PATHOPHYSIOLOGICAL INDICATORS OF PREGNANCYINDUCED HYPERTENSION IN GHANAIAN WOMEN

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DECLARATION

The research reported in this thesis was done at Lister Hospital and Fertility Centre, La General Hospital and Ridge Regional Hospital all of Accra, Ghana. This research has not been presented for any other degree.

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ABSTRACT

Pregnancy-Induced Hypertension (PIH) is the abnormal increase in blood pressure (BP) of pregnant women who do not have pre-gestational chronic hypertension or renal diseases or proteinuria before the final half of gestation, but present with high BP and proteinuria in the final half of pregnancy which normalizes postnatally. PIH ranges from high BP without proteinuria, through high BP with proteinuria and multiorgan dysfunction to high BP with proteinuria, multiorgan dysfunction with seizures. PIH is usually diagnosed when BP rises above 140/90 mmHg.Ten percent (10%) of complications of pregnancy are as a result of hypertension and this accounts for the increased risk of adverse foetal, neonatal and maternal outcomes. This necessitates early diagnosis to avert these fatal outcomes. This study sought to find the biomarkers that would assist in the early diagnosis of pregnancy-induced hypertension in Ghanaian women. To achieve this, the following specific objectives were set: The determination of the concentrations of biomarkers of systemic inflammation, endothelial injury and systemic oxidative stress in PIH and controls;the evaluation of the relationship of hepcidin levels with iron regulation and systemic inflammation in PIH and controls; the determination of the concentrations of Soluble urokinase plasminogen activator receptor, Interlukin-6 and C-reactive protein in PIH and healthy pregnancy were studied in order to evaluate the best marker for the characterization of the inflammatory status during pregnancy and the determination of serum lipids levels and its correlation with C-reactive protein, Interlukin-6, 8-isoprostagladin F2α and fibronectin in PIH.This research took place at the antenatal clinics of Ridge Regional Hospital, Accra, La General Hospital, Accra and Lister Hospital and Fertility Centre, Accra, Ghana from June, 2014 to July, 2015. This study involved forty-eight (48) women with gestational hypertension, fifty-seven (57) with preeclampsia, eighteen (18) with eclampsia and forty-five (45) normotensive pregnant women (controls) in at least their second trimester of gestation. All participants were within 18yrs to 40 yrs of age and with singleton pregnancy based on ultra-sound results. After ethical approval and informed consent had been obtained, blood (ie. EDTA whole blood, heparinized-plasma and serum) and urine samples of participants were obtained for biochemical, haematological and urine analysis. There were significantly higher levels of markers of systemic inflammation : IL-6 (19.60±10.32pg/ml vs 13.85±2.80pg/ml, p=0.04), CRP (3.31±2.81ng/L vs 0.98±0.05ng/L, p<0.0001), suPAR (2.04±0.66pg/ml vs 1.57±0.56pg/ml, p=0.03), endothelial injury: FN (21.87±11.95ng/ml vs 13.85±2.80ng/ml, p=0.01) and systemic oxidative stress: 8-iso-PGF2α (43.03±27.29pg/ml vs 5.55±5.33pg/ml, p=0.03) in PIH

women compared to controls respectively.The results of the level of hepcidin in relation to iron homeostasis and systemic inflammation among the participants indicates significant increase in the levels of hepcidin (7.72±1.07 vs 6.46±0.82, p<0.0001), ferritin (183.0±156.2 vs 37.1±30.5, p<0.0001), IL-6 (19.60±10.32 vs 13.85±2.80, p=0.04) and CRP (3.31±2.81 vs 0.98±0.05, p<0.0001) in the PIH women compared to the normotensive ones respectively. Whereas there was significantly lower iron (85±39.09 vs 138±30.33, p<0.0001) and TIBC (308.9±95.29 vs 360±68.0, p=0.0013) levels in the PIH compared to normotensive women respectively. Although the concentrations of all the inflammatory markers (ie.CRP, IL-6 and suPAR) were significantly higher in PIH women compared to the controls, suPAR stood out as the best inflammatory biomarker for distinguishing PIH women from the controls using its AUC of ROC: 0.71 (95% CI = 0.56-0.87; p = 0.0217) and its stability. After assessing the lipid profile among these women, there was a significant increase in triglycerides (2.19±0.93mmol/L vs 1.73±0.71mmol/L, p=0.003) and HDL-cholesterol (1.14±0.34mmol/L vs 1.01±0.30mmol/L, p=0.03) in the PIH women compared to the controls respectively. Triglyceride correlated positively with IL-6 in both preeclampsia (r = 0.65, p < 0.05) and eclampsia (r = 0.58, p < 0.05) subjects whereas triglyceride correlated positively with fibronectin in only eclamptic women (r = 0.75, p < 0.05). A positive correlation was also shown between total cholesterol and 8-iso-PGF2α in eclamptic women(r = 0.61, p < 0.05). The findings of this study indicate that pregnant women attending antenatal clinic need to be screened for iron levels, oxidative stress (8-iso-PGF2α), dyslipidaemia (lipid profile), endothelial dysfunction (fibronectin) and inflammation (suPAR). Also these analytes can be used in the management of PIH since they can be used to assess the prognosis of this systemic maternal syndrome.

DEDICATION

This work is dedicated to my beloved late grandmother Mrs Theresa Hope Conduah aka Auntie Adina without whose mentorship and love this pursuit of knowledge would not have come to fruition.

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LIST OF ABBREVIATIONS

4-AP – 4-Aminophenazone

8-iso-PGF2α – 8-isoprostaglandin F2 alpha

ADP – Adenosine diphosphate ATP

– Adenosine triphosphate

AUC – Area under curve

BP – Blood pressure

CRP – C-reactive protein

CHE – Cholesterol Esterase

CHOD – Cholesterol Oxidase

CI – Confidence interval

COX – Cyclo-oxygenase

DNA – Deoxyribonucleic acid

DBP – Diastolic blood pressure

DAP – Dihydroxyacetone phosphate

EC – Eclampsia

ELISA – Enzyme-linked immune-sorbent assay

EDTA – Ethylene diamine tetraacetic acid

FN – Fibronectin

GH – Gestational Hypertension

G3P – Glycerol-3-phosphate

GPO – Glycerol phosphate dehydrogenase

H_2O_2 – Hydrogen peroxide

H_2SO_4 – Sulphuric acid

HCT – Haematocrit

Hb - Haemoglobin

HRP2 – Histidine-rich protein 2

HDL – High density lipoprotein cholesterol

HRP – Horseradish peroxidase hs-CRP –

High-sensitivity C-reactive protein

IgG – Immunoglobin G

iNOS – Inducible nitric oxide synthase

IL-6 – Interlukin 6

IDA – Iron deficiency anaemia

LPL – Lipoprotein lipase

LDL – Low density lipoprotein cholesterol

M - Molar

MCH – Mean cell haemoglobin

MCHC – Mean cell haemoglobin concentration

MCV – Mean cell volume

MDG – Millennium development goal

NO – Nitric oxide

POD – Peroxidase

PLT - Platelet

PE – Preeclampsia

PIH – Pregnancy-induced hypertension

ROS – Reactive oxygen species

ROC – Receiver Operator Characteristic RBC

– Red blood cell

suPAR – Soluble urokinase plasminogen activator receptor

SD – Standard deviation

SBP – Systolic blood pressure

TC – Total cholesterol

TS - Transferrin

TIBC – Total iron-binding capacity

TG – Triglyceride

UIBC – Unsaturated iron-binding capacity uPAR

– Urokinase plasminogen activator receptor

VLDL – Very low density lipoprotein cholesterol

WBC – White blood cell

Chapter 1

INTRODUCTION

1.1 GENERAL INTRODUCTION

Hypertension is implicated in most of the complications of pregnancy and is mostly responsible for maternal mortality, as well as other serious effects on pregnancy outcomes (NHBPEP, 2000). Pregnancy-induced hypertension (PIH) has been a health problem for ages; but, the pathophysiology of this condition remains multifactorial thus making it difficult for effective clinical intervention. In 1978, Chesley was the first to do an extensive epidemiologic work on PIH and since then, several researches have been done to unearth the myth surrounding PIH. In 1739, the term eclampsia was used for the first time to differentiate the acute form of convulsion from epilepsy (Chesley, 1974). Historic literature on eclampsia is very pauce dispite the dramatic nature of the condition, and this may be so because historically midwives were responsible for delivery and doctors were mostly males so did not participate (Carty *et al.*, 2010). Carty *et al.* (2010) described a state of proteinuria that was observed for the first time in eclamptic women in the mid-nineteenth century and shygmographic tracings were used to record high blood pressure thereafter. Also Chesley (1984) reported that in 1894, pregnant women presenting with high blood pressure and proteinuria without eclamptic seizures were described as preeclamptic.

Senah (2003) in his review stated that the death of a woman as a result of pregnancy-related complications is seen as a tragic event in all Ghanaian societies and sometimes required the elaborate ritual purification of the whole society. He gave an example of pregnant women in Osu, a suburb of Accra traditionally being given a ritual sea bath soon after the burial of a pregnant woman. He also noted that in all Ghanaian societies maternal death is considered unclean and therefore expectant women were given strict dietary and behavioural codes to ensure safe delivery of children without deformities. In a research review Senah (2003) noted that a publication by Rosenfield and Maine, (1985) on maternal deaths was the advent of global attention to the issue. In this publication Rosenfield and Maine noted that maternal mortality was worsening in developing countries and that much attention has to be given to existing programs to reduce the rates. This same year 1985, in Zaria, Northern Nigeria,

Harrison analysed 22,774 consecutive hospital births and found out that the mortality due to childbirth was very high (Harrison, 1985). He also brought to bear that social and cultural factors were very important in finding the cause of the high death rate and complications in pregnancy and delivery in Nigeria. Senah (2003) reported in his review that sub-Saharan African women had a lifetime risk of 1 in 21 of dying due to childbirth which is 400 times higher compared to that of their counterparts in Western Europe or North America. He also noted that maternal death is still a challenge in developing countries and this could be due to the medical bias used in addressing the problem. Senah (2003) noted that the cause of the problem was not only medical but could be lifestyle and economical and any interventions designed against it must consider these dynamics. To buttress this point, Senah (2003) referred to Dubos (1965) who propounded that the virulence of specific causative organisms is not responsible for the occurrence and prognosis of diseases but rather the lifestyle of people.

Campbell *et al.* (1985) observed that once a woman suffered pregnancy-induced hypertension she is prone to developing PIH in future pregnancies but with better prognosis of the condition. This finding was corroborated by other studies by Sibai *et al.* (1986) and Hargood and Brown (1991) which showed that about half of women who had PIH in their first pregnancy suffered the condition again in their second pregnancy but the less severe form. In the 1978 review on PIH by Chesley, he observed that about a tenth of the women studied had recurrent eclampsia which forms 5% of the total subsequent pregnancies, and one third had recurrent gestational hypertension or preeclampsia which forms 27% of the total subsequent pregnancies (Chesley, 1978). Sibai *et al*., (1992) studied some eclamptic women and observed that those who suffered eclampsia before the second half of pregnancy had a higher recurrence risk with poorer prognosis than those who presented with the condition in the final half of pregnancy. They also found out that women who suffered recurrent PIH had a greater risk of PIH than those who had healthy first pregnancy.

There is an ongoing debate on whether developing PIH could predispose one to future chronic hypertension. Most studies done on PIH showed that women who have the syndrome have higher average blood pressure and are more prone to developing chronic hypertension later in life than those who had healthy gestation (Sibai *et al.*, 1986; Nisell *et al.*, 1995). Thus there is a greater risk of developing PIH in women who have a history of

PIH pregnancies (Nisell *et al.*, 1995). Eclamptic women who have a history of PIH pregnancies are more likely to develop chronic hypertension in future and that it is dependent on the period of gestation that it occurs, that is the earlier in gestation it occurs the higher the prevalence of chronic hypertension in future (Sibai *et al.*, 1992). In a later work by Zhang *et al.* (2001) they also confirmed that first time pregnancy-induced hypertensive women have higher risk of recurrence of PIH and this is dependent on how early it is experienced. They reported that primigravid women have a lesser risk for increased BP without proteinuria than for increased BP with proteinuria and multiorgan dysfunction and the risk increased in those who had pre-gestational chronic hypertension who developed proteinuria and multiorgan dysfunction. However, late onset eclamptic women who have no recurrent PIH are comparable to those without a history of PIH (Sibai *et al.*, 1992). It is generally thought that PIH occurs in nulliparous women and when it occurs in parous women then it is recurrent but Rasmussen *et al.* (2000) observed that it is not always the case and that parous women may also experience preeclampsia for the first time.

1.2 EPIDEMIOLOGY OF PREGNANCY-INDUCED HYPERTENSION

1.2.1 INCIDENCE AND PREVALENCE OF PREGNANCY-INDUCED HYPERTENSION

Senah (2003) in his review noted that about half a million women worldwide loose their lives from pregnancy-related complications and majority of them are in the third world. He also reported that in Ghana there is a high loss of maternal lives due to adverse pregnancy outcomes. He tried to give a reason for this high rate to be the result of the complex biological and cultural indices that determine maternal mortality. Obed and Patience (2006) noted that pregnanacy-induced hypertension accounts for approximately 9% maternal and perinatal morbidity worldwide and 7.03% for Ghana. Owiredu *et al.* (2012) reported that there were great differences among the reported incidence of pregnancy-induced hypertension cases worldwide. Thus the World Health Organisation in 1988 sought to find the reason for these differences in this reportage and therefore postulated that these differences could be due to the different definitions of PIH, population dynamics, socioeconomic and obstetric status, incidence of PIH, or usage and provision of antenatal clinics (WHO, 1988).WHO, 1988 in a controlled population based study found the incidence of clinically recognized hypertension during pregnancy to vary from 1.2% to

31.0% between countries and the variation when using a strict definition of proteinuric hypertension to be from 1.5% to 8.3% (WHO, 1988).

Kyle *et al.* (1995) in clinically controlled trials observed the incidences of gestational hypertension and preeclampsia in first time pregnant women who were non-diabetic and normotensive to be 5 % to 9 % and 5 % to 7 % respectively. An observation made by Zhang *et al.* (1997) was that the occurrence of PIH in nulliparous women was 4-5folds that of multipara women. Conde-Agudelo and Belizán (2000) noted that nulliparity increases the risk of developing preeclampsia and therefore the parity of subjects can have an effect on the incidence report. However, they reported that there is a variation in the incidence of preeclampsia in nulliparous women and Brown and Buddle (1997) speculated that the reason for this variation could be the difference in diagnostic criteria for preeclampsia used for these studies. Douglas and Redman (1994), reported the incidence of eclampsia in the United Kingdom to be 0.049% pregnancies whereas Saftlas *et al.* (1990) and Zhang *et al.* (2003) reported 0.056% and 0.1% pregnancies respectively in the United States of America. Ekholm *et al.* (1999) also found the incidence of eclampsia in Finland to be 0.024% whereas the incidence of eclampsia in Tehran was 0.1% (Sh *et al.*, 2001). Three different studies done in Sri Lanka on the incidence of eclampsia reported these results: a recent incidence of 0.28% was reported by (Jayawardana and Fernando, 1995) in Peradeniya in Central Sri Lanka, whilst in Galle in Southern Sri Lanka (Goonewardene and Sirisena, 1985) had earlier reported an incidence of 0.38% which was higher but the initial study by (Jegasothy *et al.*, 1983) in Jaffna in the North of Sri Lanka reported a much higher incidence of 0.66%. Since not all preeclamptic women do develop the worst form which is eclampsia the low incidence rates in these studies is to be expected (Owiredu *et al.*, 2012).

Hypertension is implicated in a tenth of complications of pregnancy and is responsible for most of the adverse foetal, neonatal and maternal outcomes (Duley, 2009; Steegers *et al.*, 2010). North *et al.* (2011) corroborated this by reporting that about 10% of pregnancies may present with high blood pressure and preeclampsia is responsible for 2-8% of complications of pregnancy found in the developed countries with 5% being primigravid women. As a result of the differences in definition of the condition and the challenge with measuring of BP, diagnosis becomes difficult and therefore accounts for the different incidences reported for the condition.

1.2.2 RISK FACTORS OF PREGNANCY-INDUCED HYPERTENSION

Factors that can predispose pregnant women to an abnormal increase in their blood pressure have been well documented (Steegers *et al.*, 2010). Factors that increase risk include nulliparity, previous episode of PIH,partner change,many years between pregnancies, kidney disease, and the presence of antiphospholipid antibodies (Steegers *et al.*, 2010). Owiredu *et al.* (2012) observed on the contrary that nulliparity was not a risk factor but obesity, family history of preeclampsia, condom and contraceptive usage, partner change were risk factors. Lie *et al.* (1998) reported that there is a paternal risk of reoccurrence of preeclamptic pregnancies in different women impregnanted by the same man thus suggesting paternal genetic factors in preeclampsia. Lisonkova and Joseph (2013) reported a relation of early-onset PIH with African-American race, chronic hypertension and congenital anomalies whilst late-onset preeclampsia is associated with younger maternal age, nulliparity, and diabetes mellitus. The risk of body weight causing preeclampsia has been controversial (Valensise *et al.*, 2008; Lisonkova and Joseph, 2013) but Roberts *et al.* (2011) noted that obesity has the tendency to increase the risk of all "forms" of preeclampsia. The report from Sukalich *et al.* (2006) seems to confirm that pre-gestational obesity is a strong independent condition that can pre-dispose women to PIH but the mechanism underlying this is not understood and the prevalence is 10% of obese pregnant women.

The following are risk factors adapted from Sibai (2005) and Turner (2010) :

1) Maternal obstetric factors: Women below 18yrs and above 40yrs (Esen *et al.*, 2003) who are nulliparous or have a history of preeclampsia are at risk of PIH.Also women who have multi-foetal gestation, molar pregnancy or those who go through assisted conception procedures are equally at risk.

2) Maternal comorbid conditions: Women who pre-gestationally are suffering from chronic hypertension, diabetes, infections, rheumatic, vascular, endothelial and renal diseases or are obese are highly at risk of developing PIH.

3) Maternal genetic factors**:** Women whose weight were below normal at birth or possess anti-phospholipid antibodies and have protein C resistance are prone to

developing PIH. Also women of the African-American race or those who have a first-degree relative who has suffered PIH stand the chance of suffering PIH.

4) Paternal obstetric factors: Low frequency of unprotected coitus, primipaternity, those with the history of having fathered a preeclamptic pregnancy in another woman or paternity by a male born from a preeclamptic pregnancy can lead to PIH.

1.3 PREGNANCY-RELATED HYPERTENSIVE CONDITIONS

During the first trimester of a healthy pregnancy the blood pressure (BP) of pregnant women are generally low as a result of the enlargement of the vasculature caused by the secretion of local mediators such as prostacyclin and nitric oxide (James and NelsonPiercy, 2004). There is usually a 10mm Hg drop in diastolic blood pressure by 13–20 weeks gestation (Wood and Sibai, 1996) and this drop continues until a nadir is reached in the second trimester. Then there is a systematic rise until delivery when it normalizes and then there is an immediate fall in blood pressure but then rises steadily within the first week after birth (Wood and Sibai, 1996). Pregnant women with normal BP may suffer high BP without proteinuria during puerperium, and this may be due to a degree of vasomotor instability (James and Nelson-Piercy, 2004).

Due to the paucity of information on the etiology of PIH, there are different definitions and classifications thus generating controversy when it comes to signs and symptoms used for diagnosis (Zhang *et al.*, 1997). Clinicians and Research Scientist worldwide mostly use two definitions of PIH in their work. One was proposed by the United States of America National High Blood Pressure Education Program Working Group and this is also known as the American College of Obstetricians and Gynecologists definition (NHBPEP, 2000) and the other by the International Society for the Study of Hypertension in Pregnancy (ISSHP) also known as the international definition (Davey and MacGillivray, 1988). The international definition was modified by Redman and Jefferies (1988) and is known as the Oxford definition which is currently being used frequently. PIH may be categorized as high BP without proteinuria, high BP plus proteinuria, and high BP plus proteinuria and seizures. The challenges in diagnosing PIH go beyond its definition but also the lack of general consensus on which phase of Korotkoff sound to use for the determination of the diastolic pressure (Davey and MacGillivray, 1988; NHBPEP, 2000). A difference of 6-10mmHg in

DBP was observed in about a third of participants when decreasing sound instead of no sound was used in its measurement and 5-18% had DBP differences exceeding 10 mmHg (Johenning and Barron, 1992). According to current research the use of the "disappearance of sound" as a measure of DBP is better (Johenning and Barron, 1992; López *et al.*, 1994), but not practicable to use in large sample size research since standardisation would be a challenge. Factors like size of sphygmomanometer cuff , posture of patient and lack of strict adherence to BP measurement protocols which may affect BP levels have not been resolved (Zhang *et al.*, 1997). The recent rise in the use of automated BP monitors has also compounded the sources of variation in BP results. PIH is usually diagnosed as pregnant women in their second trimester and above presenting with an abnormal rise in BP (Cnossen *et al.*, 2006) with or without proteinuria and their urine protein levels are usually measured using the dipstick method but the reliability of this method is questionable (Meyer *et al.*, 1994).

Table 1.1 HYPERTENSIVE DISORDERS OF PREGNANCY (adapted from Karthikeyan and Lip (2007))

OBSERVATIONS	**CHRONIC HYPERTENSION**	**GESTATIONAL HYPERTENSION**	**PREECLAMPSIA - ECLAMPSIA**
Increase in blood pressure	Before first half of pregnancy	Second half of pregnancy	Second half of pregnancy
Level of protein in urine	NORMAL	NORMAL	INCREASED
Blood volume	NORMAL	NORMAL	DECREASED
Platelets level	NORMAL	NORMAL	DECREASED
Liver insults	NO	NO	YES
Kidney insults	NO	NO	YES
Uric acid levels	NORMAL	NORMAL	INCREASED

Clinical Symptoms	NO	NO	YES

The information available for the classification and diagnostic definitions for the categorization of PIH is vague and thus poses a challenge to doctors and research scientist (Chappell *et al.*, 1999). Due to the lack of consensus on terminologies, the same term could mean different disorders depending on the author and this causes a lot of difficulties for counselors and public health practitioners who are responsible for recording adverse findings (Zhang *et al.*, 1997). The Council of the International Society for the Study of Hypertension in Pregnancy decided to resolve these issues by reconciling the different definitions proposed by the different groups who had done researches on hypertension (Zhang *et al.*, 1997).

Table 1.2 THE DIFFERENT DEFINITIONS OF PREGNANCY-INDUCED HYPERTENSION adapted from Zhang *et al.* (1997).

TYPE OF PIH	NHBPEP	ISSHP	Oxford Version
Gestational hypertension	Transient high blood pressure	Increased BP only	Nonproteinuric preeclampsia
Preeclampsia	Increased BP with increased urine protein and oedema	Proteinuric high blood pressure	Proteinuric preeclampsia
Eclampsia	Preeclampsia with seizure	Undefined	undefined

PIH =Pregnancy-Induced Hypertension; NHBPEP=National High Blood Pressure Education Program; ISSHP=International Society for the Study of Hypertension in Pregnancy.

1.3.1 CHRONIC HYPERTENSION

Chronic hypertension is characterized by pre-gestational high blood pressure or high BP observed for the first time before the second trimester of gestation which persists after birth (NHBPEP, 2000). Barton *et al.* (1997) and Wood and Sibai (1996) noted in their studies that obese women, older women and black women were prone to chronic hypertension and Sibai *et al.*, (1998) put the percentage of pregnancies that present with chronic hypertension at 1-5%. Due to the normal decreasing trend of BP in early pregnancy, undiagnosed

hypertensive women could appear normotensive thus masking pre-existing hypertension (McCowan *et al.*, 1996; Brown *et al.*, 2000) and which subsequently could be misdiagnosed as gestational hypertension. Thus sometimes it is after the blood pressure fails to normalize several months post partum as expected that a diagnosis is made and rarely does preeclampsia present before the first half of gestation and may also be misdiagnosed as pregestational high blood pressure (Walker, 2000). The tendency of preeclampsia with placental abruption and foetal growth restriction is approximately doubled when there is chronic hypertension (Saudan *et al.*, 1998). However, when women with chronic hypertension are well managed, they have good clinical outcomes but there is a 46% chance of developing preeclampsia when the diastolic blood pressure is ≥ 110 mm Hg within the first half of pregnancy (McCowan *et al.*, 1996).

1.3.2 PREECLAMPSIA SUPERIMPOSED ON CHRONIC HYPERTENSION

Pregnant subjects who have pre-gestational hypertension could present with preeclampsia which may manifest symptoms that are very severe than if it had occurred independently (ACOG, 1996). There is a thin line between superimposed preeclampsia and worsening chronic hypertension. This is because the symptoms of no proteinuria within the first half of pregnancy and the sudden increase in liver enzymes, proteinuria, BP and a reduction in platelets of this pregnant woman may be difficult to differentiate (ACOG, 1996). In 2005, Sibai et al working on pregnant women with chronic hypertension noted that a quarter of the pregnant women developed superimposed preeclampsia which is a much higher risk than generally observed.

1.3.3 PREGNANCY-INDUCED HYPERTENSION

Zhang *et al.* (1997) defined pregnancy-induced Hypertension (PIH) as an increase in BP and urine protein after twenty weeks of gestation in pregnant women whom hiderto were normotensive and had no kidney disease or proteinuria before this period and these conditions resolve after delivery.PIH may also be said to be a condition that manifests high blood pressure, a negative or an increased urine protein, oedema and seizures in the second trimester and above with these manifestations resolving after delivery. Some of the adverse outcomes of this systemic maternal syndrome are premature delivery,underweight baby, retained placenta, loss of mother and child lives (Owiredu *et al.*, 2012). Although PIH has

been known for ages its etiology is still not very clear, thus making its diagnosis and treatment a big challenge for clinicians. Zhang *et al.* (1997) noted that based on empirical observations, pregnancy-induced hypertension can be subdivided based on the prognosis of levels of blood pressure and urine protein, whether there are seizures and also when the symptoms were first observed that is before, during or after delivery. It is still not clear whether the subdivisions of this condition have unique pathophysiology or are as a result of the disease prognosis.

1.3.4 GESTATIONAL HYPERTENSION

Gestational hypertension or transient hypertension occurs in the second half of gestation where there is nonproteinuric high BP in women who were having normal blood pressure before this period. An abnormal rise in BP for about a quarter of an hour is seen in the worse form of gestational hypertension which then resolves within a week after delivery (NHBPEP, 2000). The incidence of gestational hypertension is about 6–7% and the high BP normalizes after birth (Walker, 2000). There is a 15-26% tendency of gestational hypertension developing into superimposed pre-eclampsia and this risk is dependent on the time of gestation at which the hypertension developed and this risk is reduced to 10% when it is diagnosed in the latter part of pregnancy (Saudan *et al.*, 1998). Also Davis *et al.* (2007) and Barton *et al.* (1997) noted that about half of women presenting with nonproteinuric high BP will progress to develop proteinuric high BP and this could be as a result of a history of loss of pregnancy, PIH and high BP. The rise in BP with this type of PIH resolves or regularizes one and a half months postpartum (James and Nelson-Piercy, 2004). Podymow and August, (2008) observed that pregnant women presenting with nonproteinuric high BP have a high tendency of progressing to proteinuric high BP even during puerperium. They also noted that the precise diagnosis of gestational hypertension is usually made after the hypertension resolves after delivery and the laboratory tests remain normal (Podymow and August, 2008).

1.3.5 PREECLAMPSIA

Janakiraman *et al.* (2009) defined preeclampsia as a maternal syndrome, which is characterized by high blood pressure, tissue oedema, proteinuria, abnormal clotting, abnormal liver and renal functions as a result of placental involvement. Cunnigham *et al.*

(2001) explained that placental substances can cause a malfunctioning of the blood vessels of pregnant women thus leading to an increase in blood pressure. The development of an elevated blood pressure measured repeatedly four to six hours apart and the presence of protein in urine in the second half of gestation are the characteristics of preeclampsia (Munjuluri *et al.*, 2005). In most cases, these symptoms develop in the third trimester of pregnancy and usually disappear within a few weeks after delivery.

Preeclampsia forms about 5 to 10% of the complications of pregnancy and can lead to maternal and foetal morbidity (Lindheimer and Katz, 1985). Preeclampsia usually occurs in nulliparous women than in multipara ones with the maternal blood pressure normalizing after delivery but still has the potential to develop essential hypertension in future (LeLorier *et al.*, 1997). The pathophysiology of preeclampsia is a mystery, but maternal, immunological, genetic factors and placental involvement have been sited as probable causes (NHBPEP, 2000). Stenczer *et al.* (2011) in their work observed a strong indication that the etiology of proteinuric high BP may be attributed to an amplified inflammatory response in the pregnant woman, oxidative stress and abnormal production of vascular substances (ie vasoconstrictors and vasodilators).The most effective intervention for this condition is the curtailment of the pregnancy either by the induction of labour or cesarean section and the delivery of the placenta (Skjærven *et al.*, 2002).

1.3.6 ECLAMPSIA

Eclampsia is diagnosed as convulsive attack in preeclamptic women which cannot be linked to any other clinical causes (NHBPEP, 2000). A majority of eclamptic patients suffer their first seizure after delivery, although pregnancy-induced hypertension is unique to pregnancy and is supposed to resolve after delivery (Lopez-Llera, 1992; Douglas and Redman, 1994). This worse form of PIH can cause irrepairable destruction of the brain, liver and kidneys. When this condition is ill-managed it can result in coma, cerebral damage, liver rupture, renal failure, cerebral hemorrhage, pulmonary oedema, cortical blindness and maternal death, cerebral edema, cerebro vascular accident and hypertensive encephalopathy (Khatun *et al.*, 1997).

1.4 PATHOGENESIS OF PREGNANCY-INDUCED HYPERTENSION

Since the proposal of Walsh (1985) that a shift in the balance of thromboxane A2 and prostacyclin (prostaglandin I2) and trophoblastic hypoperfusion and endothelial injury

(Roberts *et al.* (1989) as the possible etiology of PIH, the myth surrounding this systemic maternal syndrome has been reduced.The pathophysiology may also differ according to when the symptoms are observed (Sibai *et al.*, 2005). Late-onset pre-eclampsia is referred to as maternal pre-eclampsia and early-onset disease is referred to as placental preeclampsia. The latter emphasizes the central role of poor placentation in early-onset disease (Redman and Sargent, 2005). The pathogenesis of pregnancy-induced hypertension is multifactorial, and many diagnostic tools have been used to assist in its early detection but none has shown the required sensitivity or specificity for it to be used in routine medical practice (Carty *et al.*, 2010).

In trying to explain the pathophysiology of preeclampsia, Redman (1991) proposed a twostage model for the development of this maternal syndrome:firstly, placental blood insufficiency due to impaired placentation and secondly, the systemic activation of the maternal endothelium due to placental products formed as a result of the earlier development. Stenczer *et al.* (2011) in their study on preeclampsia also stated that the pathophysiology of this condition could be multifactorial and could be affected by intrinsic, immunological and ecological factors. They also proposed that the prognosis of this condition comes in two phases that is the pre-clinical phase where there is poor placentation and the clinical phase where the symptoms of the condition are expressed by the mother. Disproportionate systemic inflammatory response by the mother to pregnancy with systemic oxidative stress and lack of equilibrium between vasodilators and vasoconstrictors have been linked to the pathogenesis of this condition (Stenczer *et al.*, 2011). Many theories have been suggested to be the bridge between these two stages but Redman and Sargent (2005) and Cindrova-Davies (2009) noted that prominent amongst them were hypoxia or ischaemia-reperfusion (I/R) insults with a resultant excessive oxidants and placental discharge of inflammatory cytokines, reactive oxygen species (ROS), antiangiogenic factors and apoptotic/necrotic trophoblast products.

In 2007, Karthikeyan and Lip also hypothesized that this maternal syndrome may be caused by poor placentation which could lead to inflammatory signals which depends on both the genetic make-up of the foetus and the mother. Ischemia-reperfusion which is a problem with the vasculature can cause an imbalance in the generation of oxidants and antioxidants, vascular disease and immune maladaptation which may be responsible for this maternal syndrome (Lindheimer, 1993; Karthikeyan and Lip, 2007) and this is in consonance with the

earlier proposal by Redman (1991). They also hypothesized that insults in the reninangiotensin-aldosterone axis, maternal endothelial dysfunction and coagulopathies, cytokines, growth factors et cetera may be implicated in this syndrome.

1.4.1 ROLE OF THE PLACENTA IN PIH

During the first half of normal gestation, the spiral arteries change in shape in response to the invasion of extravillous trophoblasts which are formed from trophoblast of the anchoring villi resulting in the formation of dilated, inelastic vessels which have no maternal vasocontrol, thus improving placental and foetal blood supply (Brosens *et al.*, 1967) and this may extend through the decidua and myometrium (Brosens *et al.*, 1972; Khong *et al.*, 1986). In the case of this maternal syndrome, this mechanism is altered and there is impaired placentation (Brosens *et al.*, 1972) which results in the reduction of the intensity of trophoblast attack and the number of vessels invaded, thus leading to uteroplacental blood insufficiency which initiates the symptoms of this syndrome in the latter half of gestation (Brosens *et al.*, 1972; Khong *et al.*, 1986). In 2005, Redman and Sargent reported that abnormal placentation is implicated in early-onset placental preeclampsia and it occurs in the second trimester of gestation and the women have no cardiovascular risk factors (Oudejans *et al.*, 2007). Intrauterine growth retardation and abnormal placental morphology is found in placental preeclampsia (Oudejans *et al.*, 2007). Redman and Sargent (2005) and Staff *et al.* (2013) stated that there is placental involvement in hypertension in pre-eclampsia since it develops during the third trimester and resolves after delivery.

Lyall (2006), Pijnenborg *et al.* (2006) and Harris *et al.* (2009) in their studies noted that trophoblast invasion comprises of complex mechanisms that are not very well understood. In normal gestation, trophoblast invasion comprises of the differentiation of the proliferative type to the invasive one and this involves the attachment to and the breakdown of the extracellular enviroment and the movement through the decidual stroma of the placenta (Løset *et al.*, 2011). Pijnenborg *et al.* (2006) suggested that defective trophoblasts (intrinsic factors) and/or decidual environment (extrinsic factors) may lead to an impaired trophoblasts invasion. Kokkinos *et al.* (2010) hypothesized that the way trophoblast transition from the proliferative type to the invasive one is similar to that of epithelial to mesenchymal transition and tumour progression but whereas trophoblast invasion is

controlled, tumour progression is not. In a study by Zhou *et al.* (1993) it was noted that impaired trophoblast invasion that occurs in this maternal syndrome may be due to the abnormal expression of cell adhesion molecules like cadherins and intergrins. Fisher *et al.* (1989) and Librach *et al.* (1991) observed that the infiltration of the decidual tissue by the invasive trophoblast is strongly dependent on their ability to secrete matrix metalloproteinases which breaks down the extracellular matrix but in this syndrome however there is a reduced expression of matrix metalloproteinases 2,-3'-7 and -9 (Lim *et al.*, 1997; Campbell *et al.*, 2004; Reister *et al.*, 2006). Meekins *et al.* (1994) observed that irrespective of the mechanisms involved in the trophoblast attack, it is not the only determinant of malplacentation but rather suggested that there is a decline in the number of decidual and myometrial arteries being invaded from healthy to complicated pregnancies. Kim *et al.* (2003), Meekins *et al.* (1994) and Brosens *et al.* (1977), all observed that slight impairment of the remodelling of the spiral arteries can also be seen in healthy pregnancies which is contrary to most of other postulations that states that impairment of remodelling of spiral arteries occurs in preeclampsia.

1.4.2 MATERNAL FACTORS AND PIH

In normal pregnancy the vasoconstrictive effect of angiotensin II on the vasculature is not felt despite its high circulating concentrations; in PIH, however, this protection against the effects of angiotensin II is impaired, resulting in arterial vasoconstriction and an increase in blood pressure (Gant *et al.*, 1973). This was corroborated in 2009 by Kanasaki and Kalluri that comparing normal pregnancy with pre-eclampsia, vascular resistance is increased in the foeto-placental circulation and the vessels are constricted, plasma volume is lowered, renin expression is increased and plasma renin activity is also lowered in preeclampsia which leads to high blood pressure (Shah, 2005; Kanasaki and Kalluri, 2009). Angiotensin II also causes oxidative stress and renal inflammation by stimulating superoxide formation and increasing chemokine release (Mehta and Griendling, 2007).

Table 1.3: Maternal haemodynamics in normal and pre-eclamptic pregnancies adapted from Tuuri (2014)

	Normal pregnancy versus non-pregnant	**Pre-eclampsia versus normal pregnancy**	**Reference**

Blood Pressure	Slightly decreased	Increased strongly	Kanasaki and Kalluri ,2009
Mean Arterial Pressure	Decreased	Increased	Rang et al., 2002
Heart Rate	Increased	Decreased	Rang et al., 2002
Cardiac Output	Increased	Reduced	Rang et al., 2002
Systemic Vascular Resistance	Decreased (Vascular Dilation)	Increased strongly (Vascular contraction)	Rang et al., 2002, Kanasaki and Kalluri ,2009
Plasma volume	Increased	Decreased	Rang et al., 2002, Kanasaki and Kalluri ,2009
Uterine Blood Flow	Increased	Reduced	Thornburg et al., 2000, Shah DM, 2005
Renal Plasma Flow	Increased	Decreased	Thornburg et al., 2000, Moran et al.,2003
Renin-Angiotensin System	Increased	Reduced	Shah DM, 2005

1.4.3 GENETIC FACTORS AND PIH

In 2005, Roberts and Gammill proposed that the pathophysiology of PIH could be attributed to the interaction of placental blood insufficiency and intrinsic factors that predisposes the pregnant women to PIH (Roberts and Gammill, 2005). This is in consonance with PIH, as a result of repeated malplacentation and consequent deferred appearance of intrinsic or ecological vulnerability for endothelial injury. It has been proposed that the etiology of PIH could be genetic, an example being the gene for thrombophilia, (Chappell *et al.*, 2002; Mello *et al.*, 2005). Endothelial dysfunction gene: endothelin-1 , (Bakketeig *et al.*, 1979) is responsible for how a mother respond to an impairment in the function of the placenta.Since hypertension has genetic predispositioning, common risk factors like advanced maternal age and diabetes mellitus may trigger the genetic expression of endothelin-1 which would lead to PIH. Lie *et al.* (1998) reported that there is a paternal risk of reoccurrence of preeclamptic pregnancies in different women impregnanted by the same man thus

suggesting paternal genetic factors in proteinuric high BP. However, a father whose genetic profile predisposes him to cause PIH would only do so through the fetus or the placenta (Rasmussen and Irgens, 2008).

1.4.4 IMMUNOLOGICAL FACTORS AND PIH

Pregnancy-induced hypertension has an immunological basis, since it occurs mainly in nulliparous women and rarely affects parous women unless there is partner change (Robillard *et al.*, 1993). In their report, Robillard *et al.* (1994) stated that there is a strong converse relationship between the frequency of coitus by couples and the probability of the woman presenting with PIH. This means that the higher the frequency of unprotected sexual intercourse the less likelihood that the woman may suffer PIH due to contact of spermatozoa with the female genital tract. Earlier studies by Marti and Herrmann (1977) and Klonoff-Cohen *et al.* (1989) observed that the longer the length of preconceptional sexual cohabitation and the episodes of unprotected intercourse, the less likely that the woman would develop PIH. This risk reduction may be related to sperm inoculation, since spermatozoa possesses isoantigenic properties (Fellous and Dausset, 1970) so when it is absorbed by the endometrium it inoculates the woman with paternal histocompatibility antigen. This would elicit a maternal immune response that would tolerate the foetus or recognize it as "self" due to its possession of the paternal histocompatibility antigens (Marti and Herrmann, 1977). This concept has been supported by the results of several studies suggesting that repeated exposure to father's spermatozoa prior to conception may reduce the risk of pregnancy-induced hypertension in the first pregnancy (Marti and Herrmann, 1977). However, Mills *et al.* (1991) reported no association when they tried to replicate these results using data from two cohort studies.

In 2002, Dekker noted that there were two schools of thoughts regarding the aetiology of preeclampsia and these were the vascularists who propounded ischaemia-reperfusion as the cause, and the other who also see preeclampsia as a condition that results from the mother seeing its foetus as foreign. Preeclampsia occurs mainly in nulliparous women and this could be due to the maternal immune system reacting to a genetically foreign foetus (Dekker, 2002). The assumption is that in primigravid women, they are not familiar with the foetus and may see it as "non-self" but in future pregnancies it adapts better to the foetus (Do *et al.*, 2002). Furthermore, there may be partner specificity, which strengthens the

argument that preeclampsia results from a relative failure to induce maternal tolerance of paternal antigens that are most significant to preeclampsia (Cunnigham *et al.*, 2001).

The foetal genome is made up of equal halves from both parents but the foetus synthesizes antigens that are recognized as foreign by the maternal immune system, which normally should be rejected by the mother but this generally does not occur. To explain this occurrence of immune relationship between mother and foetus for a successful pregnancy out-come, Acromite *et al.* (1999) proposed the concept of the "foetal allograft".

They hypothesized that:

1) Lack of foetal immunogenicity

2) Decrease in maternal immunity during pregnancy

3) Placenta serving as an immune barrier

Of these three hypotheses, the third which suggests that the placenta creates an immune barrier between mother and foetus is the most widely considered. This barrier was initially thought not to play any role in the immunological dynamics of the mother but later the placenta was found to participate in this dynamics. It is presumed that foetal cells and molecules are released into the maternal blood during placentation and these foetal materials come into contact with the whole maternal immune system and this mechanism seems to be responsible for the maternal immune tolerance of the foetus (Acromite *et al.*, 1999).

Abnormal maternal immunological response to foeto-paternal antigens has been implicated in abnormal placentation (Belfort *et al.*, 2002). The high occurrence of hypertension and proteinuria in multiple pregnancies, molar pregnancies and multi-foetal pregnancies indicates that the level of foeto-paternal antigen and the quantity of trophoblast have a pathological role in this maternal systemic syndrome (Taylor, 1997). Several epidermiological observations implicate immunological factors in the pathogenesis of this maternal syndrome but this does not hold for nulliparous women and women with gestational hypertension (Campbell *et al.*, 1985; Misra and Kiely, 1997).Thus one may conclude that preeclampsia and gestational hypertension are different disorders with differing aetiologies. Li and Wi (2000) observed in a study that, change in partner increases

the prevalence of preeclampsia in multigravid women. An observation that women who have no history of preeclampsia in their first birth, have a 30% tendency of presenting with proteinuric high BP in future pregnancy after changing partners whereas that is not the case when they do not change partners. However, those having a history of preeclampsia in their first birth are less likely to develop the syndrome in future pregnancy after changing partners. These findings corroborate the assumption that a healthy pregnancy is a sign of maternal immune acceptance of the foetus, whereas in preeclamptic women, this immunological tolerance is impaired (Belfort *et al.*, 2002).

1.4.5 OXIDATIVE STRESS AND LIPID PEROXIDES IN PIH

Reactive radical species are formed normally as by-products of aerobic organism metabolic activities but their destructive effects are fine tuned by their bodies' efficient antioxidant defence system. However, when their production overwhelms the antioxidant defence system the normal physiological equilibrium is offset and thus a deleterious distress occurs which is commonly referred to as oxidative stress (Basu, 2004). Burton and Jauniaux (2011), also defined oxidative stress as the lack of equilibrium between oxidant and antioxidants where the oxidants are produced in excess or the anti-oxidants are deficient and this imbalance is responsible for the pathogenesis of PIH. Song *et al.* (2010) noted that the excess ROS oxidizes DNA, lipid, and proteins to produce their oxidized forms (ie. oxidized DNAs, lipid peroxides, and oxidized proteins respectively) which negatively affect their physiological functions. Excessive generation of oxidants may cause proteinuric hypertension and the biomarkers of the resultant excess oxidants have been reported to be marked in women suffering from this condition (Roberts *et al.*, 2010).

Nitric oxide (NO) which has vasodilatory and platelet-aggregation-inhibitory functions is implicated in the development of PIH when it is deficient during pregnancy (Morrow *et al.*, 1990). This vasodilatory function of nitric oxide is inhibited when there is an amplification of lipid peroxidation which is implicated in the pathophysiology of hypertension in PIH (Williams and De Swiet, 1997; Gratacós, 2000). In 2003, Basu confirmed that during pregnancy large amounts of free radicals (eg. Superoxide and hydroxyl) and ROS(eg.Singlet oxygen and hydrogen peroxide) are produced and induced by hypoperfusion, placental ischemia and inflammatory processes thus causing a significant decline in the synthesis of NO due to the inactivation of the enzyme: inducible nitric oxide synthase (iNOS) (Basu,

2003). Also Basu (2003) noted that the metabolic products (eg.Malondialdehyde and conjugated diene) of lipoperoxidation could inactivate the enzyme responsible for manufacturing nitric oxide thus causing a decline in its levels which would lead to excessive production of oxidants.

There are imperical evidences that suggest that isoprostanes are genuine indicators of the production of excessive oxidants and free radical-provoked deterioration of lipids (Roberts and Morrow, 2000; Basu, 2004). 8-iso-prostaglandin-F2α (8-iso-PGF2α) is a subunit of the F2-isoprostane group and it is synthesized in vivo by the lipid deterioration of arachidonic acid through a free radical-mediated method free of COX pathway (Roberts and Morrow, 2000; Basu, 2004). 8-iso-PGF2α plays a role in smooth muscle cell growth, platelets activation, vaso-constriction and also impairs the endothelial cell barrier function (Hart *et al.*, 1998). 8-iso-prostaglandin F2α is significantly elevated in most of oxidative stress related conditions and its evaluation can serve as a gold standard for the in vivo investigation of free radical-mediated deterioration of lipid (Morrow *et al.*, 1990; Morrow, 2000; Basu, 2003; Basu, 2004; Basu and Helmersson, 2005). The basal concentration of 8iso-PGF2α is considerably higher than that of the enzymically produced prostaglandin F2α and its free form in body fluids can easily be measured as an oxidative stress biomarker using sensitive and specific analytical methods (Morrow *et al.*, 1992; Södergren *et al.*, 2000). The plasma levels of 8-iso-PGF2α is directly proportional to the extent of lipid deterioration and the production of free radicals in patients with conditions that have bad prognosis (Massey and Nicolaou, 2013). In 1995, Morrow et al found out that patients with oxidative stress related conditions have high levels of circulating and urinary 8-iso-PGF2α. Other studies have also found high levels of lipid peroxides and free 8-iso-PGF2α in the placenta after delivery of women with PIH whereas the picture is different when it comes to those with healthy pregnancies (Staff *et al.*, 1999a; Staff *et al.*, 1999b). The increase in 8iso-PGF2α indicates the level of the breakdown of lipid as a consequence of excessive free radicals generation which would affect various tissues and the vasculature (Staff *et al.*, 1999a).

1.4.6 INFLAMMATION AND PIH

Maternal immune tolerance is developed during normal pregnancy to avoid the immunological rejection of the foetus and this is achieved by the shift from Th1 to Th2

immunity to create an anti-inflammatory state (Toldi *et al.*, 2011). Pregnancy-induced hypertensive women tend to loose this immune tolerance and develop systemic inflammation which tends to be considered as the dominant cause of this maternal systemic syndrome (Challis *et al.*, 2009). This same observation had earlier been made by Redman *et al.* (1999) that preeclampsia is developed as a result of cytokine-mediated excessive maternal inflammatory response. Saito *et al.* (1999) also observed that the systemic inflammation seen in proteinuric high BP is characterized by lack of Th2 immunity skewness and therefore the prominence of Th1 immunity and pro-inflammatory cytokines.

For early diagnosis and effective treatment of this maternal systemic syndrome, there is the need for very sensitive inflammatory biomarker to identify patients at potential risk of developing clinical symptoms. Soluble urokinase plasminogen activator receptor (suPAR) is the free form of the urokinase-type plasminogen activator receptor (uPAR) which is a membrane protein derived from the cleavage and release of the membrane-bound uPAR, and may be found in plasma, blood, cerebrospinal fluid and urine (Thunø *et al.*, 2009). Prior to this observation Huai *et al.* (2006) had noted that soluble urokinase type plasminogen activator receptor (suPAR) is the free form of the uPAR which is generally produced by cell-mediated form of response to antigens. suPAR as a biomarker is being widely used for the screening for the presence of inflammatory response as observed by Kofoed *et al.* (2006). Toldi *et al.* (2011) observed an elevation of plasma suPAR levels in response to increased activation of the immune system and increased inflammatory response which are features of this maternal systemic syndrome. This is corroborated by the findings of increased suPAR levels in cases of cancer, infectious diseases as well as in autoimmune disorders and in all of these conditions suPAR levels significantly correlate positively with the severity of the disease(Østergaard *et al.*, 2004). Thus determining suPAR levels can assist in assessing disease prognosis and treatment (Østergaard *et al.*, 2004; Ostrowski *et al.*, 2005; Kofoed *et al.*, 2006). An inflammatory biomarker stability testing done by Kofoed *et al.* (2006) showed suPAR to be comparatively highly stable confirming results obtained from earlier studies (Riisbro *et al.*, 2000). Soluble urokinase type plasminogen activator receptor's (suPAR) levels are not altered even when specimen undergo several freezing and thawing circles, thus making it the biomarker of choice for systemic inflammation (Kofoed *et al.*, 2006).

In 2003, Ridker P observed a subclinical elevation of the acute phase protein:highsensitivity C-reactive protein (hsCRP) which is a signal of systemic low-grade inflammation in non-pregnant subjects (Ridker, 2003). There is a disproportionate response to systemic inflammation by the mother usually at the latter half of healthy gestation (Sibai, 2005) but in pre-eclamptic pregnancy this inflammatory response is amplified (Sibai *et al.*, 2005). There is an increase in the levels of proinflammatory cytokines in overt preeclampsia: C-reactive protein - CRP (Teran et al., 2001), hs-CRP (Ertas *et al.*, 2010) and interlukin-6 (IL-6) (LaMarca *et al.*, 2007). In the placental, renal, and vascular tissues, tumour necrosis factor alpha triggers the endothelin system which is responsible for endothelial injury and IL-6 activates the renin-angiotensin system (RAS) which regulates the level of blood pressure (LaMarca *et al.*, 2007). These cytokines are responsible for maintaining the intergrity of the endothelium (LaMarca *et al.*, 2007). Inflammation is important in the mechanism through which overweight women become prone to proteinuric high BP (Wolf *et al.*, 2001). Abnormal adipokine production and infiltration of inflammatory cells characterize dysfunctional adipose tissue. Visceral fat produces more CRP and inflammatory cytokines than subcutaneous fat, and contributes more to oxidative stress (Roberts *et al.*, 2011). C-reactive protein (CRP) levels are frequently elevated in hypertension (Chae *et al.*, 2001), and raised CRP precedes blood pressure (BP) elevation (Sesso *et al.*, 2003), meaning inflammation plays an active role in the pathophysiology of the hypertensive syndrome.

Since pregnancy-induced hypertension is multifactorial, several investigations have been done to unearth the pathogenesis of this condition. One of these investigations is the role that impairments of iron homeostasis play in the pathogenesis of PIH. In 2002, Rayman and his team did a work on preeclamptic women and found out that the plasma iron concentrations, ferritin, and saturation of transferrin were higher as compared to healthy pregnant women, whereas their total iron binding capacity (TIBC), unsaturated iron binding capacity and apotransferrin were comparatively lower and these findings were confirmed in a later work by Basher and Deb (2006). This hyperferromia can lead to the production of reactive oxygen species (ROS) through Fenton's reaction which can subsequently lead to lipid peroxidation and endothelial cell injury in preeclampsia (Rayman *et al.*, 2002). However, the inflammatory state in preeclampsia is expected to cause hypoferromia which could lead to inflammation-induced anaemia (Balla *et al.*, 2007) which is contrary to the

findings by Rayman *et al.* (2002) and Basher and Deb (2006). Hepcidin, an acute phase protein is the regulator between iron homeostasis and inflammation and it acts by reducing the absorption of intestinal iron and the release of iron from enterocytes and macrophages through internalization and degradation of ferroportin (Nemeth and Ganz, 2009). Hepcidin expression is regulated by several factors but inflammatory signals such as interleukin-6 (IL-6) and high concentrations of iron are its primary triggers (Nemeth and Ganz, 2009). Thus there is a pathogenic cascade that starts with the inflammation producing IL-6 which triggers the production of hepcidin which in turn leads to hypoferremic state causing anaemia of inflammation (Nemeth and Ganz, 2009).

1.4.7 ROLE OF ENDOTHELIUM DYSFUNCTION IN PIH

Endothelium is a highly specialized lining of the blood vessels that functions as an active metabolic boundary separating blood and the tissues. It has the following functions: the proper functioning of the blood vessels, it is responsible for the nature of the blood vessels and also takes part in the process of coagulopathies (Chhabra, 2009). The endothelium is responsible for the synthesis and release of secretions that are responsible for the variation of the size of blood vessels, growth regulators and other factors that regulate these tasks in reponse to various mechanical and chemical stimuli (Chhabra, 2009). The malfunctioning of the lining of the blood vessels may result from the loss of equilibrium in the secretions that are responsible for: the variation in the size of the blood vessels, factors that support growth or otherwise and factors that promote plague formation in the blood vessels or otherwise (Quyyumi, 1998). The integrity and function of the lining of the blood vessels are essential for the maintenance of vascular haemostasis and blood pressure control (Blann, 2003). The vascular endothelium is responsible for the reduction in blood pressure and peripheral resistance in healthy pregnancy although nitric oxide has also been implicated in these changes (Chhabra, 2009). Nitric oxide (NO) is responsible for the maintenance of vascular tone and its insufficiency is responsible for the endothelial dysfunction seen in hypertension (Chhabra, 2009). This leads to an imbalance in the processes responsible for vascular homeostasis thus causing vasoconstriction and impaired vascular function (Chhabra, 2009). The excessive production of free radicals has been implicated in the development of this imbalance which may have resulted from systemic and localized inflammatory responses (Watson *et al.*, 2008).

In 2005, Khan and his team suggested that endothelial dysfunction may precede conception and thus make the women vulnerable to pregnancy-induced hypertension or the pregnancy may later cause impairment in endothelial function that may increase their chances of heart disease later (Khan *et al.*, 2005). The etiology of this maternal systemic syndrome is still a challenge since it has multifactorial pathophysiology due to the multiple risk factors (Sibai *et al.*, 2005) but the malfunctioning of the endothelium is key to the presentation of preeclampsia. Endothelial injury has been implicated in placental and systemic circulations in pre-eclampsia (Solomon and Seely, 2006), but the target organ is maternal endothelium (Young *et al.*, 2010). Endothelial dysfunction impairs renal function and increases total peripheral resistance (TPR) causing hypertension (Gilbert *et al.*, 2008). Proteinuria, microangiopathic haemolytic anaemia and organ hypoperfusion in pre-eclampsia is as a result of endothelial dysfunction (Solomon and Seely, 2006). Endothelial dysfunction is implicated in hypertension irrespective of whether it is caused by endocrine or renal processes or any other secondary causes (Watson *et al.*, 2008).

During healthy pregnancy lipoproteins' levels increases but this rise is doubled in PIH (Mitsuaki *et al.*, 1981; Uotila *et al.*, 1993). This increase in lipoproteins' levels damages the endothelium causing a rise in blood pressure and urine protein which are cardinal signs of pregnancy-induced hypertention (Winkler *et al.*, 2003). In PIH, the damage to the endothelium leads to an impairment of its function which results in the damage to multiple systems of the body (Dutta, 2001). Robson (1999) reiterated that an impaired lipid homeostasis is central to the development of this systemic maternal syndrome. In 2009, Chhabra noted that it is difficult to assess endothelial dysfunction and the best method for its assessment is the intra-arterial injection of acetylcholine to observe how the vasculature would response but this method is not suitable for large clinical studies (Chhabra, 2009). Cellular fibronectin is produced by endothelial cells (Mao and Schwarzbauer, 2005) and its plasma levels are elevated in response to damage of vascular tissue, after inflammation, chronic diseases (Peters *et al.*, 2003; Castellanos *et al.*, 2004) and also in women with preeclampsia (Campbell and Campbell, 1983; Friedman *et al.*, 1995).

1.5 PROBLEM STATEMENT

- Pregnancy-induced hypertension is the commonest pregnancy complication encountered in Ghanaian hospitals to date and it is the second highest cause of maternal mortality in Ghana (Owiredu et al., 2012).
- Though there have been earlier studies on oxidative stress, inflammation and endothelial dysfunction in PIH, most of these studies used markers that are saddled with drawbacks.
- In addition, studies on iron homeostasis and PIH are not well elucidated.

1.6 JUSTIFICATION

- There is the need to meet the third Sustainable Development Goal (SDG) of the agenda 2030, which is good health and wellbeing to help reduce maternal mortality
- Understanding the pathophysiological indicators of PIH would help in its early prediction and management
- There is paucity of data with respect to iron haemostasis and PIH amongst Ghanaian pregnant women.
- There are major drawbacks associated with available pathophysiological indicators of PIH.

1.7 AIM

This study sought to find pathophysiological indicators of Pregnancy-Induced Hypertension in Ghanaian women.

1.8 SPECIFIC OBJECTIVES

1. To evaluate the concentrations of the indicators of systemic inflammation: **IL-6, CRP, suPAR**, endothelial injury: **fibronectin** and systemic oxidative stress: 8-isoProstaglandin F2α (**8-iso-PGF2α**) in PIH and controls.

2. To determine the concentration of hepcidin and markers of iron metabolism , systemic inflammation and complete blood count in PIH and controls.

3. To evaluate suPAR as a possible marker for the characterization of the inflammatory status during pregnancy as against IL-6 and CRP.

4. To determine the serum lipids levels and its correlation with markers of inflammation, endothelial dysfunction and oxidative stress

Chapter 2 MATERIALS AND METHODS

2.1 RESEARCH SETTING

This was a cross-sectional study conducted amongst pregnant Ghanaian women attending antenantenal care at the Lister Hospital and Fertility Centre, La General Hospital and Ridge Regional Hospital, all in Accra, Ghana from June, 2014 to July, 2015.

Lister Hospital and Fertility Centre is a privately owned International Hospital which delivers 24-hours services in General Medicine, Surgery, Consultancy, Assisted Conception, X-ray and Laboratory Diagnostics. La General Hospital is a state owned District Hospital that delivers services in General Medicine and Public Health. Ridge Regional Hospital is a state owned referral hospital which serves as a referral centre for clinics and District Hospitals in the Greater Accra Region.

2.2 RESEARCH PARTICIPANTS

Pregnancy-Induced Hypertension was defined as a condition that manifests High blood pressure, a negative or an increased urine protein, oedema and seizures in the second trimester and above with these manifestations resolving after delivery (Cnossen *et al.*, 2006). One hundred and twenty-three (123) fasting Ghanaian pregnant women aged between 18yrs and 40yrs (Escen *et al.*, 2003) with singleton pregnancies as determined by Ultrasound scan in their second trimester and above of gestation presenting with pregnancyinduced hypertension (PIH) and forty-five (45) normotensive pregnant women who were also fasting, aged between 18yrs and 40yrs (Escen *et al.*, 2003) with singleton pregnancies as determined by Ultrasound scan in their second trimester and above of gestation were recruited as cases and controls respectively for this study.The study participants signed informed approval forms before being recruited. The Committee on Human Research Publication and Ethics (CHRPE) of the School of Medical Science, KNUST-Kumasi and the Komfo Anokye Teaching Hospital (KATH), Kumasi granted ethical clearance for the study **(CHRPE/AP/035/14 & CHRPE/AP/333/14).**

2.2.1 Exclusion criteria

Participants who were non-fasting or had multi-foetal gestation, chronic hypertension, renal disorder, multigravida, first trimester of gestation, liver disorder, maternal or foetal infection and foetal congenital anomaly were excluded from this study.

2.2.2 Sub-types of PIH (NHBPEP, 2000)

Pregnancy-induced hypertension was sub-divided into three groups as follows:

- Preeclampsia (PE) was defined as a continual high blood pressure with urine protein level of at least 2+ on dipstick testing
- Eclampsia (EC) was defined as preeclamptic women presenting with oedema and convulsions/coma during or soon after pregnancy
- Gestational hypertension (GH) or transient hypertension was defined as a continual high blood pressure with negative urine protein on dipstick testing.

2.2.3 Sample Size

The target population was pregnant women attending antenatal clinic. The necessary minimum sample size for the study was calculated to be 123 women, based on the assumption that 12.14% of the female population experienced pregnancy induced hypertension (Owiredu *et al.*, 2012), with an expected difference of 5% between the sample and the general population and a type I error (α) of 0.05.

$$n = \frac{z^2(1-p)p}{d^2}$$

Where n = minimum sample size; Z = standard normal variance=1.96 to obtain a power of 95% confidence interval (β=5%) and a type 1 error probability of 5%; d=Absolute standard error=0.05; p=prevalence=12.14%.

2.2.4 Research Participants Groupings

The research participants were grouped according to five types of modalities; the three types of hypertension in pregnancy, hypertensives put together as a group and one without hypertension as follows:

Type 1: Participants presenting with negative urine protein (**GH**) - 48
Type 2: Participants presenting with abnormal levels of protein in urine (**PE**) - 57
Type 3: Participants presenting with high level of protein in urine and seizures (**EC**) - 18
Type 4: The first three types grouped as **PIH** – 123 (i.e. Types 1+2+3)
Type 5: Normotensive women with healthy pregnancy (**Controls**) - 45

2.3 MEASUREMENT OF BLOOD PRESSURE

Blood pressures of the participants were taken between 7.00a.m and 10.00a.m in the morning by a state registered nurse using the right size of sphygmomanometer cuff and a stethoscope with participants sitting upright. Measurements were taken after the participants were well rested as recommended by the American Heart Association (Kirkendall *et al.*, 1967). The diastolic pressure was determined using the absence of sound that is the phase V of Korotkoff sound. The average of three readings with a five (5) minute rest period inbetween-readings was recorded to the nearest 2.0 mm Hg. A participant was said to be hypertensive when two readings of blood pressure were abnormally high on at least two separate visits (Egerman, 2001).

2.4 BLOOD AND URINE SAMPLES COLLECTION

Venous blood (10ml) was drawn after 8-12 hours fast and urine (20ml) was collected from each of the pregnant women who fell within the four depicted groups. Rubber tourniquet was applied around the arm above the elbow for less than one minute and the ante cubital fossa was cleaned with 70% methylated spirit. Phlebotomy was performed using 19G needles fixed on 10 ml syringes and 10 ml of subject's blood was taken from the ante cubital vein. 4.0 ml of blood was put into a tube containing ethylene diamine tetraacetic acid (EDTA) as an anticoagulant, 3.0 ml of blood was put into another tube containing Lithium-heparin as an anticoagulant and the remaining 3.0 ml of the blood was put into a plain tube.

The blood in the plain tube was allowed to clot and span at a centrifugal speed of 1500 g for five minutes and the serum was frozen at -80^0 C until assayed, whilst the EDTA anticoagulated blood and Lithium-heparin anticoagulated blood were mixed very well to prevent clotting using a mechanical mixer Assistent® (Karl Hecht GmbH & Co KG, Stettener Strabe 22-24, 97647 Sondheim v.d. Rhön, Germany). The tube containing the Lithium-heparin anticoagulated blood was then span at a centrifugal force of 2000 g for five minutes and the plasma was frozen at -80^0 C until assayed.

The lipid profile and renal dysfunction markers were measured using the serum. The plasma was used for the iron studies, hepcidin, endothelial dysfunction marker, oxidative stress marker and inflammatory markers. The EDTA anticoagulated blood was used for the haematological parameters.

The urine was used for urine protein and urine microalbumin analyses.

2.5 BIOCHEMICAL ASSAYS

The iron and lipids studies were assayed using the BT 5000® Random Access Chemistry Analyzer (Biotecnica Instruments S.pA, Via Licenza, 18, 00156-Rome, Italy) and the LABKIT® chemistry reagent kits (CHEMELEX, S.A. Pol. Ind. Can Castells-C/Industry Ship 113 J, 08420 Canovelles-Barcelona, Spain).

The inflammatory markers, endothelial dysfunction marker, renal dysfunction marker and hepcidin were analysed using the Stat Fax 303 Plus Microplate Reader (Awareness Technology Incorporated, Palm City, Florida, USA) and the Elabscience® ELISA kits (Elabscience Biotechnology Company Limited, WuHan, China).

The oxidative stress marker was assayed using the Stat Fax 303 Plus Microplate Reader (Awareness Technology Incorporated, Palm City, Florida, USA) and the YH Biosearch® ELISA kit (Shanghai Yehua Biological Technology Company Limited, Shanghai, China).

2.5.1 IRON STUDIES

2.5.1.1 Principle and Methodology for Iron (Perrotta, 1984)

The iron was dissociated from transferrin-iron complex in weakly acid medium. The liberated iron was then reduced into the bivalent form by means of ascorbic acid to Ferous ions which gave a coloured complex with FerroZine.

$$Transferrin(Fe^{3+})_2 + e^- \xrightarrow{Ascorbic\ acid} 2Fe^{2+} + Transferrin$$

$$Fe^{2+} + {}_{FerroZine} \rightarrow Coloured\ complex$$

The intensity of the colour formed was proportional to the iron concentration in the sample.

2.5.1.2 Principle and Methodology for Transferrin (TRF) (Dati *et al.*, 1996)

Transferrin forms insoluble complexes with anti-transferrin antibodies. These complexes cause an absorbance change dependent on the transferrin concentration that can be quantified by comparison with a calibrator of known transferrin concentration.

2.5.1.3 Principle and Methodology for Ferritin (Cook *et al.*, 1974)

Ferritin forms insoluble complexes with anti-ferritin antibodies. These complexes cause an absorbance change dependent on the ferritin concentration that can be quantified by comparison with a calibrator of known ferritin concentration.

2.5.1.4 Principle and Methodology for Total iron-binding capacity (TIBC) (Perrotta, 1984)

Transferrin was saturated with excess of Fe^{3+} and the unbound portion was precipitated with magnesium carbonate. The total amount of iron was then determined. The difference between the total iron-binding capacity (TIBC) and initial iron yields the unsaturated ironbinding capacity.

2.5.1.5 Principle and Methodology for hepcidin (Hepc)

The Elabscience® assay kits (Elabscience Biotechnology Company Limited, WuHan, China) was used in determining the plasma hepcidin (Hepc) using the double antibody sandwich enzyme-linked immunosorbent assay (ELISA) method. The manufacturer's protocol for the performance of the assay was rigorously followed. The principle underlying

the assay is the simultaneous binding of hepcidin to two identical antibodies; one solid phase on a microwell plate and the other in solution with Avidin-horseradish peroxidase (HRP). 100µl of calibrators and plasma were dispensed into their corresponding microwells in ready-to-use microwell plates containing the solid phase anti-hepcidin and warmed at 37^0 C for 90 minutes. The liquid was removed and 100 µl of (1:100 dilution) of Biotinylated detection antibody was pipetted into the microwells and warmed at 37^0 C for 1 hr. The biotinylated detection antibody in the microwells was then removed and the microwells rinsed three times with 350 µl wash buffer. After rinsing, 100µl of (1:100 dilution) antihepcidin-HRP conjugate was added to each microwell and warmed at 37^0 C for 30 min. The anti-hepcidin-HRP conjugate in the microwells were then removed and the microwells rinsed five times with 350 µl wash buffer.

An enzymatic reaction was initiated by the addition of 90 µl chromogen into each microwell and then warmed at 37^0C for 15 min in the dark. Only those wells that contained the hepcidin, biotinylated detection antibody and Avidin-HRP conjugate appeared blue. The reaction was halted with the addition of 50µl of 0.15 M Sulphuric acid (H_2SO_4) and the colour turned yellow. The concentration of the end-point colour (yellow) developed corresponded to the quantity of hepcidin. Absorbances were measured at 450nm and the concentrations calculated from a standard curve generated using calibrators of known concentrations in a Stat Fax 303 Plus Microplate Reader (Awareness Technology Incorporated, Palm City, Florida, USA). Within-assay coefficient of variation and analytic sensitivity of hepcidin were <10% and 9.38ng/ml respectively as indicated by the manufacturer.

2.5.2 LIPID STUDIES

2.5.2.1 Principle and Methodology for Total cholesterol (TC) (Naito, 1984)

Cholesterol esterase (CHE) was used to liberate cholesterol and free fatty acids from Cholesterol esters. Cholesterol oxidase (CHOD) then converted Cholesterol to 4Cholestenone and hydrogen peroxide. Hydrogen peroxide (H_2O_2) then reacted with 4aminophenazone (4-AP) and phenol in the presence of peroxidase (POD) to give Quinonimine (red coloured dye).

$$Cholesterolesters + H_2O \xrightarrow{CHE} Cholesterol + fatty\ acids$$

$$Cholesterol + O_2 \xrightarrow{CHOD} 4\text{-}Cholestenone + H_2O_2$$

$$2H_2O_2 + Phenol + 4\text{-}AP \xrightarrow{POD} Quinonimine + 4H_2O$$

The intensity of the colour formed was proportional to the cholesterol concentration.

2.5.2.2 Principle and Methodology for Triglyceride (TG) (Bucolo and David, 1973)

Triglycerides incubated with lipoprotein lipase (LPL) liberated glycerol and free fatty acids. Glycerol was converted to glycerol-3-phosphate (G3P) and adenosine-5-diphosphate (ADP) by glycerol kinase and ATP. Glycerol-3-phosphate (G3P) was then converted by glycerol phosphate dehydrogenase (GPO) to dihydroxyacetone phosphate (DAP) and hydrogen peroxide (H_2O_2). Hydrogen peroxide (H_2O_2) reacted with 4-aminophenazone (4-AP) and pchlorophenol in the presence of peroxidase (POD) to give Quinonimine (red coloured dye).

$$Triglycerides + H_2O \xrightarrow{LPL} Glycerol + free\ fatty\ acids$$

$$Glycerol + ATP \xrightarrow{Glycerol\ kinase} G3P + ADP$$

$$G3P + O_2 \xrightarrow{GPO} DAP + H_2O_2$$

$$H_2O_2 + 4\text{-}AP + p\text{-}Chlorophenol \xrightarrow{POD} Quinonimine + H_2O$$

The intensity of the colour formed was proportional to the triglyceride concentration.

2.5.2.3 Principle and Methodology for High-density lipoprotein cholesterol (HDL) (Naito, 1984)

Direct determination of High density lipoprotein cholesterol (HDL) was done in two steps that is the elimination of lipoprotein and the measurement of HDL.Firstly, anti-lipoprotein antibody bound to low density lipoprotein, very low density lipoprotein and chylomicrons

other than high density lipoprotein cholesterol. Secondly, the enzymes selectively reacted with the cholesterol present in the HDL particles and thus only HDL cholesterol was subjected to cholesterol measurement. The primary reading was done at 600 nm and the secondary at 700 nm.

2.5.2.4 Low-density lipoprotein cholesterol (LDL) determination

LDL was calculated from the Friedwald's equation in accordance to the manufacturer:

LDL (mmol/l) = TC – HDL – TG / 2.2

2.5.3 ESTIMATION OF INFLAMMATORY MARKERS

2.5.3.1 Principles and Methodologies for inflammatory markers

The Elabscience® ELISA kits (Elabscience Biotechnology Company Limited, WuHan, China) were used in determining the plasma Soluble urokinase-type plasminogen activator receptor (suPAR), Interlukin-6 (IL-6) and C-reactive protein (CRP) using the double antibody sandwich enzyme-linked immunosorbent assay (ELISA) technique. The manufacturer's protocols for the performance of the assays were rigorously followed. The principle underlying the assays were the simultaneous binding of inflammatory markers to two identical antibodies; an immobilized one on a microwell plate and the other in solution with Avidin-horseradish peroxidase (HRP).100 µl of calibrators and plasma were pipetted into their corresponding microwells in ready-to-use microwell plates containing the solid phase anti-inflammatory marker IgG antibodies and warmed at 37^0 C for 90 minutes.The liquid was removed and 100 µl of (1:100 dilution) of Biotinylated detection antibody was pipetted into the microwells and warmed at 37^0 C for 1 hr. After this period the Biotinylated detection antibody in the wells were then removed and the microwells rinsed three times with 350 µl wash buffer. After the rinsing, 100µl of (1:100 dilution) anti-inflammatoryHRP conjugate was added to each microwell and warmed at 37^0 C for 30 min. The antiinflammatory-HRP conjugate in the wells were then removed and the wells rinsed five times with 350 µl wash buffer.

An enzymatic reaction was initiated by the addition of 90µl chromogen into each microwell and then warmed at 37^0 C for 15 min. Only those wells that contained the inflammatory marker, biotinylated detection antibody and Avidin-HRP conjugate appeared blue. The

reaction was halted with the addition of 50µl of 0.15 M Sulphuric acid (H_2SO_4) and the colour turned yellow. The concentration of the end-point colour (yellow) corresponded to the quantity of inflammatory marker. Absorbances were measured at 450nm and the concentrations calculated from a standard curve generated using calibrators of known concentrations in a Stat Fax 303 Plus Microplate Reader (Awareness Technology Incorporated, Palm City, Florida, USA). Within-assays coefficient of variations was < 10% for suPAR, IL-6 and CRP. The sensitivities of the tests were 0.1 ng/ml for suPAR, 9.37pg/ml for IL-6 and 0.469ng/ml for CRP as indicated by the manufacturer.

2.5.4 ESTIMATION OF ENDOTHELIAL CELL INJURY MARKER

2.5.4.1 Principles and Methodologies for Fibronectin (FN)

The Elabscience® ELISA kit (Elabscience Biotechnology Company Limited, WuHan, China) was used in determining the plasma fibronectin (FN) using the double antibody sandwich enzyme-linked immunosorbent assay (ELISA) technique. The manufacturer's protocol for the performance of the assay was rigorously followed. The principle underlying the assay was the real-time binding of fibronectin to two identical antibodies; one in a solid phase in a microwell plate and the other in solution with Avidin-horseradish peroxidase (HRP). 100 µl of calibrators and plasma were pipetted into their corresponding wells in ready-to-use microwell containing the solid phase anti-fibronectin IgG antibodies and warmed at 37^0 C for 90 minutes. The liquid was removed and 100µl of (1:100 dilution) of Biotinylated detection antibody was pipetted into the microwells and warmed at 37^0 C for 1hr. After this period the Biotinylated detection antibody in the microwells were then removed and the microwells rinsed thrice with 350 µl wash buffer. After rinsing, 100 µl of (1:100 dilution) anti-fibronectin-HRP conjugate was added to each microwell and warmed at 37^0 C for 30 min. The anti-fibronectin-HRP conjugate in the microwells were then removed and the wells rinsed five times with 350 µl wash buffer.

An enzymatic reaction was initiated by the addition of 90 µl chromogen into each microwell and then warmed at 37^0 C for 15 min. Only those microwells that contained the fibronectin, biotinylated detection antibody and Avidin-HRP conjugate appeared blue. The reaction was halted with the addition of 50µl of 0.15 M Sulphuric acid (H_2SO_4) and the colour turned yellow. The concentration of the end-point colour (yellow) developed corresponded to the

quantity of fibronectin. Absorbances were measured at 450nm and the concentrations calculated from a standard curve generated using calibrators of known concentrations in a Stat Fax 303 Plus Microplate Reader (Awareness Technology Incorporated, Palm City, Florida, USA). Within-assay coefficient of variation and the analytic sensitivity of the assay were <10 and 0.94ng/ml respectively as indicated by the manufacturer.

2.5.5 ESTIMATION OF OXIDATIVE STRESS MARKER

2.5.5.1 *Principle and Methodology for oxidative stress marker*

The YH Biosearch® assay kit (Shanghai Yehua Biological Technology Company Limited, Shanghai, China) was used in determining the plasma 8-iso-prostaglandin F2α using the double antibody sandwich enzyme-linked immunosorbent assay (ELISA) technique. The manufacturer's protocol for the performance of the assay was rigorously followed. The principle underlying the assay is the simultaneous binding of 8-iso-prostaglandin F2α to two monoclonal antibodies; an immobilized one on a microplate and the other a soluble one conjugated with Streptavidin-horseradish peroxidase (HRP). 50 µl of calibrator (biotin antibodies had united in advance in calibrator) and 40 µl aliquots of plasma were dispensed into their respective wells then 10 µl anti-8-iso-PGF2α IgG antibodies and 50 µl Streptavidin-horseradish peroxidase (HRP) were also added, mixed very well and incubated for 60 minutes at 37^0C. After this period the contents of the microwells were then removed and the microwells rinsed five times with 350 µl wash buffer and blotted. An enzymatic reaction was initiated by firstly adding 50 µl of chromogen A then the same quantity of chromogen B to each well, mixed very well and incubated at 37^0 C for 10 minutes. Only those wells that contained the 8-iso-prostaglandin F2α, anti-8-iso-PGF2α IgG antibodies and Streptavidin-horseradish peroxidase (HRP) conjugate appeared blue. The reaction was halted with the addition of 50 µl of 0.15 M Sulphuric acid (H_2SO_4) and the colour turns yellow. The concentration of the end-point colour developed corresponded to the quantity of 8-iso-prostaglandin F2α. Absorbances were measured at 450 nm and the concentrations calculated from a standard curve generated using calibrators of known concentrations in a Stat Fax 303 Plus Microplate Reader (Awareness Technology Incorporated, Palm City, Florida, USA). Within-assay coefficient of variation and analytic sensitivity of 8-isoprostaglandin F2α were <10% and 0.241pg/ml respectively as indicated by the manufacturer.

2.5.6 ESTIMATION OF RENAL FUNCTION MARKERS

2.5.6.1 Principle and Methodology for Cystatin C (Cys-C)

The Elabscience® ELISA kit (Elabscience Biotechnology Company Limited, WuHan, China) was used in determining the serum Cystatin C (Cys-C) using the double antibody sandwich enzyme-linked immunosorbent assay (ELISA) technique. The manufacturer's protocols for the performance of the assays were rigorously followed. The principle underlying the assay is the real-time binding of hepcidin to two identical antibodies; one in the solid phase on a microwell plate and the other in solution with Avidin-horseradish peroxidase (HRP). 100 µl of calibrators and serum were distributed into their corresponding wells in ready-to-use microwell plates containing the solid phase anti-Cystatin C and warmed at 37^0 C for 90 minutes. The liquid was removed and 100 µl of (1:100 dilution) of Biotinylated detection antibody was pipetted into the microwells and incubated at 37^0C for 1hr. After this period the diluted Biotinylated detection antibody in the microwells were removed and the microwells were rinsed three times with 350 µl wash buffer. After washing, 100 µl of (1:100 dilution) anti-Cystatin C-HRP conjugate was added to each well and warmed at 37^0 C for 30 min. After this period the anti-Cystatin C-HRP conjugate in the microwells were then removed and the microwells rinsed five times with 350 µl wash buffer.

An enzymatic reaction was initiated by the addition of 90 µl chromogen into each microwell and warmed at 37^0 C for 15 min in the dark. Only those wells that contained the Cystatin C, biotinylated detection antibody and Avidin-HRP conjugate appeared blue. The reaction was halted with the addition of 50µl of 0.15 M Sulphuric acid (H_2SO_4) and the colour turned yellow. The concentration of the end-point colour (yellow) developed corresponded to the quantity of Cystatin C present in the serum. Absorbances were measured at 450nm and the concentrations calculated from a standard curve generated using calibrators of known concentrations in a Stat Fax 303 Plus Microplate Reader (Awareness Technology Incorporated, Palm City, Florida, USA). Within-assay coefficient of variation and analytic sensitivity of Cystatin C were <10% and 0.94ng/ml respectively as indicated by the manufacturer.

2.5.6.2 Principle and Methodology for urine micro-albumin (MA)

Urine micro-albumin was determined using the dip-stick qualitative method (DURUI™, DURUI Industerial Company Limited. 95 Yunhe Street, New & High Technology Development Zone.Changchun, Jilin 130012 People's Republic of China). The manufacturer's instructions were strictly adhered to. The principle of the assay is based on the protein "error of indicators". Anion in the specific pH indicator (sulfone phthalein dye which is highly sensitive for micro-albumin) is attracted by cation on the micro-albumin molecule and makes the indicator further ionized, which changes its colour from light-blue to blue. Early morning urine was collected into clean dry plastic containers and fresh strip was dipped into it up to the test area, for not more than two seconds. Excess urine was removed by draining the strip along the brim of the container making sure the test area does not touch the container. This was done to prevent excess urine on the strip from reacting with chemicals between adjacent pads leading to incorrect results. The test result was read by holding the strip horizontally and compared with the colour chart on the strip container under good lighting condition.

2.6 HAEMATOLOGICAL ASSAYS

2.6.1 *Methodology for Full blood count (FBC)*

The Sysmex® 500i haematological analyser (Sysmex Corporation, 1-5-1WakinohamaKaigandori, Chuo-ku, Kobe 651-0073, Japan) was used to measure the haematological parameters at a room temperature of 15-30^{0}C. The EDTA anticoagulated whole blood was mixed very well and the manufacturer's instructions for operating this haematology analyser were strictly adhered to.

2.6.2 *Principle and Methodology for Malaria Antigen Test (MAT)*

The CareStart™ malaria antigen rapid test (Access Bio Incorporated, 5 Clyde Road. Suite A, Somerset, New Jersey 08873, United States of America) was used for malaria investigation. The principle of the test is the qualitative detection of histidine-rich protein 2 (HRP2) antigen of the Plasmodium falciparum. 5µl of EDTA anticoagulated whole blood was dropped onto sample well of the test cassette, then 3 drops of assay buffer was added

and the result read after 20minutes. The presence of two pink lines in the results window is a positive case and the presence of only the control line is a negative case.

2.7 URINALYSIS

2.7.1 *Principle and Methodology for urine protein*

Urine protein was determined using the dip-stick qualitative method (DURUI™, DURUI Industerial Company Limited. 95 Yunhe Street, New & High Technology Development Zone. Changchun,Jilin 130012 People's Republic of China). The manufacturer's instructions were strictly adhered to. The principle of the assay is based on the protein "error of indicators". Anion in the specific pH indicator is attracted by cation on the protein molecule and makes the indicator further ionized, which changes its colour from yellow to blue-green. Early morning urine was collected into clean dry plastic containers and fresh strip was dipped into it up to the test area, for not more than two seconds. Excess urine was removed by draining the strip along the brim of the container making sure the test area does not touch the container. This is done to prevent excess urine on the strip from reacting with chemicals between adjacent pads leading to incorrect results. The test result was read by holding the strip horizontally and compared with the colour chart on the strip container under good lighting condition.

2.8 STATISTICAL ANALYSIS

Continuous variables are expressed as their mean ± SD, while categorical variables were expressed as proportion. Comparisons of the women with PIH (gestational hypertension, preeclampsia and eclampsia separately and combined) against the control group were performed using unpaired t tests or chi-square tests where appropriate. Association between variables was assessed using linear regression or correlation analysis where appropriate. A level of $p < 0.05$ was acceptable as statistically significant unless otherwise stated. The Receiver Operator Characteristic (ROC) analysis was done to assess the diagnostic ability of some selected analytes. A level of $p < 0.05$ was acceptable as statistically significant unless otherwise stated.

Reference intervals for some selected analytes were determined using "robust method" (CLSI Guidelines C28-A3) because the participants were less than one hundred and twenty.

The reference limits were estimated using bootstrapping (percentile interval method) (Efron and Tibshirani, 1994), test for outliers were done with the method based on Reed *et al.* (1971) and normality was tested using the D'Agostino-Pearson test for Normal distribution.

Table 2.1: Reference intervals of some selected analytes as determined from the control subjects

Variables	Mean	Median	SD	Reference Interval
Hepcidin (ng ml^{-1})	6.46	6.30	0.82	4.67-8.30
IL-6 (pg ml^{-1})	13.85	12.50	2.80	8.36-19.34
Fibronectin (ng ml^{-1})	9.71	10.08	3.97	0.84-18.55
suPAR (ng ml^{-1})	2.10	2.10	0.74	0.48-3.74
8-iso-PGF2α (pg ml^{-1})	3.63	3.92	1.14	1.43-6.53

SD = standard deviation, IL-6 = Interleukin 6 and suPAR =soluble urokinase plasminogen activator receptor, 8-iso-PGF2α = 8-iso-prostaglandin F2α

GraphPad Prism version 6.00 for windows (GraphPad software, San Diego California USA, www.graphpad.com) and MedCalc Statistical Software version 14.8.1 (MedCalc Software bvba, Ostend, Belgium; http://www.medcalc.org; 2014) were used for these statistical analyses.

Chapter 3

RESULTS

None of the participants had malaria and renal dysfunction (Cystatin C and urine micro-albumin were used as biomarkers to rule out renal dysfunction).

3.1 HEPCIDIN CONCENTRATIONS AND IRON HOMEOSTASIS IN STUDIED PARTICIPANTS

3.1.1 Demographic, haematological and biochemical parameters of the studied participants

3.1.1.1 Control

The demographic, heamatological and biochemical features of the participants are shown in Table 3.1. Blood pressure as measured by the SBP and DBP, the heamatological parameters, the iron status markers, the inflammatory marker (i.e. CRP, and IL-6) as well as hepcidin concentration were all within the normal range among the normotensive women with healthy pregnancy (Control).

Table 3.1 represent the results of the studied population when stratified based on the type of PIH.

3.1.1.2 Gestational Hypertension

The age and gestational age (31.9 ± 5.44 yr and 36.3 ± 4.77 wks respectively) of consented women with gestational hypertension were significantly older in age as well as gestational week ($p<0.0001$ and $p<0.0001$ respectively) than the normotensive women with healthy pregnancy (26.2 ± 4.23 yr and 27.5 ± 6.11 wks respectively). As shown in Table 3.1, the haematological parameters; total WBC and MCHC were considerably increased in GH than the normotensive women with healthy pregnancy but the RBC, Hb, HCT as well as MCV were considerably decreased in GH than those with normal pregnancy without hypertension. However, the mean platelet count and MCH were similar between the women with GH and those without hypertension (Table 3.1).

The iron haemostasis markers: plasma iron and total iron binding capacity (TIBC) were considerably reduced in GH than the normotensive women with healthy pregnancy. Whereas

plasma ferritin was significantly higher among the women with GH, plasma transferrin level was similar to the normotensive women with healthy pregnancy. The mean hepcidin level among the women with GH (7.30 ± 0.71 ng/ml) was significantly higher (p = 0.002) as compared to the normotensive women with healthy pregnancy (6.463 ± 0.82 ng/ml) (Table 3.1).

CRP was significantly higher in GH than the normotensive women with healthy pregnancy however IL-6 was similar to the control group (Table 3.1).

3.1.1.3 Preeclampsia

The patients with preeclampsia were older in age and gestational age as compared to the normotensive women with healthy pregnancy (30.35 ± 6.54yr vs 26.2 ± 4.2yr respectively) and (36.51 ± 3.84wks vs 27.5 ± 6.11wks respectively) respectively. The mean values of WBC and MCHC of the preeclamptic patients were higher as compared to the normotensive women with healthy pregnancy. Though the mean values of RBC, Hb, MCH and platelets count of the preeclamptic women were lower than that of the normotensive women with healthy pregnancy they were statistically not significant. However, Hct and MCV mean values were considerably decreased in preeclampsia compared to the normotensive women with healthy pregnancy (Table 3.1).

Comparing the mean plasma iron and total iron binding capacity (TIBC) values of preeclamptic women to that of the normotensive women with healthy pregnancy, they were significantly lower, whereas their mean plasma ferritin values were elevated. The mean transferrin values of women with preeclampsia though higher than that of the normotensive women with healthy pregnancy (227.9 ± 53.79 mg/dl vs 212 ± 38.9 mg/dl respectively), they were not statistically significant (p = 0.0981). Women with preeclampsia had higher mean plasma value of hepcidin than that of the normotensive women with healthy pregnancy (Table 3.1).

The inflammatory cytokines CRP and IL-6 levels in participants with this condition were higher than the normotensive women with healthy pregnancy (Table 3.1).

3.1.1.4 Eclampsia

Eclamptic women were comparatively older than the normotensive women with healthy pregnancy and also had a higher mean gestational age. The normotensive women with healthy pregnancy had

lower mean WBC and MCHC values than those with eclampsia but though the eclamptic women had higher mean MCH values than the controls they were not statistically significant. Whereas the mean Hct, MCV and platelet count values were considerably lower in the eclamptic than the normotensive women with healthy pregnancy, RBC and Hb mean values though also lower comparatively were not statistically significant (Table 3.1).

Plasma iron and total iron-binding capacity mean values in eclamptic patients were lower than the normotensive women with healthy pregnancy (65 ± 34.76 µg/dl vs 138±30.33 µg/dl respectively and 261.2 ± 80.82 µg/dl vs 320 ± 88.2µg/dl respectively). Mean plasma transferrin values though lower in eclamptic women as compared to the normotensive women with healthy pregnancy was not statistically significant. Plasma hepcidin mean values of eclamptic patients were higher than that of the normotensive women with healthy pregnancy healthy (Table 3.1).

CRP and IL-6 mean values were increased in patients with eclampsia as compared to the normotensive women with healthy pregnancy (Table 3.1).

3.1.1.5 Pregnancy-Induced Hypertension

The mean age and gestational weeks of the normotensive women with healthy pregnancy healthy were lower than those with pregnancy-induced hypertension. As shown in Table 3.1, the mean values of WBC and MCHC of pregnancy-induced hypertensive patients were elevated than those with healthy pregnancy. Pregnancy-induced hypertensive women had significantly lower mean RBC, Hb, Hct and MCV values than the normotensive women with healthy pregnancy, but though their mean MCH and platelets values were also lower they were not statistically significant (Table 3.1).

The mean iron and total iron-binding capacity values of pregnancy-induced hypertensive women were comparatively lower than that of the normotensive women with healthy pregnancy healthy. Plasma transferrin mean value of pregnancy-induced hypertensive patients though higher than that of the normotensive women with healthy pregnancy was not statistically significant but the mean ferritin value of pregnancy-induced hypertensive women was statistically higher comparatively with the control. There were elevated levels of hepcidin in participants presenting with PIH compared to the controls (7.72 ± 1.07ng/ml vs 6.463 ± 0.82ng/ml respectively) (Table 3.1).

The mean serum CRP and plasma IL-6 values were considerably higher in pregnancy-induced hypertensive women than the control (Table 3.1).

3.1.2 General trends of Haematologic and Biochemical parameters amongst the studied participants

Generally, the mean age of pregnancy-induced hypertensive women were higher than those with uncomplicated pregnancy without hypertension but those with eclampsia were the youngest. An increase in the severity of pregnancy-induced hypertension leads to a relative decrease in the mean values of platelets count and an increase in the mean values of leucocytes count, IL-6, hepcidin and CRP but the mean CRP value in preeclampsia is comparatively lower than that of GH and eclampsia. There is a decrease in the mean plasma iron, TIBC and transferrin concentrations as pregnancy-induced hypertension progresses, thus a corresponding decrease in the mean haemoglobin concentrations results. Ferritin mean values though generally increased in pregnancyinduced hypertension were lower in preeclampsia (Table 3.1).

Table 3.1: Demographic, haematological and biochemical parameters of the studied participants stratified by PIH

VARIABLE	CONTROL	GH	P VALUE	PE	P VALUE	EC	P VALUE	PIH	P VALUE
AGE (yrs)	26.2 ± 4.23	31.9 ± 5.44	< 0.0001	30.35 ± 6.54	0.0004	28.67 ± 4.53	0.0448	30.72 ± 5.93	< 0.0001
GEST. AGE(wks)	27.5 ± 6.11	36.3 ± 4.77	< 0.0001	36.51 ± 3.84	< 0.0001	36.22 ±3.00	< 0.0001	36.40 ± 4.10	< 0.0001
SBP(mmHg)	109 ± 8.74	159 ± 15.7	< 0.0001	158.9 ± 13.64	< 0.0001	156.6 ± 9.91	< 0.0001	158.6 ± 13.96	< 0.0001
DBP(mmHg)	64.7 ± 8.69	97.4 ± 8.64	< 0.0001	97.95 ±10.86	< 0.0001	97.39 ± 7.11	< 0.0001	97.63 ± 9.48	< 0.0001
WBC(*10^3/µl)	1.76 ± 0.70	10.3 ±3.64	< 0.0001	12.33 ± 7.00	< 0.0001	12.82 ± 3.30	< 0.0001	11.60 ± 5.50	< 0.0001
RBC(*10^6/µl)	4.14 ± 0.49	3.72 ± 0.82	0.0039	4.034 ± 0.768	0.4223	3.803 ± 0.973	0.0727	3.878 ± 0.83	0.0472
Hb(g/dl)	10.9 ± 1.35	9.98 ± 2.18	0.0196	10.29 ± 1.882	0.081	10.37 ±2.479	0.3017	10.18 ±2.084	0.0383
HCT (%)	37.7 ± 4.39	31.6 ± 6.75	< 0.0001	32.95 ± 6.267	< 0.0001	31.92 ± 7.757	0.0004	32.26 ± 6.664	< 0.0001
MCV(fL)	91.4 ± 8.73	85.4 ± 7.46	0.0005	82.38 ± 9.729	< 0.0001	85.18 ±11.24	0.0213	83.96 ± 9.201	< 0.0001
MCH(pg)	26.4 ±2.77	27.0 ±2.58	0.2871	25.71 ± 2.706	0.2125	27.62 ± 3.030	0.1292	26.49 ± 2.789	0.8477
MCHC(g/dl)	28.9 ± 1.22	31.6 ± 2.18	< 0.0001	31.35 ± 2.555	< 0.0001	32.58 ±2.493	< 0.0001	31.65 ± 2.422	< 0.0001
PLT(10^3/µl)	174.00 ± 44.06	168.60 ± 72.18	0.6843	160.50 ± 40.30	0.1554	142.90 ±36.41	0.0302	161.80 ± 56.54	0.2204
IRON(µg/dl)	138 ± 30.33	93 ± 30.99	< 0.0001	80 ± 44.76	< 0.0001	65 ± 34.76	< 0.0001	85 ± 39.09	< 0.0001
TS (mg/dl)	212 ± 38.9	232 ± 69.4	0.0911	227.9 ± 53.79	0.0981	210.4 ± 26.89	0.8713	227.0 ± 57.75	0.1092
FERRITIN(ng/ml)	37.1 ± 30.5	152.2 ± 138.7	< 0.0001	183 ± 164	< 0.0001	280.4 ± 156.2	< 0.0001	183.0 ± 156.2	< 0.0001
TIBC(µg/dl)	360 ± 68.0	320 ± 88.2	0.0172	314.7 ±102.0	0.0125	261.2 ± 80.82	< 0.0001	308.9 ± 95.29	0.0013
HEPCIDIN(ng/ml)	6.463 ± 0.82	7.30 ± 0.71	0.002	7.66 ± 1.19	0.001	8.02 ± 1.21	< 0.0001	7.72 ± 1.07	< 0.0001
CRP(ng/L)	0.98 ± 0.05	3.67 ± 2.14	< 0.0001	2.839 ± 2.281	0.0029	5.611 ± 2.852	< 0.0001	3.309 ± 2.814	< 0.0001
IL-6 (pg/ml)	13.85 ± 2.8	13.97 ± 4.84	0.939	21.27 ± 11.52	0.0332	23.57 ± 16.44	0.0463	19.60 ± 10.32	0.0433

GH: Gestational Hypertension; PE: Preeclampsia; EC: Eclampsia; PIH: Pregnancy-induced Hypertension; GEST. AGE: Gestational Age; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; WBC: White Blood Cells; RBC: Red Blood Cells; Hb: Haemoglobin; HCT: Haematocrit; MCV: Mean Cell Volume;

MCH: Mean Cell Haemoglobin; MCHC: Mean Cell Haemoglobin Concentration; PLT: Platelets; TS: Transferrin; TIBC: Total Iron Binding Capacity; CRP: Creactive protein; IL-6: Interlukin-6; P VALUE < 0.05: Statistically significant.

3.1.3 Prevalence of anaemia, iron deficiency and iron deficiency anaemia amongst the studied participants

As shown in Table 3.2, the prevalence of anaemia as defined by haemoglobin concentration was 46.7%. However, the prevalence of iron deficiency increase from 0.0% using plasma iron concentration to 2.2% using plasma transferrin concentration through 17.8% using plasma ferritin concentration to 22.2% using total iron binding capacity among healthy women with uncomplicated pregnancy without hypertension. Iron deficiency anaemia also increased from 0.0% using plasma iron and plasma transferrin as diagnostic indicator via 8.9% using total iron binding capacity to 11.1% when plasma ferritin was applied as diagnostic indicator among healthy women with uncomplicated pregnancy without hypertension (Table 3.2).

3.1.3.1 Gestational Hypertension

When the studied population was classified based on the type of PIH, the prevalence of anaemia (60.4%) using Hb concentration was not significantly ($p = 0.1838$) different from the healthy women with uncomplicated pregnancy without hypertension. Irrespective of the variable that was used as a marker of iron deficiency anaemia, the proportion of women with GH who developed iron deficiency anaemia as compared to the normotensive women with healthy pregnancy was similar (Table 3.2).

From Table 3.2, the ratio of GH who developed iron deficiency when iron, transferrin and TIBC were used as diagnostic indicators was not significantly different from the proportion of the healthy women with uncomplicated pregnancy without hypertension. However, using ferritin, the prevalence of iron deficiency among women with GH (2.1%) was significantly ($p = 0.0105$) lower than the prevalence of iron deficiency among healthy women with uncomplicated pregnancy without hypertension (17.8%).

3.1.3.2 Preeclampsia

The prevalence of anaemia using Hb concentration was similar ($p = 0.2593$) in preeclampsic women (57.9%) when compared to the normotensive women with healthy pregnancy (46.7%). However, when plasma iron and ferritin were used as marker of iron deficiency anaemia, those with preeclampsia had significantly ($p = 0.0003$) higher prevalence of IDA as defined by plasma iron (24.6%) and significantly ($p = 0.0461$) lower prevalence of IDA as defined by plasma ferritin

(1.8%) as compared to the healthy women with uncomplicated pregnancy without hypertension (0.0% and 11.1% respectively). Transferrin and TIBC concentrations were similar in the two groups using unpaired *t*-test (Table 3.2).

Also, the proportion of the participants with iron deficiency when plasma iron and ferritin were used as markers was significantly (p = 0.0001) higher using plasma iron (28.1%) and significantly (p = 0.0161) lower using plasma ferritin (3.5%) as compared to the healthy women with uncomplicated pregnancy without hypertension (0.0% and 17.8% respectively). Transferrin and TIBC concentrations were similar in the two groups using unpaired *t*-test (Table 3.2).

3.1.3.3 Eclampsia

From Table 3.2, the prevalence of anaemia using Hb concentration was not significantly (p = 0.8109) different when women with eclampsia (50.0%) was compared to the healthy women with uncomplicated pregnancy without hypertension (46.7%). But, when plasma iron was used as marker of iron deficiency anaemia, those with eclampsia had significantly (p = 0.0231) higher prevalence of IDA (11.1%) as compared to the healthy women with uncomplicated pregnancy without hypertension (0.0%). Ferritin, transferrin and TIBC did not show any significant difference among the two groups using unpaired *t*-test (Table 3.2).

Similarly, the proportion of the participants with iron deficiency when plasma iron was used as marker was significantly (p = 0.0050) higher (16.7%) as compared to the healthy women with uncomplicated pregnancy without hypertension (0.0%). Ferritin, transferrin and TIBC concentrations were similar in the two groups using unpaired *t*-test (Table 3.2).

3.1.3.4 Pregnancy-Induced Hypertension

When the studied population was pooled together as PIH, the prevalence of anaemia (57.7%) using Hb concentration was also not significantly (p = 0.2023) different from the healthy women with uncomplicated pregnancy without hypertension (46.7%). However, when plasma iron and ferritin were used as markers of iron deficiency anaemia, those with PIH had significantly (p = 0.0051) higher prevalence of IDA as defined by plasma iron (15.5%) and significantly (p = 0.0064) lower prevalence of IDA as defined by plasma ferritin (1.6%) as compared to the healthy women with

uncomplicated pregnancy without hypertension (0.0% and 11.1% respectively). Transferrin and TIBC concentrations were similar in the two groups using unpaired *t*-test (Table 3.2).

Also, the proportion of the participants with iron deficiency when plasma iron and ferritin were used as markers was significantly (p = 0.0023) higher using plasma iron (17.9%) and significantly (p = 0.0004) lower using plasma ferritin (2.4%) as compared to the healthy women with uncomplicated pregnancy without hypertension (0.0% and 17.8% respectively). Transferrin and TIBC concentrations were similar in the two groups using unpaired t-test (Table 3.2).

3.1.3.5 Trend of iron deficiency and iron deficiency anaemia amongst the studied participants

Generally, the prevalence of iron deficiency decreases using plasma ferritin, transferrin and TIBC as the severity of the disease progress from the control to eclampsia. However, the prevalence of iron deficiency using plasma iron increases from 0.0% among the control group through 6.3% among the women with GH to 28.1% among women with pre-eclampsia before decreasing to 16.7% among women with eclampsia (Table 3.2).

Also, the prevalence of iron deficiency anaemia generally decreases using serum ferritin, transferrin and TIBC as the severity of the disease progress from the control to eclampsia. IDA as defined by plasma iron however increases from 0.0% among the control group through 6.3% among the women with GH to 24.6% among women with pre-eclampsia before decreasing to 11.1% among women with eclampsia (Table 3.2).

Table 3.2: Prevalence of anaemia, iron deficiency and iron deficiency anaemia amongst the studied participants classified by PIH

VARIABLE	CONTROL	P VALUE	GH	P VALUE	PE	P VALUE	EC	P VALUE	PIH	P VALUE
ANAEMIA	21(46.67%)			0.1838		0.2593		0.8109		0.2023
IRON DEFICIENCY										
IRON(µg/dl)	0(0.00%)		3(6.25%)	0.0882	16(28.07%)	0.0001	3(16.67%)	0.005	22(17.89%)	0.0023
FERRITIN(ng/ml)	8(17.78%)		1(2.08%)	0.0105	2(3.51%)	0.0161	0(0.00%)	0.0556	3(2.44%)	0.0004
TRANSFERRIN(mg/dl)	1(2.22%)		1(2.08%)	0.9632	0(0.00%)	0.258	0(0.00%)	0.5238	1(0.81%)	0.4558
TIBC(µg/dl)	10(22.22%)		9(18.75%)	0.6781	12(21.05%)	0.8866	1(5.56%)	0.1154	22(17.89%)	0.5262
IDA										
IRON(µg/dl)	0(0.00%)		3(6.25%)	0.0882	14(24.56%)	0.0003	2(11.11%)	0.0231	19(15.45)	0.0051
FERRITIN(ng/ml)	5(11.11%)		1(2.08%)	0.0766	1(1.75%)	0.0461	0(0.00%)	0.1405	2(1.63%)	0.0064
TRANSFERRIN(mg/dl)	0(0.00%)		1(2.08%)	0.3303	0(0.00%)		0(0.00%)		1(0.81%)	0.5441
TIBC(µg/dl)	4(8.89%)		5(10.42%)	0.8033	3(5.26%)	0.472	1(5.56%)	0.6584	9(7.31%)	0.7356

	(n=48)	**(n=57)**	**(n=18)**	**(n=123)**
(n=45)	29(60.42%)	33(57.89%)	9(50.00%)	71(57.72%)

GH: Gestational Hypertension; PE: Preeclampsia; EC: Eclampsia; PIH: Pregnancy-induced Hypertension; TIBC: Total Iron Binding Capacity; IDA: Iron Deficiency Anaemia; P VALUE < 0.05: Statistically significant.

3.1.4 Association between hepcidin, haematological and biochemical parameters amongst the studied participants

3.1.4.1 Gestational Hypertension

For every 1 ng/ml increase in hepcidin, SBP significantly decreased by 8.9 mmHg ($r^2 = 0.31$; $p < 0.05$), haemoglobin concentration decreased by 1.4 g/dL ($r^2 = 0.24$; $p < 0.05$) and platelet decreased by 70.3 x 10^3/µl ($r^2 = 0.38$; $p < 0.01$) amongst the women with GH (Table 3.3).

3.1.4.2 Preeclampsia

Among the women with preeclampsia (Table 3.3), for every 1 ng/ml increase in hepcidin, DBP significantly decreased by 3.9 mmHg ($r^2 = 0.16$; $p < 0.05$) and ferritin significantly increased by 53.4 ng/ml ($r^2 = 0.20$; $p < 0.05$).

3.1.4.3 Eclampsia

From Table 3.3, for every 1 ng/ml increase in hepcidin, ferritin significantly increased by 34.0 ng/ml ($r^2 = 0.17$; $p < 0.05$), TIBC decreased by 42.4 µg/dl ($r^2 = 0.40$; $p < 0.01$) and CRP increased by 0.5 ng/L ($r^2 = 0.09$; $p < 0.05$) among the women with eclampsia.

3.1.4.4 Pregnancy-Induced Hypertension

Combining the GH, pre-eclampsia and eclampsia groups as PIH, it was realized that, for every 1 ng/ml increase in hepcidin, DBP significantly decreased by 3.3 mmHg ($r^2 = 0.14$; $p < 0.01$), ferritin significantly increased by 49.6 ng/ml ($r^2 = 0.14$; $p < 0.01$), TIBC decreased by 27.0 µg/dl ($r^2 = 0.14$; $p < 0.01$) and CRP increased by 0.7 ng/L ($r^2 = 0.08$; $p < 0.05$) (Table 3.3).

Table 3.3: Linear regressional analysis between hepcidin, haematological and biochemical parameters amongst the studied participants classified by PIH

VARIABLE	GH		PE		EC		PIH	
	β	r^2	β	r^2	β	r^2	β	r^2
AGE	-2.74	0.12	1.03	0.00	-0.45	0.01	-0.92	0.04
GEST. AGE(WKS)	0.63	0.01	0.73	0.08	0.85	0.12	0.73	0.05
SBP(mmHg)	**-8.90***	0.31	-1.38	0.01	2.24	0.07	-1.82	0.03
DBP(mmHg)	-4.92	0.16	**-3.94***	0.16	-1.42	0.06	**-3.29****	0.14
WBC(*10^3/µl)	-0.11	0.00	1.03	0.15	-0.35	0.02	0.65	0.04
RBC(*10^6/µl)	-0.42	0.14	-0.02	0.00	0.07	0.01	-0.05	0.00
Hb(g/dl)	**-1.40***	0.24	-0.03	0.00	0.18	0.01	-0.09	0.00
HCT(%)	-3.49	0.16	-0.20	0.00	0.06	0.00	-0.49	0.01
MCV(fL)	0.05	0.00	-0.43	0.00	-0.85	0.01	-0.25	0.00
MCH(pg)	-0.71	0.03	0.12	0.00	0.00	0.00	0.15	0.00
MCHC(g/dl)	-0.78	0.07	0.18	0.01	0.45	0.05	0.26	0.02
PLT(*10^3/µl)	**-70.25****	0.38	0.93	0.00	-6.66	0.01	-8.73	0.02
IRON(µg/dl)	37.86	0.04	8.59	0.02	-5.48	0.03	-2.38	0.00
TRANSFERRIN(mg/dl)	2.92	0.00	-0.59	0.00	0.04	0.00	-3.75	0.00
FERRITIN(ng/ml)	38.50	0.05	**53.42***	0.20	**34.00***	0.17	**49.55****	0.14
TIBC(µg/dl)	-31.02	0.06	-8.03	0.02	**-42.37****	0.40	**-26.99****	0.14
CRP(ng/L)	0.41	0.02	0.33	0.03	**0.50***	0.09	**0.65***	0.08
IL-6 (pg/ml)	5.37	0.00	-35.30	0.07	-1.56	0.00	-23.47	0.04

GH: Gestational Hypertension;PE: Preeclampsia; EC: Eclampsia;PIH: Pregnancy-induced Hypertension;GEST. AGE: Gestational Age;SBP: Systolic Blood Pressure;DBP: Diastolic Blood Pressure;WBC: White Blood Cells;RBC: Red Blood Cells;Hb: Haemoglobin;HCT: Haematocrit; MCV: Mean Cell Volume;MCH: Mean Cell Haemoglobin;MCHC: Mean Cell Haemoglobin Concentration;PLT: Platelets;TIBC: Total Iron Binding Capacity;CRP: C-reactive protein;IL-6: Interlukin-6; ***** *Correlation is significant at 0.05 level;* ****** *Correlation is significant at 0.01 level.*

3.2 INFLAMMATORY MARKERS (suPAR, IL-6 AND CRP) LEVELS IN STUDIED PARTICIPANTS

The inflammatory markers increased significantly amongst PIH compared to the controls.

3.2.1 Gestational Hypertension

As shown in Table 3.4, the mean±SD of the CRP of the women with GH (3.67 ± 2.14 ng/L) was considerably (P<0.0001) raised than the healthy women with uncomplicated pregnancy without hypertension (0.98 ± 0.05 ng/L). The mean±SD of the IL-6 as well as suPAR level among the women with GH (13.97 ± 4.84 pg/ml and 1.92 ± 0.43 ng/ml respectively) were not significantly (p = 0.9390 and p = 0.1091 respectively) different from the healthy women with uncomplicated pregnancy without hypertension (13.85 ± 2.8 pg/ml and 1.57 ±0.56 ng/ml respectively).

3.2.2 Preeclampsia

The average levels of C-reactive protein, interlukin-6 as well as suPAR among the women with preeclampsia (2.84 ± 2.28 ng/L, 21.27 ± 11.52 pg/ml and 2.07 ± 0.82 ng/ml respectively) were significantly higher (p = 0.0029, p = 0.0332 and p = 0.0442 respectively) than the healthy women with uncomplicated pregnancy without hypertension (0.98 ± 0.05 ng/L, 13.85 ± 2.8 pg/ml and 1.57 ±0.56 ng/ml respectively) (Table 3.4).

3.2.3 Eclampsia

When the mean±SD of the CRP, IL-6 as well as suPAR level among eclamptic women were compared to the normotensive women with healthy pregnancy, those with eclampsia (5.61 ± 2.85 ng/L, 23.57 ± 16.44 pg/ml and 2.1 ± 0.64 ng/ml respectively) had significantly higher (p < 0.0001, p = 0.0463 and p = 0.0392 correspondingly) of CRP, IL-6 and suPAR as compared with the healthy women with uncomplicated pregnancy without hypertension (0.98 ± 0.05 ng/L, 13.85 ± 2.8 pg/ml and 1.57 ±0.56 ng/ml respectively) (Table 3.4).

3.2.4 Pregnancy-Induced Hypertension

The mean±SD of IL-6 level of the women with PIH (19.60 ± 12.32 pg/ml) was similar (P = 0.1033) to the normotensive women with healthy pregnancy (13.85 ± 2.8 pg/ml). The mean±SD of the

CRP as well as suPAR level among the women with PIH (3.309 ± 2.814 ng/L and 2.04 ± 0.66

Results

ng/ml respectively) was considerably raised ($p < 0.0001$ and $p = 0.0313$ correspondingly) than the healthy women with uncomplicated pregnancy without hypertension (0.98 ± 0.05 ng/L and 1.57 ±0.56 ng/ml respectively) (Table 3.4).

3.3 ASSOCIATION BETWEEN SOLUBLE UROKINASE PLASMINOGEN ACTIVATOR RECEPTOR (SUPAR), INTERLUKIN-6 (IL-6) AND C-REACTIVE PROTEIN (CRP) AMONGST THE STUDIED PARTICIPANTS

3.3.1 Gestational Hypertension

When suPAR was regressed against the other inflammatory markers, it was revealed that, for every 1 ng/ml increased in suPAR, IL-6 significantly increased by 37.1 pg/ml ($r^2 = 0.14$; $p < 0.05$) among the women with GH but CRP showed no response (Table 3.5).

3.3.2 Preeclampsia

Among the women with preeclampsia (Table 3.5), when suPAR concentration was regressed against the other inflammatory markers, it was realized that, for every 1 ng/ml increased in suPAR, CRP significantly increased by 20.8 ng/L ($r^2 = 0.19$; $p < 0.05$) and IL-6 significantly increased by 33.5 pg/ml ($r^2 = 0.13$; $p < 0.05$).

3.3.3 Eclampsia

From Table 3.5, when suPAR concentration was regressed against CRP and IL-6, the results indicate that, for every 1 ng/ml increased in suPAR, CRP significantly increased by 21.0 ng/l ($r^2 = 0.15$; $p < 0.05$), and IL-6 significantly increased by 20.9 pg/ml ($r^2 = 0.16$; $p < 0.05$) among the women with eclampsia.

3.3.4 Pregnancy-Induced Hypertension

Combining the GH, pre-eclampsia and eclampsia groups as PIH in Table 3.5, when their suPAR concentration was linearly regressed against CRP and IL-6, the results indicates that, for every 1 ng/ml increase in suPAR, CRP significantly increased by 26.3 ng/l ($r^2 = 0.18$; $p < 0.05$), and IL-6 significantly increased by 30.8 pg/ml ($r^2 = 0.19$; $p < 0.05$) among the women with PIH.

Table 3.4: Inflammatory markers amongst the studied participants classified by PIH

VARIABLE	CONTROL	GH	P VALUE	PE	P VALUE	EC	P VALUE	PIH	P VALUE
CRP(ng/L)	0.98 ± 0.05	3.67 ± 2.14	< 0.0001	2.839 ± 2.281	0.0029	5.611 ± 2.852	< 0.0001	3.309 ± 2.814	< 0.0001
IL-6 (pg/ml)	13.85 ± 2.8	13.97 ± 4.84	0.939	21.27 ± 11.52	0.0332	23.57 ± 16.44	0.0463	19.60 ± 10.32	0.0433
suPAR (ng/ml)	1.57 ±0.56	1.92 ± 0.43	0.1091	2.07 ± 0.82	0.0442	2.1 ± 0.64	0.0392	2.04 ± 0.66	0.0313

GH: Gestational Hypertension; PE: Preeclampsia; EC: Eclampsia; PIH: Pregnancy-induced Hypertension; CRP: C-reactive protein; IL-6: Interlukin-6; suPAR: Soluble Urokinase Plasminogen Activator Receptor; P VALUE < 0.05: Statistically significant.

Table 3.5: Linear regressional analysis between suPAR and CRP and IL-6 amongst the studied participants

VARIABLE	GH		PE		EC		PIH	
	B	r^2	β	r^2	β	r^2	β	r^2
CRP(ng/L)	0.15	0.01	**20.80***	0.19	**21.01***	0.15	**26.30***	0.18
IL-6 (pg/ml)	**37.12***	0.14	**33.50***	0.13	**20.90***	0.16	**30.77***	0.19

*GH: Gestational Hypertension; PE: Preeclampsia; EC: Eclampsia; PIH: Pregnancy-induced Hypertension; CRP: C-reactive protein; IL6: Interlukin-6; suPAR: Soluble Urokinase Plasminogen Activator Receptor; ***** Correlation is significant at 0.05 level.*

3.3.5 Receiver Operator Characteristic analysis of the inflammatory markers amongst the studied population

3.3.5.1 Gestational Hypertension

ROC curve analyses showed a 0.55 area under the curve for CRP (95% CI: 0.44-0.66, p = 0.344); a 0.68 area under the curve, at a confidence interval of 95% within the range of 0.52-0.85 and at a significance of p = 0.0130 for IL-6 and a 0.67 area under the curve for suPAR at a confidence interval of 95% within the range of 0.45-0.90 at a significance of p = 0.1570 as indicated in Figure 3.1.

At the cut-off of > 1.50 ng/L for CRP the sensitivity and specificity of CRP in differentiating between GH and healthy pregnancies is 54.17% and 55.56% respectively. However, at the cut-off of > 19.15 pg/ml and > 2.44 ng/ml for IL-6 and suPAR respectively, the sensitivities were 15.38% and 9.09% respectively and specificities were 100.00% each as shown in Table 3.6.

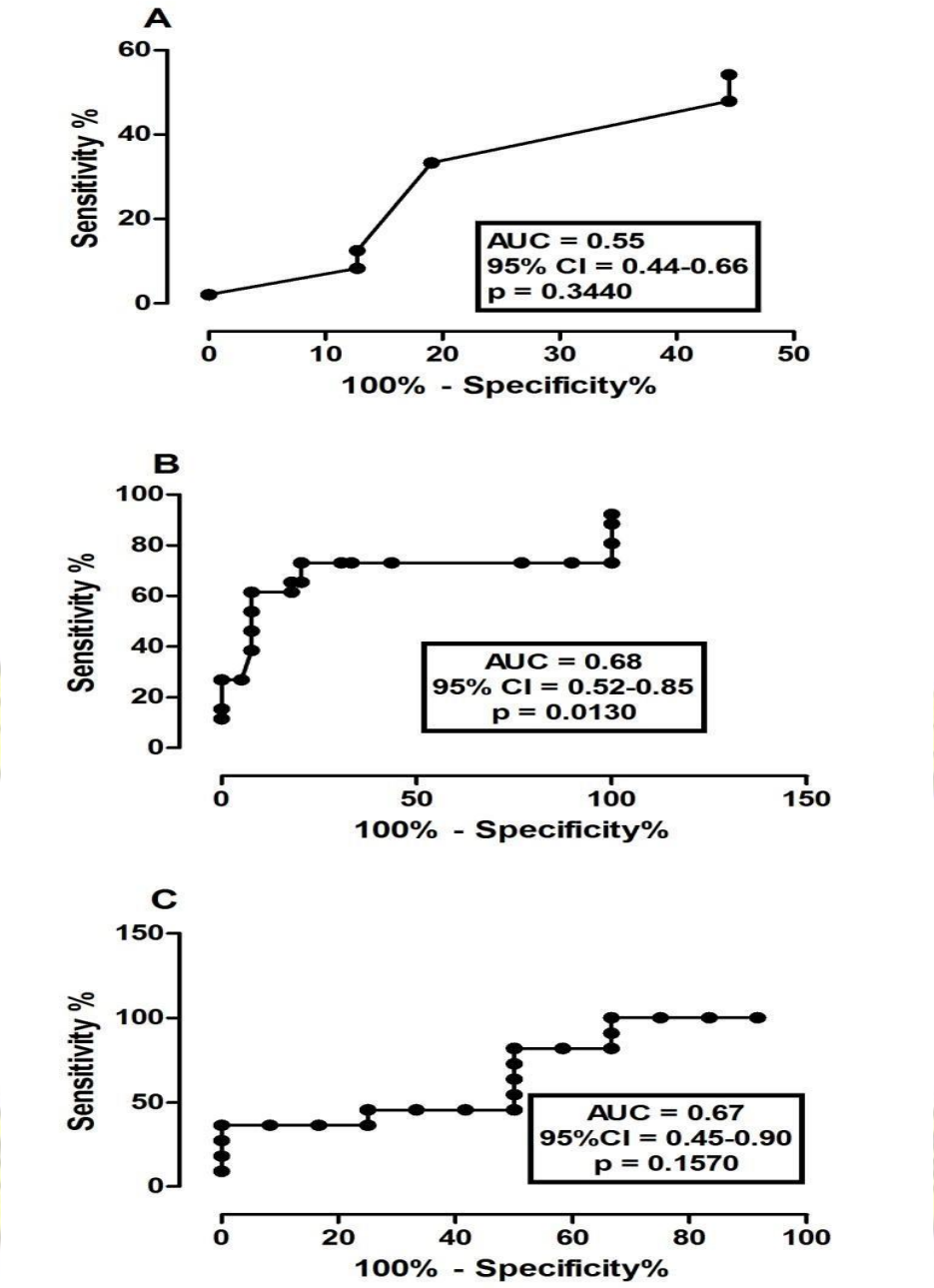

Figure 3.1: Receiver Operator Characteristic analysis of serum C-reactive Protein (A), plasma Interlukin-6 (B) and plasma Soluble Urokinase Plasminogen Activator Receptor (C) level in Healthy Pregnancies and Gestational Hypertension. AUC: Area Under Curve; CI: Confidence Interval.

3.3.5.2 Preeclampsia

As shown in Figure 3.2 the ROC curve analyses showed a 68% area under the curve for CRP (95% CI: 0.55-0.81, p = 0.0089); a 74% area under the curve (95% CI: 0.61-0.87, p = 0.0015) for IL-6 and a 81% area under the curve for suPAR (95% CI: 0.68-0.94, p = 0.0004).

Among the women with preeclampsia, the sensitivities of CRP, IL-6 and suPAR were 35.48%, 56.76% and 12.12% respectively at the cut-offs of > 2.00 ng/L, > 19.52 pg/ml and > 3.75 ng/ml respectively. Each of them indicated specificity of 100.00% at the corresponding cut-off values (Table 3.6).

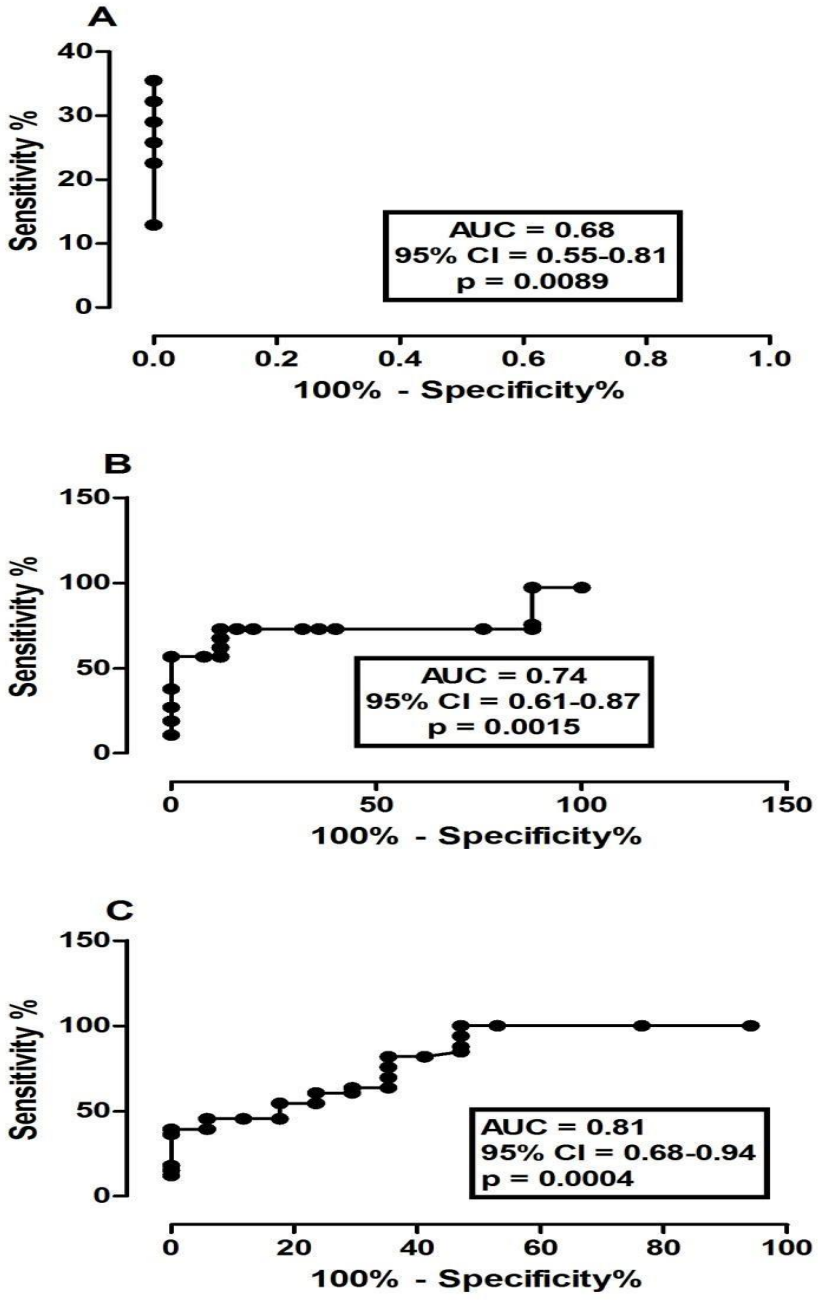

Figure 3.2: Receiver Operator Characteristic analysis of serum C-reactive Protein (A), plasma Interlukin-6 (B) and plasma Soluble Urokinase Plasminogen Activator

Receptor (C) level in Healthy Pregnancies and Preeclampsia. AUC: Area Under Curve; CI: Confidence Interval.

3.3.5.3 Eclampsia

ROC curve analyses as presented in Figure 3.3 showed a 79% area under the curve for CRP (95% CI: 0.67-0.91, p = 0.0001); a 83% area under the curve (95% CI: 0.70-0.96, p = 0.0002 for IL-6 and a 84% area under the curve for suPAR (95% CI: 0.71-0.97, p = 0.0009).

Among the women with eclampsia, the sensitivities of CRP, IL-6 and suPAR were 58.62%, 68.00% and 7.69% respectively at the cut-offs of > 1.50 ng/L, > 18.55 pg/ml and > 3.18 ng/ml respectively. Whereas IL-6 indicated a specificity of 68.47%, CRP and suPAR indicated specificities of 100.00% each at the corresponding cut-off values (Table 3.6).

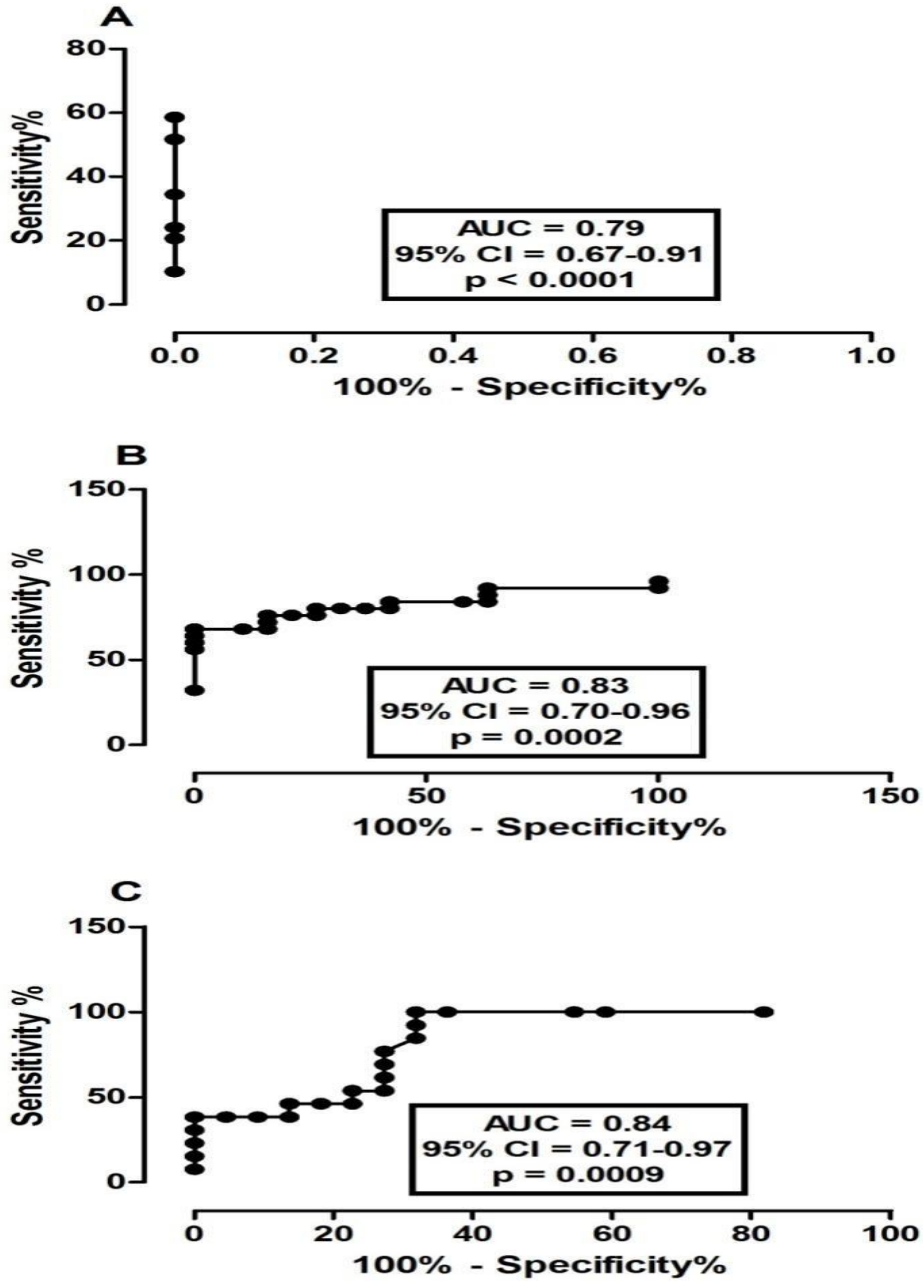

Figure 3.3: Receiver Operator Characteristic analysis of serum C-reactive Protein (A), plasma Interlukin-6 (B) and plasma Soluble Urokinase Plasminogen Activator Receptor (C) level in Healthy Pregnancies and Eclampsia. AUC: Area Under Curve; CI: Confidence Interval.

3.3.5.4 Pregnancy-Induced Hypertension

The results of ROC curve analyses were a 56% area under the curve for CRP (95% CI: 0.47-0.65, p = 0.1696); a 75% area under the curve (95% CI: 0.66-0.83, p < 0.0001) for IL-6 and a 71% area under the curve for suPAR (95% CI: 0.56-0.87, p = 0.0217) as indicated in Figure 3.4.

From Table 3.6, the sensitivities of CRP, IL-6 and suPAR in differentiating between PIH and healthy pregnancies are 50.00%, 47.73% and 7.02% respectively at the cut-off of > 1.50 ng/L, > 19.15 pg/ml and > 3.96 ng/ml respectively. Whereas CRP gave a specificity of 55.56%, IL-6 and suPAR indicated specificities of 100.00% each at the corresponding cut off values (Table 3.6).

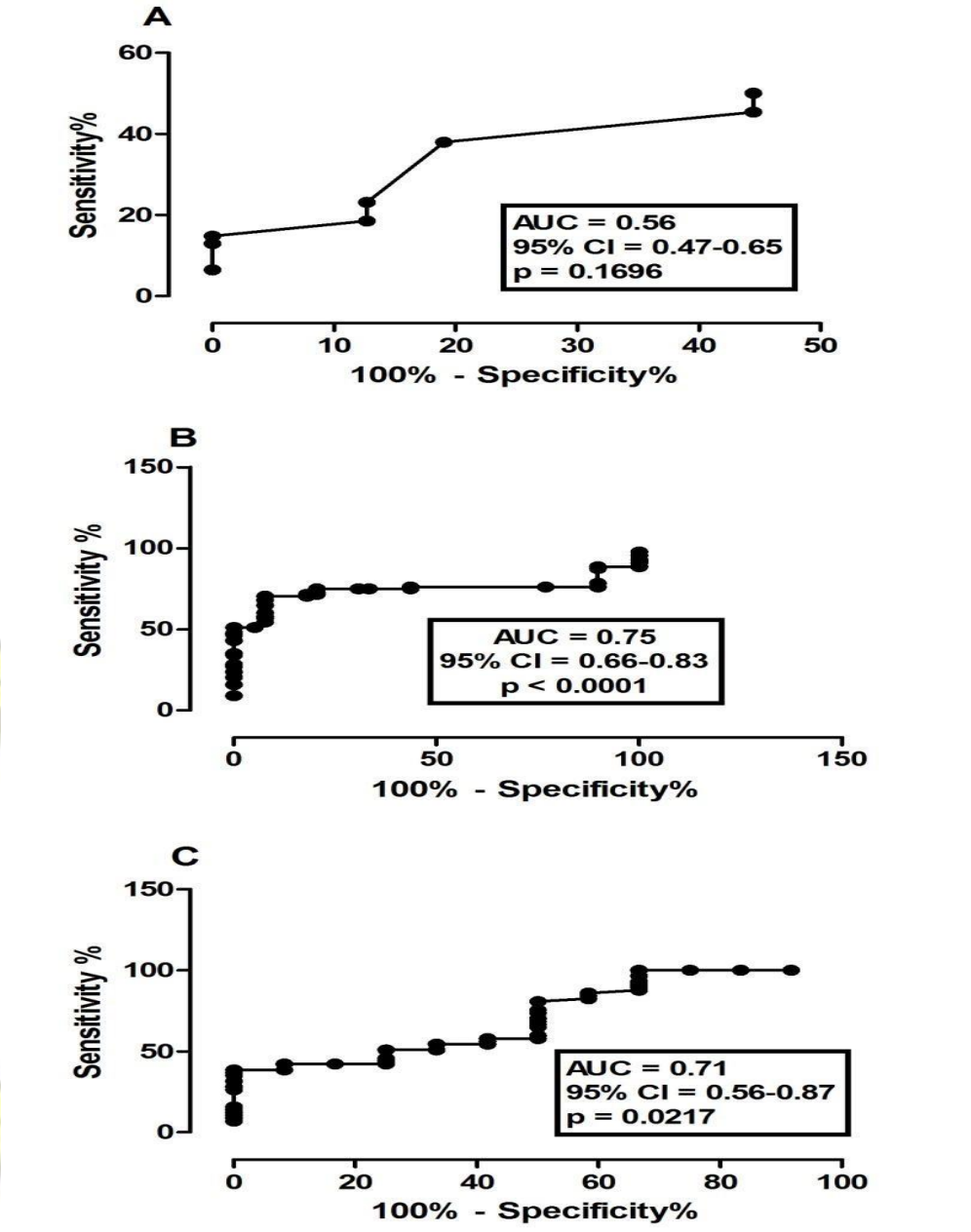

Figure 3.4: Receiver Operator Characteristic analysis of serum C-reactive Protein (A), plasma Interlukin-6 (B) and plasma Soluble Urokinase Plasminogen Activator Receptor (C) level in Healthy Pregnancies and Pregnancy-induced Hypertension. AUC: Area Under Curve; CI: Confidence Interval.

Table 3.6: Sensitivity and specificity of the inflammatory markers in differentiating between healthy pregnancies and hypertensive pregnancies

VARIABLE	CUT- OFFS	SENSITIVITY (%)	95% CI	SPECIFICITY (%)	95% CI
GH					
CRP(ng/L)	> 1.50	54.17	39.17% to 68.63%	55.56	42.49% to 68.08%
IL-6 (pg/ml)	> 19.15	15.38	4.36% to 34.87%	100.00	90.97% to 100.00%
suPAR (ng/ml)	> 2.44	9.09	0.23% to 41.28%	100.00	73.54% to 100.00%
PE					
CRP(ng/L)	> 2.00	35.48	19.23% to 54.63%	100.00	92.13% to 100.00%
IL-6 (pg/ml)	> 19.52	56.76	39.49% to 72.90%	100.00	86.28% to 100.00%
suPAR (ng/ml)	> 3.75	12.12	3.40% to 28.20%	100.00	80.49% to 100.00%
EC					
CRP(ng/L)	> 1.50	58.62	38.94% to 76.48%	100.00	92.13% to 100.00%
IL-6 (pg/ml)	> 18.55	68.00	46.50% to 85.05%	89.47	66.86% to 98.70%
suPAR (ng/ml)	> 3.18	7.69	0.20% to 36.03%	100.00	84.56% to 100.00%
PIH					
CRP(ng/L)	> 1.50	50.00	40.22% to 59.78%	55.56	42.49% to 68.08%
IL-6 (pg/ml)	> 19.15	47.73	36.96% to 58.65%	100.00	90.97% to 100.00%
suPAR (ng/ml)	> 3.96	7.02	1.945% to 17.00%	100.00	73.54% to 100.00%

GH: Gestational Hypertension; PE: Preeclampsia; EC: Eclampsia; PIH: Pregnancy-induced Hypertension; CRP: C-reactive protein; IL6: Interlukin-6; suPAR: Soluble Urokinase Plasminogen Activator Receptor; CI: Confidence Interval; %: Percentage.

3.4 INFLAMMATORY, ENDOTHELIAL DYSFUNCTION AND OXIDATIVE STRESS MARKERS AMONGST STUDIED PARTICIPANTS

All the subjects were ranging in age from 18 to 40 years (Escen et al., 2003) with a mean±SD age of 30.72 ± 5.93 yr. The gestational age also ranges from 22 weeks to 42 weeks with a mean±SD of
36.40 ± 4.10 weeks.

3.4.1 Biochemical profile of the studied population

The platelets and biochemical characteristics of the four groups of participants and one group of control subjects when stratified based on the type of PIH are summarized in table-3.7.

3.4.1.1 Control

The platelets, inflammatory markers, endothelial dysfunction marker (FN) as well as oxidative stress marker (8-iso-PGF2α) were all within the normal range among the normotensive women with healthy pregnancy (Control) (Table 3.7).

3.4.1.2 Gestational Hypertension

The mean platelets count of women with GH was similar to those with healthy pregnancy. As shown in Table 3.7, whereas the mean values of TG and HDL were significantly increased ($p=0.0152$, $p=0.0595$ respectively) among the women with GH (2.16±1.00mmol/L and 1.16±0.43mmol/L respectively) as compared to the normotensive women with healthy pregnancy (1.73±0.71mmol/L and 1.01±0.30mmol/L respectively), the increase in TC and LDL among the GH women as compared to normotensive women with healthy pregnancy were similar (Table 3.7).

The inflammatory markers (CRP and IL-6) in GH were raised than those with healthy pregnancy, however, it was only CRP (3.67±2.14 ng/L) that was significantly higher ($p< 0.0001$) when compared with the control (0.98±0.05 ng/L) (Table 3.7).

The mean value of fibronectin, a marker of endothelial dysfunction was similar between the women with GH and the normotensive women with healthy pregnancy. The oxidative stress marker 8-iso-PGF2α mean value was significantly higher ($p=0.0549$) in GH (33.86±22.50pg/ml) as compared to the normotensive women with healthy pregnancy (5.55±5.33pg/ml) (Table 3.7).

3.4.1.3 Preeclampsia

Women presenting with preeclampsia had similar mean platelet count to that of the controls. Mean values of TG (2.19±0.96mmol/L) and HDL (1.12±0.23mmol/L) of the preeclamptic patients were significantly raised (p=0.0112 and p=0.0592 correspondingly) than the normotensive women with healthy pregnancy (1.73±0.71mmol/L and 1.01±0.30mmol/L correspondingly) whereas the TC and LDL mean values of the preeclamptic women were similar to the control (Table 3.7).

The mean values of inflammatory cytokines CRP (2.84±2.28 ng/L), and IL-6 (21.27±11.52 pg/ml), endothelial dysfunction marker (FN) (22.41±12.68 ng/ml) and oxidative stress marker (8-iso-PGF2α) (37.86±22.88 pg/ml) were significantly increased (p=0.0032, p=0.0332, p=0.0201 and p=0.0295 correspondingly) in preeclamptic women than in the controls (0.98 ± 0.05 ng/L, 13.85±2.80 pg/ml,13.85±2.80 ng/ml and 5.55±5.33 pg/ml respectively) (Table 3.7).

3.4.1.4 Eclampsia

Statistically eclamptic women showed significantly reduced (p=0.0302) mean platelet count 142.90±36.41*10^3/µl) as compared to women with uncomplicated pregnancy without hypertension (174.00±44.06*10^3/µl). The TC (5.25±0.86mmol/L) and TG (2.21±0.58mmol/L) mean values of women with eclampsia were significantly raised (p=0.044 and p= 0.0173 correspondingly) compared with women with uncomplicated pregnancy without hypertension (4.55±1.08mmol/L and 1.73±0.71mmol/L respectively) but though their mean HDL and LDL values were also higher than the control women they were not statistically significant (Table 3.7).

The women who had eclampsia had significantly higher mean values of CRP (5.61±2.85 ng/L, p< 0.0001), IL-6(23.57±16.44pg/ml, p=0.0463), FN (25.28±14.35ng/ml, p=0.0065) and 8-iso-PGF2α (55.35±36.47pg/ml, p=0.0175) as compared to those with uncomplicated pregnancy without hypertension CRP (0.98 ± 0.05 ng/L), IL-6 (13.85±2.80 pg/ml), FN (13.85±2.80ng/ml) and 8-isoPGF2α (5.55±5.33pg/ml) respectively (Table 3.7).

3.4.1.5 Pregnancy-Induced Hypertension

As shown in Table 3.7, pregnancy-induced hypertensive women and the normotensive women with healthy pregnancy had similar mean platelet count. Although pregnancy-induced hypertensive women generally had significantly higher lipid profile that is TC (4.92±1.066mmol/L, p= 0.0595),

TG (2.19±0.93mmol/L, p=0.0033) and HDL (1.14 ±0.34mmol/L, p=0.0251) mean values than women with uncomplicated pregnancy without hypertension (4.55±1.08mmol/L, 1.73±0.71mmol/L and 1.01±0.30mmol/L respectively) their mean LDL (2.62±0.83mmol/L) value was not significant (Table 3.7).

The mean serum CRP (3.31±2.81 ng/L, p < 0.0001), and IL-6 (19.60±10.32 pg/ml, p= 0.0433) values were significantly increased in patients with pregnancy-induced hypertension as compared to the women with uncomplicated pregnancy without hypertension (0.98 ± 0.05 ng/L and13.85±2.80 pg/ml respectively) (Table 3.7).

Women presenting with PIH showed significantly higher levels of FN (21.87±11.95ng/ml, p= 0.0067) and 8-iso-PGF2α (43.03±27.29pg/ml, p=0.0278) as compared to those who had uncomplicated pregnancy without hypertension (13.85±2.80ng/ml and 5.55±5.33pg/ml respectively) as shown in Table 3.7.

3.4.1.6 General trends of lipids and other biochemical parameters amongst the studied participants

Generally, there was a decrease in mean platelet count of participants presenting with PIH compared to normotensive women with healthy pregnancy but there is a relative reduction within the PIH patients as the condition worsens. There is a marked increase in the lipid profile (TC, TG, HDL & LDL) mean values of PIH patients as the condition progresses as compared to the controls. Also there is a general rise in the levels of inflammatory cytokines (CRP & IL-6), endothelial dysfunction marker (FN) and oxidative stress marker (8-iso-PGF2α) in PIH patients as compared to those with uncomplicated pregnancy without hypertension as the prognosis of PIH gets bad (Table 3.7).

Table 3.7: Distribution of lipid profile and other biochemical markers amongst the studied participants

VARIABLE	CONTROLS	GH	P VALUE	PE	P VALUE	ECLAMSIA	P VALUE	PIH	P VALUE
PLT(*10^3/µl)	174.00±44.06	168.60±72.18	0.6843	160.50±40.30	0.1554	142.90±36.41	0.0302	161.80±56.54	0.2204
TC(mmol/L)	4.55±1.08	4.81±1.25	0.2892	4.94±0.87	0.0691	5.25±0.86	0.044	4.92±1.066	0.0595
TG(mmol/L)	1.73±0.71	2.16±1.00	0.0152	2.19±0.96	0.0112	2.21±0.58	0.0173	2.19±0.93	0.0033
HDL(mmol/L)	1.01±0.30	1.16±0.43	0.0595	1.12±0.23	0.0592	1.17 ±0.35	0.1003	1.14 ±0.34	0.0251
LDL(mmol/L)	2.52±0.63	2.64±0.94	0.5194	2.60 ±0.80	0.6337	2.60±0.60	0.6627	2.62±0.83	0.5273
CRP(ng/L)	0.98 ± 0.05	3.67±2.14	< 0.0001	2.84±2.28	0.0032	5.61±2.85	< 0.0001	3.31±2.81	< 0.0001
IL-6(pg/ml)	13.85±2.80	13.97±4.84	0.939	21.27±11.52	0.0332	23.57±16.44	0.0463	19.60±10.32	0.0433
FN(ng/ml)	13.85±2.80	17.65±6.63	0.1037	22.41±12.68	0.0201	25.28±14.35	0.0065	21.87±11.95	0.0067
8-iso-PGF2α (pg/ml)	5.55±5.33	33.86±22.50	0.0549	37.86±22.88	0.0295	55.35±36.47	0.0175	43.03±27.29	0.0278

GH: Gestational Hypertension; PE: Preeclampsia; EC: Eclampsia; PIH: Pregnancy-induced Hypertension; PLT: Platelets; TC: Total Cholesterol; TG: Triglyceride; HDL: High Density Lipoprotein; LDL: Low Density Lipoprotein; CRP: C-reactive protein; IL-6: Interlukin-6; FN: Fibronectin; 8-iso-PGF2α:8-iso-Prostaglandin F2α; P VALUE < 0.05: Statistically significant.

3.4.2 Receiver Operator Characteristic analysis of the inflammatory markers, endothelial dysfunction marker and oxidative stress marker amongst the studied participants

3.4.2.1 Gestational Hypertension

Figure 3.5 shows ROC curve analyses with a 55% area under the curve for CRP (95% CI: 0.440.66, p = 0.3440); a 68% area under the curve, at a confidence interval of 95% within the range of 0.52-0.85 and at a significance of p = 0.0130 for IL-6 and a 66% area under the curve for FN (95% CI: 0.49-0.83, p = 0.0697) and a 61% area under the curve for 8-iso-PGF2α (95% CI: 0.45-0.77, p=0.1871).

At the cut-off of > 1.50 ng/L for CRP the sensitivity and specificity of CRP in differentiating between GH and healthy pregnancies is 54.17% and 55.56% respectively. However, at the cut-off of > 19.15 pg/ml for IL-6 the sensitivity is 15.38% and the specificity is 100.00%. Using FN and 8iso-PGF2α as markers of establishing the difference between GH and healthy pregnancy at cut-offs (>18.67 ng/ml and >7.79 pg/ml respectively) their respective sensitivity and specificity are (35.00% and 36.00% respectively) and (73.91% and 72.00% respectively) as shown in Table 3.8.

As shown in Table 3.9, there is no significant relationship between the lipid profile and platelet, inflammatory markers, endothelial dysfunction marker as well as oxidative stress marker.

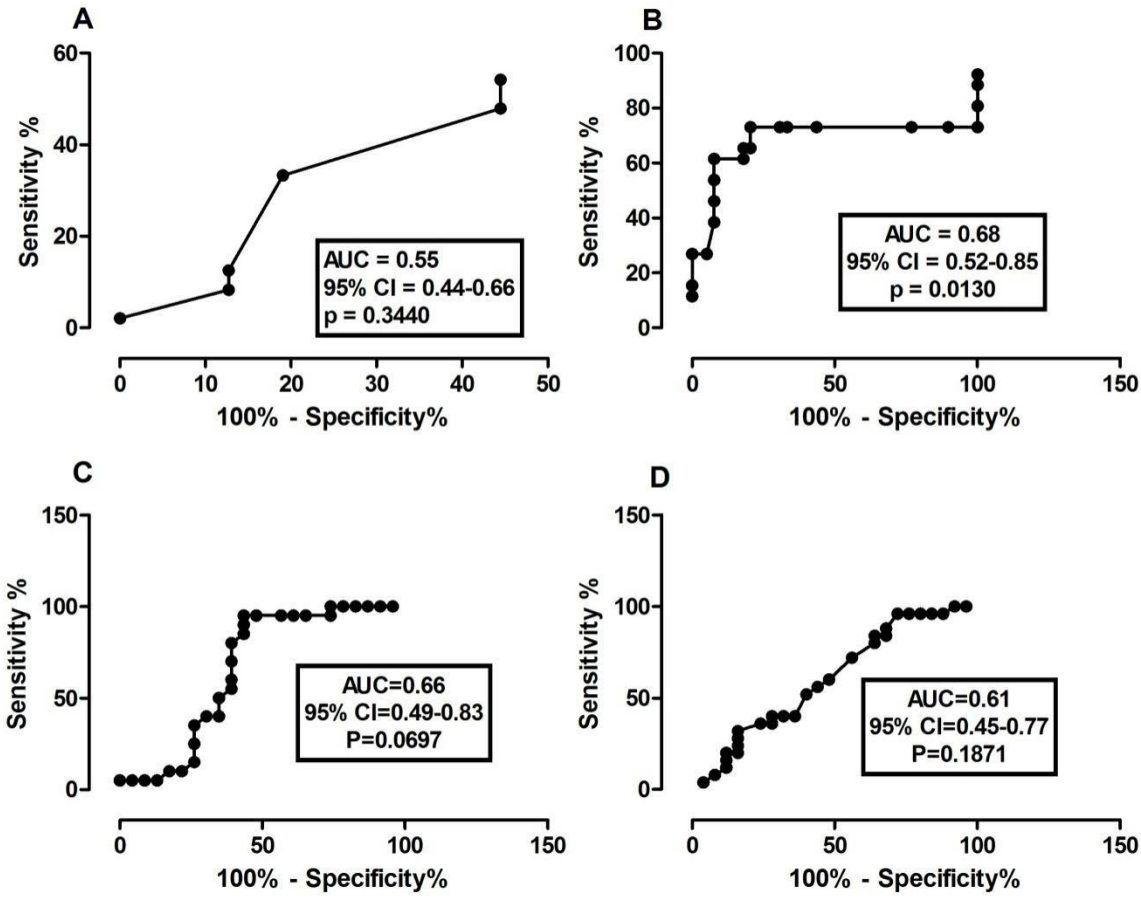

Figure 3.5: Receiver Operator Characteristic analysis of serum C-reactive Protein (A), plasma Interlukin-6 (B), plasma Fibronectin (C) and plasma 8-iso-Prostaglandin F2 alpha (D) level in Healthy Pregnancies and Gestational Hypertension

3.4.2.2 Preeclampsia

As shown in Figure 3.6, the ROC curve analyses showed a 68% area under the curve for CRP (95% CI: 0.55-0.81, p = 0.0089); a 74% area under the curve, at a confidence interval of 95% within the range of 0.61-0.87 and at a significance of p = 0.0015 for IL-6 and a 66% area under the curve for FN (95% CI: 0.48-0.83, p = 0.0923) and a 74% area under the curve for 8-iso-PGF2α (95% CI: 0.58-0.90, p=0.0094).

Among the women with preeclampsia, the sensitivity of CRP and IL-6 are 35.48% and 56.76% respectively at the cut-offs of > 2.00 ng/L and > 19.52 pg/ml respectively. Each of them indicated specificity of 100.00% at the corresponding cut-off values. Whereas at cut-offs of >18.06 ng/ml and >6.63 pg/ml respectively for FN and 8-iso-PGF2α, they showed sensitivity of (30.77% and 50.00% respectively) and specificity of (75.00% and 85% respectively) in distinguishing between PE and healthy pregnancy (Table 3.8).

Generally, there is no significant correlation between the lipid profile and platelet, inflammatory markers, endothelial dysfunction marker as well as oxidative stress marker, except for triglyceride that showed considerable positive relationship with IL-6 ($r = 0.65$; $p < 0.05$) (Table 3.9).

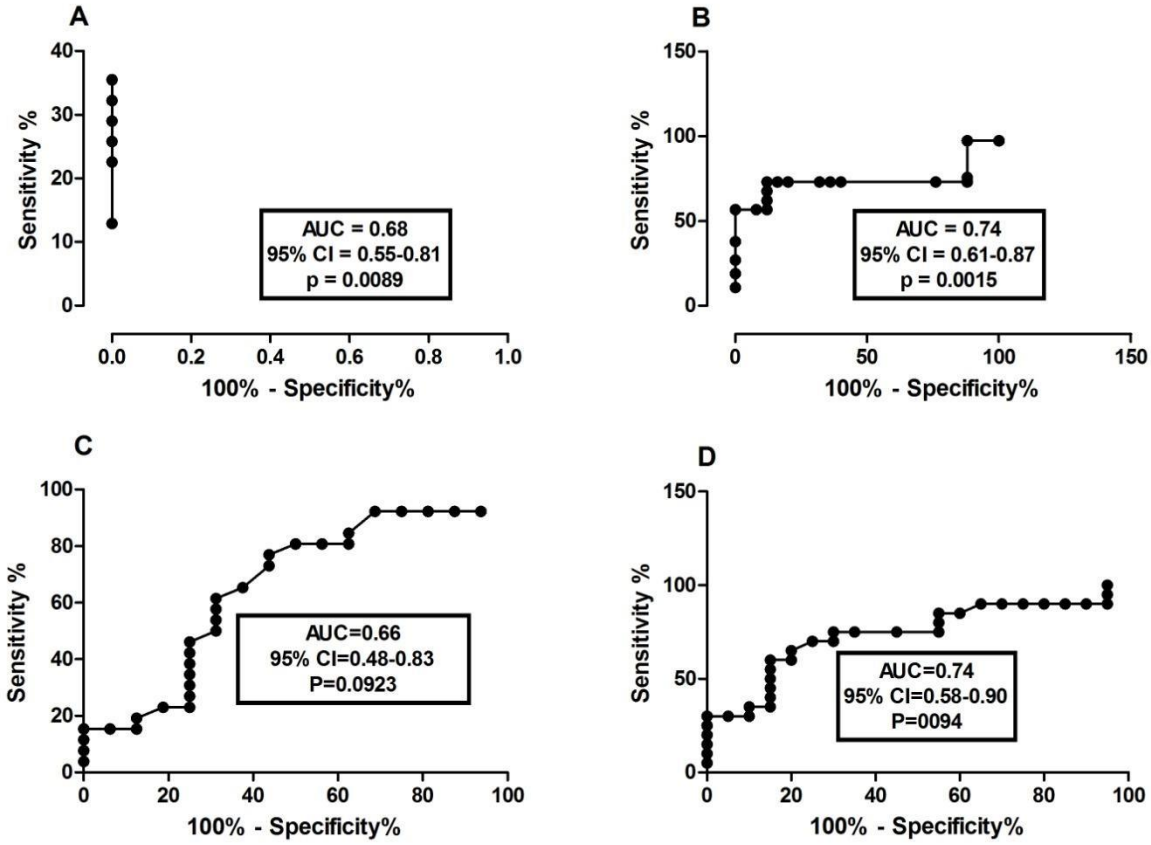

Figure 3.6: Receiver Operator Characteristic analysis of serum C-reactive Protein (A), plasma Interlukin-6 (B), plasma Fibronectin (C) and plasma 8-iso-Prostaglandin F2 alpha (D) level in Healthy Pregnancies and Preeclampsia

3.4.2.3 Eclampsia

ROC curve analyses as presented in Figure 3.7 showed a 79% area under the curve for CRP (95% CI: 0.67-0.91, p = 0.0001); a 83% area under the curve, at a confidence interval of 95% within the range of 0.70-0.96 and at a significance of p = 0.0002 for IL-6 and a 70% area under the curve for FN (95% CI: 0.53-0.86, p = 0.0251) and a 76% area under the curve for 8-iso-PGF2α at a confidence interval of 95% within the range 0.64- 0.88 at a significance of p=0.0007.

Differentiating between eclamptic women and healthy pregnant women, the sensitivity of CRP and IL-6 were 58.62% and 68.00% respectively at the cut-offs of > 1.50 ng/L and > 18.55 pg/ml respectively. Whereas IL-6 indicated a specificity of 68.47%, the CRP indicated specificity of 100.00% at the corresponding cut-off values. Also using FN and 8-iso-PGF2α as markers of differentiation at cut-offs of (>18.08 ng/ml and >6.03 pg/ml respectively), their sensitivity and specificity were (41.67% and 58.82% respectively) and (71.43% and 79.17% respectively) respectively as shown in Table 3.8.

Although there is generally, no significant correlation between the lipid profile and platelet, inflammatory markers, indicators of endothelial malfunction and overproduction of oxidants, there is significant positive relationship between triglyceride and interlukin-6 ($r=0.58$, $p<0.05$) and with FN ($r=0.75$,$p<0.05$).Also TCHOL has a significant positive relationship with 8-iso-PGF2α ($r=0.61$,$p<0.05$) (Table 3.10).

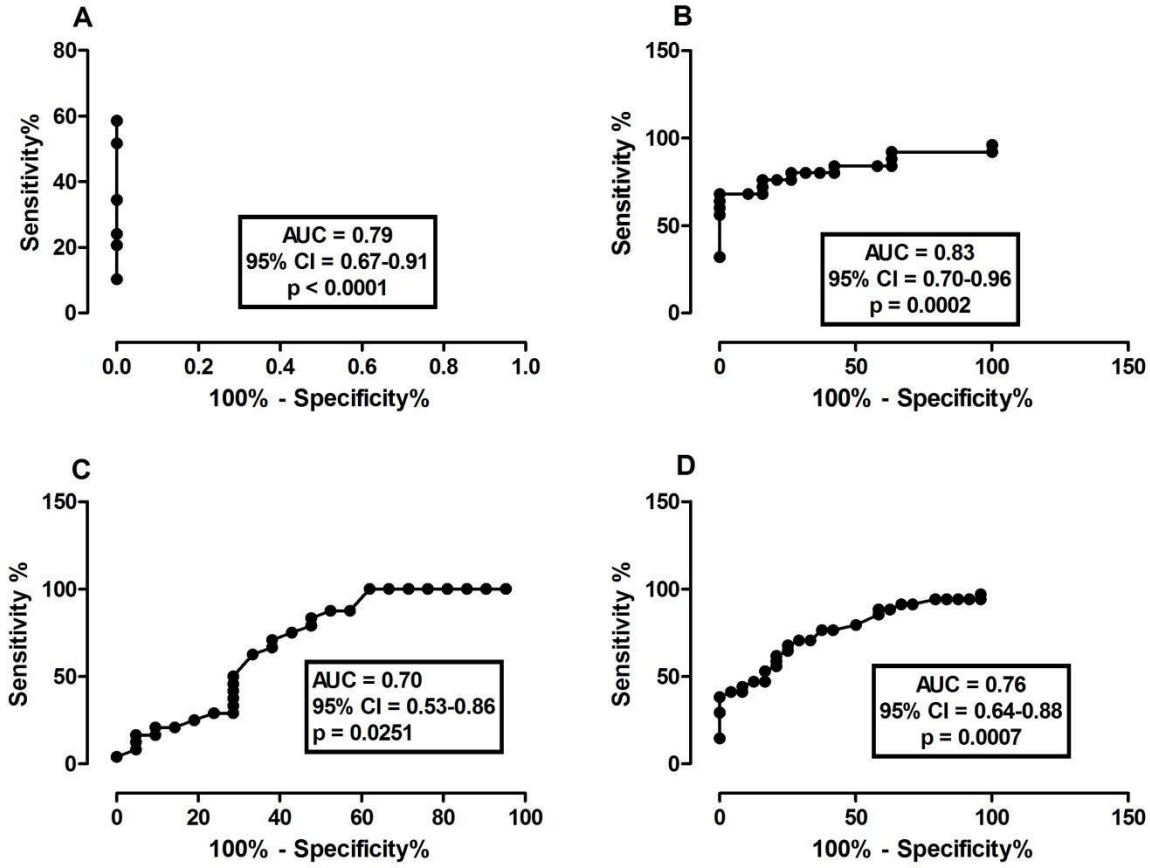

Figure 3.7: Receiver Operator Characteristic analysis of serum C-reactive Protein (A), plasma Interlukin-6 (B), plasma Fibronectin (C) and plasma 8-iso-Prostaglandin F2 alpha (D) level in Healthy Pregnancies and Eclampsia

3.4.2.4 Pregnancy-Induced Hypertension

The results of ROC curve analyses as shown in figure 3.8 were 56% area under the curve for CRP at a confidence interval of 95% within the range of 0.47-0.65 and at a significance of p = 0.1696; a 75% area under the curve (95% CI: 0.66-0.83, p < 0.0001) for IL-6 and a 66% area under the curve for FN at a confidence interval of 95% within the range of 0.52-0.80 at a significance of p = 0.0229 and a 65% area under the curve for 8-iso-PGF2α (95% CI: 0.52-0.78, p=0.0241).

From Table 3.8, the sensitivity of CRP and IL-6 in differentiating between PIH and healthy pregnancies is 50.00% and 47.73% respectively at the cut-offs of > 1.50 ng/L and > 19.15 pg/ml respectively. Whereas CRP indicated a specificity of 55.56%, IL-6 indicated specificity of 100.00% at the corresponding cut-off values. Also the sensitivity and specificity of FN and 8-isoPGF2α at cut-offs (>18.57 ng/ml and >7.05 pg/ml correspondingly) in showing the difference between PIH and healthy pregnancy were (34.29% and 48.10% respectively) and (73.91% and 72.00% respectively) respectively.

Platelet, inflammatory markers, endothelial dysfunction marker as well as oxidative stress marker showed no significant correlation with the lipid profile as shown in Table 3.10.

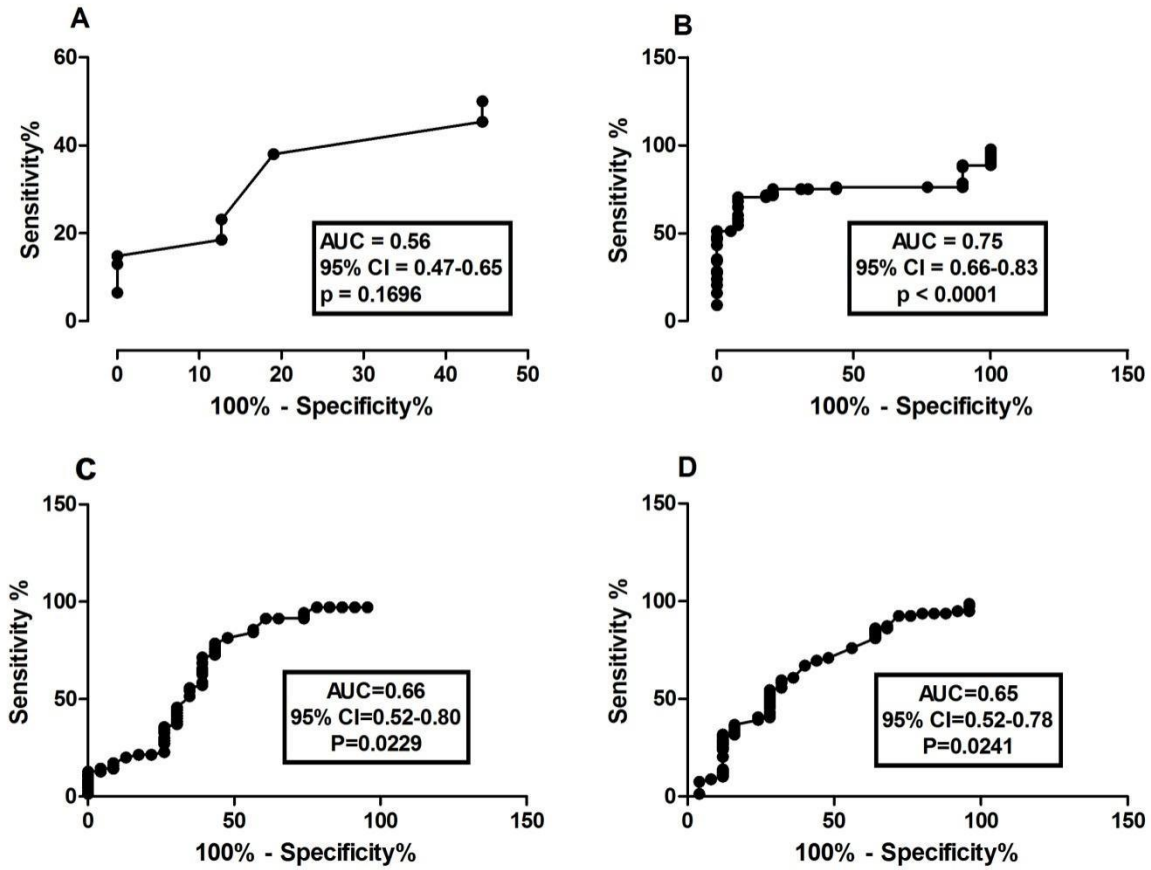

Figure 3.8: Receiver Operator Characteristic analysis of serum C-reactive Protein (A), plasma Interlukin-6 (B), plasma Fibronectin (C) and plasma 8-iso-Prostaglandin F2 alpha (D) level in Healthy Pregnancies and Pregnancy-induced Hypertension

Table 3.8: Sensitivity and specificity of inflammatory markers, endothelial dysfunction marker and oxidative stress marker of the studied participants

VARIABLE	CUTT OFFS	SENSITIVITY (%)	95% CI	SPECIFICITY (%)	95% CI
GH					
CRP(ng/L)	> 1.50	54.17	39.17% to 68.63%	55.56	42.49% to 68.08%
IL-6 (pg/ml)	> 19.15	15.38	4.36% to 34.87%	100.00	90.97% to 100.00%
Fibronectin(ng/ml)	> 18.67	35.00	15.39% to 59.22%	73.91	51.59% to 89.77%
8-iso-PGF2α (pg/ml)	> 7.79	36.00	17.97% to 57.48%	72.00	50.61% to 87.93%
PE					
CRP(ng/L)	> 2.00	35.48	19.23% to 54.63%	100.00	92.13% to 100.00%
IL-6 (pg/ml)	> 19.52	56.76	39.49% to 72.90%	100.00	86.28% to 100.00%
Fibronectin(ng/ml)	> 18.06	30.77	14.33% to 51.79%	75.00	47.62% to 92.73%
8-iso-PGF2α (pg/ml)	> 6.63	50.00	27.20% to 72.80%	85.00	62.11% to 96.79%
EC					
CRP(ng/L)	> 1.50	58.62	38.94% to 76.48%	100.00	92.13% to 100.00%
IL-6 (pg/ml)	> 18.55	68.00	46.50% to 85.05%	89.47	66.86% to 98.70%
Fibronectin(ng/ml)	> 18.08	41.67	22.11% to 63.36%	71.43	47.82% to 88.72%
8-iso-PGF2α (pg/ml)	> 6.03	58.82	40.70% to 75.35%	79.17	57.85% to 92.87%
PIH					
CRP(ng/L)	> 1.50	50.00	40.22% to 59.78%	55.56	42.49% to 68.08%
IL-6 (pg/ml)	> 19.15	47.73	36.96% to 58.65%	100.00	90.97% to 100.00%
Fibronectin(ng/ml)	> 18.57	34.29	23.35% to 46.60%	73.91	51.59% to 89.77%

8-iso-PGF2α (pg/ml)	> 7.05	48.10	36.71% to 59.64%	72.00	50.61% to 87.93%

Table 3.9: Correlational analysis between lipid profile and inflammatory, endothelial dysfunction and oxidative stress markers amongst women with gestational hypertension and preeclampsia

	GH				PE			
VARIABLE	**TC** (mmol/L)	**TG** (mmol/L)	**HDL** (mmol/L)	**LDL** (mmol/L)	**TC** (mmol/L)	**TG** (mmol/L)	**HDL** (mmol/L)	**LDL** (mmol/L)
PLT(*10^3/µl)	0.08	-0.16	0.05	0.19	0.16	-0.22	0.18	0.19
CRP(ng/L)	0.15	0.03	0.04	0.20	0.23	0.10	0.10	0.16
IL-6(pg/ml)	0.41	0.04	0.32	0.40	0.36	**0.65***	0.34	0.29
FN(ng/ml)	0.21	0.15	0.29	0.18	0.49	0.54	0.02	0.21
8-iso-PGF2α (pg/ml)	0.33	0.06	0.39	0.31	0.32	0.60	0.24	0.40

*GH: Gestational Hypertension; PE: Preeclampsia; TC: Total Cholesterol; TG: Triglyceride; HDL: High Density Lipoprotein; LDL: Low Density Lipoprotein; PLT: Platelets; CRP: C-reactive protein; IL-6: Interlukin-6; FN: Fibronectin; 8-iso-PGF2α:8-iso-Prostaglandin F2 alpha; * Correlation is significant at 0.05 level.*

Table 3.10: Correlational analysis between lipid profile and inflammatory, endothelial dysfunction and oxidative stress markers amongst women with eclampsia and pregnancy-induced hypertension

	EC				PIH			
VARIABLE	**TC** (mmol/L)	**TG** (mmol/L)	**HDL** (mmol/L)	**LDL** (mmol/L)	**TC** (mmol/L)	**TG** (mmol/L)	**HDL** (mmol/L)	**LDL** (mmol/L)
PLT(*10^3/µl)	-0.28	-0.21	0.23	0.13	0.07	-0.14	0.05	0.18
CRP(ng/L)	0.61	0.47	0.01	0.15	0.17	0.10	0.02	0.15
IL-6(pg/ml)	0.35	**0.58***	0.15	0.24	0.08	0.20	0.11	0.08
FN(ng/ml)	0.32	**0.75***	0.40	0.10	0.16	0.06	0.06	0.14
8-iso-PGF2α (pg/ml)	**0.61***	0.15	0.29	0.23	0.29	0.04	0.16	0.31

EC: Eclampsia; PIH: Pregnancy-induced Hypertension; TC: Total Cholesterol; TG: Triglyceride; HDL: High Density Lipoprotein; LDL: Low Density Lipoprotein; PLT: Platelets; CRP: C-reactive protein; IL-6: Interlukin-6; FN: Fibronectin; 8-iso-PGF2α:8-iso-Prostaglandin F2 alpha; ******* *Correlation is significant at 0.05 level.*

Chapter 4 DISCUSSION

Pregnancy-Induced hypertension (PIH) affects approximately 12.14% primigravid women in Ghana, and is responsible for their ill-health and death as well as that of their foetuses (Owiredu *et al.*, 2012). This systemic disorder is characterized by the development of a maternal syndrome that includes hypertension, proteinuria, oedema and vascular abnormalities. In most cases, these symptoms develop in the third trimester of pregnancy and usually disappear within one week after delivery. Annually greater than 500,000 pregnancy-related deaths occur globally and about 99% of them in developing countries (WHO, 1991). It is clear that PIH is a complicated condition that affects many organs through an amplified systemic inflammation, oxidative stress and injury to the endothelium. Due to its complex nature there is a difficulty when dealing with this condition either with its diagnosis and treatment or research work. The only way of alleviating the symptoms of PIH is the removal of the placenta thus when a woman develops PIH before she is due the only option available to obstetricians is the management of the condition until she delivers safely (von Dadelszen *et al.*, 2011). This approach to managing PIH does not guarantee the survival of the mother and the foetus due to the paucity of known management protocols. Therefore, any approach that would assist in the early prediction of this systemic maternal syndrome and avert its fatal consequences on the mother and her foetus would be clinically beneficial.

4.1 HEPCIDIN CONCENTRATION AND IRON HOMEOSTASIS IN STUDIED PARTICIPANTS

This study described hepcidin concentrations in PIH compared to healthy pregnant women. Hepcidin's associations with iron homeostasis, complete blood cell count), and with markers of systemic inflammation (IL-6, CRP), which enhances its expression was determined

It was generally observed that women with conditions of PIH including GH, PE and EC were much older than women without these conditions (Table 3.1). Advanced maternal age increases the potential of her developing pregnancy-induced hypertension (Zhang *et al.*, 1997). It may be attributed to the fact that as one ages, one becomes more prone to non-communicable diseases like hypertension, type II diabetes, heart diseases and osteoporosis. Thus, PIH will be expected to be pronounced in older women who have

an increased risk to this condition as has been reported by several studies (Ros *et al.*, 1998; Jacobsson *et al.*, 2004; Duckitt and Harrington, 2005). However, the severe form of PIH which is eclampsia happens to have affected the younger participants of this study and this seems to reflect the findings of Klungsøyr *et al.* (2012), which showed that the rates of preeclampsia increased more over time among younger than older women.

The impairment of iron homeostasis is among several factors that have been implicated in the pathogenesis of PIH (Toldi *et al.*, 2011). Rayman *et al.*, 2002 and Basher and Deb, 2006 found an increase in plasma iron , ferritin, and transferrin in PIH, whereas TIBC, UIBC and apotransferrin declined in PIH when compared to healthy pregnant women. To explain the role of hyperferremia in PIH, Rayman *et al.* (2002) explained that this increase in plasma iron concentration leads to the production of free radicals containing oxygen also referred to as ROS through the Fenton's reaction, and these ROS exacerbate lipid peroxidation and endothelial cell injury which are implicated in the pathogenesis of PIH. However, plasma iron concentrations are expected to decrease due to the ongoing inflammation in PIH as suggested by Balla *et al.* (2007) that chronic inflammation decreases iron availability leading to inflammation-induced anemia. Thus a hypoferremic state is expected in PIH instead of increased plasma iron concentrations as was the findings by Toldi *et al.* (2011). This study showed a contrary picture of a lower plasma iron concentration in PIH as compared to the healthy pregnant women but higher ferritin and transferrin concentrations in PIH than the controls (Table 3.1) as was the finding by (Rayman *et al.*, 2002) but it was only ferritin that was statistically significant. This study also showed a decrease in TIBC in PIH as compared to the healthy pregnant women just as was shown by Basher and Deb (2006).

It is generally known that during pregnancy women expend a lot of iron and will require iron supplements to compensate for the increased demand. However, significant decrease in plasma iron levels among women with PIH is remarkable, due to the essential role iron plays in the body, especially in pregnant women where they are essential for early placental development which maintains pregnancy and provides nutrients and oxygen to the developing foetus (Koenig *et al.*, 2014).It has been reported that systemic inflammation results in hypoferremia through a mechanism that leads to an upsurge of hepcidin (Nemeth and Ganz, 2006; Koenig *et al.*, 2014) which is proposed

to be the key regulator of iron metabolism (Koenig *et al.*, 2014). The relationship between iron homeostasis and inflammation is hepcidin, an acute phase peptide which controls the movement of iron from within the cells into plasma and other fluids outside the cells through ferroportin that has a receptacle for hepcidin and is the only known protein responsible for the movement of iron from the cells into extracellular fluids in vertebrates (Nemeth and Ganz, 2009). Ferroportin is expressed on cells that are responsible for iron metabolism in the body and these are duodenal enterocytes responsible for absorbing dietary iron, macrophages in the liver and the spleen where old erythrocytes are recycled, hepatocytes responsible for iron storage and placental trophoblasts transferring iron to the fetus during pregnancy (Donovan *et al.*, 2005). It acts by reducing intestinal iron absorption and iron release from enterocytes and macrophages through internalization and degradation of ferroportin (Nemeth and Ganz, 2009). Therefore, the hypoferremia seen in PIH in this study could be attributed to the high plasma hepcidin mean values which could be accountable for the high ferritin seen in this study. Hepcidin expression is regulated by several factors but it is primarily triggered by inflammatory signals, such as IL-6 (Wrighting and Andrews, 2006). Hepcidin is also increased in inflammation and infection, and it is presumed that this regulation evolved as a host defense mechanism to deny the microorganisms of iron which they need for their survival (Nemeth and Ganz, 2009). There was a general rise in plasma hepcidin concentration as the prognosis of PIH worsened that is eclampsia which is the worst form of PIH had the highest plasma hepcidin concentration followed by preeclampsia and gestational hypertension which is the mild form of PIH (Table 3.1). This could be attributed to an increase in inflammation as this maternal systemic syndrome worsens and this is depicted by the respective rise in the inflammatory marker IL-6 which is a primary trigger of hepcidin (Toldi *et al.*, 2011).

In this study, it was observed that indicators of inflammation interlukin-6 and CRP considerably increased in women with PIH and its associated conditions while among healthy pregnant women without PIH, their levels were within normal range (Table 3.1). This finding supports several reports which point out a possible association between PIH and inflammation (Teran *et al.*, 2001; Steegers *et al.*, 2010). The condition of inflammation that is associated with PIH as observed in this study, will lead to the creation of a hypoferremic state (decreased blood iron levels) as observed in this study. A decrease in plasma iron levels will lead to a commensurate decrease in TIBC as

observed in our study (Table 3.1), due to the positive correlation existing between plasma iron levels and TIBC. Additionally there is increase demand for iron during inflammation for the synthesis of haematopoietic cells including inflammatory cells (Kramer *et al.*, 2002), which are required to initiate and maintain inflammation. As a consequence, there is an increased consumption of iron, thus leading to a decreased plasma iron and consequently a decrease in TIBC among pregnant women with PIH and its associated diseases. This study did not carry out a single experiment to establish the relationship between hepcidin and inflammation as conducted in some studies (Nemeth and Ganz, 2006; Koenig *et al.*, 2014), but from this study, it can also be inferred that a positive correlation exists between inflammation and increased hepcidin levels as we found that the inflammatory markers IL-6 and CRP which were increased among women with PIH also had increased hepcidin levels compared to control groups.

Serum ferritin is the commonest biomarker that has been used to assess iron status in most studies done in the developing countries but the results has been questionable (Cardoso *et al.*, 1994). The stability of ferritin is not altered when one is on oral iron therapy but is increased when there is inflammation. Stained bone marrow examination is the most reliable way of determing the levels of iron stores in pregnancy (Letsky, 1991). A novel biomarker transferrin receptor which has a high level of stability in chronic disease and pregnancy would be perfect for the assessment of iron stores in pregnancy (Carriaga *et al.*, 1991; Baynes *et al.*, 1994). Even though ferritin levels have been found to influence the levels of serum iron (Walters *et al.*, 1973) such that increased ferritin levels will consequently increase serum iron levels, its not the case in this study. It was observed that PIH participants with increased ferritin levels rather had low levels of plasma iron (Table 3.1). This outcome may be as a result of the continuous intake of iron supplements by these pregnant women which are stored as ferritin, and thus increases the ferritin levels. However, due to the increased consumption of iron by these subjects, serum iron will be consumed readily while ferritins are gradually depleted. Also the high plasma hepcidin concentration would lead to an increase in the internalization and degradation of ferroportin which is an iron transporter, thus impairing the release of stored iron (ferritin) from the cells into plasma leading to hypoferremia and high ferritin concentration as seen in this study (Table 3.1).

It is not surprising therefore that there is a progressive increase in plasma ferritin concentration as the syndrome progresses from gestational hypertension through preeclampsia to eclampsia.

Anaemia in pregnancy is a state of low haemoglobin concentration below 11 g/dL or a packed cell volume below 0.33 in the early and latter parts of gestation, while in the mid-term a value of 10.5 g/dL is used (WHO, 1968; CDC, 1989). The pathophysiology of gestational anaemia in the third world is probably multifactorial (Watson-Williams, 1968; Baker, 1981; Masawe, 1981; Fleming, 1989) and these may include lack of iron and folate through malnutrition, abnormal haemoglobin diseases and also as a result of infection or parasitic infestation.Anaemia is very common in pregnancy than in nonpregnant women (WHO, 1992). The decrease in the level of blood in pregnant women is a challenge in most third world countries and when the prognosis is bad is highly responsible for the death of mothers (Usanga *et al.*, 1994; van den Broek and Letsky, 2000; WHO, 2001).Even the mild to moderate form of anaemia affects the socio-economical and health status of pregnant women (Haas and Brownlie, 2001). Pregnant women with severe anaemia are very likely to require blood transfusion during delivery (Zucker *et al.*, 1994), and therefore expose them to blood borne pathogens (Lackritz, 1997). Ross and Thomas (1996) estimated maternal deaths due to anaemia in sub-Saharan Africa to be around 20%c which corroborates the observation made by Harrison (1975) in an earlier work that anaemia accounts for 20% of maternal deaths in Africa.

This study found out that women with PIH and its associated conditions showed increased WBC and MCHC but decreased RBC, Hb, HCT, MCV and platelets compared to healthy women with normal uncomplicated pregnancy (Table 3.1). There have been conflicting results pertaining to the complete blood counts of women with PIH, due to the difference between methods and/or equipment used for automated blood count (Ceyhan *et al.*, 2006). Makuyana and colleagues (Makuyana *et al.*, 2001) observed no significant variation in the haematological variables Hb, WBC, RBC, MCV and platelet count in 38 PE and 72 normal women. However, it can be reasoned out that since PIH has been shown to be associated with inflammation (Teran *et al.*, 2001; Steegers *et al.*, 2010), it is logical to have an increased WBC count among patients with this condition compared to healthy pregnant women without any

conditions of PIH as found in this study. This leucocytosis in PIH could be as a result of physiologic stress induced by the pregnant state (Fleming, 1975) and there is usually neutrophilia on differential counts which is likely due to impaired neutrophilic apoptosis in pregnancy (Gatti *et al.*, 1994).

The comparatively lower red blood cell indices (RBC, Hb, HCT,MCV) that is anaemia and low platelets count found in PIH in this study (Table 3.1) could be attributed to the haemodilution that occurs during pregnancy that is whilst plasma volume seems to double during pregnancy the corresponding increase in red cell volume is half of the plasma volume increase (Letsky, 1980; Letsky, 1995). Shehata *et al.* (1999) also in their study found that there is "gestational thrombocytopenia" which they attributed to haemodilution and increased platelets activation and clearance during pregnancy. This haemodilution is caused by the increase in renin activity and the reduction in atrial natriuretic peptide level as a result of the pregnancy leading to systemic vasodilation and increase in vascular capacitance. This causes a plasma volume elevation due to underfilled vascular system rather than actual blood volume elevation which elicits an opposite response in renin and atrial natriuretic peptide (Barriga *et al.*, 1994; Ajzenberg *et al.*, 1998). Contrary to the findings of this research, other studies have shown that platelets are key in the pathophysiology of high BP with proteinuria (Boriboonhirunsarn *et al.*, 1995) and thus, increasing platelet levels will lead to worsening disease outcomes. In view of this, it is appropriate to infer that women with conditions of PIH will have increased platelet counts as found in the studies by Neiger and colleagues (Neiger *et al.*, 1991) who reported that thrombocytosis was observed in sixty-seven PE women but not in seventy-one normotensive women with healthy pregnancy. Additionally, the levels of platelets observed in GH and PE were similar in a study conducted by same authors (Neiger *et al.*, 1991) which was also noted in this study.

The World Health Organisation in 1992 found the prevalence of anaemia in preganacy to be highest in Asia, followed by Africa and then Latin America. The prevalences of anaemia in pregnancy were comparatively better in the developed countries and stood at less than 20% of women. Developing countries have a challenge of exact anaemia in pregnancy prevalence figures due to the biase in the published rates which are gathered from antenatal clinics. This poses a challenge to clinicians and researchers when the prevalence of anaemia in pregnancy is needed for their work. In this study the

prevalences of anaemia in GH, PE, EC and PIH using Hb were 60.42%, 57.89%, 50.00% and 57.72% respectively (Table 3.2), which fall within the range of the estimated prevalence of anaemia in pregnancy in Africa (ie. 35% to 56%) (WHO, 1992). The anaemia in pregnancy prevalences of the subgroups of PIH in this study was not statistically significant.

Iron deficiency is more common among pregnant women in some developing countries, than normal iron levels (Beard, 2000). Hidden iron deficiency; that is low iron level that does not result in anemia is as common as iron deficiency anaemia thus its frequency is double that of iron deficiency anaemia (Suominen *et al.*, 1998; Asobayire *et al.*, 2001; Mehta, 2004; Monarrez-Espino *et al.*, 2004). The prevalence of iron deficiency in PIH found in this work was lower (ie. 17.89%) (Table 3.2) than the average reported by WHO (ie. 50%) and this can be attributed to the iron supplement policy adopted by the Ghana Health Service for all women attending antenatal clinic. This has been the results in the developed countries where food fortified with iron has led to a dramatic decrease in the prevalence of IDA (Cook *et al.*, 1986; Kazal Jr, 2002; Lynch, 2005). Iron deficiency is still a worldwide problem despite the advancements in modern medical practice, improved nutritional discipline and the use of oral iron supplements. The hemochromatosis genes which help to increase iron absorption and could prevent iron deficiency are found in very few of the world populace (Le Gac and Férec, 2005) and are not as harmful as previously thought (Beutler *et al.*, 2003) The women with healthy uncomplicated pregnancy in this study did not suffer iron deficiency (Table 3.2) and this could be as a result of the normal hepcidin level and thus the availability of ferroportin to transport iron from the cells into plasma.

Iron-deficiency anemia (IDA) is a state of low hemoglobin level in combination with red blood cells which are abnormally small in size and a depleted iron reserve (Denic and Agarwal, 2007). Anaemia due to lack of iron may still persist irrespective of a daily oral iron therapy (Denic and Agarwal, 2007). The body adjusts itself to the lack of iron in normal pregnancy by increasing iron absorption (Svanberg, 1975; Letsky, 1980; Whittaker *et al.*, 1991), but there is malabsorption due to the high phytate based grain diets eaten in the tropics (Letsky, 1980). The prevalence of IDA in developing countries is marked in pregnancy than in those not pregnant (Denic and Agarwal, 2007). The occurrence of IDA (15.45%) in PIH found in this study is about a fourth of the finding

(56%) by Denic and Agarwal (2007). As explained earlier this could be as a result of the government of Ghana's intervention in giving iron supplements to all pregnant women attending antenatal clinic. This finding is not the same as the observation made by Beard (2000) that most pregnant women in the Indian subcontinent had anaemia due to low level of iron than normochromasia (WHO, 1992; Beard, 2000) just as in women in some African countries (Asobayire *et al.*, 2001). This high occurrence of anaemia as a result of low iron level could depict the likelihood of the inablility of man to adjust to this condition (Cook *et al.*, 1986). There was no IDA in the women with healthy uncomplicated pregnancy in this study (Table 3.2) and this could be as a result of comparatively lower level of inflammation occurring thus having normal levels of hepcidin which leads to availability of iron.

4.2 INFLAMMATORY MARKERS (SUPAR, IL-6 AND CRP) LEVELS IN STUDIED PARTICIPANTS

This research estimated the concentration of suPAR in comparison with interlukin-6 and CRP in PIH and healthy pregnancy in order to evaluate suPAR as a possible marker for the characterization of the inflammatory status during pregnancy. Its diagnostic accuracy for distinguishing PIH women and healthy controls, based on the degree of the systemic inflammation was determined.

Inflammation is strongly implicated in the pathophysiology of pregnancy-induced hypertension. Generalized reaction to the presence of systemic inflammation is a characteristic observed in hypertension in pregnancy (Redman *et al.*, 1999; Challis *et al.*, 2009). High concentrations of inflammatory markers like CRP and IL-6 have been reported in several conditions like chronic heart failure, viral infections, bacterial infections and different forms of cancers have been thoroughly documented (McIntyre *et al.*, 1997; Chen *et al.*, 2014; Garcia-Anguita *et al.*, 2015; Przepiera-Bwdzak *et al.*, 2015). Increased suPAR levels have been associated with immune response as found in viral, parasitic and bacterial infections like Human immunodeficiency virus infections, malaria and tuberculosis respectively, and different forms of cancers such as breast, prostate and ovarian cancers (Sidenius *et al.*, 2000; Balabanov *et al.*, 2001; Ostrowski *et al.*, 2005; Gupta *et al.*, 2015). Eugen-Olsen *et al.* (2002) in a study demonstrated the effectiveness of suPAR as an indicator of inflammation as well as a predictor of adult onset diabetes mellitus, cardiovascular disease, malignancies and death in healthy

individuals. Marked increase in suPAR levels positively correlated with an increased risk of the above mentioned conditions (Eugen-Olsen *et al.*, 2002).

This research observed a marked level of plasma suPAR in PIH than the normotensive women with healthy pregnancy (Table 3.4). This observation may be ascribed to the elevated levels of immune activity in pregnancy-induced hypertension as opposed to the immune adaptation seen in healthy pregnancy. This result is in agreement with previous finding by Toldi *et al.* (2011). This study also indicated that there was corresponding increase in the other inflammatory markers CRP and IL-6 with PIH as compared to Healthy Pregnancy which is consistent with earlier works by Takacs *et al.* (2003); Batashki *et al.* (2005); Rebelo *et al.* (2013). There were relatively increased levels in all the inflammatory markers measured as the condition progresses from gestational hypertension to preeclampsia and then to eclampsia. This is because of the increased severity of Pregnancy-induced hypertension as it progresses to eclampsia (Martin *et al.*, 1999). Early diagnosis of PIH is vital in order to prevent maternal systemic symptoms that may result in increased morbidity and mortality to both mother and foetus (Toldi *et al.*, 2011). Therefore, the reliable detection of systemic inflammation will be an essential tool for the prevention of the progression of the PIH into severe state, and plasma suPAR level might be one of the potentially reliable pregnancy inflammatory markers. PIH is also characterized by an increased level of immune activation due to the impaired expansion of the immune tolerance characteristic for healthy pregnant women. It seems that the predictive value of suPAR is unspecific to respective disorders, as it reflects the overall systemic inflammation and immune activation universally observed in several pathological conditions. Imperatively, marked level of suPAR signifies how bad a disease is (Østergaard *et al.*, 2004; Ostrowski *et al.*, 2005).

The observation made in this study provided evidence for increased suPAR levels in pregnancies complicated with PIH. In this disorder, early diagnosis is of great importance to prevent the development of maternal systemic symptoms that culminate in increased maternal and fetal morbidity and mortality. The reliable detection of maternal systemic inflammation would enable healthcare providers to take preventive steps via early treatment of the condition. Laboratory markers for routine screening of PIH are needed to identify patients at potential risk suPAR might be a good candidate

due to its stability in plasma samples. The ROC curve analysis also provided essential evidence to the effect that plasma suPAR measurement is superior to CRP but not IL-6 in differentiating healthy pregnancies from patients with PIH (Figure 3.3). This notwithstanding, suPAR could still be a valuable tool in differentiating healthy pregnant women from PIH women and this is supported by the stability of suPAR in plasma sample. In contrast with CRP, the current standard indicator of lowgrade inflammation suPAR concentrations in healthy individuals are stable, independent of having eaten or not (Sier *et al.*, 1999). suPAR concentration in plasma is stable even after several freezing and thawing sessions (Riisbro *et al.*, 2000).

4.3 INFLAMMATORY, ENDOTHELIAL DYSFUNCTION AND OXIDATIVE STRESS MARKERS AMONGST STUDIED PARTICIPANTS

The lipid profile of PIH women were determined and compared with normotensive women with healthy pregnancy and the relationship or connection between TC, TG & HDL and GH, PE, EC & PIH was established. The lipid profiles of PIH were also correlated with CRP, IL-6, 8-iso-PGF2α and FN.

Normal human pregnancy results in physiologic hyperlipidemia and for that matter hypertriglyceridemia (Peschke *et al.*, 1999; Enquobahrie *et al.*, 2004). Indeed, some researches reported up to about two to three folds increase in TG in healthy pregnancy comparatively to non-pregnant healthy women (An-Na *et al.*, 1995).
Hyperoestrogenemia which is characteristic of normal pregnancies (induction of hepatic triglyceride biosynthesis) (Glueck *et al.*, 1975) in addition to pregnancy related lipogenesis and fat storage in preparation for a growing foetus (Kaaja *et al.*, 1995), increased synthesis of VLDLs (resulting from a feedback response to increased lipolysis as a result of increased insulin resistance during pregnancy)(Kaaja *et al.*, 1995), increased activity of hepatic lipase and decreased catabolism of lipid at adipose tissue level (Brizzi *et al.*, 1999) have been attributed for the observable hypertriglyceridemia in normal pregnancies (Aziz and Mahboob, 2007).

However, evidence available suggests that dyslipidemia resulting in increased maternal systemic inflammation together with disproportionate generaion of oxidants and endothelial injury/dysfunction are central to the cause and severity of PIH (Roberts *et al.*, 1989; Redman and Sargent, 2005). In this study, there was increased TG

concentration among PIH women, with concentrations increasing from GH through to Eclampsia (Table 3.7), indicating the TG concentrations increase with disease severity. The observed increase in TG concentrations in PIH and its subgroups is in consonance with findings from other studies carried out in various populations around the world (Cekmen *et al.*, 2003; Enquobahrie *et al.*, 2004). Consequently, the reduction in lipolytic activities and the upregulation of placental VLDL receptors of mothers with healthy pregnancies, there is the movement of lipoproteins that are saturated with TG to the fetoplancental unit. This is not the case in some pregnancies where the excessive formation of feotoplancental blood vessels are impaired, thus triggering a rise in the production of maternal TG (Chappell and Morgan, 2006; Prasad *et al.*, 2009). Further, the decreased catabolism of TG-rich lipoproteins and the supposed resultant decline in lipoprotein breakdown leads to the buildup of lipoproteins saturated with TG in the mother's blood (Chappell and Morgan, 2006; Prasad *et al.*, 2009). These may induce platelet activation and aggregation as well as endothelial dysfunction, hence the major clinical features of PIH. This rise in TG found in PIH results in its accumulation in the blood vessels such as the spiral arteries of the uterus and are implicated in the injury of the endothelium through the production of LDL (Sattar *et al.*, 1997; Brizzi *et al.*, 1999).

Another school of thought hypothesized that the observed hypertriglyceridemia in PIH could probably be as a result of the increased demand for the limited amount of the rate limiting enzyme that regulates its utilization by Chylomicron and LDL. Chylomicron clearance usually takes place in two steps; triglyceride breakdown by lipoprotein lipase and its utilization by the liver. Therefore when any of these two steps are delayed there is a resultant buildup of remnants in the plasma and is usually implicated in the hypertriglyceridemia resulting in plague formation which is linked with PIH (Noris *et al.*, 2005; Estari *et al.*, 2009). Hyperlipidaemia is paramount in the inhibition of the action of the secretion which promotes the dilation of the endothelium (Williams and De Swiet, 1997; Gratacós, 2000).Presumably, all these circumstances may play roles in the pathogenesis of hypertension in PIH.

This research observed similar TC concentrations in the PIH groups except eclampsia and this is in line with the earlier observations made by(Khong *et al.*, 1986; Kaaja *et al.*, 1995; Sattar *et al.*, 1997)). However, TC was observed to have increased in PIH patients in studies by Phalak and Tilak (2012) and Epstein and Ross (1999). Abnormal

lipid metabolism has been seen to be associated with PIH therefore increased TC in EC but not in other PIH groups could be seen as a reflection of disease severity. Also, a significant increase in HDL was observed in PIH in this research and this is agreeable with the observations made by Bai *et al.* (2002) among pregnant women in China. It however conflicts with reports by (Vani *et al.*, 2015), who found HDL to be higher in normal pregnancy than in PIH. They explain that, in PIH, there is a reduction in levels of Oestrogen which regulates the generation of TG and HDL and inhibits serum LDL formation (Srinivas *et al.*, 2009). They also added that, the reduced concentration of HDL in this systemic maternal syndrome is not only due to the reduction in the levels of oestrogen but also as a result of insulin resistance (Goonewardene and Sirisena, 1985). The increase in HDL in PIH in this study seems to be adaptational in PIH patients, protecting them against the cardiovascular risk of hypertension (Irinyenikan *et al.*, 2013).

An abnormal change in the structural integrity of the endothelium due to hyperlipidaemia is cardinal to the development of hypertension (Elzen *et al.*, 1996; Sattar *et al.*, 1997). Also the excessive generation of free radicals could lead to the malfunctioning of the lining of the blood vessels due to lipid peroxidation. In this study, endothelial injury as measured by the levels of fibronectin was significantly increased among PIH women (Table 3.7). The observed high FN in this research is in consonance with the observations made by (Frohlich, 1989; Lockwood and Peters, 1990; Powers *et al.*, 2008). Hepatocytes and endothelial cells are the main source of plasma fibronectin production therefore any injury to these cells would reflect in its level and serve as a sign of its disturbance (Roberts *et al.*, 1989; Ballegeer *et al.*, 1992; Pipkin, 1995). It has been proposed that hypertension in pregnancy may be caused by an amplified inflammation which results in the modification of a number of plasma secretions that control endothelial integrity (Williams and De Swiet, 1997; Gratacós, 2000). When there is a damage or disturbance in the structural integrity of the endothelium,platelets clump together resulting in thrombocytopenia and plague formation disorder with contractions of the blood vessels and increased clotting (Gratacós, 2000; Cekmen *et al.*, 2003). Hence the increased levels of FN as well as the fall in platelet count (EC) as observed in this study (Table 3.7). The increase in FN and the fall in platelet count from GH to EC show that endothelial injury increases with diseases severity.

Vasoconstriction, damage to the endothelium and systemic inflammation are implicated in the cause of hypertension in pregnancy (Challis *et al.*, 2009).

The lack of equilibrium in the production of oxidants and antioxidants seems to be pivotal in the pathogenesis of PIH (Boutet *et al.*, 2009; Burton and Jauniaux, 2011). Excess ROS interact with DNA, lipid, and proteins to produce oxidized DNAs, lipid peroxides, and oxidized proteins respectively and usually negatively affect their physiological functions (Song *et al.*, 2010). Overproduction of oxidants result in the methodical insults to lining of blood vessels and numerous parts of the human anatomy (Cekmen *et al.*, 2003). 8-iso-PGF2α is a prostaglandin-like product synthesized in the body by the peroxidation of arachidonic acid through a free radical-facilitated nonenzymatic reaction (Morrow *et al.*, 1992). 8-iso-PGF2α causes blood vessels to reduce in size, helps in the development of smooth muscle, stimulates platelets to aggregate and provokes the malfunctioning of the boundary formed by the lining of the blood vessels (Patrono and FitzGerald, 1997; Hart *et al.*, 1998). Increased concentrations of 8iso-PGF2α are observed in persons suffering from deadly diseases which are caused by the excessive formation of reactive oxygen species (Morrow *et al.*, 1995). The observed marked 8-iso-PGF2α found in this research conforms to the results obtained in other studies (Barden *et al.*, 1996; Staff *et al.*, 1999b) which support the notion that the overproduction of free radicals result in dyslipidaemia which is central to the development of PIH (GH, PE and EC). Several other researches have demonstrated large quantities of lipid peroxides and placental 8-iso-PGF2α in PIH pregnancies after delivery unlike the placenta of a healthy pregnancy (Staff *et al.*, 1999a; Staff *et al.*,

1999b). There was a progressive increment in 8-iso-PGF2α observed in this research, with the highest in the EC group than in PE and GH. This result suggests that as the level of oxidation of lipids increases the prognosis of this disorder worsens. Thus the increase in 8-iso-PGF2α is caused by excessive free radicals oxidation of lipids which affects the lining of the blood vessels (Staff *et al.*, 1999a).

Furthermore, Isoprostanes (including 8-iso-PGF2α) encourage the production of inositol-triphosphate and the growth of smooth muscles of the blood vessels and stimulate the secretion of endothelin (Fukunaga *et al.*, 1993; Fukunaga *et al.*, 1994). High concentrations of endothelin have been reported in proteinuric hypertension,

which is seen as a consequence of injury to the lining of the blood vessels (Taylor *et al.*,
1990; Nova *et al.*, 1991). The natural functions of 8-iso-PGF2α implicates it in the symptoms seen in PIH and these are increase in blood pressure, the malfunctioning of the lining of blood vessels of the kidney and placenta and the seizures in eclampsia (Morrow *et al.*, 1995; Bachi *et al.*, 1996). The consequence of the impairment of the activity of the enzyme: copper-zinc superoxide dismutase (Cu-Zn SOD) which has an antioxidative property is the rise in plasma levels of 8-iso-PGF2α (Morrow *et al.*, 1995). Therefore, the findings of a reduction in the activity of this enzyme in the placentas of PIH women (Wang and Walsh, 1996) suggests a pronounced production of placental 8iso-PGF2α in PIH. This could be the explanation for the increased plasma levels of 8iso-PGF2α seen in PIH women in this research. The biological actions of 8-iso-PGF2α on endothelial cell dysfunction could also account for the increased FN concentrations in PIH as observed in this study.

The contribution of endothelial dysfunction can be viewed in a larger context as part of the inflammatory network. Hence, it is inevitable that, on average, all the indicators of inflammation which are normally subclinically high in pregnancy are amplified in PIH (Mantovani and Dejana, 1989; Redman and Sargent, 2005). This study showed a significant increase in the inflammatory markers i.e. CRP and IL-6 among PIH patients as observed earlier by (Belo *et al.*, 2003; Kumru *et al.*, 2006; Bernardi *et al.*, 2008; Guven *et al.*, 2009; Mori *et al.*, 2010; Thilaganathan *et al.*, 2010; Catarino *et al.*, 2012). Normal pregnancy evokes a mild systemic inflammatory response. This response, which varies from woman to woman, is wide-ranging and by the third trimester involves inflammatory leukocytes, endothelial activation, the acute phase response, and metabolic features of systemic inflammation. Again, in PIH, the blood insufficiency experienced by the placenta results in the excessive formation of free radicals and proinflammatory cytokines, which may provoke the malfunctioning of the lining of the blood vessels of the mother (Benyo *et al.*, 2001; Mehendale *et al.*, 2008; Järvisalo *et al.*, 2006). IL-6 is one of the indicators of inflammation, that is implicated in immune activation in PIH and C-reactive protein (CRP) increases rapidly when triggered by an inflammatory stimulus (Gruys *et al.*, 2005; Sharma *et al.*, 2007). Hence, an increase in CRP and IL-6 in PIH as observed in this study (Table 3.7) supports reports of PIH as a widespread maternal systemic inflammatory response.

Chapter 5

CONCLUSION

5.1 GENERAL CONCLUSIONS

- High hepcidin and ferritin concentrations were observed in PIH women compared to controls. This may be accountable for the low iron level responsible for hypoxia in PIH.
- There was increased inflammation in PIH than the normotensive as evidenced by markedly elevated suPAR, IL-6 and CRP levels.
- The observed lower variability and higher stability of suPAR however, makes it a better marker for characterizing inflammatory status during pregnancy.
- The hypertriglycerimia and high level of High Density Lipoprotein cholesterol concentrations in PIH compared to controls could be accountable for the inflammation in PIH.
- There was increased oxidative stress as evidenced by increase in 8-iso-PGF2α among PIH compared to the controls.
- This comparatively increased 8-iso-PGF2α and fibronectin values observed in PIH than the controls could account for the endothelial injury in PIH.

5.2 RECOMMENDATIONS

- There is the need to include the measurement of soluble transferrin receptor antibody (iron deficiency marker), 8-iso-PGF2α, suPAR, fibronectin and lipid profile amongst pregnant women attending antenatal clinic.
- Also these analytes can be used in the management of PIH since they can be used to assess the prognosis of this systemic maternal syndrome.
- Further studies should estimate ferroportin and soluble transferrin receptor antibody levels to enrich the research.
- Maternal health education should be intensified.

- More resources should be availed for more research into the pathophysiology of PIH to assist in reducing its prevalence.

5.3 LIMITATION

- Dietary information on study participants was not obtained, since a high phytate content diet could impair dietary iron absorption.

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