (ELISA)

To measure the serum levels of the selected biomarkers, a duo set kit for the biomarkers were purchased from R & D systems (Minneapolis, USA). The manufacturer's steps in running the assay were followed. The summary of the analytes used is shown in table 2.

For coating, a 96-well microplate was coated with 50µl of the capture antibody of each biomarker at a working concentration of 4ug/ml. The plate was incubated overnight at room temperature.

The coated plate was aspirated and washed using a wash buffer (1x Phosphate Buffer Saline (PBS) and 0.05% tween 20) by dipping the plate in a basin containing the wash buffer. The wells were aspirated after swirling the plate for some time. This step was repeated 3 more times to ensure that the plate was well washed.

The microplate was patted dry and the wells were blocked with 3% reagent diluent (3% BSA in PBS, pH 7.2-7.4, 0.2 µm filtered) for a minimum of 1 hour. The aspiration and washing steps were repeated after blocking to prepare the plate for sample addition.

Samples and standards for each biomarker were diluted with 1x reagent diluent (1% BSA in PBS, pH 7.2-7.4, 0.2 µm filtered) to the desired concentration (Table 1). Sample addition (100µl) was done and plates were incubated and placed on a shaker and incubated for 2 hours. The aspiration and washing steps were repeated after blocking to prepare the plate for sample addition. The detection antibody (100µl) was added to the plates at the desired concentration, placed on a shaker and incubated for 2 hours (for the addition of detection for the PAI-1 2% of heat-activated normal goat serum was added to the detection). After the detection incubation, the plates were aspirated and washed 4 times. The plates were patted dry for the Streptavidin-HRP addition. 100µl of Streptavidin-HRP was added to each well of the 96 well plates in a dark environment. The plates were wrapped in foil and placed on the shaker for 20 minutes. The plates were aspirated and washed 4 times. 100µl of substrate solution to each well. Incubate for 20 minutes. The assay was stopped with H_2SO_4 after incubating for 15 minutes for Neropilin-1, 20 minutes for PAPP-A, and 30 minutes for BNP and PAI-1. The optical density of each plate was read using the microplate reader at 450nm.

Table 2: Summary of Analytes for Sandwich ELISA Assay

Analyte	**Plasma Dilution**	**Standards Dilution**	**Detection Range**
Neuropilin-1	1:200	3-fold	31.2-2000pg/ml
N Terminal prohormone -Brain Natriuretic Peptide	1:50	3-fold	312-1000pg/ml
Pregnancy Associated Plasma Protein -A	1:1	3-fold	0.8-50ng/ml

Plasminogen Activator Inhibitor -1	1:50	3-fold	0.3-21pg/ml

3.11 Data Management and Analysis

The data entry was done in RedCap (version 8.10.20-© 2020 Vanderbilt University) and exported to excel for data cleaning and management. The data was exported into Stata/IC 15.0 Stata Corp, College Station, TX for further analysis.

A normality test was done to identify data that were normally distributed using the Shapiro-Wilk test. Frequency and proportion were used to analyse haemoglobin genotypes. The sociodemographic data and clinical features were summarised using median, mean, range and standard deviation. ANOVA was used for analysing continuous variables that were normally distributed and the Fischer's exact test was used to analyse categorical variables. In comparing two variables the Mann-Whitney test was used whereas the Kruskal Wallis test was used for statistical significance for variables with more than two categories. multivariate logistic regression was performed to assess if the biomarkers were associated with preeclampsia among hemoglobinopathy patients at a p-value of 0.05 and a 95% confidence interval.

3.12 Ethical Consideration

3.12.1 Ethical Approval

Before the commencement of the study, ethical approval was sought from the Institutional Review Board of the NMIMR. The NMIMR IRB (IRB# 00001276)), is registered with OHRP (FWA 00001824). Approval was also sought from the KBTH - IRB (sampling site).

3.12.2 Informed/Voluntary consent

Written informed consent was sought before enrolling participants. Participation was strictly voluntary and any participant was free to withdraw from the study at any time. Translation of the study in the local language to the participants was done when necessary

3.12.3 Phlebotomy of Venous Blood

Risks are related to the phlebotomy of venous blood, which is required for the study. These are generally minor – consisting primarily of pain/trauma at the site. Only qualified phlebotomists were used for blood collection to minimize the risk associated with the blood sampling procedure.

3.12.4 Volume of blood to be drawn from pregnant women

Pregnant women were assessed by midwives and obstetricians for anaemia and any contraindications before blood was drawn. A blood volume of 5ml which was different from blood drawn for routine diagnosis was collected and this did not pose any harm to participants.

3.12.4 Safety procedures

Samples were collected using sterile, single-use disposable equipment such as butterfly needles.

3.12.5 Confidentiality

Data collected in this study was sensitive. Data were handled anonymously and all information remained confidential. Confidentiality as part of the acceptable standard practice in the NMIMR using non-identifiable study identification codes was ensured. This was applied to the storage of hard copies of the data collected in a locked cabinet.

CHAPTER FOUR

4.0 Results

Table 3 shows the background and clinical characteristics of the study participants. In total, one hundred and eight (108) women were recruited for this study. 18.52% (20/108) of the women were hemoglobinopathy patients diagnosed with preeclampsia 21.30% (23/108) of the women had preeclampsia complicated pregnancy, 35.19% (38/108) of the women had hemoglobinopathy only and 25% (27/108) had no hemoglobinopathy. The mean age of all the participants was 29±5.7years and it ranges from 18 to 43years.

4.1 Background and Clinical Characteristics of Participants.

Among the 4 comparison groups, women with preeclampsia complicated pregnancy only had the highest mean age of study participants was 31±4.5years but there was no significant difference between the ages among the study groups (Table 3). A total of 46 of the participants had data on body mass index (BMI). Women with hemoglobinopathy (HbP) only had the highest median BMI of 28.5kg/m^2 ranging from 22.55kg/m^2 to 43.95kg/m^2. This was not significant showing no difference among the groups. Systolic (SBP) and diastolic (DBP) blood pressure was high among the groups with PE. Women with both HbP and PE had a median SBP of 116mmHg (93-146) mmHg and a median DBP of 73mmHg (50-103) mmHg which was the highest among the four comparison groups. Women who were normotensive and had normal haemoglobin genotypes had the lowest median SBP and DBP [SBP: 100 (90-120) mmHg, DBP: 65 (50-90) mmHg]. These differences were significant (SBP: p=0.002, DBP: p=0.020). Proteinuria was present in women with HbP and women with preeclampsia only. This was significant. This can be seen in table 3.

Table 3: Background and Clinical Characteristics of the Study Participants

Characteristics	**Total Number of Participants (N=108)**	**Hemoglobinopathy (n=43)**		**Normal Hemoglobin (n=65)**		**P-value**
		PE (n=20)	**No PE (n=23)**	**PE (n=38)**	**No PE (n=27)**	
		n (%)	**n (%)**	**n (%)**	**n (%)**	
Maternal age (years), Mean, ±SD	108	29.55±6.0	29.47±6.1	31±4.5	29.63±6.2	0.7679
BMI (kg/m^2) Median(range)	46	28.3(20.3-33.2)	27.7 (20.5-36.9)	28.5 (22.5-43.9)	24.5 (21.1-31.9)	0.5050
Gravidity	108					0.3630[a]
Primigravida		6(30.0)	8(21.0)	4(17.4)	10(37.0)	
Multigravida		14(14.0)	30(79.0)	19(82.6)	17(62.96)	
Parity	108					0.4860[a]
Nullipara		13(65.0)	18(47.37)	8(34.8)	13(48.2)	
Primipara		2(10.0)	9(23.68)	6(26.1)	6(22.2)	
Multipara		4(20.0)	11(28.95)	9(39.1)	8(29.6)	
Grand multipara		1(5.0)	0(0)	0(0)	0(0)	
Blood Pressure at First Visit	107					
Median Systolic blood Pressure at first visit(mmHg)		116 (93-146)	100 (90-194)	115 (90-171)	100 (90-120)	0.0016
Median Diastolic Blood Pressure at first visit(mmHg)		73(50-103)	66(50-82)	70(60-120)	65(50-90)	0.0204

*: significant P -value less than 0.05, [a] : Fischer's exact test, [b] : chi-square test, [c] : ANOVA

Characteristics	Total Number of Participants (N)	Hemoglobinopathy		Normal Hemoglobin		P-value
		PE n (%)	No PE n (%)	PE n (%)	No PE n (%)	
Proteinuria	99					<0.001*[a]
Negative		0(0)	33(91.7)	0(0)	25(100.0)	
Trace		2(11.1)	3(8.3)	1(5.0)	0(0)	
1+		3(16.67)	0(0)	8(40.0)	0(0)	
2+		8(44.4)	0(0)	4(20.0)	0(0)	
3+		3(16.7)	0(0)	4(20.0)	0(0)	
4+		2(11.1)	0(0)	3(15.0)	0(0)	
Prior Abortion	106					0.1630[a]
Yes		11(55.0)	7(25.93)	6(27.3)	7(25.9)	
No		9(45.0)	26(70.3)	16(72.3)	20(74.1)	
Previous Caesarean Section	106					0.120[a]
Yes		1(5.26)	12(31.58)	5(21.7)	4(15.4)	
No		18(94.7)	26(68.42)	18(78.3)	22(84.6)	

*: significant P -value less than 0.05, [a] : Fischer's exact test, [b] : chi-square test, [c] : ANOVA

4.2 Confirmation of Hemoglobin genotypes of patients

From gel electrophoresis, it was found that the majority 54.6% (49/108) of the patients had normal hemoglobinopathy genotypes and the frequent haemoglobin genotype that was the HbAA genotype (figure 4). After confirmation, some misclassifications of patients' sickling status were detected. The confirmation tests reclassified 0.08% of the participants with initial HbAA genotype at recruitment and 2.8% of the participants with initial haemoglobin genotype HbSS at recruitment were also reclassified. Participants with other hemoglobinopathies were also detected. The other haemoglobin genotypes detected included SCA.

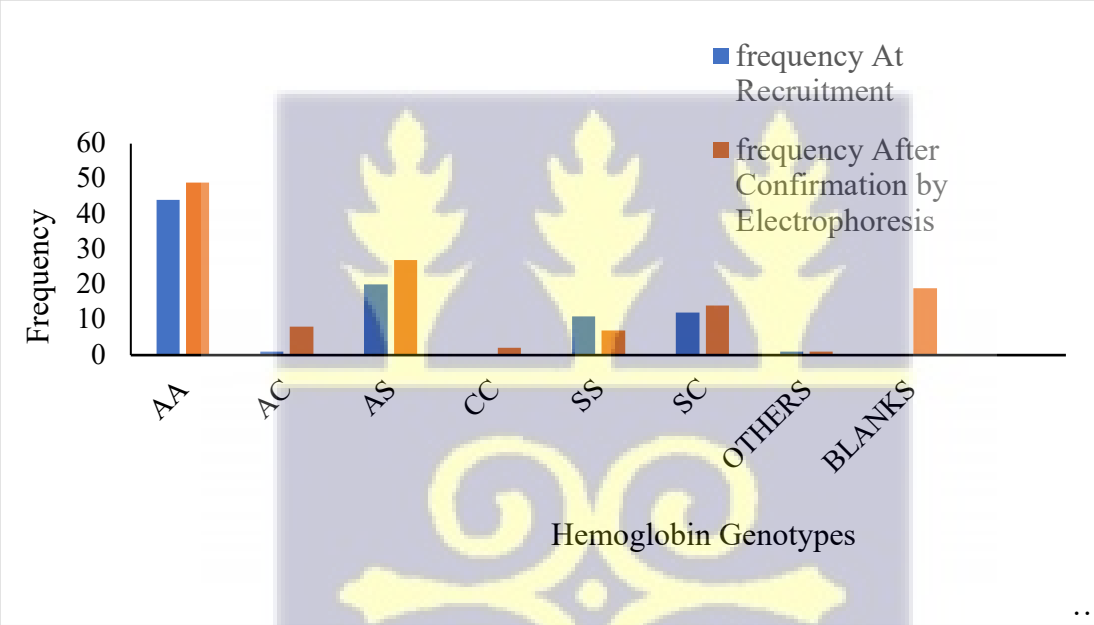

Figure 4: Frequency of Hemoglobin genotype of study participants

4.3 Maternal Birth Outcome of Study Participants

Details of birth outcomes are represented in table 4. The median gestational age at delivery was found to be significantly low in women with both hemoglobinopathies and preeclampsia (36(26-42)) compared to the other comparison groups [HbP/No PE: (38(35-41)), No HbP/PE: (37(28-41)), No HbP/No PE: (40(34-42)); $p<0.0001$]. Delivery by caesarean section was dominant among the different modes of delivery in women with PE and HbP (78.9%) as well as women with HbP only (63.2%) and PE only (87.0%) whereas vaginal delivery was the dominant mode of delivery in women with no HbP and no PE (46.2%) and this was statistically significant ($p=0.009$). All the women in the comparison groups were alive after delivery. All women with both HbP and PE and women with no HbP and PE did not experience any birth complications while 5.4% of women with HbP only and 17.4% of women with PE only experienced birth complications during delivery($p=0.036$). significant ($p=0.009$). All the women in the comparison groups were alive after delivery. All women with both HbP and PE and women with no HbP and PE did not experience any birth complications while 5.4% of women with HbP only and 17.4% of women with PE only experienced birth complications during delivery($p=0.036$).

Table 4: Maternal Birth Outcome of Study Participants

Characteristics	**Total Number of Participants (N)**	**hemoglobinopathy**		**Normal Hemoglobin**		**P-value**
		PE n (%)	**No PE n (%)**	**PE n (%)**	**No PE n (%)**	
Median Gestational Age at Delivery	96	36 (26-42)	38 (35-41)	37 (28-41)	40 (34-42)	0.0001*
Mode of Delivery	106					0.009*[a]
Vaginal		4 (21.1)	14 (36.8)	2 (8.7)	13 (50.0)	
Cesarean Section		15 (78.9)	24 (63.2)	20 (87.0)	12 (46.2)	
Instrumental		0 (0)	0 (0)	1 (4.3)	1 (3.9)	
Mother Alive after Delivery	107					0[a]
Yes		20 (100.0)	23 (100.0)	38 (100.0)	26 (100.0)	
HELLP Syndrome After Delivery	106					1.000[a]
Yes		0 (0.0)	1 (2.6)	0 (0.0)	0 (0.0)	
No		20 (100.0)	37 (97.4)	22 (100.0)	26 (100.0)	
Birth Complication	106					0.036*[a]
Yes		0 (0.0)	2 (5.4)	4 (17.4)	0 (0.0)	
No		20 (100.0)	35 (94.6)	19 (82.6)	26 (100.0)	

*: significant P -value less than 0.05, [a] : Fischer's exact test, [b] : chi-square test, [c] : ANOVA

4.4 Perinatal outcome among study Participants

Details of the perinatal outcome among the study participants are described in table 5. There were clinically significant findings among the four comparison groups (HbP/no PE, HbP/PE, no HbP/PE, and no HbP/ no PE) concerning the frequency of NICU admissions, stillbirth and sex of the baby. The frequency of low birth weight was 61.1% highest among women with both HbP and PE whereas it was lowest among women with no HbP and no PE. The mean Apgar score at 1 minute was low in the preeclamptic groups (HbP/PE and no HbP/PE). The lowest mean APGAR score which as 6.00±2 was in the HbP and PE. At 5 minutes after birth, the mean Apgar score was high in the comparison groups with the children born by women with no HbP and no PE recording the highest mean Apgar score of 8.54±1.2. the frequency of NICU admissions was high in the preeclamptic groups. NICU admissions are most frequent (65%) in babies of women with both HbP and PE.

Table 5: Perinatal outcome among the Study Participants

Characteristics	Total Number of Participants (N)	Hemoglobinopathy		Normal Hemoglobin		P-value
		PE n (%)	No PE n (%)	PE n (%)	No PE n (%)	
Still Birth	107					0.034*[b]
Yes		2(10.0)	0(0.0)	0(0.0)	0(0.0)	
No		18(90.0)	37(100.0)	23(100.0)	26(100.0)	
Sex	104					0.0120*[a]
Male		15(79.0)	22(59.5)	7(31.8)	11(42.3)	
Female		4(21.05)	15(40.5)	15(68.2)	15(57.7)	
Baby's Birthweight (kg)	103					0.000*[a]
<2.5		11(61.1)	4(10.8)	10(45.4)	0(0.0)	
>2.5		7(38.9)	33(89.2)	12(54.6)	26(100.0)	
Mean Apgar Score	105					
Mean Apgar Score at 1 minute		6.00±2.2	7.14±1.4	6.26±1.7	7.35±1.2	0.0018*
Mean Apgar Score at 5 minutes		7.32±2.3	8.22±1.2	7.48±1.7	8.54±1.2	0.0017*
NICU Admission	107					0.000*[b]
Yes		13(65.0)	7(18.4)	12(52.2)	3(11.5)	

No	7(35.0)	31(81.6)	11(47.8)	23(88.5)

*: significant P -value less than 0.05, [a] : Fischer's exact test, [b] : chi-square test, [c] : ANOVA

4.5 Serum Levels of Selected Biomarkers Among Study Participants.

There was no significant difference between serum levels of NTpro-BNP among the comparison group. Among the women with HbP, mean serum levels of NTpro-BNP were higher in the preeclamptic group (40.76±21.6pg/ml, p=0.9309) compared to the non-preeclamptic group. The same finding was observed in normal haemoglobin women. Between the comparison groups, the highest mean serum level of NTpro-BNP was found in women with both HbP and PE and the lowest was in women with normal pregnancy.

The serum levels of PAI-1 were significantly different among the groups statistically. Serum levels of PAI-1 were extremely high among the HbP and PE women (135.18±157.1pg/ml, p= 0.0105). Among the women with hemoglobinopathies, the highest serum levels were seen among those with preeclampsia compared to those not diagnosed with preeclampsia. Among the women without HbP, it was observed that those that had PE had the highest serum level of PAI-1(62.04±57.3pg/ml p=0.0105). Across the comparison groups, the highest level of PAI-1 was recorded in women with both HbP and PE.

Although there were no significant differences between the mean serum levels of PAPP-A, across the comparison groups, the women with HbP diagnosed with PE had a low mean serum level of PAPP-A (0.86±0.7ng/ml, p=0.4563). Within the HbP group, those without PE had a high serum level of PAPP-A. Among the normal haemoglobin group, the women diagnosed with preeclampsia had a high serum level of PAPP-A compared to those without PE (1.03±0.7ng/ml, p= p=0.4563).

The highest median serum level of Neuropilin-1 was 1918pg/ml which ranges from 78.5pg/ml to 3134pg/ml in women with PE complicated pregnancy. Among the hemoglobinopathy group,

women without PE had a median serum level of 1618.9pg/ml ranging from 63.9pg/ml to 6658.5pg/ml.

The difference between the groups was found not to be statistically significant(p=0.8649). This can be seen in Table 6 below.

Table 6: Level of Selected Circulating Biomarkers (NTpro-BNP, PAI-1, PAPP-A and Neuropilin-1 among Hemoglobinopathy and normal Hemoglobin women

Level of Selected Circulating Biomarkers	**hemoglobinopathy**		**Normal Hemoglobin**		**P-value**
	PE	**No PE**	**PE**	**No PE n (%)**	
Mean NTpro-BNP (pg/ml)	40.76±21.6	40.29±27.8	39.9±22.3	36.78±19.4	0.9309[c]
Mean PAI-1 (pg/ml)	135.18±157.1	50.41±14.4	62.04±57.3	49.76±30.9	0.0105*[c]
Mean PAPP-A (ng/ml)	0.86±0.7	1.12±0.82	1.03±0.7	0.9±0.54	0.4563[c]
Median Neuropilin-1 (pg/ml)	1593.7 (329-50000)	1618.9 (63.9-6658.5)	1918(78.5-3134.1)	1748(52.7-7012.6)	0.8649[c]

*: significant P -value less than 0.05, [a] : Fischer's exact test, [b] : chi-square test, [c] : ANOVA

4.6 Correlation of the Selected Circulating Biomarkers and Clinical parameters of Pregnancy.

The correlation between the selected circulating biomarkers and clinical parameters is shown in table 7. There was a significant difference between the DBP of the mother at the first prenatal visit and preeclampsia diagnosis though the correlation was weak (r=0.1993, p=0.0456; r=0.4036, p=0.0080). No significant correlation was found between the serum levels of PAPP-A, Neuropilin-1, and PAI-1 and the clinical characteristics measured although an inversely weak correlation was seen in some.

Table 7: Correlation of the Selected Circulating Biomarkers and Clinical parameters of Pregnancy

Clinical Features	Spearman's Correlation Coefficient	P-value
NTpro-BNP		
SBP at First Prenatal Visit	-0.024	0.8115
DBP at First Prenatal Visit	0.1994	0.0456*
Gestational age at the first prenatal visit	0.0028	0.9779
SBP at PE Diagnosis	0.2276	0.1472
DBP at PE Diagnosis	0.4036	0.0080*
PAPP-A		
SBP at First Prenatal Visit	0.0063	0.9485
DBP at First Prenatal Visit	-0.0311	0.7506
Gestational age at the first prenatal visit	0.0558	0.5701
SBP at PE Diagnosis	-0.1155	0.4445
DBP at PE Diagnosis	-0.1240	0.4115
Neuropilin-1		
SBP at First Prenatal Visit	-0.0095	0.9250
DBP at First Prenatal Visit	0.0665	0.5085
Gestational age at the first prenatal visit	-0.0112	0.9123
SBP at PE Diagnosis	0.2494	0.1113
DBP at PE Diagnosis	-0.0015	0.9927
PAI-1		
SBP at First Prenatal Visit	0.0108	0.9182
DBP at First Prenatal Visit	-0.0356	0.7348
Gestational age at the first prenatal visit	-0.0925	0.3804
SBP at PE Diagnosis	0.0727	0.6646
DBP at PE Diagnosis	0.0905	0.5889

*: significant P-value less than 0.05

4.7 Factors Predicting Preeclampsia in Women with Hemoglobinopathies

Based on the test of association done all values that were significantly associated with the comparison groups were included in multiple logistic models to determine the biomarker more reliable for predicting preeclampsia in people with hemoglobinopathy. In the multivariate model, adjusting for age gravidity, parity, gestational age, baby's sex, and baby's weight it was

found that none of the markers showed any significance in predicting preeclampsia in hemoglobinopathy pregnant women. This is shown in table 8. After adjusting for all other confounding factors, gravidity was a significant predictor of preeclampsia in pregnant women with hemoglobinopathy [AOR: 0.29, 95% CI: 0.090-0.91, p=0.034].

Table 8: Factors Predicting Preeclampsia in Women with Hemoglobinopathies

Characteristics	Adjusted Odds Ratio	95% Conf. Interval	P-value
NTpro-BNP	1.08	0.99-1.18	0.081
PAPP-A	0.93	0.18-4.83	0.935
Neuropilin-1	1.00	0.99-1.00	0.937
PAI-1	0.96	0.91-1.00	0.062
Age	1.42	0.94-2.13	0.096
Gravidity	0.29	0.090-0.91	0.034*
Parity	0.31	0.05-2.02	0.219
SBP at first Antenatal Visit	1.02	0.94-1.11	0.605
DBP at first Antenatal Visit	0.90	0.77-1.04	0.147
Gestational age at Delivery	0.58	0.33-1.01	0.056
Mode of Delivery			0.14
Vaginal	1.00		
Cesarean Section	0.10	0.00-2.11	
Instrumental	1.27	0.21-7.37	
Mean Apgar Score			0.793
Apgar Score at 1 minute	1.00		
Apgar Score at 5 minutes	0.69	0.09-4.91	
Baby's Birthweight			0.253
>2.5	1.00		
<2.5	0.14	0.00-4.17	
Baby's Birthweight	14.29	1.23-165.55	0.033*
Admission to NICU			0.019*
No	1.00		
Yes	0.01	0.00-0.49	
Sex			0.008*
Male	1.00		
Female	113.57	3.44-743742.64	

*: significant P -value less than 0.05

CHAPTER FIVE

5.0 Discussion

Preeclampsia is a leading cause of hypertensive disorders among pregnant women. Although a lot of studies have been carried out in search of the best method to predict, diagnose, treat and prevent it, none has been successful. This is mainly because of the poor understanding of the aetiology and the multifactorial nature of the disease. To contribute to knowledge about the disease in high-risk groups, this study set out to investigate the serum levels of selected biomarkers (NTpro-BNP, PAI-1, Neuropilin-1 and PAPP-A) in pregnant women with hemoglobinopathies diagnosed with preeclampsia compared with those without preeclampsia and normal haemoglobin pregnant women with preeclampsia and those without preeclampsia. From this study, we found that among the serum levels of the selected biomarkers measured the serum level of PAI-1 was significantly high among pregnant women with hemoglobinopathy and preeclampsia. We also found that there was no significant association between the serum levels of NTpro-BNP, PAI-1, Neuropilin-1 and PAPP-A and hemoglobinopathy and preeclampsia.

Demographic parameters were compared among the comparison groups, mean age, parity, gravidity, gestational age at first prenatal visit, previous abortion and previous caesarian section. The mean age of study participants, pregnant women with preeclampsia but without hemoglobinopathy was similar to the mean age of pregnant women with preeclampsia in a study by Udenze et al., (2017). It was also found that the mean age was higher than the mean age of preeclamptic women reported in a study by Kumari et al., (2017). This difference could be attributed to the study area and sample size used for the study. Also, the type of study may influence the mean age. The mean age of the study shows that most of the participants were in the early reproductive age and were not approaching menopause. Some studies have shown

that women in their mid-30s were at more risk of preeclampsia than those below that age(Adu-Bonsaffoh et al., 2014). More so, generally among all the comparison groups, the majority of the study participants were nulliparous. More so, the majority of the study participants were women with hemoglobinopathy only and one pregnancy. This study was similar to that of (Kumari et al., 2017) and (Wilson et al., 2012a) which also had the majority of their study participants being nulliparous.

In this study, the majority of the participants had haemoglobin genotype HbA. Among the women with abnormal hemoglobinopathies, those with sickle cell traits dominated. A study by Acquah and colleagues, (2020), which looked at hemoglobinopathies in patients with asymptomatic carriage of *Plasmodium falciparum* in the same population and study area also found that people with the HbAS genotype dominated in their study population. They found some misclassification of the haemoglobin genotypes which corroborate with the findings of this study. These differences can be attributed to the techniques used in identifying the haemoglobin genotypes.

In assessing, the levels of the selected biomarkers it was found that the mean level of NTpro-BNP was high in all the preeclamptic groups. It was also higher in women with hemoglobinopathy and preeclampsia than in women with preeclampsia only this difference was not significant. Similar findings were seen in studies by Sadlecki et al., (2016) but the differences in their mean were significant. Although the pattern in the mean levels of NTpro-BNP was similar among the comparison groups in our study and that of the study by Kumari et al., (2017), their findings were significant whereas ours was not significant. This variation could be attributed to the type of tests that were carried out in both studies. In the study by Kumari et al., (2017) the Roche CARDIAC NTpro-BNP kit was used to measure the levels of NTpro-BNP in plasma samples whereas in this study we used the sandwich ELISA method with a duo set kit from R&D systems. NTpro-BNP helps to promote diuresis, natriuresis,

vasodilation of the systemic and pulmonary vasculature, and reduction of circulating levels of endothelin and aldosterone. The findings from the study imply that during preeclamptic pregnancy stress placed on the cardiomyocytes to pump more blood at a higher pressure to supply the fetus with sufficient blood oxygen is needed. Women with both conditions (HbP and PE) will have higher levels because having a hemoglobinopathy, especially sickle cell disease puts you at a higher risk of pulmonary hypertension so the cardiomyocytes may be more stressed when preeclampsia sets in. The use of a validated biomarker such as brain uretic peptide could provide important prognostic and diagnostic information on the prevalence of pulmonary hypertension in sickle cell disease patients (Machado R.F., Hildesheim M., Mendelsohn L., Alan T., Remaley E., Gregory J. K, 2008).

The mean serum level of PAPP-A was found to be low in women with preeclampsia and hemoglobinopathies. In the women with hemoglobinopathy, those with PE complicated pregnancy had the lowest level of PAPP-A but in the women, with normal haemoglobin group, those with PE complicated pregnancy had low levels of PAPP-A. This finding was in contrast with studies by Lau et al., (2012) and Smith et al., 2002). PAPP-A is a glycoprotein produced in the placenta, which is present in maternal circulation in rising concentrations during pregnancy. Utilizing its proteolytic activity, PAPP-A functions as a regulatory protein in the insulin-like growth factor system which is known to play a significant role in the regulation of fetal growth and the formation of the placenta (Kirkegaard et al., 2010). It was discovered in a study conducted by Yaron et al., (2002) that low levels of PAPP-A are indicative of an imminent fetal loss, chromosomal abnormalities and adverse pregnancy outcomes. Low levels of PAPP-A lead to adverse pregnancy outcomes because PAPP-A is produced by the trophoblast and low levels thereof may be a sign of abnormal placental function that manifests also in other placental-related complications such as miscarriage and fetal growth restriction (Yaron et al., 2002). Some studies conducted have shown strong evidence that the levels of

PAPP-A were increased in pre-eclamptic women as compared to normotensive pregnant women(Peeva et al., 2019). It was also found that pregnant sickle cell disease patients produced lower levels of PAPP-A as compared with normal pregnant patients although the reason why was unclear (Peeva et al., 2019).

PAI-1 serum levels were found to be high in preeclamptic groups and extremely high in women with hemoglobinopathy diagnosed with preeclampsia and this finding was significant implying that there was a difference in the level of PAI-1. In studies by (Said et al., 2012) and (Udenze et al., 2017) who segregated it by the first and second trimester, the trend was similar in preeclamptic pregnancy compared to normotensive pregnancy. Plasminogen activator inhibitor-1 (PAI-1) is a member of the superfamily of serine-protease inhibitors (or serpins), and the principal inhibitor of both the tissue-type and the urinary-type plasminogen activator, the two plasminogen activators able to activate plasminogen.(Matteo, Cesari; Marco, Pahor; Raffaele, 2011). Plasminogen is converted by the action of plasminogen activators such as tissue-type plasminogen activators and urokinase-type plasminogen activators. PAI-1 works to suppress fibrinolysis interacts with vascular cells and regulates cell replication and angiogenesis (Matteo, Cesari; Marco, Pahor, Raffaele, 2011). In a study that compared the mRNA expression of PAI-1 and tPA in maternal plasma among pregnant women with and without preeclampsia, it was found that there was a significant increase in mRNA expression in maternal pregnancies complicated by preeclampsia as compared to normotensive age-matched controls (Purwosunu et al., 2007). Some studies show that the level of PAI-1 is elevated in sickle cell disease patients as compared to normal patients(Patel et al., 2010). This was evident in the levels seen among women with hemoglobinopathy and PE.

Neuropilin-1 in hemoglobinopathy patients was high in those who had no preeclampsia whereas in the normal haemoglobin group it was high in the preeclamptic patients. In a study by (Xu et al., 2016). It was observed that low levels of Neuropilin -1 were found in patients

although this observation was in placental tissues of pregnant women. The low level of Neuropilin-1 was seen in those that had hemoglobinopathies only. NRP-1 has been shown to mediate angiogenesis and vasculogenesis by acting as a $VEGF_{165}$ receptor that guides the formation of nerve fibre in the developing nervous system by acting as a receptor for semaphorins (Kawasaki et al., 1999). According to a study conducted by Xu et al., (2016) it was observed that pregnant female mice models with pre-eclampsia expressed lower levels of NRP-1 as compared to control mice. This led to the suggestion that the absence or low levels of NRP-1 may worsen the case of pre-eclampsia. This is obvious in the patients with HbP and PE.

Though weak significant correlation was seen between NTpro-BNP and diastolic blood pressure during 1st prenatal visit and at diagnosis. Studies by Udenze et al., (2017) showed a positive correlation between PAI-1 and systolic blood pressure and diastolic blood pressure but in our study, an inverse correlation was seen.

In a multivariate model to assess if the biomarkers could predict PE in women with hemoglobinopathy adjusting for clinical characteristics in this study, it was found that none of the markers measured showed any significance in predicting PE although they all showed high odds. Gravidity showed significance in predicting PE in women with hemoglobinopathy diagnosed with PE whereas baby sex and birthweight determined possible prenatal outcomes due to preeclampsia in women with hemoglobinopathy diagnosed with PE. Research by Yaron et al., (2002) has shown that maternal and perinatal outcomes such as miscarriage and fetal growth restrictions were placental-related complications that occur when there is a low level of one of the biomarkers, PAPP-A. This implies that the significant odds seen in perinatal and maternal outcomes after adjusting for levels of the selected biomarkers and other confounding factors show that the biomarkers are possible determinants of perinatal and maternal outcomes in pregnant women with both hemoglobinopathy and preeclampsia.

5.1 Limitation of the Study

The unavailability of complete data for most of the pregnancies, especially among comparison women, could potentially introduce some level of bias in the study. To minimize this bias, it was ensured that variables with responses from 80% of the observations were used.

Selection bias was one of the limitations of this study because the outcome was a rare condition. To minimize this, one participant from each comparison group was picked in a week.

The generalization of the results of this study is limited because participants were drawn from only one institution. A large sample size of participants from different institutions would have given a different picture.

CHAPTER SIX

6.1 Conclusion

In conclusion, this study set out to determine the serum levels of NTpro-BNP, PAPP-A, Neuropilin-1 and PAI-1 in peripheral blood of pre-eclampsia patients with hemoglobinopathy and those without preeclampsia. From the study, there were variations in the concentrations of the selected biomarkers measured in pregnant women with hemoglobinopathies diagnosed with PE compared to the comparison groups. This variation was only significant in the serum levels of PAI-1. This implies that using the levels of PAI-1 could help predict PE in hemoglobinopathy patients.

More so, there were misclassifications of patients' sickling status. The confirmation tests reclassified some participants with initial haemoglobin genotype of HbAA genotype and others of HbSS at recruitment.

There was a positive but poor correlation between diastolic blood pressure at the first prenatal visit and PE diagnosis.

More so, an association was found between both perinatal and maternal outcomes and HbP and PE pregnancy. This is evident that perinatal and maternal outcomes are adverse pregnancy outcomes of HbP and PE pregnancy.

All biomarkers investigated did not show any promising ability in predicting preeclampsia in women with HbP and PE and women without HbP and PE but the rather possible determination of the perinatal and maternal outcome of pregnancy could be predicted.

6.2 Recommendations

1. The Government should collaborate with therapeutic companies to explore the therapeutic potential of the PAI-1 biomarker since it is showing a significant level of variation among HbP and PE patients compared to normal haemoglobin patients.
2. The Ghana Health Service should create awareness about the risk of developing preeclampsia when one has hemoglobinopathy genotypes
3. The Ghana Health Service should educate expectant mothers on the perinatal outcomes and the necessity of attending antenatal when they are at high risk.
4. A longitudinal study with a larger sample size in various institutions should be studied to have a broader picture.

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7.0 APPENDICES

Appendix 1

Questionnaire

The Role of Epidermal Growth Factor-like Domain 7 (EGFL7) in Sickle Cell Disease Patients Diagnosed with Preeclampsia
ASSESSMENT/QUESTIONNAIRE
IA.1 Study ID
number……|__|__|__|__|__|__|__|
IA.2 Hospital Folder Number ……..……...|__|__|__|__|/|__|__|
IA.3 Date of admission hospital……. ___/___/___
Inclusion Criteria ..... Tick which one
IC.1 Preeclampsia with sickle cell/hemoglobinopathy trait patients (SS, SC, AS AC) ……. |__|
****IMPORTANT RECRUIT THESE AT ALL TIMES******
Controls (to be recruited as instructed each week)
IC.2 Normal pregnancy…….
|__|
IC.3 Preeclampsia complicated pregnancy…….…. |__|
IC.4 Normal pregnancy without preeclampsia but the mother with SS, SC, AS or AC……..… |__|
General Exclusion Criteria
GE.1 History of diabetes mellitus (gestational diabetes is not excluded) (1=yes, 2=no) ……|___|
GE.2 heart disease (1=yes, 2=no) ……..……..…..
|___|
GE.3 Pre-existing renal disorders except sickle cell nephropathy are included (1=yes, 2=no) .....|___|
GE.4 Cancer (1=yes, 2=no) ……..…….....
|___|
GE.5 Hematologic disorders other than hemoglobinopathies (1=yes, 2=no) ……...|___|
GE.6 Immunologic disorders (1=yes, 2=no) ……..……....
|___|
GE.7 Sepsis/infection (1=yes, 2=no)
…….....|___|
GE.8 Chronic hypertension (without SS) (1=yes, 2=no) ……....
|___|
If the answer to any of the above questions is yes, do not recruit into the study!
*****CONSENT REQUIRED****** please indicate if obtained (1=yes, 2=no)
…….…… |___|
GE.9 Name of staff or MD who obtained consent. _____
2
IA.1 Study ID number……. ……..|__|__|__|__|__|__|__|
IA.2 Hospital Folder Number……..... |__|__|__|__|/|__|__|
General Information (Maternal-get from medical folder/records) ... if not applicable—9999
GI.1 Age (years at last six months) ……. |__|__|.|__|
GI.2 Pregnancy history
GI.2a Gravidity (≥ 1) ……...|___|
GI.2b Parity (≥ 0) ……. |___|
GI.2c Prior Abortion (1=yes, 2=no) ……. |___|
GI.2d If yes indicate number……..…..... |___|
GI.3 Previous caesarean section (1=yes, 2=no) ……..... |___|

GI.3a If yes indicate number.…… |___|
GI.4 Sickling status sodium metabisulfite (1=positive, 2=negative) …… …..|___|
GI.5 Haemoglobin genotype (1=SS, 2= SC, 3= AS, 4=AC, 5=AA, 6= Other-Specify) ……. |___|
GI.6 Blood group (1=O, 2= AB, 3= A, 4=B,) ……..|___|
GI.7 Rhesus factor (1= negative, 2= positive) .....|___|
GI.8 G6PD status (normal=1, partial defect=2, full defect=3) …… |___|
Prenatal Visit (if the information is available) ……. if not applicable—9999……. |_____|
PV.1 Date of first prenatal visit……___/___/___
PV.2 Gestational age at first prenatal visit (in weeks) …… |___|
PV.3 Expected date of delivery……___/___/___
PV.4 Weight of mother at first prenatal visit (in kg) ……||___|___|. |__|
PV.5 Height mother at first prenatal visit (in cm) ……|__|__|__|
PV.6 BMI at first prenatal visit (kg/cm2) …… _____
PV.7 Blood pressure (mmHg) at first prenatal visit……..…|__|__|__|/|__|__|__|
PV.8 Preeclampsia diagnosed at prenatal visit (1=yes, 2=no) ……..……..……|___|
PV.9 If yes, a state gestational age preeclampsia diagnosis was made at the prenatal visit
in weeks (or 9999 if not applicable) ……..……..……|___|
PV.10 Date of prenatal visit preeclampsia diagnosis was made (9999 if not applicable) ___/___/___
Admission for Delivery
AD.1 Date of peripheral blood collection ..... ___/___/___
AD.2 Time of peripheral blood collection ..... ___: ___hrs
AD.3 Date of placental tissue collection ..... ___/___/___
AD.4 Time of placental tissue collection ..... ___: ___hrs
Physical Exam on labour ward admission, Vital Signs and Laboratory Results
PE.1 Blood pressure (mmHg) initial presentation to the Korle Bu hospital……|__|__|__|/|__|__|__|
PE.2 Preeclampsia (1=yes, 2=no) ……..…|__|
PE.3 Gestational age at diagnosis if yes above (weeks)……..……|__|
PE.4 Blood pressure at diagnosis of preeclampsia (9999=NA) (mmHg)……. |__|__|__|/|__|__|__|
PE.5 Is the patient on the antihypertensive drug? (1=yes, 2=no, 9999= N/A) .....|__|
PE.6 State antihypertensive drug prescribed if yes above (Nifedipine =1, Methyldopa =2,
Labetalol =3, Hydralazine =4, Other =5) …… _____
NB: PE.1 and PE.4 may be the same thing if diagnosed with preeclampsia at presentation
3
IA.1 Study ID number…… …..…..|__|__|__|__|__|__|__|__|
IA.2 Hospital Folder Number……..... |__|__|__|__|/|__|__|
Past Medical History
PM.1 Low dose aspirin taken for preeclampsia pre-admission (1=yes, 2=no) ……...|___|
PM.2 History of sickle cell nephropathy (1=yes, 2=no, 9999=N/A) ……..……...|___|
Delivery Information
DI.1 Date of delivery…….... __/___/___
DI.2 Gestational age at delivery (weeks)……..……|___|
DI.3 Mode of delivery (Vaginal=1, Caesarean=2, Instrumental delivery=3) ……..... |___|
DI.4 Blood pressure after delivery……..…|__|__|__|/|__|__|__|
Maternal birth outcome
MB.1 Mother lived (1=yes, 2=no) ……..……..…|___|
MB.2 Sickle cell crisis (1=yes, 2=no) …… …..……...|___|
MB.3 HELLP syndrome (1=yes, 2=no) .....|___|
MB.4 Other birth complications. (1=yes, 2=no) ……...……...…… |___|
MB.4a If yes name birth complication_____
Perinatal outcome of a baby
PO.1 Baby lives at birth. (1=yes, 2=no) …… |___|
PO.1 Twin babies = Baby A (1=yes, 2=no) …… …..……..|___|
= Baby B (1=yes, 2=no) ……...…… |___|
PO.2 Stillbirth (1=yes, 2=no) ……..……|___|

PO.2 Twin babies = Baby A (1=yes, 2=no) ……. …..……|___|
= Baby B (1=yes, 2=no) ……...…… |___|
If yes; tick appropriate
PO.2a Macerated stillbirth (in utero) ……..…… |___|
PO.2a If twins, Baby A……..…… |___| Baby B……..…… |___|
PO.2b FSB (during labour/delivery) ……..……..... |___|
PO.2b If twins, Baby A……..…… |___| Baby B……..…… |___|
PO.3 Sex (1=M, 2=F) ……..…… |___|
PO.3 Sex If twins (1=M, 2=F) …. Baby A……..…… |___| Baby B……. |___|
PO.4 Apgar at 1 minute…….... ___/___
If twins…….....Baby A___/___.....Baby B___/___
PO.5 Apgar at 5 minutes…… ___/___
If twins…….....Baby A___/___.....Baby B___/___
PO.6 Birth weight at one decimal place (kg)……..…. ….…...|___| |___|
If twins…….....Baby A |___|.|___|.....Baby B |___|.|___|
PO.7 Head circumference (cm)……..…_____
If twins…….....Baby A _____.....Baby B _____
PO.8 Length of baby (cm)……..…..._____
If twins…….....Baby A _____.....Baby B _____
PO.9 NICU admission (1=yes, 2=no) ……..……..……|___|
If twins…….....Baby A |___|.....Baby B |___|
……PO.9a If yes, state reason for NICU admission_____
4
IA.1 Study ID number…… ……..|__|__|__|__|__|__|__|
IA.2 Hospital Folder Number…….…….... |__|__|__|__|/|__|__|
LABORATORY RESULTS (Maternal)
LR.1a Mother received a blood transfusion before sampling? (1=yes, 2=no) ……..…|___|
LR1b Mother received fresh frozen plasma before sampling? (1=yes, 2=no) ……..…|___|
Full Blood Count Results:
LR.2a Haemoglobin (Hb. g/dL) ……...|__|__|.|__|
LR.2b RBC (x10^{12}/L)
…….....|__|.|__|__|
LR.2c HCT (%)
…….……|__|__|.|__|
LR.2d MCV (fL)……..……
|__|__|.|__|
LR.2e MCH (pg)……..……|__|__|.
|__|
LR.2f MCHC (g/dL) ……..……..…|__|__|.|__|
LR.2g RDW_SD (fL)……..……....…|__|__|.
|__|
LR.2h RDW_CV (%) ……..……|__|__|.|__|
LR.2i PLT(x10^{9}/L)
…….……|__|__|__|
LR.2j PDW (fL) ……..……
…..|__|__|.|__|
LR.2k MPV (fL)…...……..……....…|__|__|.
|__|
LR.2l P_LCR (%) ……..……....…|__|__|.
|__|
LR.2m PCT (%) …...……..……....
|__|.|__|__|
LR.2n WBC (x10^{9}/L) …...……...……...……....…|__|__|.
|__|

LR.2o NEUT#.....|__|__|. |__|__|
LR.2p NEUT%.....|__|__|.
|__|
LR.2q LYM# .....|__|__|.
|__|__|
LR.2r MONO#.....|__|. |__||__|
LR.2s MONO%.....|__|__|.
|__|
LR.2t EO#.....|__|.
|__|__|
LR.2u EO%.....
|__|.|__|
LR.2v BASO#.....|__|.
|__|__|
LR.2w BASO%.....
|__|.|__|
LR.3 Urine Protein (1= negative, 2= Trace, 3= 1+, 4= 2+, 5= 3+, 6=4+) .....|___|
Liver function test:
LR.4a Total bilirubin (mg/dL) .....
LR.4b Total bilirubin (SI) (umol/L) .....
LR.4c Direct bilirubin (mg/dL) .....
LR.4d AST (SGOT) (U/L) .....
LR.4e ALT (SGPT) (U/L) .....
LR.4f ALK Phos. (g/L) .....
LR.4g GGT (U/L).....
LR.4h Total Protein (g/dL) .....
LR.4i Total Protein (SI) (g/L) .....
LR.4j Albumin (g/dL) .....
LR.4k Albumin (SI) (g/L) .....
5
IA.1 Study ID number..... |__|__|__|__|__|__|__|__|
IA.2 Hospital Folder Number..... |__|__|__|__|/|__|__|
Renal function test (blood urea and electrolytes):
LR.5a Sodium (mmol/L) .....
LR.5b Potassium (mmol/L) .....
LR.5c Chloride (mmol/L) .....
LR.5d Urea (SI) (mmol/L) .....
LR.5e Creatinine (SI) (mmol/L) .....

AN. Additional Notes