METABOLIC SYNDROME, OXIDATIVE STRESS AND PUTATIVE RISK FACTORS AMONGST GHANAIAN WOMEN PRESENTING WITH PREGNANCY-INDUCED HYPERTENSION

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DECLARATION

The experimental work described in this thesis was carried out at the Department of Molecular Medicine, SMS, KNUST. This work has not been submitted for any other degree and the contents of this thesis are my work.

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ABSTRACT

Pregnancy-Induced Hypertension, one of the commonest complications of pregnancy, is an important cause of maternal and perinatal morbidity and mortality. It is of great concern to obstetricians due to its sudden onset and non availability of a definitive cure. This current study, therefore, seeks to establish the incidence and prevalence rates of PIH among Ghanaian women obtaining antenatal care at the Komfo Anokye Teaching Hospital. It also seeks to determine the probable cause(s) or risk factors of PIH among Ghanaian women; develop appropriate screening modalities for the early detection and management of PIH; and the elucidation of the role of the Metabolic Syndrome as well as oxidative stress in the pathogenesis of PIH. A case-control study was conducted among pregnant women visiting Komfo Anokye Teaching Hospital (KATH), Kumasi, Ghana between November, 2006 and December, 2007. Thirty women with preeclampsia, seventy with gestational hypertension and fifty normotensive pregnant women (controls) in the second half of pregnancy were recruited for this study. After ethical approval and informed consent had been obtained, information on socio-demographic characteristics, medical history and previous obstetric history were obtained by face-to-face interviews from the respondents and assessed through medical records. Blood pressure, anthropometric measurements, urine sample for urine protein and blood sample were taken for some biochemical and hormonal analysis. Data was obtained from the Reproductive Health Unit of the hospital for the determination of percentage incidence and prevalence for the year under assessment. An incidence of 5.46, 6.01, and 11.46% for GH, PE and PIH respectively was obtained for 2006. In the year 2007, percentage incidences for the various clinical conditions were, 8.01, 9.03 and 17.04% respectively. The prevalence for the years under review was 5.87, 6.55 and 12.42% correspondingly. Additionally, increased prevalence for all three conditions in the rainy season was observed for the year under review as compared to the dry season. To assess risk factors for hypertensive pregnancy data was obtained from the questionnaire administered to the participants. Age was a significant risk factor for the development of PIH (PE+ GH); both the young and older mothers were at increased risk of the condition. Underweight [OR

7.5; 95%CI 0.4-150.4] and obesity [OR 4.7; 95%CI 1.7-12.7] increased the risk of Pregnancy-Induced Hypertension and the risk was even higher for the development of PE. Women without formal education or those who have attained only basic education were also at risk of developing PIH; intake of alcoholic beverages and high salt consumption increased the risk of developing PIH. Nulliparous women were protected from the risk of developing PE [OR 0.02; 95%CI 0.0-0.4] from this study. Family history of hypertension predisposed women to PIH [OR 5.5; 95%CI 2.0-15.1], GH [OR 10.6; 95%CI 3.6-31.4] and PE as well [OR 6.0; 95%CI 1.8-19.5]. Although, a history of abortion conferred some form of protection against preeclampsia and gestational hypertension, the number required varied in both cases. The use of contraceptives in either the male [OR 5.6; 95%CI 1.2-25.2] and the female [OR 1.7; 95%CI 0.8-3.5] partner similarly increased the risk of PIH. Partner change as well as placental hormonal imbalance (hPL) is also associated with increased risk of the clinical conditions. After assessing lipid peroxidation and oxidative stress among these women, there was a significant increase in triglycerides and LDL-cholesterol in the subject groups compared to the control. MDA, the lipid peroxidation marker among the PIH subjects was significantly increased as compared to the normotensive pregnant women (controls). A significant positive correlation between MDA and blood pressure (SBP and DBP) was also observed. There was a significant increase in the prevalence of the metabolic syndrome among the PIH (PE+GH) subjects as compared to the normotensive pregnant women (controls) using both the National Cholesterol Education Program (NCEPIII) and WHO criteria. From this study, the prevalence of GH, PE and PIH were 5.87, 6.55 and 12.42% respectively and with seasonal variations in their occurrence. The findings of this study suggests that, besides maternal aberrations posing risk factors for PIH, partner and placental roles could also be linked to the aetiology of Pregnancy-Induced Hypertension and these risk factors should be screened for during antenatal visits. This study also clearly indicates that Ghanaian women presenting with PIH are very prone to dyslipidaemia as well as lipid peroxidation, this might in part explain the oxidative stress and endothelial vascular dysfunction observed in these group of women. Ghanaian

women presenting with PIH are also extremely prone to the development of the metabolic syndrome, thus the indices must be screened for during antenatal care.

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Linda Ahenkorah

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ABBREVIATIONS

3,5-DHBS	3,5-dichloro-2-hydroxybenezene
4-AAP	4-aminoantipyrine
ADP	Adenosine-5-diphosphate
AHA	American Heart Association
AI	Atherogenic Index
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
ATP	Adenosine triphosphate
BCRCP	British Columbia Reproductive Care Program
BMI	Body Mass Index
CG	Control Group
CIs	Confidence Intervals
CK	Creatine Kinase
DAP	Dihydroxyacetone Phosphate
DBP	Diastolic blood pressure
DNA	Deoxyribonucleic Acid
ECV	Extracellular volume
EGIR	European Group for the study of Insulin Resistance
ELISA	Enzyme- Linked Immunosorbent Assay
FAS	Foetal Alcohol Syndrome
FBS	Fasting Blood Sugar
G-3-P	Glycerol -3- phosphate
G-6-PDH	Glucose -6-phosphate dehydrogenase
GH	Gestational Hypertension

GK	Glycerol Kinase
GP	Gestational Period
GPO	Glycerophosphate Oxidase
HCl	Hydrochloric acid
HDL	High Density Lipoprotein
HELLP	Haemolysis elevated liver enzymes low platelet count syndrome
HK	Hexokinase
hPL	Human Placental Lactogen
Ht	Height
HUVECS	Human Umbilical Vein Endothelial Cells
IFCC	International Federation of Clinical Chemist
IL-6	Interleukin 6
IUGR	Intra-Uterine Growth Reduction
KATH	Komfo Anokye Teaching Hospital
LDH	Lactate Dehydrogenase
LDL	Low Density Lipoprotein Cholesterol
M. age	Maternal age
MDA	Malondialdehyde
mRNA	Messenger Ribonucleic Acid
NaCl	Sodium Chloride
NAD	Nicotinamide Adenine Dinucleotide
NCEP	National Cholesterol Education Program
NO	Nitric Oxide
NOS	Nitric Oxide synthase
ORs	Crude Odds Ratios
PCOS	Polycystic Ovarian Syndrome
PE	Preeclampsia

PI	Ponderal Index
PIH	Pregnancy-Induced Hypertension
PMI	Placental Malaria Infection
PUFA	Poly-unsaturated fatty acid
SBP	Systolic blood pressure
SEM	Standard Error of the Mean
SES	Socio-Economic Status
SHR	Spontanoeusly Hypertensive Rat
TC	Total Cholesterol
TG	Triglycerides
Tn I	Troponin I
TNF-α	Tumour Necrosis Factor- alpha
USA	United States of America
VEGF	Vascular Endothelial Growth Factors
VLDL	Very Low density Lipoprotein
WC	Waist Circumference
WHO	World Health Organization
WHR	Waist-to-hip ratio
WHtR	Waist-to Height Ratio
Wt	Weight

Chapter 1

INTRODUCTION

1.1 GENERAL INTRODUCTION

Throughout the world 585,500 women die every year as a result of Pregnancy and Childbirth. Over 98% of all maternal mortality occurs in developing countries (Abouzahr & Royston, 1991; Bates *et al.*, 2008). The most common cause of these maternal deaths are complications of pregnancy and child birth such as, haemorrhage, sepsis, complications of unsafe abortions, hypertension disorders of pregnancy and obstructed labour (WHO, 1994). In developing countries, 17% of direct obstetric deaths are as a result of hypertension (Maine, 1987). Maternal and perinatal morbidity and mortality as a result of hypertensive pregnancy are major health problems in developing countries like Ghana. Studies carried out by OseiNketiah, (2001) revealed that in Ghana, 40% of maternal deaths are as a result of hypertensive pregnancy, antepartum haemorrhage and post partum haemorrhage.

Hypertensive pregnancy has been documented as a complication of pregnancy for centuries but its aetiology remains obscure to date. The occurrence of fits in pregnant women has been documented as early as 4th Century B.C. by Hippocrates (O'Dowd & Philipp, 1994), hence the condition termed ECLAMPSIA, a Greek word which literally means ''shine forth'', depicting an abrupt development. It was also recognized that hypertension and albuminuria herald the onset of fits in these pregnant women as such the term PREECLAMPSIA was devised. The term pregnancy-induced hypertension (PIH) is most widely used currently, (Lewis & Chamberlain, 1990) since not all preeclamptic women subsequently develop eclampsia.

Preeclampsia is a clinical condition of pregnancy characterized by hypertension, and proteinuria. It is a multisystem disorder affecting nearly every organ and system in the human body. Different studies clearly show that the uteroplacental blood flow, its vascular resistance, endothelial integrity, endothelial damage, the platelets and coagulation system, and neutrophils all interact in preeclampsia. It is most probable that unless more is known about the dynamics of uteroplacental blood flow, flow behaviour and its influence on the vascular endothelium, confusion and inconsistency will continue. It is therefore, understandable why this condition is sometimes referred to as ''the disease of theories''.

1.2 HISTORY

Historically, the word ECLAMPSIA dates from the 17th century. It is derived from a Greek word meaning ' to shine forth ' due to the visual phenomenon accompanying the condition. The associated seizures were believed to be due to blood poisoning or toxins derived from the pregnancy; hence it was termed toxaemia of pregnancy. Contemporary researchers attempted to clarify the disease process based on observed pathophysiological changes (Sengupta, 1996).

In 1781, Alexander Hamilton, described eclampsia as a condition associated with seizures. In 1896 when the sphygmomanometer was invented, arterial hypertension was found to be associated with eclampsia. Uteroplacental ischaemia and infarction reduction in maternal uteroplacental blood flow and uterine distension leading to hypertension and proteinuria through utero-renal reflex all have been implicated in preeclampsia (Berger & Cavanagh, 1963; Browne & Veall, 1953; Sengupta, 1996). Later, with the advancement of science, the importance was laid more on genetic, hematological, biochemical, hormonal and immunological factors (Lyall & Greer, 1994). Browne (1958), clarified most of the pathophysiological changes associated with the disease, linking it with cortisone hormone imbalance and this hormone was implicated for all the

changes. Hypoproteinaemia and vitamin deficiency have also been implicated in the aetiology of this condition (Chaudhuri, 1970). Recently, some studies have also related vitamin and calcium dysregulation with preeclampsia (Belizan *et al.*, 1991).

However, none of the reported dysregulation has been proven in a systematic study till date, even though preeclampsia has been associated with changes in the levels of lipoproteins, calcium and some vitamins.

Wilson *et al.,* (1992) suggested activation of the coagulation system. The entire hematological changes are the secondary effect consequent to the primary vascular or endothelial damage. The reasons for the endothelial damage are not known (Lyall & Greer, 1994). Petrucco (1974) and others, have revealed a diminution in spontaneous lymphocytic transformation. Endothelin-1 gene expression is increased in placental villous tissue of preeclamptics, contributing to placental vasoconstriction and vascular insufficiency (McMahon *et al.*, 1993). Chesley *et al.,* (1962) have concluded that preeclampsia could be due to a simple recessive trait. A multifactorial interaction in the aetiopathogenesis of preeclampsia has been proposed which takes into account the steroidal hormone-micronutrient interaction as the key process involved in the endothelial-trophoblastic proliferation and interactions (Sengupta & Guha, 1994).

1.3 HYPERTENSIVE DISORDERS OF PREGNANCY

Hypertensive disorders of pregnancy continue to be one of the largest causes of maternal and foetal mortality and morbidity and affects 3% to 10% of all pregnancies worldwide (Granger *et al.*, 2001). Hypertension is a common clinical complication during pregnancy. It is usually defined as systolic blood pressure of at least 140 mmHg and/or diastolic blood pressure of at least 90 mmHg (Cnossen *et al.*, 2006). Hypertension is reported to account for 15% of all antenatal hospitalizations for pregnancy complications in the United States (Scott *et al.*,

1997). Depending on the region, between 9.1% (Africa, Asia), 16.1% (developed countries), and 25.7% (Latin America) of maternal deaths may be attributed to pregnancy associated hypertension (Khan *et al.*, 2006). About 18% of foetal deaths are associated with hypertensive disorders (Cnossen *et al.*, 2006). The diagnostic criteria for disorders of hypertension in pregnancy are not presently consistent and there are a number of different systems made known by major working groups and international societies. Inspite of this, advancement toward merging the classification has been made and the major consensus statements now agree on most of the terminology. The National High Blood Pressure Education Program Working Group (2000) has classified hypertensive disorders of pregnancy as chronic hypertension, preeclampsia superimposed on chronic hypertension, preeclampsia-eclampsia, gestational hypertension (pregnancy-induced hypertension).

1.3.1 *CHRONIC HYPERTENSION*

Chronic hypertension, also called essential hypertension, is arterial hypertension of unidentified cause. Chronic hypertension is defined by elevated blood pressure that predates the pregnancy, and it is documented before 20 weeks gestation, or is present 12 weeks after delivery (National High Blood Pressure Education Group, 2000). Chronic hypertension affects about 1-5 % of pregnant women (Sibai *et al.*, 1998) with higher occurrence in obese women, older women and black women (Barton *et al.*, 1997; Sibai, 1996). Studies have also established an increased risk of chronic hypertension in women, after years of pregnancy complicated with Pregnancy-Induced Hypertension or preeclampsia (Nisell *et al.*, 1995; Shammas & Maayah, 2000). Similarly, chronic hypertension is associated with increased risks of preeclampsia and abruptio placentae, as well as increases in neonatal mortality and morbidity (McCowan *et al.*, 1996; Sibai, 1996). Many individuals with chronic hypertension usually will have a positive family history

of hypertension as well as its complications, including congestive heart failure, coronary artery disease, stroke, and renal dysfunction.

1.3.2 *PREECLAMPSIA SUPERIMPOSED ON CHRONIC HYPERTENSION*

Superimposed preeclampsia similar to chronic hypertension is associated with significantly increased risks of maternal and foetal death, foetal growth restriction, and placental abruption (August *et al.*, 2004). The incidence of superimposed preeclampsia in chronic hypertension ranges from 4.7 to 18.4% for mild hypertension (DBP >90 mmHg) (Chesley, 1978; Sibai & Anderson, 1986; Sibai *et al.*, 1983) up to 54% to 100% for severe hypertension (DBP >100 mmHg) (Rey & Couturier, 1994). It has been noted that, black women with chronic hypertension in pregnancy have a higher incidence of superimposed preeclampsia and premature babies compared to white women with chronic hypertension (Rey *et al.*, 1997). August *et al.*, (2004) developed a predictive model for superimposed preeclampsia in women with a diagnosis of chronic hypertension, during pregnancy and after delivery, based on these risk factors:

Blood pressure, ≥140/90 mmHg measured either before pregnancy or in the first trimester of pregnancy, serum uric acid level, >3.6 mg/dl; and plasma renin activity, <4 ng/mL/min. The biochemical parameters should be determined at 20 weeks of gestation. However, women without these risk factors might still have a possibility of the development of superimposed preeclampsia. August *et al.,* (2004) reported a higher probability for women presenting with one or more of these risk factors given that 16% of women with one of the risk factors as above had a probability of 30% to 40%; whereas women with two risk factors had a probability of 62%; and women with all three risk factors had a probability of 86%. Although blood pressure of 140/90 mmHg is related to a high risk of superimposed preeclampsia among women with chronic hypertension, a good number of women develop superimposed preeclampsia between blood pressure of 130/80 mmHg and 137/88 mmHg (August *et al.*, 2004).

1.3.3 *PREGNANCY-INDUCED HYPERTENSION*

Pregnancy–Induced Hypertension (PIH) is defined as the occurrence of hypertension after 20 weeks of gestation in a woman without prior hypertension (National High Blood Pressure Education Group, 2000). It is usually defined as systolic blood pressure of at least 140 mmHg and/or diastolic blood pressure of at least 90 mmHg (Cnossen *et al*., 2006). When accompanied by proteinuria, the disorder is termed preeclampsia and when it is without significant proteinuria it is termed gestational or transient hypertension (National High Blood Pressure Education Group, 2000).

1.3.4 *GESTATIONAL HYPERTENSION*

Gestational Hypertension (GH) is defined as onset of hypertension, (systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg) after 20 weeks of gestation in the absence of significant proteinuria and is generally characterized by good maternal and foetal outcomes (Brown & Buddle, 1995; Davey & MacGillivray, 1988; Helewa *et al*., 1997). A rise in blood pressure of at least 25 mmHg systolic or 15 mmHg diastolic during pregnancy, even if the absolute blood pressure level was less than 140/90 mmHg, was also included in past definitions, (National High Blood Pressure Education Group, 1990). Usually in gestational hypertension the hypertension resolves to normal within 3 months postpartum (Brown & Buddle, 1995) although these women may be inclined to essential hypertension later in life. Gestational Hypertension (GH), a generally more benign disorder is diagnosed when blood pressure is elevated in the absence of features of preeclampsia and when other systemic manifestations are absent. GH is occasionally called mild preeclampsia (Australasian Society for the Study of Hypertension in Pregnancy, 1993). The distinction between preeclampsia and gestational hypertension is made by the presence and magnitude of proteinuria (Seely & Solomon, 2003). The high false-positive rate of 26 to 83% with ''1+'' proteinuria (Brown & Buddle, 1995; Meyer *et al*., 1994; Saudan *et al*., 1998) has led to the use of ''2+'' on urinary dipstick testing as the

cutoff for proteinuria (Davey & MacGillivray, 1988; Sibai *et al.*, 2000). Therefore during classification, pregnant women presenting with ''1+''proteinuria, trace or negative 24-hour urinary protein measurement are considered as having gestational hypertension. It has been established that women with gestational hypertension and ''1+'' proteinuria are more likely to develop severe hypertension or severe maternal disease than those women with negative or trace proteinuria on dipstick (North *et al.*, 1999).

1.3.5 *PREECLAMPSIA*

Preeclampsia (PE) is an intriguing disease, whose aetiology has remained obscure for centuries. For decades now, theories to explaining the aetiology of the disease have been put forward by various authorities. Although, complications of hypertensive disorders of pregnancy have been recognized for centuries, the cause(s) are still poorly understood. Preeclampsia is usually defined as systolic blood pressure of at least 140 mmHg and/or diastolic blood pressure of at least 90 mmHg, accompanied by proteinuria first detected after 20 weeks gestation (Cnossen *et al.*, 2006). Previously, the definition included a rise in blood pressure from preconception or first trimester values of more than 25–30 mmHg systolic and/or 15 mmHg diastolic (Seligman, 1987).

Some authorities have classified preeclampsia as mild or severe in some classification systems (Australasian Society for the Study of Hypertension in Pregnancy, 1993), but the definition of mild preeclampsia is still conflicting. This may lead to confusion between mild preeclampsia and gestational hypertension, and for this reason the latest (Australasian Society for the Study of Hypertension in Pregnancy, 1993) classification does not stratify or classify preeclampsia (Brown *et al.*, 2000). Preeclampsia is a systemic condition associated only with pregnancy, whose hallmarks are high maternal blood pressure, proteinuria and severe fluid retention. Other systemic manifestations include disseminated intravascular coagulation, haemolysis, elevated liver function test and rarely

seizures (Seely & Solomon, 2003). It is a common complication of pregnancy affecting about 5 to 10% of all pregnancies (Lindheimer & Katz, 1985). Preeclampsia can be life-threatening for both mother and foetus, additionally it accounts for inductions of labour and caesarean sections performed in many hospitals. Commonly, preeclampsia occurs in first pregnancies than subsequent pregnancies with the mother's blood pressure returning to normal after delivery (LeLorier *et al.*, 1997) although they may equally be predisposed to essential hypertension later in life. Potential causes and mechanisms behind preeclampsia remain unknown, but maternal, immune, genetic factors and placenta have been implicated (National High Blood Pressure Education Group, 2000).

1.3.6 *ECLAMPSIA*

Eclampsia which is a rare but more severe form of PIH is defined as seizures in a pregnant woman with preeclampsia in the absence of known or subsequently diagnosed convulsive disorder (Villar *et al.*, 2006). Eclampsia is the occurrence of generalised convulsions during pregnancy, labour, or within seven days of delivery which is not caused by epilepsy or other convulsive disorders. Eclamptic seizures are relatively rare and occur in less than 1 % of women with preeclampsia (Witlin & Sibai, 1998). Beck and Menezes (1981) established that 7% of deaths due to eclampsia were more attributable to haemolysis, elevated liver enzymes, and low platelet count (HELLP syndrome), a modification of severe hypertension that results in multiorgan failure. The HELLP syndrome, the definition of which may vary slightly from country to country, is usually specified by the presence of an abnormal peripheral blood smear, with schistocytes and/or burr cells; serum lactate dehydrogenase, bilirubin, and transaminase more than twice the upper limit of normal, and platelet count less than $100x10^9/L$. Eclampsia is a progressive state of preeclampsia. Normally this stage of the condition would not be reached unless if, the expectant woman has

not taken her antenatal visits seriously or in the absence of antenatal visits or late reporting to hospitals.

1.4 PATHOGENESIS AND PATHOPHYSIOLOGY OF PREGNANCYINDUCED HYPERTENSION

1.4.1 *Historical Perspective*

The specific aetiology of preeclampsia has evaded scientists and physicians from the time of Hippocrates and since then, various models have been advanced. The symptoms of preeclampsia and eclampsia were previously described by the ancient Greeks (Chesley, 1978) but eclampsia (preeclampsia with seizures) was differentiated from epilepsy first in 1739 by the French obstetrician Sauvages, and later termed eclampsia parturentium (Chesley, 1984). In 1843, proteinuria was observed, and led to the notion that eclampsia depended on uraemic poisoning caused by impaired renal excretion. After the detection of eclamptic hypertension at the end of the nineteenth century, the disease was considered a manifestation of essential hypertension, brought to light and particularly coloured by pregnancy (Chesley, 1984). However, it was identified that preeclampsia occurred only in the presence of placental tissue, together with cases of retained placental tissue and hydatiform mole, where the foetus is absent (Roberts & Redman, 1993). Therefore, pathology of the placenta was earlier considered to be the causative factor. In 1967, Robertson and Brosens described specific structural modifications of the uteroplacental unit in preeclampsia (Robertson *et al*., 1967) and until recently, unsuccessful placentation has been regarded an essential feature for preeclampsia to develop.

To date, the pathogenesis of hypertensive pregnancy still remains uncertain; it has been said to be multifaceted and includes immune, genetic, placental abnormalities and environmental factors. Kopcow & Karumanchi, (2007) documented the immunological basis of preeclampsia. Chelbi and Vaiman, (2008) hypothesized that genetic, epigenetic and environmental factors are

involved in the pathogenesis of preeclampsia. Additionally, it has been reported that there is decreased formation of vasodilators such as nitric oxide (Granger *et al.*, 2001; Mitchell *et al.*, 2007) and prostacyclin (Granger *et al.*, 2001) in women who present with gestational hypertension and preeclampsia. Mitchell *et al.*, (2007) has also reported that increased reactive oxygen species (superoxide and peroxynitrite) production and decreased bioavailability of the endothelial nitric oxide (NO) synthase (eNOS), cofactor tetrahydrobiopterin (BH4) could add to maternal endothelial dysfunction in rats with pregnancy-induced hypertension and the numerous characteristics of preeclampsia.

It has also been suggested that preeclampsia is a two-stage disease (Roberts, 2000). The first stage is asymptomatic and is characterized by abnormal placental development during the first trimester leading to placental insufficiency and the release of disproportionate amounts of placental materials into the maternal circulation. This in turn leads to the second, symptomatic stage, where the pregnant woman develops characteristic hypertension, renal impairment, and proteinuria and also at increased risk for the HELLP syndrome (haemolysis, elevated liver function enzymes and low platelets), eclampsia, and other end organ damage (Hladunewich *et al.*, 2007). All these may contribute to endothelial dysfunction typical of gestational hypertension and preeclampsia and this endothelial dysfunction may in turn, trigger several critical features of preeclampsia, including vasoconstriction, hypertension, loss of the usual pregnancy-associated refractoriness to pressor effects of angiotensin II, increased platelet aggregation and proteinuria (National High Blood Pressure Education Group, 2000).

Hypertensive pregnancy particularly, preeclampsia, a multisystem disorder with varying progression resulting in signs and symptoms which require prompt management is associated with generalized vasospasm and progressive participation of essential organs such as the liver, kidney, brain and blood

systems. Some data have suggested that abnormalities of the placenta are the primary cause of preeclampsia, because placental delivery reverses the symptoms of preeclampsia, suggesting that the placenta has a central role in the condition. Additionally, women with increased placental tissue for gestational age, such as those with hydatiform moles and twin pregnancies, have an increased incidence of preeclampsia.

1.4.2 *PLACENTAL INVOLVEMENT*

It is reasonable to assume that the placenta plays a vital role in the pathogenesis of the disease on the basis that the only definitive treatment remains delivery of the placenta. Pathologic examination of placentae from preeclamptic pregnancies generally reveals placental infarcts and sclerotic narrowing of arteries and arterioles, with typical reduced endovascular invasion by cytotrophoblasts and insufficient remodeling of the uterine spiral arterioles (Lim *et al.*, 1997). There is a 30 – 80% incidence of growth restriction in relationship with preeclampsia (BCRCP, 2006).

In normal pregnancies, the uteroplacental spiral arteries go through major remodelling by invading cytotrophoblast, and the muscular, intimal and endothelial layers of the spiral arteries are replaced by trophoblast (Brosens, 1977; Robertson *et al.*, 1967). Thus the arteries are transformed to high flow; low resistance vessels capable of meeting the requirements of the foetus, and these physiological modifications include the arterial segments of the inner third of the myometrium (Fig.1.1). Preeclampsia is linked with a failure of trophoblast to transform the spiral arteries; the number of transformed arteries is decreased, and the transformation does not reach the myometrial segments of the arteries (Pijnenborg *et al.*, 1991; Robertson *et al.*, 1967). There is histological confirmation that in women who later develop preeclampsia there has been defective penetration by the cytotrophoblast, so that these maternally derived arteries keep the musculoelastic elements of their walls (Brosens, 1977). Unsuccessful placentation may cause decreased placental blood flow, and consequent under

perfusion of the placenta may stimulate hypoxic injuries and produce toxic metabolites. The prevailing hypothesis over the last decade has been that toxic metabolites from a hypoxic placenta released to the maternal circulation may lead to the generalized syndrome of preeclampsia (Roberts & Redman, 1993). It has also been observed that, maldeveloped placentas are also seen in cases of foetal growth restriction devoid of preeclampsia (Khong *et al.*, 1986). Additionally, most babies born after preeclamptic pregnancies usually have an appropriate birth weight for their gestation, which strongly suggests primarily normal placentas. It has therefore been proposed that, the critical factor for the development of preeclampsia may be the maternal response to a normal as well as a maldeveloped feto-placental unit (Ness & Roberts, 1996) .

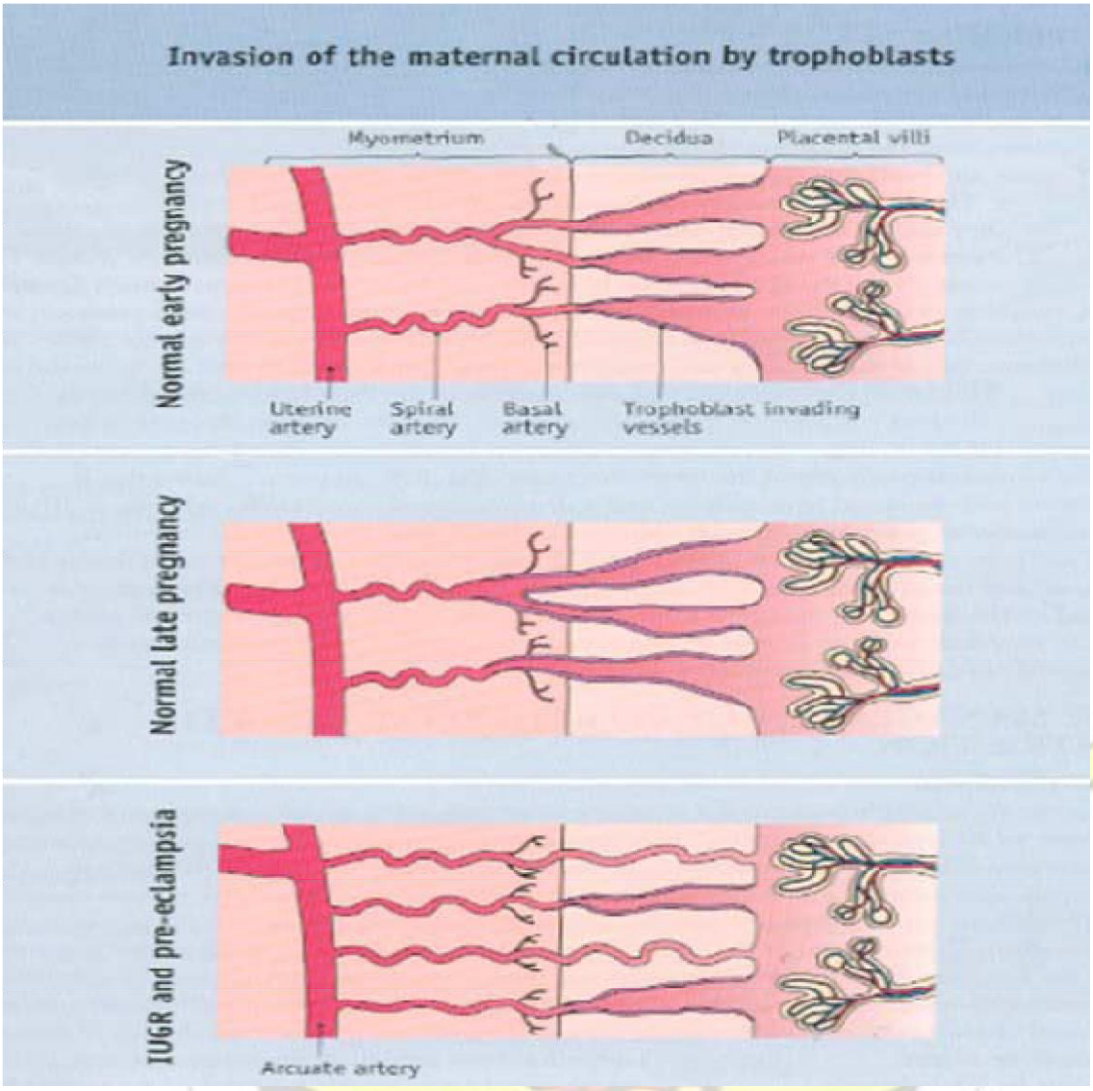


Figure 1.1 **Invasion by trophoblasts.**

Upper panel shows normal early pregnancy with invasion to level of the decidua. Middle panel shows invasion to level of myometrium following second wave of trophoblast invasion. Lower panel shows inadequate implantation of IUGR and preeclampsia. Broken lines indicate trophoblast invading blood vessels (Source: Mosby's Color Atlas and Text of Obstetrics and Gynecology, Fig. 7.8, pg. 160).

The endothelial disturbance within the placenta will influence platelet aggregation, vasospasm and thrombosis. The balance between endothelial release of vasoconstrictors such as endothelin and thromboxane, and vasodilators

such as prostacyclin, is modified in preeclampsia in favour of vasoconstriction. The combined outcome of the above changes is a decline in uteroplacental perfusion, predominantly later in pregnancy. This is believed to trigger the release of factor(s) into the maternal circulation (Belfort *et al.*, 2002).

1.4.3 *CIRCULATING FACTORS*

There is widespread information that implicates the presence of a circulating factor obtained from the placenta that targets the endothelial cell in preeclampsia and stimulates widespread activation. The theory and the nature of the circulating factor(s) and its association to preeclampsia has been examined extensively in a variety of in vivo and in vitro studies. An early study to examine the existence of circulating factors was documented over two decades ago and is extensively recognized for revitalizing the concept of toxaemia (Rodgers *et al.*, 1988) of pregnancy. This study demonstrated that serum obtained from women with preeclampsia was cytotoxic to cultured human umbilical vein endothelial cells (HUVECS) when compared with control sera from normotensive pregnant women. The cytotoxic effect of serum obtained before delivery was greater than that collected postpartum; this swift disappearance of cytotoxic activity suggested that the supposed factor(s) had a short half-life and provided confirmation that the factors were linked to products of conception. This cytotoxic effect was also demonstrated when a combination of antenatal sera from normal pregnant women and women with preeclampsia was used, thus lending credence to the existence of an active circulating factor as opposed to a relative absence of a protective factor (Belfort *et al.*, 2002). It has now been established that the circulating factor(s) induce an alteration in endothelial function instead of gross morphological injury and cell death, as was originally proposed (Tsukimori *et al.*, 1992). A number of circulating factors with precise characteristics have been implicated as precursors for this role.

These characteristics include the ability to pass liberally into the maternal circulation, amplify cellular permeability and probably cell turnover, modify prostacyclin and nitric oxide production and promote a functional change in the response to endothelium-dependent agonists (Belfort *et al.*, 2002).

1.4.3.1 Vascular Endothelial Growth Factors (VEGFs)

VEGFs comprise a family of glycoproteins that comprise of five VEGF isoforms and the homologous placental growth factor. They express their biological activity of advancing blood vessel permeability, endothelial cell growth, and angiogenesis by binding to one of two receptors: foetal liver tyrosine-like (flt-1) receptor and a kinase domain receptor (Terman *et al.*, 1992; Vaisman *et al.*, 1990). VEGF has a hydrophobic secretory signal sequence and makes use of an in vitro effect specific to vascular endothelial cells at concentrations comparable to circulating levels found in vivo (Ferrara *et al.*, 1992). VEGF has been shown to stimulate an increase in vascular permeability through the protein kinase C pathway, an ability which has been demonstrated by plasma from women with preeclampsia (Haller *et al.*, 1998).Varying reports have been documented with regards to the concentration of circulating VEGF levels in women presenting with preeclampsia. Several studies have reported that serum VEGF levels are significantly elevated in patients with preeclampsia (Baker *et al.*, 1995; Sharkey *et al.*, 1996). However, Lyall *et al.,* (1997) measured serum VEGF concentrations in women with preeclampsia, normotensive pregnant women and in non-pregnant controls. They reported that levels were significantly lower in normotensive pregnant women than in non-pregnant women and was further reduced in preeclampsia. An explanation for the obvious inconsistency was offered by the demonstration that quantification of VEGF in pregnancy is influenced by interferences from binding proteins.

The origin of the elevated circulating levels of VEGF in pregnancies complicated by preeclampsia is still uncertain. However, it has been documented that VEGF

is expressed in placental tissue and when trophoblast cells are cultured in hypoxic conditions, VEGF production is increased (Belfort *et al.*, 2002). Although, studies on placental tissue are unclear; some have demonstrated that the expression of VEGF mRNA is decreased in pregnancies complicated by preeclampsia (Lyall *et al.*, 1997), whereas others, using immunohistochemical techniques have reported that staining for VEGF is increased in pregnancies complicated by preeclampsia (Simmons *et al.*, 2000). On the other hand, VEGF production may increase in response to vascular endothelial cell injury as it has recently been demonstrated that VEGF produced in vascular smooth muscle may contribute to the commencement of endothelial repair (Tsurumi *et al.*, 1997). As endothelial cell damage is common in preeclampsia, this may result in an increase in both local and circulating concentrations of VEGF. Probably, this implies that the increased levels of VEGF detected in preeclampsia may participate in the pathogenesis of vascular damage.

1.4.3.2 Neurokinin B

Neurokinin B is a neuropeptide and one of three known mammalian tachykinins. The tachykinins are normally confined to the nervous tissue and exert their effects peripherally by release from nerve endings and activation of the neurokinin receptors, NK1, NK2, and NK3. Neurokinin B binds preferentially to the NK3 receptor, activation of which has been demonstrated to stimulate hypertension through contraction of the rat portal vein and mesenteric vasculature (D'OrleansJuste *et al.*, 1991) and amplification of canine heart rate (Thompson *et al.*, 1998). It has been documented that the syncytiotrophoblast of the human placenta expresses neurokinin B mRNA (Page *et al.*, 2000). Additionally, it has been shown that plasma levels of the peptide are significantly increased in women with preeclampsia in comparison to the levels in normal pregnant women (Belfort *et al.*, 2002).

Page *et al.,* (2000) theorized that, in response to placental ischemia resulting from defective trophoblastic invasion, placental production of neurokinin B elevates, in order to increase blood pressure and correct the hypoperfusion of the fetoplacental unit. The succeeding stimulation of the neurokinin B receptors is hypothesized to result in constriction of the mesenteric vascular bed and the portal veins, leading to an elevation in blood pressure, damage to the liver and kidneys and the symptoms of abdominal pain. Decline in blood flow to the liver is presumed to lead to a buildup of undetoxified metabolites, such as lipid peroxides, which may enhance endothelial cell damage and dysfunction. Also, Page *et al.,* (2000) suggested that in severe cases of preeclampsia, levels of neurokinin B may be adequate to stimulate peripheral NK1 receptors on platelets and neutrophils and thus contribute to the other symptoms of preeclampsia linked with activation of these cells. Futhermore, it has been hypothesized that increased secretion of neurokinin B may possibly predate the advancement of clinical signs and symptoms of preeclampsia and thus elevated levels of neurokinin B in early pregnancy may categorize pregnancies likely to develop preeclampsia (Belfort *et al.*, 2002). However, the findings of their study await corroboration or refutation in longitudinal studies.

1.4.3.3 The Role of Oxidative Stress and Lipid Peroxides

Oxidative stress is a pathological state in which pro-oxidants dominate over antioxidants. The ensuing rise in the formation of reactive oxygen species can damage cell membranes, proteins and DNA. These observations have led to the budding hypothesis signifying that reduced placental perfusion generates oxidative stress and leads to general endothelial dysfunction in preeclampsia. Wang & Walsh (1996) have shown that enzyme activities and mRNA expression of the placental antioxidant enzymes superoxide dismutase and glutathione peroxidase were significantly decreased in placentas from women with pregnancies complicated by preeclampsia in contrast to those from normotensive pregnant women. This presupposes that there will be an irregular increase in

placental production of lipid peroxides in preeclampsia. In addition, indicators of lipid peroxidation, including malondialdehyde (Hubel *et al.*, 1996) and 8epiprostaglandin-F2 (Barden *et al.*, 1996) are elevated in the plasma of women with preeclampsia.

Additionally, it has been reported that both the water soluble antioxidant, ascorbic acid and the lipid-soluble antioxidants alpha tocopherol and beta-carotene levels are decreased in the plasma of women with preeclampsia compared to those of normal pregnant women. This suggests that antioxidant nutrients may be overutilized in preeclampsia to counteract free radical–mediated cell disturbances, resulting in a reduction in antioxidant plasma levels in this pathology (Mikhail *et al.*, 1994). The amplification in lipid peroxide formation by the placenta in preeclampsia may account for several of the pathological changes observed in the diseased condition. Lipid peroxides formed at a primary site may build up in lipoproteins and be transported throughout the circulation due to their higher halflife. Lipid peroxides have in addition been noted to stimulate smooth muscle contractions in an array of isolated arterial preparations. Furthermore, elevation of circulating levels of lipid peroxide products induced by deprivation of vitamin E in rats produced an increased pressor responsiveness to angiotensin II and a decrease disolated mesenteric artery relaxation to acetylcholine (Hubel *et al.*, 1989a).

1.4.4 *PLACENTAL MALARIA AND HYPERTENSIVE PREGNANCY*

Malaria and hypertension are two common diseases of pregnancy in the tropical areas of Africa. These two conditions have physiopathologic similarities such as placental ischemia, endothelial dysfunction, and production of proinflammatory cytokines (Challier & Uzan, 2003). As result of these similarities, a probable association between malaria and hypertension in pregnancy has been proposed. Wickramasuriya in 1936, reported an ‘‘epidemic of toxaemic pregnancies

(preeclampsia) subsequent to the wake of the malaria epidemic'' (Wickramasuriya, 1936).

Placental parasitaemia is characterized by sequestration of parasites in the placenta, which results in placental ischemia, elevated production of proinflammatory cytokines, and increases in endothelial dysfunction cytokines (Challier & Uzan, 2003). A probable mechanism is that Placental Malaria Infection (PMI) leads to placental ischemia and loss of placental integrity, which results in endothelial cell dysfunction and cytokine activation (Brabin & Johnson, 2005).

Brabin (1983) reported that placental parasitaemia occurs twice as often in first pregnancies compared to later pregnancies in women living under conditions suitable for malaria transmission. Indeed, the placenta has been implicated in the pathogenesis of preeclampsia, and preeclampsia is a key cause of maternal mortality in both developed and developing countries (Harrison, 1985), preeclampsia results in intrauterine growth restriction (Roberts & Lain, 2002). Placental malaria is also known as a leading cause of foetal growth restriction and increasing maternal mortality (Brabin *et al.*, 2004b). Inspite of their health implications, little data is obtainable with regards to their relationship.

Preeclampsia is generally characterized by a shallow, incomplete endovascular cytotrophoblast invasion, inadequate uterine arterial transformation, maternal endothelial cell dysfunction which can be systemic, and a partner-specific protective effect (Roberts & Lain, 2002). Brabin, (1983) has indicated that the peak prevalence of P. *falciparum* parasitaemia occurs between 13 and 18 weeks gestation in women living under holoendemic environment for malaria. This corresponds to the time when endovascular cytotrophoblastic invasion of spiral arteries occurs, as remodelling is frequently complete by 20–22 weeks gestation (Pijnenborg *et al.*, 1981). In addition to occlusion caused by retention of parasitized erythrocytes, PMI occurring at this time may interfere with normal

cytotrophoblast invasion and transformation of the maternal vasculature, thus leading to impaired placental development and function (Brabin & Johnson, 2005).

Dorman *et al.,* (2002) utilized continuous wave Doppler ultrasound to establish whether abnormal uterine artery flow velocity waveforms, indicative of failed trophoblast invasion, were associated at 32–35 weeks gestation with malaria infection. Abnormal waveforms were indeed significantly related to malaria after controlling for preeclampsia (relative risk 2.11; 95% CI, 1.24–3.59). These results suggest that uteroplacental haemodynamics were altered in the presence of falciparum malaria, and that a preeclampsia-like process had taken place. Both preeclampsia and PMI have been associated with an enlargement of the placenta (Brabin *et al.*, 2004b; Coonrod *et al.*, 1995), and may contribute to reduced placental perfusion (Roberts & Lain, 2002). Reduced placental perfusion is consequently a common link between malaria and preeclampsia and, for both these conditions, several factors related to trophoblast migration and invasiveness can be implicated (Brabin & Johnson, 2005).

Maternal endothelial dysfunction is a typical feature of preeclampsia, but whether it is a cause or consequence of the disorder, is still uncertain (Brabin & Johnson, 2005). Women presenting with preeclampsia have increased levels of numerous markers of endothelial activation (Roberts & Lain, 2002), several of which are vasoconstrictors and procoagulants advancing the formation of microthrombi. Some researchers have put forward that preeclampsia represents a pregnancyinduced inflammatory response leading to maternal endothelial cell dysfunction (Meekins *et al.*, 1994; Redman *et al.*, 1999). Circulatory factors released secondary to poor placental perfusion may improve this response which is characterized by a more pronounced Th1 and innate immune phenotype, including raised levels of IL-12 and tumour necrosis factor-alpha (TNF-α) (Saito & Sakai, 2003; Sargent *et al.*, 2003), comparable to that observed in placental

malaria (Fievet *et al*., 2001; Fried *et al*., 1998). Therefore, abnormal placentation (a conditioning factor) interrelates with an inflammatory factor (a precipitating factor) to cause the syndrome (Lala & Chakraborty, 2003). This suggests that placental pathology alone is inadequate to induce preeclampsia without the added occurrence of a precipitating factor(s) (Roberts & Lain, 2002).

Other precipitating factors leading to endothelial activation have been suggested, principally, oxidative stress and the production of reactive oxygen species (Roberts & Hubel, 1999). The essential role of oxidative stress and inflammatory cytokines is now well established in the host response to infection, including malaria. The malperfused placenta may participate in the generation of these reactive oxygen species. Generation of the highly reactive hydroxyl ion (OH^-) radical is reliant on iron availability, and extracellular haemoglobin released during malaria haemolysis would augment release of iron and generate free haem and lipid hydroperoxides (Brabin & Johnson, 2005).

The vascular endothelium of the placenta could be a vital association between sequestered adhering P. *falciparum* parasites and induction of preeclampsia in women with poor placental perfusion. Loss of endothelial cell integrity associated with parasite adhesion could stimulate endothelial cell activation. Increased endothelial leakage has been illustrated in preeclampsia in non-malarious areas (Campbell & Campbell, 1983) and umbilical cord parasitaemia, signifying placental leakage, is well described in placental malaria (Tobian *et al*., 2000). The incidence of malaria-associated foetal anaemia (Brabin *et al*., 2004a) could also relate to loss of endothelial cell integrity. Malarial placental endothelial cell dysfunction related to high parasite densities might predispose to preeclampsia in some pregnant women. The swift development of the endothelial anomaly could explain the involvement of the more severe form of the disease, eclampsia, in women with malaria but who have low malarial immunity (Wickramasuriya, 1936). Amplified shedding of syncytiotrophoblastic debris,

which may be a provoking stimulus for preeclampsia (Sargent *et al.*, 2003) would be expected to be significantly increased in placental malaria. Acute symptomatic malaria usually occurs only amongst women with low pre-pregnancy malarial immunity, whereas most women living under holoendemic malarious conditions are asymptomatic during pregnancy, despite placental infection. The level of acquired systemic malarial immunity before pregnancy could sway the probability of preeclampsia, as well as the extent of placental involvement (Brabin & Johnson, 2005).

1.4.5 *MATERNAL FACTORS*

The abnormal placentation that results from failure of trophoblast remodeling of uterine spiral arterioles is thought to lead to the release of secreted factors that go into the maternal circulation, resulting in the clinical signs and symptoms of preeclampsia. All of the clinical symptoms of preeclampsia can be attributed to glomerular endotheliosis, increased vascular permeability, and a systemic inflammatory response that results in end organ damage and/or hypoperfusion. These clinical manifestations usually occur after the 20th week of pregnancy (Hladunewich *et al.*, 2007). Cellular and molecular constituents of the immune system may play a central role in the control of normal trophoblast invasion (Redman *et al.*, 1999), and abnormal maternal immune response to the invading trophoblast has been considered a contributory factor for shallow trophoblast invasion (Redman *et al.*, 1999)

Accommodation to normal pregnancy includes a decrease in both systolic and diastolic blood pressure as a result of a decrease in systemic vascular resistance secondary to vasodilation (Hladunewich *et al.*, 2007). Relaxin, released from the ovaries under the control of Human Chorionic Gonadotrophin, upregulates nitric oxide synthase (NOS) (Fujiyama *et al.*, 2001). NOS, is the enzyme that produces nitric oxide (NO) from arginine, through the endothelial endothelin B receptor (Jeyabalan *et al.*, 2003). In preeclampsia, derangement of endothelial-derived

vasoactive factors is believed to result in the predominance of substances that are vasoconstrictors (endothelin, thromboxane A2) over vasodilators (NO, prostacyclin). Hypertension, defined as repeat blood pressure measurements ≥140/90 mmHg, may result from abnormal vasoconstriction (Hladunewich *et al.*, 2007). It is therefore possible that the hypertension as seen in hypertensive pregnancy may be a result of abnormalities such as abnormal vasoconstriction.

Essential hypertension has consistently been associated with increased risk of preeclampsia (Sibai *et al.*, 1998; Sibai *et al.*, 1983), and as measured at first antenatal visit, increased blood pressure within the normal range, also increases the risk (Eskenazi *et al.*, 1991; Sibai *et al.*, 1997; Sibai *et al.*, 1995; Stone *et al.*, 1994). Metabolic disorders, including obesity, dyslipidaemia, insulin resistance and diabetes (Ahenkorah *et al.*, 2008; Kaaja *et al.*, 1999; Sibai *et al.*, 1995; Stone *et al.*, 1994; Turpin *et al.*, 2008) are all associated with preeclampsia. Also, increased frequency of thrombophilic disorders is also observed in preeclampsia (van Pampus *et al.*, 1999). Recently, several authors have reported increased frequency in preeclampsia of certain genotypes engaged in vascular disease and remodelling, volume regulation, and blood pressure control. Candidate genes comprise the T235 allele for angiotensinogen, factor V Leidenmutation, and homozygosity for methylene tetrahydrofolate reductase (Dizon-Townson *et al.*, 1996; Kupferminc *et al.*, 2000; O'Shaughnessy *et al.*, 1999). Although some of these associations have been difficult to reproduce (Grandone *et al.*, 1997; Morgan & Ward, 1999) it has been proposed that genes involved in circulatory and metabolic regulation may also be involved in the development of preeclampsia (Morgan & Ward, 1999).

1.4.6 *GENETIC FACTORS*

A familial factor has been documented in the pathogenesis of preeclampsia for several years. 15A familial gene factor predisposition for preeclampsia has been documented (Cincotta & Brennecke, 1998) confirming that genetic factors may

contribute to its development. Epidemiological studies have also established a 3 to 4 fold increase in the incidence of preeclampsia in first-degree relatives of affected women (Arngrimsson *et al.*, 1990; Chesley & Cooper, 1986). The risk of preeclampsia may be two-fold higher among daughters of women who have experienced the disease in contrast to women without history of preeclampsia (Mogren *et al.*, 1999). Additionally, in women with severe preeclampsia, the prevalence of preeclampsia and eclampsia among their daughters was significantly higher than among their daughters-in-law (Arngrimsson *et al.*, 1990). Thereby a mother to daughter genetic predisposition is suggested, which proposes a model of mitochondrial inheritance of preeclampsia, as put forward by Folgero *et al.,* (1996).

On the other hand, the single-gene model of inheritance that best explains the overall frequency of preeclampsia is the presence of homozygosity for the same recessive gene both in the mother and the foetus (Liston & Kilpatrick, 1991). Accordingly, these foetuses would be homozygous for the recessive allele, which they would pass to their male and female offspring. Thus, both male and female offspring would share an increased risk of having a pregnancy complicated by preeclampsia. Furthermore, in a study among offspring of cases of preeclampsia that included all degrees of severity, both males and females were more likely than controls to have a child that was the product of a preeclamptic pregnancy (Esplin *et al.*, 2001). A population-based study in Norway among men who had children with more than one woman give additional support to the observation of a paternal contribution to the risk of preeclampsia. This study showed that men who fathered a child with a woman whose pregnancy was complicated by preeclampsia were nearly twice as likely to experience preeclampsia with another woman as men without such history (Lie *et al.*, 1998).Thus, there are suggestions that the maternal and paternal contribution to the inheritance of preeclampsia may vary according to the severity of the pathology.

Overall, a variety of conditions may herald the syndrome of preeclampsia, and most likely preeclampsia results from a combination of placental, immunologic, maternal, paternal, modifier genes and environmental factors. Still, there is little information as to whether these diverse conditions are associated with specific clinical manifestations of the syndrome. However, if specific patterns of associations exist between maternal risk factors and clinical subtype of preeclampsia, this may help in clarifying the underlying heterogeneous pathogenesis (Zhang *et al.*, 1997).

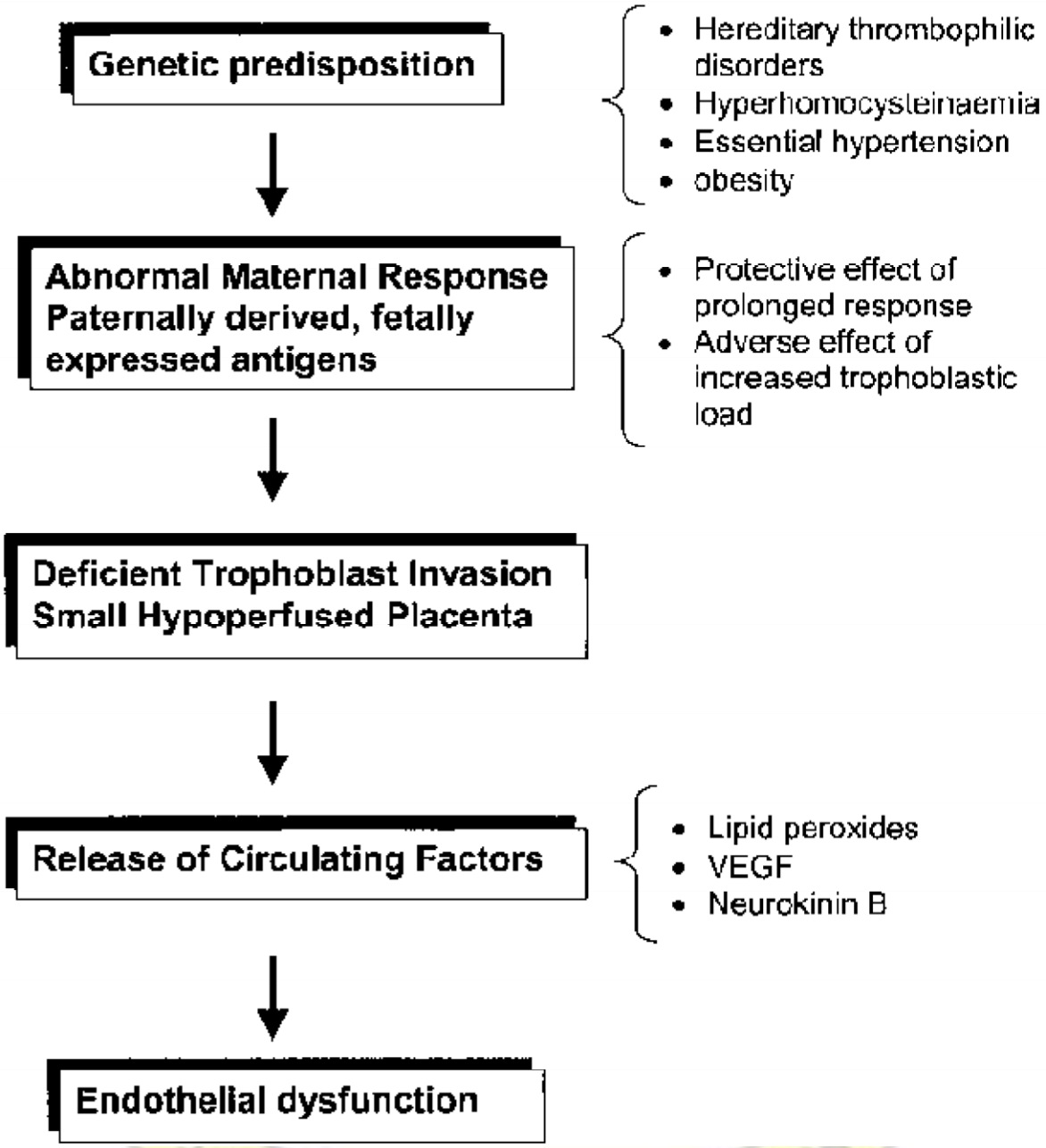


Figure 1.2 The proposed pathogenesis of preeclampsia.

Source: Belfort, Thornton & Saade, The Aetiology of Preeclampsia: In Hypertension in Pregnancy, 2002.

1.4.7 *ENDOTHELIUM INVOLVEMENT*

Indications for endothelial participation in preeclampsia abounds. The best characterized morphological abnormality of this syndrome, once assumed to be pathognomonic of the condition, glomerular endotheliosis, involves endothelial cells (Spargo *et al.*, 1959). Preeclampsia is associated with a loss of endothelial cell integrity and a resultant increase in vascular permeability (Campbell & Campbell, 1983). There is also overabundance of data from the study of circulating endothelial cell markers in preeclampsia. Levels of fibronectin, factor VIII antigen, von Willebrand factor, tissue plasminogen activator, and plasminogen activator inhibitor-1 have been found to be elevated in the circulation of women with preeclampsia (Campbell & Campbell, 1983; Friedman *et al.*, 1995). Although these substances are synthesized by several cell types, they are all produced in the vascular endothelium. Furthermore, several studies have reported increased levels of circulating endothelin-1 in preeclampsia (Mastrogiannis *et al.*, 1991; Taylor *et al.*, 1990), which may be a sign of increased synthesis by activated endothelial cells (MacCumber *et al.*, 1989; Yoshimoto *et al.*, 1990).

An intact endothelium is a vital component of the coagulation system (Roberts & Redman, 1993). More sensitive indicators of coagulation abnormalities are present in a high proportion of women with this disorder and include an altered ratio of factor VIII–related antigen to coagulation activity (Redman *et al.*, 1977), a reduction in the platelet count (Redman *et al.*, 1978) and an increase in the levels of plasma βthromboglobulin. One of the most striking and consistent pathophysiological abnormalities of women with preeclampsia is an increased sensitivity to pressor agents such as angiotensin II (Gant *et al.*, 1973). The mechanism underlying both the suppressed response to pressor agents in normal pregnancy, and the increased sensitivity observed in preeclampsia is yet to be fully elucidated. However, there is well-documented evidence that endothelium-

dependent relaxation of resistance in arteries is impaired in preeclampsia. The vascular endothelial cell appears to be the target of the disease process in preeclampsia. A variety of studies have reported evidence of widespread endothelial cell activation and impaired endothelialdependent responses (Belfort *et al.*, 2002). Many of the clinical manifestations of preeclampsia can be accounted for by activation of this cell type. However, the exact nature of the activation and subsequent function of the endothelial cell in vivo has not been fully elucidated (Belfort *et al.*, 2002).

1.4.8 *IMMUNOLOGICAL FACTORS*

The fundamental cause for the failure of trophoblast invasion in preeclampsia is unidentified. It has been suggested that disordered placentation might be a sign of an abnormal maternal immunological response to foetal antigens derived from the father. This has been suggested due to the increased prevalence of preeclampsia in multiple pregnancies, molar pregnancies, and those associated with increased placental mass which implies that foetal antigen load and trophoblast volume have a pathological role in this disorder (Taylor, 1997). Available widespread epidemiological evidence to associate immunological factors in the aetiology of preeclampsia and other studies have confirmed this observation and furthermore have demonstrated that the association does not hold for nulliparity and gestational hypertension (Campbell *et al.*, 1985; Misra & Kiely, 1997). This might indicate that preeclampsia and gestational hypertension are different disorders with differing aetiologies.

Research has revealed that change in partner increases the prevalence of preeclampsia in multigravid women. In a cohort study conducted by Li & Wi (2000) based on 140,147 women with two consecutive births during 1989–1991 the following observations were made. Among the women without any history of preeclampsia in the first birth, changing partners resulted in a 30% increase in the risk of preeclampsia in the subsequent pregnancy when compared to those

who did not change partners. However, among women with preeclampsia in the first birth, changing partners resulted in a 30% reduction in the risk of preeclampsia in the subsequent pregnancy. These findings corroborate the hypothesis that a normal pregnancy is a sign of a state of tolerance to the foreign paternally derived antigens of the foetus, whereas in preeclamptic women, this immunological tolerance is impaired (Belfort *et al.*, 2002).

Similarly, some studies have revealed that the occurrence of preeclampsia may be related to the duration of prior exposure to paternal antigens in sperm. It has been suggested (Morcos *et al.*, 2000) that the use of barrier methods of contraception which reduces exposure to sperm and seminal fluid are associated with an increased risk of developing preeclampsia during the subsequent pregnancy. Whereas, oral sex, and the swallowing of seminal fluid is correlated with a reduced incidence of preeclampsia (Koelman *et al.*, 2000). This implies that during a protracted sexual relationship, women develop an immune response against paternal antigens expressed on spermatozoa or in seminal fluid, which is possibly impaired in women using barrier methods of contraception and enhanced by oral exposure. This thus, induces a tolerance that immunologically protects against exposure to paternally derived foetal antigens in subsequent pregnancies (Belfort *et al.*, 2002).

1.5 BIOCHEMICAL ASPECTS OF HYPERTENSIVE PREGNANCY

1.5.1 *THE HELLP SYNDROME*

Over the past 20 years a cohort of women with severe preeclampsia presenting with haemolysis, elevated liver function tests and thrombocytopenia (low platelets) has come to be widely known as having the HELLP syndrome (Weinstein, 1982). These groups of women are often symptomatic, often presenting with upper abdominal pain, nausea, and vomiting (Belfort *et al.*, 2002). Although multiple specific thresholds of serum chemistry abnormality exist for the HELLP syndrome, the transaminase elevations are characteristically mild to

moderate (>10 times normal), there is evidence of haemolysis (elevated lactate dehydrogenase and bilirubin), and platelet counts are generally below 100,000/mm^2. On liver biopsy, the HELLP syndrome is characterized by deposition of fibrin in the periportal regions and sinusoids, focal parenchymal necrosis, but no inflammatory infiltration (Belfort *et al.*, 2002; Rolfes & Ishak, 1986). HELLP syndrome is a serious pregnancy disorder characterized by laboratory evidence of abnormal interaction between the microvasculature and circulating constituents of blood. This produces thrombocytopenia, microangiopathic haemolytic anaemia, and the release of cellular breakdown products, particularly from the liver, including transaminases and lactate dehydrogenase (Belfort *et al.*, 2002).

Basically, there are three general diagnostic criteria for the laboratory diagnosis of HELLP syndrome (Magann & Martin, 1995; Sibai, 1990a). Initially, most patients show microangiopathic changes, which are evident on peripheral bloodsmear but do not essentially, correlate with the clinical signs of disease severity such as degree of hypertension and/or proteinuria. The fragmented red cells observed are termed schistocytes, burr cells, and fragmentocytes. The extent of microangiopathic haemolytic anemia seems to correlate with the extent of small vessel participation and the subsequent endothelial dysfunction. As a result of the haemolysis, serum indirect bilirubin and total lactate dehydrogenase increase and serum haptoglobin decreases (Hamm *et al.*, 1996; Martin *et al.*, 1991) in these women.

The presence of elevated liver enzymes is the second criterion for HELLP syndrome and it is indicative of hepatic cell injury and dysfunction. Much attention is paid to elevated transaminases for this criterion, principally aspartate aminotransferase (AST) (Kew, 2000). Both AST and alanine aminotransferase (ALT) are present in high concentration in hepatocytes, leaking into the circulation when hepatocytes or their cell membranes are damaged (Kew, 2000).

ALT is the more specific marker of hepatocellular injury, since it is confined to the cytoplasm of hepatocytes. Serum concentrations of AST and ALT are raised in nearly all forms of liver disease. Serum levels are highly variable and are determined by the intracellular concentration and source of the enzymes, the amount leaked from the cell, and the rate of clearance from the circulation. Serum concentrations are thus not necessarily a reliable index of the severity of hepatic injury, and neither of the aminotransferases alone is an ideal marker of hepatocyte injury. During active HELLP syndrome the ratio of AST to ALT is often 2:1 or higher, whereas with disease recovery the ratio returns to 1:1 or even reverses (Belfort *et al.*, 2002; Martin *et al.*, 1991).

The third criterion for HELLP syndrome is thrombocytopenia. It is the quickest laboratory change of the disease that can be detected by most available laboratory testing (Martin *et al.*, 1991). The accepted definition of thrombocytopenia is a platelet count ≤150,000/mL, subdivided into three subgroups of 100,000 to 150,000/mL for mild thrombocytopenia, 50,000 to 100,000/mL for moderate, and ≤50,000/mL for severe thrombocytopenia. Nonetheless, a threshold of ≤100,000/mL is also diagnostic of HELLP syndrome. This is partly to distinguish HELLP syndrome from the more benign gestational thrombocytopenia, and in part because the threshold for significant threat to the mother and foetus with HELLP syndrome most likely exists at a platelet count <100,000/mL (Sibai, 1990a).

Inspite of the extensive knowledge about the HELLP syndrome, it is not the only cause of hepatocellular dysfunction in late pregnancy. Severe folic acid deficiency has been confused with HELLP syndrome and has resulted in preterm delivery with poor neonatal outcome. In megaloblastic anaemia associated with early pregnancy anaemia, thrombocytopenia, elevated liver enzymes, and abdominal pain, a bone marrow evaluation maybe required to make the diagnosis. This is

because in severe disease, red cell folate levels may lag behind the clinical course, and if there is concomitant iron deficiency megaloblastosis may be masked (Walker *et al.*, 1997).

1.5.2 *ELECTROLYTES AND HYPERTENSIVE PREGNANCY*

Some studies have proposed that irregularities of intracellular cation metabolism may take part in the pathophysiology of preeclampsia (David-Dufilho *et al.*, 1992; Sanders *et al.*, 1999). Although, most biochemical and haematological laboratory parameters change during pregnancy, data on cation patterns during pregnancy are conflicting mainly due to variations in methods, and most reports have examined very few cations in preeclampsia (Ebose *et al.*, 2007).

The idea of derangements in intracellular pH, calcium and magnesium ([Ca2+]i and [Mg2+]i respectively) homeostasis being involved in the pathogenesis of hypertensive pregnancy, derives in part from the established roles of these cations in contractility of smooth muscles and in cellular energy metabolism. The exact nature of their involvement however, remains speculative (Ebose *et al.*, 2007).

1.5.2.1 INTRACELLULAR CALCIUM

Available literature on the role of plasma calcium in the aetiology of pregnancyinduced hypertension has been inconsistent. Whereas some studies suggested a decrease, others found no change (Haller *et al.*, 1989; Hojo *et al.*, 1999; Zemel, 1990). Futhermore, several irregularities in calcium metabolism have been described in women with preeclampsia (Hojo *et al.*, 1999; Kisters *et al.*, 1998). Singh *et al.,* (1993) reported that serum calcium was significantly reduced during the third trimester in women with pregnancy-induced hypertension. Ionized calcium was also found to be significantly lower in hypertensive women as compared to that in normotensive women (Varner *et al.*, 1983). Attention has been drawn to the need to find a reasonable explanation for the role of calcium in

preeclampsia over the past few years especially because such disturbances in calcium metabolism have not been linked to chronic hypertension (Kosch *et al.*, 2000; Krari & Allain, 1991). Hojo *et al.,* (1999) reported an increased [Ca2+]i of lymphocytes in preeclampsia but not in chronic hypertension and suggested that a calcium deficit leading to an increased intracellular free calcium concentration during late pregnancy contributes to the pathogenesis of preeclampsia. These dramatic changes in plasma calcium could be due to sequestration of Ca^{2+} in cell membranes and intracellular compartments of certain tissues in preeclampsia (Ebose *et al.*, 2007).

A significant increase in erythrocyte membrane calcium of preeclamptic women compared to healthy relevant controls was reported by Kosch *et al.,* (2000). Similarly, an increased calcium concentration has been observed in placental tissue from preeclamptic patients (Pitkin, 1985). The increased calcium in tissues of preeclamptics supports the concept of a peculiar alteration in calcium flux triggered by an event which could be related to the increased pressor activities in preeclampsia (Ebose *et al.*, 2007). Most probably lymphocytes, platelets, and some other cell types are induced to mobilize plasma Ca^{2+} into the cytoplasm in preeclampsia. Intracellular free calcium is an important second messenger which controls the initiation of numerous cellular and subcellular processes (Rasmussen, 1986) including vascular smooth muscle contraction, secretion, metabolism, neuronal excitability, cell proliferation and cell death. Ebose *et al.,* (2007) have suggested that the marked intracellular calcium elevation in preeclampsia might be due to a decrease in intracellular cyclic adenosine mono-phosphate (cAMP).

1.5.2.2 MAGNESUIM

Despite the fact that, much is known regarding the pathways responsible for the regulation of intracellular calcium, the factors regulating magnesium remain poorly defined (Oshima *et al.*, 2000). Magnesium participates in the maintenance

of cellular ionic balance, a role it performs in union with other ions such as sodium, potassium, and calcium (Gums, 2004). Villanueva *et al.,* (2001) found that abnormalities in the calcium and magnesium metabolism may participate in the pathogenesis of preeclampsia. Studies have shown that increases in extracellular magnesium modulate the platelet regulation of cytosolic calcium through inhibition of the $Na+/Ca^{2+}$ exchanger. Kisters *et al.,* (2000) reported a markedly lowered cellular phosphate and magnesium concentrations and an altered ATP metabolism in aortic smooth muscle cells, suggesting a membrane defect or a magnesium deficit in hypertensive cells. Indeed, before the use of the magnesium therapy for treatment of preeclampsia, changes in circulating magnesium and calcium levels in women with preeclampsia have been reported (Ganzevoort, 2002;

Halhali *et al.*, 2001).

Although, some studies have reported that magnesium levels in hypertensive pregnancies and normal pregnancies do not differ significantly (Handwerker *et al.*, 1995; Kashyap *et al.*, 2006; Sanders *et al.*, 1999), others have reported that serum magnesium levels could be significantly elevated in preeclamptic women relative to women with uncomplicated pregnancy (Villanueva *et al.*, 2001). However, most findings support the hypothesis that low levels of magnesium may possibly play a role in the aetiology of preeclampsia (Sukonpan & Phupong, 2005). A deficiency of intracellular Mg^{2+} in muscle tissue may be associated with impaired glucose utilization, and if such defect also exists in resistant blood vessels, the elevated resistance may lead to increased blood pressure (Ng *et al.*, 1992) as observed in hypertensive pregnancies. In a study conducted by Ebose *et al.,* (2007), the finding of low levels of magnesium in the smooth muscle tissue of preeclamptic rats as compared to pregnant controls coupled with a significant increase in systolic blood pressure in the preeclamptic rats, corroborates this hypothesis.

1.5.2.3 SODIUM AND CHLORIDE

High sodium intake has been reported to be associated with higher blood pressure levels among persons consuming low-calcium diets especially (Hamet *et al.*, 1991). The impact of dietary NaCl on blood pressure may be affected by consumption of potassium or calcium. The urine sodium-potassium ratio is a stronger correlate of blood pressure than either sodium or potassium alone (INTERSALT Cooperative Research Group., 1988; McCarron *et al.*, 1984). Elevated intracellular sodium content has been documented in preeclampsia (Forrester & Alleyne, 1980). Kashyap *et al*. (2006), have indicated there is a highly significant elevation in the plasma sodium level in the preeclamptic patients as compared to uncomplicated pregnancies. However, according to Matsumoto *et al.,* (1998) and Brown *et al* (1988) the serum concentrations of sodium are lowered in preeclampsia. Indeed, high sodium intake has been reported to be associated with higher blood pressure, however, the mechanisms by which salt intake raises the blood pressure remains speculative (de Wardener *et al.*, 2004). It has been suggested that an acute increase in sodium input can induce a rise in blood pressure by either raising plasma sodium even when the extracellular fluid volume (ECV) is falling (Friedman, 1990a; Friedman, 1990b), or by increasing the ECV even when the plasma sodium is falling (de Wardener *et al.*, 2004). This implies that severe alterations in plasma sodium and ECV are individually capable of separately controlling the blood pressure. Although the change in ECV may have a pressor effect, the associated rise in plasma sodium itself may also cause the blood pressure to rise (de Wardener *et al.*, 2004). In an experiment conducted on spontaneously hypertensive rat (SHR) an increase in sodium concentration > 5 mmol/L induces pressor effects on the brain and on the renin-angiotensin system. Such a rise can also induce changes in cultured vascular tissue similar to those that occur in the vessels of human and animal models on a high sodium diet, independent of the blood pressure, suggesting that a small

increase in plasma sodium may be part of the mechanisms whereby dietary salt increases the blood pressure (de Wardener *et al*., 2004).

Some studies have also shown that dietary salt also increases the mass of the left ventricular wall, stiffens conduit arteries (Safar *et al*., 2000), thickens and narrows arteries (Illyes *et al*., 2000) independent of the blood pressure. Hypertension results from the interplay of internal derangements (primarily in the kidney) and the external environment. The inability of the kidney to excrete sodium exacerbates the rise in arterial pressure and other damaging cardiovascular effects of dietary salt (de Wardener *et al*., 2004). The impairment of the kidneys can either be genetic, as seen in essential hypertension and the spontaneously hypertensive rat (SHR) (Woolfson, 1990), or it can be superimposed, as seen in obesity, (Hall, 2003) or renal disease.

1.5.2.4 POTASSIUM

Potassium, the main intracellular cation, has usually been viewed as a minor factor in the pathogenesis of hypertension. However, abundant evidence indicates that a potassium deficit has a critical role in hypertension and its cardiovascular sequelae (Adrogue & Madias, 2007). Available literature gives indications of varying levels of plasma potassium and hypertensive disorders of pregnancy. While, some reports failed to show any significant difference (Kashyap *et al*., 2006; Singh, 1993), others report elevation of plasma potassium in these subjects. Handwerker *et al*., (1995) also reported a highly significant elevation in the plasma K+ level and the K+/IMg2+ ratio in preeclamptic patients as compared to uncomplicated pregnancies.

Early reports of the vasodilatory or blood-pressure–lowering properties of both potassium depletion and potassium supplementation (Meneely *et al*., 1957) delayed the recognition of the toxic effects of potassium depletion to the blood vessels (Adrogue & Madias, 2007). Potassium restriction causes a deficit in

cellular potassium which triggers cells to gain sodium in order to preserve their tonicity and volume (Adrogue & Madias, 2007). These deficits in potassium, sodium and chloride results in contraction of both the intracellular and extracellular compartments, thereby stimulating a decrease in blood pressure (Meneely *et al.*, 1957; Ray *et al.*, 1991). Studies in rats showed that the pressor effect of potassium depletion requires increased consumption of sodium chloride (e.g., 4.5 g of sodium chloride per 100.0 g of dietary intake) (Dahl *et al.*, 1972) to normalize the system. Additionally, population studies have shown an inverse relation of potassium intake to blood pressure, the prevalence of hypertension, or the risk of stroke (Vupputuri *et al.*, 2003).

1.6 INCIDENCE AND PREVALENCE OF HYPERTENSIVE DISORDERS OF PREGNANCY

There are varying reports on the incidence of hypertensive disorders of pregnancy worldwide and these incidence reports show great disparity. This may be attributable to differences in definition, population composition, demographic and obstetric characteristics, actual disease incidence, or access to and availability of antenatal care services (WHO, 1988). A population based international collaborative study designed to control for these factors found that clinically recognized hypertension during pregnancy varied by a factor of 25 (incidence range 1.2% to 31.0%) between countries. Even using a strict definition of proteinuric hypertension, the incidence varied by a factor of 5 (incidence range 1.5% to 8.3%) (WHO, 1988).

Hypertension complicating pregnancy (approximately 9% worldwide) has been reported to be associated with substantial maternal and perinatal morbidity and death, mostly because of preeclampsia (pure or superimposed on chronic hypertension) (Villar *et al.*, 2006). Pregnancy-Induced Hypertension complicates 510 % pregnancies in the United States and is a major cause of maternal, foetal and neonatal morbidity and mortality (Seely & Solomon, 2003). Gestational

hypertension has been reported to complicate between 4.4 and 17.5% of pregnancies, with a weighted mean of 14.6% (Hauth *et al.*, 1993; North *et al.*, 1999; Stone *et al.*, 1995). The reported incidence of preeclampsia varies between 3-10% (Mittendorf *et al.*, 1996; Redman & Jefferies, 1988; WHO, 1988) and some of this variation may be attributable to differences between study populations. In Tehran, an incidence of 3% for preeclampsia has been reported (Pyri *et al.*, 2001). In Sri Lanka, studies on hypertensive disorders of pregnancy have been reported to occur in 4.9% of pregnant women delivering in a tertiary care hospital (Jayawardana & Fernando, 1995). This study and another from the same hospital (Jayawardana & Lekamge, 1994), report the proportion of hypertensive women having preeclampsia (43.4% and 46.5% respectively) as being not much less than that of gestational hypertension (51.1% and 53.4% respectively). In Ghana, incidence of preeclampsia amongst pregnant women has been reported to be about
7.03% (Obed & Aniteye, 2006).

Nulliparity is believed to increase the risk of developing preeclampsia (CondeAgudelo & Belizan, 2000; Redman & Jefferies, 1988), thus the incidence reports would be strongly influenced by the parity of the subjects. However, the reported incidence of preeclampsia in nulliparous women also varies substantially, (Cnattingius *et al.*, 1997) and some of this variation may depend on the use of different diagnostic criteria for preeclampsia between studies (Brown & Buddle, 1997).

Similarly, eclampsia has been reported to occur in 0.049% pregnancies in the United Kingdom (Douglas & Redman, 1994), 0.056% pregnancies in United States of America (Saftlas *et al.*, 1990), 0.1% reported by (Zhang *et al.*, 2003), and in Finland 0.024% (Ekholm *et al.*, 1999), incidence of eclampsia in Tehran was 0.1% (Pyri *et al.*, 2001). The incidence is lower in Sri Lanka, where a 0.28% of pregnancies in Peradeniya in Central Sri Lanka (Jayawardana & Fernando, 1995),

0.38% of pregnancies in Galle in Southern Sri Lanka (Goonewardene & Sirisena, 1984-85), and 0.66% of pregnancies in Jaffna in the North of Sri Lanka (Jegasothy *et al.*, 1983) has been reported. The low prevalence of eclampsia is not unexpected; because not all preeclamptic women progress to the eclamptic stage.

1.7 SEASONAL VARIATIONS IN THE OCCURRENCE OF HYPERTENSIVE PREGNANCY

Seasonal factors and humidity are said to influence the incidence of hypertensive disorders of pregnancy (Bider *et al.*, 1991). A number of studies have revealed that the incidence of pregnancy-induced hypertension, preeclampsia and eclampsia is dependent on the season of delivery and or season of conception (Makhseed *et al.*, 1999; Phillips *et al.*, 2004). The hypothesis with seasonal variation is based on changes in temperature and humidity. In Ghana, more cases of eclampsia have been observed during the rainy season (Obed *et al.*, 1994). Neela *et al.*, (1993) supported the hypothesized association between increasing humidity and a lower temperature range with the increased incidence of eclampsia. Wacker *et al.*, (1998) in their study found an increased incidence of preeclampsia at the end of the dry season and in the first months of the rainy seasons. Magnus *et al.*, (2001) reported a systematic seasonal variability in the occurrence of preeclampsia with a peak in the winter months and a minimum in the summer. In contrast, Magann *et al*, (1995) and later Makhseed and co workers (1999) indicated no statistical correlation between preeclampsia and meteorological factors.

Conversely, Phillips *et al.*, (2004) identified a seasonal variation in preeclampsia that appears to be more strongly related to timing of conception than to the timing of delivery. Current studies on the seasonal occurrence of preeclampsia based on timing of delivery have been conducted in Zimbabwe, Norway, Kuwait and Israel. In Zimbabwe, a peak incidence of preeclampsia was found in women delivering in December and January. This period corresponded to the end of the dry season and the beginning of the rainy season (Wacker *et al.*, 1998). In Norway,

cases of preeclampsia peaked in the winter months (Magnus & Eskild, 2001) and similarly, the incidence of preeclampsia peaked in women delivering in November in Kuwait (Makhseed *et al.*, 1999), and between January and March in Israel (Bider *et al.*, 1991). Zahiri *et al.,* (2007) on the other hand, found no correlation between preeclampsia or eclampsia and the season and attributed their finding to the relative similarities between seasons in North of Iran i.e. relative similarities between spring and summer, and between autumn and winter (Zahiri *et al.*, 2007).

1.8 RISK FACTORS FOR DISORDERS OF HYPERTENSIVE PREGNANCY

Research into PIH has been unlimited as a result of its growing prevalence, but to date the aetiology remains unknown, however, a number of risk factors have been identified (Roberts & Lain, 2002; Zhang *et al.*, 1997). These risk factors for hypertensive pregnancy (preeclampsia and gestational hypertension) includes maternal, paternal, genetic, environmental and/or obstetric factors. Reportedly, primiparas are known to be at markedly greater risk of preeclampsia than multiparas (Chesley, 1984). Preeclampsia is reported to complicate 25-30% of nulliparous pregnancies, it is more common in nulliparous women than in multiparous women and as such the first pregnancy is understood to be a risk factor for preeclampsia (Serhal *et al.*, 2003). Lack of leisure-time physical activity early in pregnancy (Marcoux *et al.*, 1989), the use of barrier contraceptives (KlonoffCohen *et al.*, 1989), young maternal age (Saftlas *et al.*, 1990), partner change (Duckitt & Harrington, 2005; Sibai *et al.*, 1997; Trupin *et al.*, 1996); have all been reported to amplify the risk of PIH or preeclampsia. Women with hypertensive pregnancy are also reported to present with pregnancy overweight and metabolic derangement and are thought to present with a syndrome similar to the Metabolic Syndrome.

1.8.1 *THE METABOLIC SYNDROME*

1.8.1.1 Historical Background

As early as the 1960s and 1970s, researchers began to document a clustering of the elements of cardiovascular risk in certain patients. It was not until 1988 that a uniting cause Insulin Resistance was proposed and the term syndrome X applied. The term "metabolic syndrome" dates back to at least the late 1950s, but came into widespread usage in the late 1970s to depict various associations of risk factors with diabetes, that had been noted as early as the 1920s (Joslin, 1921; Kylin, 1923). The Marseilles physician Dr. Jean Vague, in 1947, made the remarkable observation that upper body obesity appeared to predispose to diabetes, atherosclerosis, gout, and calculi (Vague, 1947). Avogaro *et al.,* (1967) described six moderately obese patients with diabetes, hypercholesterolemia, and marked hypertriglyceridemia all of which improved when the patients were put on a hypocaloric, low carbohydrate diet.

In 1977, Haller used the term "Metabolic Syndrome" for associations of obesity, diabetes mellitus, hyperlipoproteinaemia, hyperuricaemia and steatosis hepatitis when relating the additive effects of risk factors on atherosclerosis (Haller, 1977). The same year, Singer used the term for associations of obesity, gout, diabetes mellitus, and hypertension with hyperlipoproteinaemia (Singer, 1977). In 1977 and 1978, Gerald B. Phillips developed the concept that risk factors for myocardial infarction concur to form a "constellation of abnormalities" (i.e., glucose intolerance, hyperinsulinaemia, hyperlipidaemia [hypercholesterolemia and hypertriglyceridemia] and hypertension) that is associated not only with heart disease, but also with aging, obesity and other clinical states. He suggested there must be an underlying linking factor, the identification of which could lead to the prevention of cardiovascular disease; he hypothesized that this factor was sex hormones (Phillips, 1977; 1978).

In 1988, Gerald M. Reaven proposed insulin resistance as the fundamental factor and named the constellation of abnormalities Syndrome X. Unfortunately,

Reaven did not include abdominal obesity, which has also been put forward as the underlying factor, as part of the condition today (Reaven, 1988). After several name changes over the past two decades, including the term diabesity used in lay publications, the name became Metabolic Syndrome. The terms "metabolic syndrome," "insulin resistance syndrome" and "syndrome X" are now used exclusively to define a constellation of abnormalities that is associated with increased risk for the development of type 2 diabetes and atherosclerotic vascular disease.

The Metabolic Syndrome is a combination of medical disorders that increase the risk of developing cardiovascular disease and diabetes. The chief components of the metabolic syndrome are central adiposity, dyslipidaemia, hypertension, and glucose intolerance, although, chronic inflammation, procoagulation, and impaired fibrinolysis are also thought to play a role.

1.8.1.2 PREVALENCE OF THE METABOLIC SYNDROME

The prevalence of the metabolic syndrome varies from place to place and differs for different ethnic groups. In the US, studies carried out by Ford *et al,* revealed that in 2000, about 64 million Americans were affected with the Metabolic Syndrome. Currently, an estimated 47 million US adults have the Metabolic Syndrome. Some experts predict that at least half of persons over age 60 would meet the criteria for this syndrome. In another survey analysis, as many as 4.2% of US adolescents aged 12 to 19 years was reported to have the disorder. Prevalence rates of Metabolic Syndrome differ across ethnic groups. The highest overall prevalence has been found in Mexican Americans, who make up a rapidly growing segment of the United States population (Ford *et al*., 2002).

1.8.1.3 CAUSES OF THE METABOLIC SYNDROME

The aetiology of the metabolic syndrome is unidentified. The pathophysiology is very complex and has only been partly clarified. Usually, it is observed in people

who are obese, advanced in age, sedentary, and have a measure of insulin resistance. The most significant factors in order are: age, genetics, sedentary lifestyle (reduced physical activity and overindulgence of caloric intake), poor diet.

There is debate regarding whether obesity or insulin resistance is the cause of the Metabolic Syndrome or if they are consequences of a more far-reaching metabolic derangement. However, the Metabolic Syndrome is not observed in the absence of insulin resistance, while obesity is not present in many individuals who present with the Metabolic Syndrome. A number of markers of systemic inflammation including C- reactive protein, are often increased, as are fibrinogen, interleukin 6 (IL–6), tumour necrosis factor- alpha (TNF-α) and others. Some have pointed to oxidative stress due to a variety of causes including increased uric acid levels caused by dietary fructose (Hallfrisch, 1990; Reiser *et al.*, 1989).

1.8.1.4 PATHOPHYSIOLOGY OF THE METABOLIC SYNDROME

The pathophysiology of the Metabolic Syndrome has only been partly elucidated but usually, there is development of visceral fat after which the adipocytes of the visceral fat increase plasma levels of TNF-α and alter levels of a number of other substances (e.g., adiponectin, resistin, PAI-1). TNF-α has been shown not only to cause the production of inflammatory cytokines, but possibly to trigger cell signalling by interaction with a TNF-α receptor that may lead to insulin resistance. An experiment with rats which were fed a diet one-third of which was sucrose has been proposed as a model for the development of the metabolic syndrome. The sucrose first elevated blood levels of triglycerides, which induced visceral fat and ultimately resulted in insulin resistance (Fukuchi *et al.*, 2004). The progression from visceral fat to increased TNF-α to insulin resistance has some parallels to human development of the Metabolic Syndrome

1.8.1.5 SIGNS AND SYMPTOMS

Common symptoms associated with the metabolic syndrome are as listed below:

- Fasting hyperglycemia — type 2 diabetes mellitus or impaired fasting glucose, impaired glucose tolerance, or insulin resistance.
- High blood pressure
- Central obesity (also known as visceral, male-pattern or apple-shaped adiposity), overweight with fat deposits mainly around the waist.
- Decreased High Density Lipoprotein Cholesterol.
- Increased Triglycerides

The signs are: elevated uric acid levels, fatty liver (especially in concurrent obesity), progressing to non-alcoholic fatty liver disease. Associated diseases are polycystic ovarian syndrome, haemochromatosis (iron overload), and acanthosis nigricans (a skin condition featuring dark patches).

1.8.1.6 CLASSIFICATION OF THE METABOLIC SYNDROME

There are many classifications for the Metabolic Syndrome, which have been put forward by varying authorities. These classifications though similar, vary slightly but it is expected that all the different types of classification would identify the same persons as having the Metabolic Syndrome. To date, no single definition or classification has been accepted but the most widely used are:

1.8.1.6.1

WHO

The World Health Organization criteria of 1999 necessitates the presence of diabetes mellitus, impaired glucose tolerance, impaired fasting glucose or insulin resistance, in addition to any two of the following: (Kuzuya *et al.*, 2002)

- Blood pressure: ≥ 140/90 mmHg
- Dyslipidaemia: triglycerides (TG): ≥ 1.695 mmol/L and high-density lipoprotein cholesterol (HDL-C) ≤ 0.9 mmol/L (male), ≤ 1.0 mmol/L (female)
- Central obesity: waist/hip ratio > 0.90 (male); > 0.85 (female), and/or body mass index > 30 kg/m^2
- Microalbuminuria: urinary albumin excretion ratio ≥ 20 mg/min or Albumin/Creatine ratio ≥ 30 mg/g

1.8.1.6.2

NCEP III

According to the National Cholesterol Education Program Adult Treatment Panel III (2001), the presence of three or more of the following: (NCEP, 2001).

- Central obesity: waist circumference ≥ 102 cm or 40 inches (male), ≥ 88 cm or 36 inches (female)
- Dyslipidaemia: TG ≥ 1.695 mmol/L (150 mg/dl)
- Dyslipidemia: HDL-C < 40 mg/dl (male), < 50 mg/dl (female)
- Blood Pressure ≥ 130/85 mmHg
- Fasting Plasma Glucose ≥ 6.1 mmol/L (110 mg/dl)

1.8.1.6.3

EGIR

The European Group for the Study of Insulin Resistance (1999) requires insulin resistance defined as the top 25% of the fasting insulin values among non-diabetic individuals plus two or more of the following:

- Central obesity: waist circumference ≥ 94 cm (male), ≥ 80 cm (female)
- Dyslipidaemia: TG ≥ 2.0 mmol/L and/or HDL-C < 1.0 mg/dl or treated for Dyslipidaemia
- Hypertension: blood pressure ≥ 140/90 mmHg or antihypertensive medication
- Fasting plasma glucose ≥ 6.1 mmol/L

1.8.1.6.4

American Heart Association/Updated NCEP

There is misunderstanding as to whether AHA/NHLBI proposed to produce a different set of guiding principle or merely revise the NCEP ATP III definition. According to Scott Grundy, University of Texas Southwestern Medical School, Dallas, Texas, the aim was just to revise the NCEP ATP III definition and not generate a novel definition (Grundy *et al*., 2004) and based on their deliberations the following was the outcome:

Elevated waist circumference:

Men — Equal to or greater than 40 inches (102 cm)

Women — Equal to or greater than 35 inches (88 cm)

Elevated triglycerides: Equal to or greater than 150 mg/dl

Reduced HDL ("good") cholesterol:

Men — Less than 40 mg/dl

Women — Less than 50 mg/dl

Elevated blood pressure: Equal to or greater than 130/85 mmHg or use of medication for hypertension

Elevated fasting glucose: Equal to or greater than 100 mg/dl (5.6 mmol/L) or use of medication for hyperglycaemia.

1.8.1.7 PREVENTION OF THE METABOLIC SYNDROME

Different strategies have been designed to prevent the development of the Metabolic Syndrome. These include increased physical activity (such as walking 30 minutes every day) (Lakka & Laaksonen, 2007) and a healthy, reduced calorie diet (Feldeisen & Tucker, 2007). A lot of research supports the value of a healthy lifestyle as above. However, one study stated that these measures are effective in only a minority of people (Katzmaryk., 2003). The International Obesity Taskforce states that interventions on a sociopolitical level are required to reduce development of the Metabolic Syndrome in populations (James, 2004). In 2007, a study of 2,375 male subjects over 20 years suggested that daily intake of a pint of milk or equivalent dairy products more than halved the risk of the Metabolic Syndrome (Snijder *et al.*, 2007). Other studies either support or conflict the findings of the study.

1.8.2 *PREGNANCY-INDUCED HYPERTENSION AND METABOLIC SYNDROME*

It is common knowledge that women who develop Pregnancy-Induced Hypertension develop a syndrome similar to the Metabolic Syndrome. These women with hypertensive complicated pregnancy, exhibit exaggeration of insulin resistance and metabolic changes (Seely & Solomon, 2003). There is

uncertainty as to the pathogenesis of these factors in hypertensive pregnancy but many have suggested that it may play a role in either disease evolution or markers underlying a disease process (Seely & Solomon, 2003). Women in whom PIH eventually develops are more likely to present with pregnancy overweight and to demonstrate, during pregnancy, some of the risk factors characterizing atherosclerosis, such as dyslipidaemia (hypertriglyceridaemia, low levels of highdensity lipoprotein (HDL) cholesterol and low-density lipoprotein (LDL) cholesterol (Belo *et al.*, 2002; Sattar *et al.*, 1997a) insulin resistance (Kaaja *et al.*, 1999; Seely & Solomon, 2003) and endothelial dysfunction (Roberts, 1998). Indeed, these metabolic aberrations (increased adiposity, hyperlipidaemia, hyperglycaemia, and elevated blood pressure) are reminiscent of the metabolic syndrome (Forest *et al.*, 2005). However geographical, social, economic and racial differences are thought to be responsible for incidence rates up to 3 times higher in some populations (Lopez-Jaramillo *et al.*, 2001; WHO, 1988). In some countries such as Columbia it is the main cause of maternal mortality. Up to 42% of maternal deaths are attributed to this disorder in Colombia (Lopez-Jaramillo *et al.*, 2001). Because of the increased risk for morbidity and mortality associated with the Metabolic Syndrome, an understanding of the dimensions of this syndrome is critical both for allocating health care and research resources and for other purposes (Ford & Giles, 2003).

1.8.3 *OXIDATION AND LIPID PEROXIDATION IN HYPERTENSIVE PREGNANCY*

Oxygen is vital for human existence. But unfortunately, like many other chemicals it can also be lethal. Very high levels of oxygen are known to hurt the eyes, lungs and the Central Nervous System. As oxygen is metabolized in the body potentially toxic intermediates are equally produced. During metabolism, as electrons are added unto oxygen, free radicals and additional substances are produced. Free radicals are molecules containing one or more unpaired electrons

that make them very reactive to other molecules. Subcellular organelle membrane and different enzymes in all tissues, including the cytochrome P-450 system in the liver, can reduce oxygen and also make free radicals. Mitochondrial respiration, detoxification of xenobiotics by the cytochrome also generates highly reactive oxygen species. Although, free radicals are beneficial in the phagocytic destruction of invading organisms and in cancer therapy, they can also cause a great deal of damage to the body and have been implicated in a lot of diseases (Diplock, 1984). Accumulated free radicals in the system cause cell injury, disease or the aging phenomenon, oxidative cellular damage. Oxidative stress is a pathological state, implicated in the aetiology of many disorders, in which pro-oxidants dominate over antioxidants. Free radicals also react with fatty acids in cell membranes generating lipid peroxides (lipid peroxidation), which in turn hinder activities of cellular enzymes or decompose into added reactive metabolites (Diplock, 1984).

Free radicals set off lipid peroxidation by attacking polyunsaturated fatty acids in cell membranes.

Lipid peroxidation is a reaction whereby molecular oxygen is incorporated into poly-unsaturated fatty acids (PUFA) to yield lipid peroxides. Lipid peroxidation mediated by free radicals is considered to be the major mechanism of cell membrane destruction and cell damage (endothelial damage) and is a key contributing factor to the pathophysiologic condition of preeclampsia (Ozan *et al.*, 2002). Lipid peroxidation of cell membrane coupled with fatty acids and cholesterol normally changes cell membrane fluidity and permeability leading to damage of the cell membrane. The free radical mediated oxidation of cell membrane lipids proceed by a chain mechanism resulting in oxidative damage. There is considerable epidemiological, laboratory and clinical evidence to suggest a significant role for free radical activation, and deficient or over utilized antioxidant defenses in the pathogenesis of PIH (Gulmezoglu *et al.*, 1997) and

excess free radicals are typically accompanied by increased utilization of antioxidants (Mikhail *et al.*, 1994).

Hubel, (1989b) has also suggested that free radical mediated peroxidation may be involved in the endothelial damage observed in preeclampsia. Several reports support this concept, including an increase in lipid peroxidation products (Ahenkorah *et al.*, 2008; Maseki *et al.*, 1981). Plasma lipid peroxidation products such as lipid peroxides have been shown to increase in PIH, superoxide anion have been shown to inactivate endothelium derived relaxing factor (Mikhail *et al.*, 1994). Lipid peroxides are known to inhibit prostacyclin synthetase and prostacyclin synthesis decreases in endothelial cell injury (Roberts & Hubel, 1999; Roberts *et al.*, 1989). These result in vascular resistance and platelet aggregation (Bussolino & Camussi, 1980) and vascular resistance and platelet aggregation are known features of PIH. It has been shown that lipid peroxidation occurs in platelet lipid membrane of preeclamptics (Garzetti *et al.*, 1993) this may predispose to the platelet aggregation observed in PIH patients.

There is increasing evidence that endothelial cell dysfunction is the primary pathophysiological mechanism which causes preeclampsia (Friedman *et al.*, 1991; Roberts *et al.*, 1989). However, the pathway mediating endothelial cell layer dysfunction still remains unclear. One hypothesis receiving amplified attention is that the endothelial dysfunction may be the result of increased oxidative stress, which is characterized by disequilibrium between oxidant and antioxidant forces in favour of oxidation. The lipid peroxidation process, initiated by the reaction of free radicals with polyunsaturated fatty acids (Hubel *et al.*, 1989b) is used as a marker of oxidant force.

Diverse studies world wide have reported elevated lipid levels in PIH patients (GH and PE) (Ahenkorah *et al.*, 2008; Forest *et al.*, 2005; Kaaja *et al.*, 1995; Sattar *et al.*, 1997a; Seely & Solomon, 2003; Turpin *et al.*, 2008). Cell membranes are

generally made up of lipid bilayers and thiol containing proteins. The unsaturated lipid component and thiol containing proteins of the cell membranes are susceptible to free radical attack. Antioxidants are compounds that dispose, scavenge and suppress the formation of free radicals, or oppose their actions (Sies, 1991). Free radicals are formed in both physiological and pathological conditions in mammalian tissues (Krishna Mohan & Venkataramana, 2007; Plaa & Witschi, 1976). But normally, defense mechanisms of the body play an important role in the form of antioxidants, making every effort to minimize the damage, as an adaptation to stressful situations. An increased disequilibrium between oxidants (free radicals) and antioxidants results in oxidative stress when the imbalance favours oxidation. The unrestrained production of free radicals is considered as an important factor in the tissue damage induced by several conditions (Sato *et al.*, 1996) as reportedly observed in PIH patients (Mutlu-Turkoglu *et al.*, 1998).

Accumulating evidence from clinical and epidemiology studies suggests that diffuse endothelial dysfunction resulting from oxidative stress, play a role in the pathogenesis of preeclampsia (Redman & Sargent, 2000; Roberts, 1998). Endothelial dysfunction may, therefore, play a pivotal role in the genesis of the multisystem disorder that develops in preeclampsia. Growing evidence indicates endothelial cell dysfunction as the primary pathophysiological mechanism leading to preeclampsia (Friedman *et al.*, 1991; Roberts, 1998). However, the pathway mediating endothelial cell layer dysfunction and preeclampsia is unidentified. But one theory receiving increased consideration is that the endothelial dysfunction may be the result of increased oxidative stress because of increasing reports of elevated free radical activity in women with PIH (Aksoy *et al.*, 2003).

1.8.4 *DIETARY SALT AND HYPERTENSIVE PREGNANCY*

There is evidence in support of the hypothesis which proposes that prolonged increases in salt intake or the habitual high intake of salt is related to rise in arterial blood pressure (Chobanian & Hill, 2000) however, the mechanisms by which salt intake raises the blood pressure still remains unclear (de Wardener *et al*., 2004). Cappuccio *et al*, (2006) showed a significant and positive relationship between the level of salt intake and both systolic and diastolic blood pressure. Hypertension involves abnormal and persistent changes in the blood pressure control mechanisms. In industrialized societies, the individual's average salt consumption is 10 g/day, and the incidence of hypertension is greater than in rural societies. A large body of evidence points to a link between dietary salt, kidney function, and hypertension (Guyton, 1991; Hall *et al*., 1980; Meneton *et al*., 2005). Various mechanisms by which salt increases blood pressure have been put forward. A decrease in the capacity of kidneys to excrete salt would cause salt and water retention, increased extracellular and plasma volume, and increased blood pressure. The kidneys' ability to excrete sodium declines gradually with age, and smaller increases in salt intake induce a rise in blood pressure. Also, with increasing age, the glomerular filtration rate is reduced, accompanied by a decline in functioning nephrons and progressive glomerulosclerosis. If, with age, salt consumption is not reduced, sodium balance is maintained by raising fractional sodium excretion, which requires elevation of blood pressure (Corman & Michel, 1987; Khalil, 2006). High salt diet may also increase renal medullary osmolality and decrease NO synthase expression (Herrera *et al*., 2006) and reduced renal medullary NO synthase activity is associated with salt-sensitive hypertension (Tian *et al*., 2003).

1.8.5 *AGE A RISK FACTOR FOR PREGNANCY-INDUCED HYPERTENSION*

Several epidemiological studies have considered the association of maternal age and the risk of Pregnancy-Induced Hypertension (gestational hypertension and preeclampsia). Inspite of this, there have been inconsistent reports on the effects of maternal age on preeclampsia. While some studies did not find age a significant risk factor (Anorlu *et al.*, 2005; Conde-Agudelo & Belizan, 2000; Eskenazi *et al.*, 1991), some studies have reported increased risk of preeclampsia in younger women who are ≤ 21 years (Sibai, 1990b) and other studies have reported an association of increased risk of preeclampsia with women who are 35 years or older (Conde-Agudelo & Belizan, 2000; Sibai, 1990b). Several studies have reported that increasing age is associated with an increased risk of gestational hypertension (Brown & de Swiet, 1999; Eras *et al.*, 2000; Hartikainen *et al.*, 1998). Teenagers and women aged 35 years and over generally have been shown to have a greater risk of adverse perinatal outcomes, including low birthweight, (Fraser *et al.*, 1995; Reichman & Pagnini, 1997) small-for-gestational age, (Cnattingius *et al.*, 1992; Fraser *et al.*, 1995), preterm birth (PTB), (Cnattingius *et al.*, 1992; Fraser *et al.*, 1995; Jacobsson *et al.*, 2004) and perinatal or infant mortality (Cnattingius *et al.*, 1992; Jacobsson *et al.*, 2004; Olausson *et al.*, 1999) and these are largely associated with Pregnancy-Induced Hypertension.

1.8.6 *ANTHROPOMETRIC INDICES* AND *HYPERTENSIVE PREGNANCY*

Anthropometric measurement is the science of measuring the human body parts for height, weight, and size of component parts, including skinfold thickness, to study and compare the relative proportions under normal and abnormal conditions. Anthropometric indices includes weight (Wt), height (Ht), ponderal index (PI), body mass index (BMI), thigh circumference/head circumference ratio (THR), waist-to-hip ratio (WHR), waist-to-height ratio, waist circumference, hip

circumference mid-arm circumference/head circumference(MAC/OFC), weight/head circumference (W/OFC), and weight/length (W/L). Anthropometric measurements are among the most frequently applied methods for assessing nutritional status in pregnant women and are recognized as an effective tool for the prevention of perinatal, morbidity and mortality, the prognosis of child health, and the promotion of women's health (Oliveira *et al.*, 2004; Padilha & Nelson, 2009) Several studies have shown an association between anthropometric indicators and pregnancy outcome.

1.8.6.1 BODY MASS INDEX (BMI)

The Body Mass Index (BMI) was invented by the Belgian, Adolphe Quetelet (17961874). It is a standardized estimate of an individual's relative body fat calculated from his or her height and weight. BMI is expressed as weight in kilograms divided by the square of height measurement in meters (Institute of Medicine, 1992; Lao & Ho, 2000). On the basis of BMI, individuals can be classified as

Underweight = <18.5 kg/m^2, Normal weight = 18.5-24.9 kg/m^2, Overweight = 2529.9 kg/m^2, Obese = BMI of 30 kg/m^2 or greater (Getahun *et al.*, 2007; WHO, 1997; WHO, 1995). Obesity has become a major health problem due to its increasing prevalence and associated morbidity and mortality (Jousilahti *et al.*, 1996; Seidell *et al.*, 1996). Correlation between BMI and obesity has been reported (Ko *et al.*, 2001). Increasing BMI is associated with a higher risk of diabetes mellitus, hypertension, and other cardiovascular risk factors in both Caucasians and Asians, such as Hong Kong Chinese (Ko *et al.*, 1999; Ko *et al.*, 1998; Srinivasan *et al.*, 1996). Obesity is characterised by an excess of body fat (BF), which is defined conventionally as BF 25 % in males and 35 % in females (young adults aged, 35 years) (Deurenberg *et al.*, 1998; Ko *et al.*, 2001). Maternal body fatness has been associated with preeclampsia for decades. Since then, various studies have examined the relation between maternal prepregnancy BMI and preeclampsia

but this relationship may have skewed these results due to sociodemographic and lifestyle variables across continents. Preeclampsia has been found to occur more commonly among women of high prepregnancy BMI in bivariate analyses (Barton *et al.*, 1997; Bowers & Cohen, 1999; Ogunyemi *et al.*, 1998). Several studies have shown a strong association between increased maternal body mass and risk of preeclampsia (Anorlu *et al.*, 2005; Bodnar *et al.*, 2005; Eskenazi *et al.*, 1991; Villamor & Cnattingius, 2006). It has also been observed that women in whom PIH develop, enter into pregnancy either overweight or obese and also demonstrate, during pregnancy, some risk factors characterizing atherosclerosis, such as dyslipidaemia (Belo *et al.*, 2002; Sattar *et al.*, 1997a), insulin resistance (Kaaja *et al.*, 1999; Seely & Solomon, 2003) and endothelial dysfunction (Roberts, 1998). These metabolic anomalies (increased adiposity, hyperlipidaemia, hyperglycemia and elevated blood pressure) are suggestive of the Metabolic Syndrome (Ahenkorah *et al.*, 2008; Turpin *et al.*, 2008).

Similar to preeclampsia, several studies have reported that increasing BMI and age are associated with an increased risk of gestational hypertension (Brown & de Swiet, 1999; Eras *et al.*, 2000; Hartikainen *et al.*, 1998). Compared with underweight women with BMI <19.8, obese women with a BMI >29 had an increased risk of gestational hypertension (Ros *et al.*, 1998). Other studies have reported a similar association between increasing BMI and increased risk (1.7- 3-fold) of developing gestational hypertension (Eras *et al.*, 2000; Hartikainen *et al.*, 1998).

1.8.6.2 WAIST-TO-HIP RATIO (WHR)

Waist-to-hip ratio (WHR) is the ratio of the circumference of the waist to that of the hips. It measures the proportion by which fat is distributed around the torso.WHR is so far the most widely used index of central fat distribution and

widely used due to its benefits in routine monitoring and assessment in patients (Caprio *et al.*, 1996). Studies concerning WHR in women have focused largely on its value as a biomarker for health and attractiveness. Men and women in various Western cultures attach more sexual attractiveness to low WHR in women (Furnham *et al.*, 1997; Singh, 1994). A WHR of 0.7 for women and 0.9 for men have been shown to correlate strongly with general health and fertility. Women with high WHR exhibit decreased fertility (Zaadstra *et al.*, 1993) more menstrual irregularities (Hartz *et al.*, 1984) and cardiovascular disease risk factors (Hartz *et al.*, 1984; Marti *et al.*, 1991; Wing *et al.*, 1991). A correlation between WHR and hypertensive pregnancy has being established. Yamamoto *et al.*, (2001) established that higher WHR is a significant predictor of the development of preeclampsia, irrespective of overall adiposity (BMI and BW).

1.8.6.3 WAIST CIRCUMFERENCE (WC)

Waist circumference is the best simple anthropometric index of abdominal visceral adipose tissue, (Pouliot *et al.*, 1994). It is accepted that the measurements of abdominal adipose tissue correlate better with cardiovascular risk factors than BMI (Ashwell & Hsieh, 2005; Expert Panel on the Identification, 1998). The correction of the waist circumference for stature or hip circumference improves its performance in the prediction of the incidence of hypertension (Ochsenbein-Kolble *et al.*, 2007). Differing values of WC have been associated with increased risk of hypertension in different populations. In men, WC cut-off points of 76, 81, 80, 83 and 87 cm provided the highest sensitivity for identifying hypertensives in Nigeria, Cameroon, Jamaica, St Lucia and Barbados, respectively. The equivalent cut-off points in women were 72, 82, 85, 86 and 88 cm (Okosun *et al.*, 2000).

1.8.6.4 WAIST-TO-HEIGHT RATIO (WHtR)

Waist-to-height ratio is another anthropometric index of abdominal adiposity marker; it is a better predictor of metabolic and cardiovascular risk than BMI, WC and WHR (Hsieh & Muto, 2005; Schneider *et al.*, 2007).

1.8.7 *JOB STRESS, EXERCISE AND HYPERTENSIVE DISORDERS OF PREGNANCY*

The occurrence of preeclampsia as well as gestational hypertension has been associated with stress at work and reduced participation in exercise during pregnancy. Some studies have reported that high job stress have a positive relationship with gestational hypertension (Landsbergis & Hatch, 1996; Marcoux *et al.*, 1989). Preeclampsia has been hypothesized as a stress-related disease and indeed epidemiologic studies show that the relative risk for preeclampsia is increased in many stressful situations (Takiuti *et al.*, 2003). Reduced levels of physical exercise have also been associated with the development of gestational hypertension (Marcoux *et al.*, 1999; Marcoux *et al.*, 1989). Similarly, moderate/high physical activity is reported to be associated with a two times increase in the risk of severe preeclampsia compared to mild activity (Spinillo *et al.*, 1995). Marcoux *et al.*, (1989) also reported that the lack of leisure-time physical activity early in pregnancy had the tendency to increase the risk of developing gestational hypertension.

1.8.8 *PARITY AND HYPERTENSIVE PREGNANCY*

Parity which refers to the number of times a woman has given birth, has for long been associated with hypertensive pregnancy. The strong relationship between parity and the clinical condition was documented over 300 years ago by Mauriceau, who indicated that ''primigravidas are at far greater risk of convulsions than multiparas.'' Several studies have corroborated this observation and others have revealed that the association does not hold for nulliparity and nonproteinuric hypertension (Campbell *et al.*, 1985; Misra & Kiely, 1997). Indeed, gestational hypertension has been reported to be more common in nulliparous

than multiparous women (1.6- to 2-fold), but the association is less remarkable than that seen in preeclampsia (Campbell & MacGillivray, 1999; Hartikainen *et al.*, 1998; Trupin *et al.*, 1996). In a Scottish study of over 130,000 pregnancies, the relative risk (RR) of gestational hypertension in nulliparous women compared to multiparas was 1.98 (95% CI 1.94–2.03) in singleton pregnancies and 1.85 (95% CI 1.55–2.21) in twin pregnancies (Campbell & MacGillivray, 1999). Among nulliparous women, gestational hypertension was more common in the first pregnancy compared to subsequent pregnancies, [odds ratio (OR) 2.29 (95% CI 1.65-3.20)] (Eras *et al.*, 2000). Indeed, preeclampsia is frequently considered as being a clinical condition of first pregnancies (Roberts & Redman, 1993). Serhal and Craft, (1987) also reported that first pregnancy is a risk factor for preeclampsia and its occurrence is more common in nulliparous than multiparous women. Suggesting that after a previous normal pregnancy, there is a markedly lower incidence of preeclampsia in subsequent pregnancies. But the protective effect of multiparity, is however lost with change of partner (Dekker, 2002). This has led to widespread epidemiological studies, and has given rise to data to implicate immunological factors in the aetiology of preeclampsia.

1.8.9 *PRIMIPATERNITY AND HYPERTENSIVE PREGNANCY*

It has been suggested that primipaternity rather than primiparity is an appropriate risk factor (Robillard *et al.*, 1999; Robillard *et al.*, 1993) for hypertensive pregnancy since some studies have shown that the protective effect of multiparity, is lost with change of partner (Belfort *et al.*, 2002). This implies that, not only are primiparas at high risk, multiparas having a child with a new father (Li & Wi, 2000; Lie *et al.*, 1998; Need *et al.*, 1983; Trupin *et al.*, 1996) are equally at a high risk for hypertensive pregnancy. Trupin *et al.*, (1996) reported that multiparous women who change their partners have a slightly higher risk of developing gestational hypertension than multiparous women with the same partner [OR 1.3 (95% CI 1.1–1.6)]. In a cohort study based on 140,147 women with

two successive births during 1989– 1991, Li and Wi (2000), established that among women without preeclampsia in the first birth, changing partners resulted in a 30% increase in the risk of preeclampsia in the subsequent pregnancy as compared with women who did not change partners. However, among women with preeclampsia in the first birth, changing partners resulted in a 30% reduction in the risk of preeclampsia in the subsequent pregnancy. Li and Wi's findings show that preeclampsia is rather a disease of primipaternity rather than primigravidity and are consistent with the hypothesis that normal pregnancy reflects a state of tolerance to the foreign paternally derived antigens of the foetus, whereas in women with preeclampsia, this immunological tolerance is impaired. Similarly, an increased risk has been noticed among women who had had artificial insemination by an unknown donor (Belfort *et al.*, 2002; Smith *et al.*, 1997).

Some studies also provide data for the existence of the supposed 'dangerous' father. Lie, *et al.,* (1998) revealed that men who fathered one preeclamptic pregnancy were nearly twice as likely to father a preeclamptic pregnancy in a different woman in spite of whether she had already had a preeclamptic pregnancy or not. Thus, mothers had a substantially increased risk in their second pregnancy (2.9%) if they became pregnant by a man who had fathered a preeclamptic first pregnancy in another woman. This risk was nearly as high as the average risk amongst first pregnancies (Belfort *et al.*, 2002; Lie *et al.*, 1998).

1.8.10 *CONTRACEPTIVE USE AND HYPERTENSIVE PREGNANCY*

Pregnancy-Induced Hypertension has long been considered to have an immunological basis, as its frequency is largely increased with primigravidae and rarely affects multigravid women unless there is a change in paternity (Robillard *et al.*, 1993). 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 & Herrmann, 1977). A prospective study of 1011 pregnant women reported a strong inverse association between the length of sexual cohabitation with the father and the risk of pregnancy-induced hypertension, (Robillard *et al.*, 1994). The risk of developing gestational hypertension or preeclampsia was increased 12-fold if the duration of sexual cohabitation before conception was less than 4 months compared to more than 12 months (Robillard *et al.*, 1994). The very high incidence (24.0%) of pregnancy-induced hypertension among new-paternity multiparous women was shown to be related to a remarkably short period of sperm exposure preceding conception, suggesting that extended duration of sexual intercourse might reduce this risk. It is therefore assumed that this may be related to the contact of spermatozoa with the female genital tract. However, it remains to be established whether the risk of developing pregnancy-induced hypertension is dependent on the type of contraception used (Gratacos *et al.*, 1996).

Furthermore, oral sex and the swallowing of seminal fluid correlated with a diminished occurrence of preeclampsia (Koelman *et al.*, 2000). Studies on the use of contraceptives as a risk factor for hypertensive pregnancy remain debatable. Some studies have reported that barrier methods or oral contraceptives do not seem to modify the risk of Pregnancy-Induced Hypertension (Gratacos *et al.*, 1996), others have reported otherwise. Klonoff-Cohen *et al.,* (1989) conducted a case-control study, evaluating the contraceptive histories of 110 primiparous women with preeclampsia along with 115 pregnant women without preeclampsia. Their data pointed towards a 2.4-fold increased risk of preeclampsia for contraceptive users who are not exposed to spermatozoa.

1.8.11 *ABORTION AND DISORDERS OF HYPERTENSIVE PREGNANCY*

Some studies have examined the effect of abortion on the incidence of pregnancyinduced hypertension (PIH) in a subsequent pregnancy (Eras *et al.*, 2000). MacGillivray, (1958) first addressed this question in a study that individually examined the effects of a full-term pregnancy and abortion on the incidence of PIH in subsequent pregnancy. He established that a full-term pregnancy conferred a protective effect and reduced the incidence of disease in subsequent pregnancy by as much as 90% whereas abortion offered a measure of protection nearing but not equivalent to that conferred by a completed pregnancy (Eras *et al.*, 2000). The protective effect of abortion has been corrobated by other studies (Abi-Said *et al.*, 1995; Sibai *et al.*, 1997; Sibai *et al.*, 1995). It has been suggested that the number of previous abortions that confers this protection varies for gestational hypertension and preeclampsia (Eras *et al.*, 2000).

1.9 AIMS AND OBJECTIVES

Pregnancy-Induced Hypertension (PIH) is one of the leading causes of maternal and foetal mortality and morbidity in the world and in Ghana today. A study by Osei-Nketiah, (2001) has shown that in Ghana, 40% of maternal deaths are as a result of hypertensive pregnancy, antepartum haemorrhage and postpartum haemorrhage. This high incidence rate can be attributed to poverty, lack of antenatal care, late visit to hospitals, illiteracy, less sensitive method for early detection of hypertensive complicated pregnancy. It is interesting to note that in our local community and among our rural folks, oedema, a common feature of PIH is attributed to the conception of a male child and as such these early signs are ignored by some expectant mothers while others would only visit the hospital a few weeks to their due date.

Based on reports of maternal, placental, endothelial dysfunction and paternal implication in the genesis of PIH, it is worth investigating these hypotheses that

have been connected to the pathogenesis of PIH among Ghanaian pregnant women. Understanding PIH would be one of the foremost targets of predicting and treating or managing preeclampsia, a more complicated form of the condition. Currently, the only definitive treatment for preeclampsia is preterm delivery of the baby when the life of either the mother or baby is threatened. Based on accumulating implicative findings for PIH and the scanty information on prevalence rates, this current study, seeks to establish the incidence and prevalence rates of PIH among Ghanaian women obtaining antenatal care at the Komfo Anokye Teaching Hospital. It also seeks to determine the probable cause(s) or risk factors of PIH among Ghanaian women; develop appropriate screening modalities for the early detection and management of PIH; and the elucidation of the role of the Metabolic Syndrome as well as oxidative stress in the pathogenesis of PIH. The specific objectives include the following:

- To investigate the prevalence and incidence of Pregnancy-Induced Hypertension, gestational hypertension as well as preeclampsia amongst women visiting the Komfo Anokye Teaching Hospital. This would give information on the occurrence rate of the clinical condition.

- To investigate prevalence of the Metabolic Syndrome amongst Ghanaian women with PIH as compared to normotensive pregnant women. This would give informed scientific knowledge about the maternal abnormalities and its role as a cause of PIH.

- To evaluate the cardiovascular risk profile amongst Ghanaian women with PIH as compared to normotensive pregnant women. This would inform if PIH compromises the cardiovascular health status of women with PIH or if a compromised cardiovascular status leads to the genesis of PIH.

- To investigate dyslipidaemia and lipid peroxidation amongst Ghanaian women with PIH as compared to normotensive pregnant women. This would give informed knowledge about the presence of possible cellular (vascular) damage in this category of women.

- To establish the correlation between Human Placental Lactogen and PIH as compared to normotensive pregnant women. This would give informed scientific knowledge about the role of placental abnormalities in the aetiology of PIH among Ghanaian pregnant women.

- To elucidate the possible risk factors associated with the development of pregnancy-induced hypertension. This would help in the development of appropriate screening modalities for early detection of PIH and preeclampsia to enhance its management.

Chapter 2 MATERIALS AND METHODS

2.1 SUBJECTS

Between November, 2006 and December, 2007 two groups of women, comprising of one hundred pregnant women with pregnancy–Induced hypertension (seventy with gestational hypertension and thirty with preeclampsia) and fifty normotensive pregnant women visiting the Obstetrics and Gynaecology Department of the Komfo Anokye Teaching Hospital in Kumasi, Ashanti Region of Ghana were consecutively selected and recruited for this study. All the subjects were Ghanaians, their participation voluntary and informed consent was obtained from each of them. The diagnosis of hypertensive complications during pregnancy was assessed by a single qualified Obstetrician/Gynaecologist using the diagnostic criteria of the National High Blood Pressure Education Program Working Group. Briefly, the presence of high blood pressure on two occasions six hours apart was considered gestational hypertension (GH) while pregnant women who had proteinuria level of 2+ positive result on a dipstick, were considered as presenting with preeclampsia (PE) (Forest *et al.*, 2005), finally, GH and PE were collectively considered as (PIH) for research purposes.

Each subject had a questionnaire-based interview, which was conducted privately and in person and lasted approximately 45 minutes. Information was obtained on maternal lifestyle factors such as smoking and alcohol consumption during pregnancy, demographic data, recent medical history, a complete present and past obstetric history (including abortions and ectopic pregnancies), contraceptive use, occupational factors, exercise and information on the parents. Each participant reported the outcomes of all previous pregnancies as livebirths, stillbirths, induced abortions, spontaneous abortions. The induced abortion was further classified into either hospital induced or self induced. The accuracy of the self-reported information on reproductive history and socio-demographic information collected

from the mother was assessed through record reviews of hospital database with a 100% rate of accuracy. All biochemical analyses were performed without knowledge of subject's clinical status by means of code numbering. The study was approved by the local Committee on Human Research Publication and Ethics (CHRPE/KNUST/KATH/15_03_08).

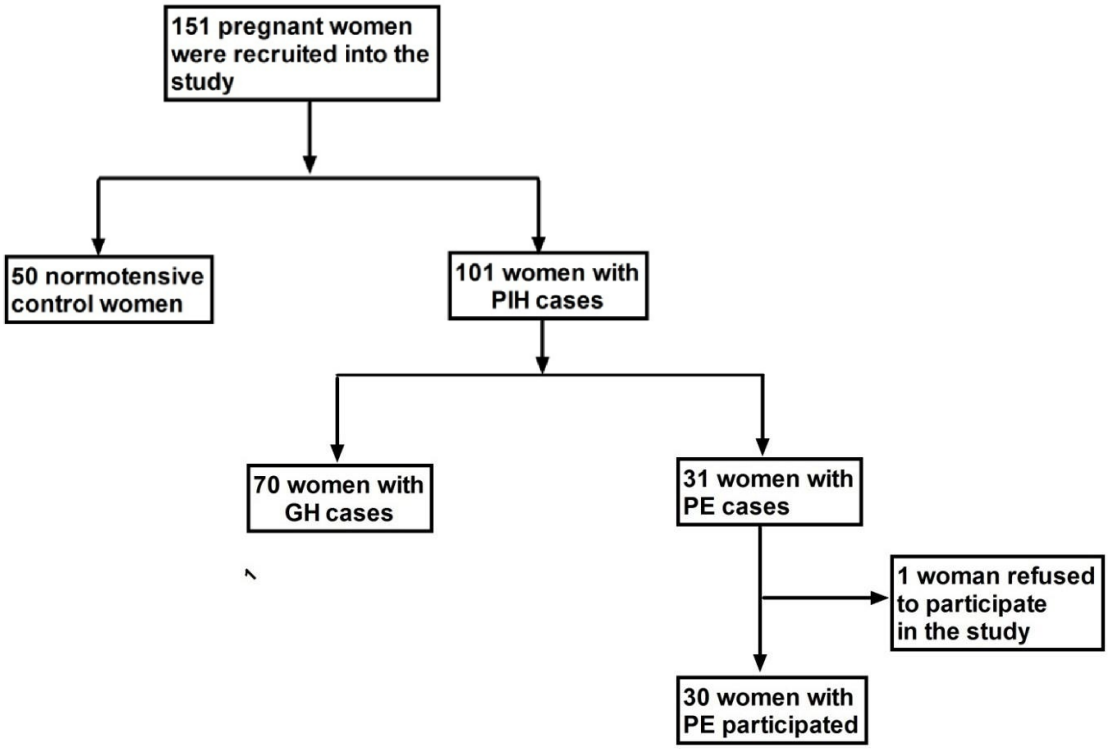


Figure 2.1 Flow diagram for the recruitment of the study subjects.

2.1.1 *Inclusion criteria*

- Pregnant women within the age of 17- 45 years.
- Pregnant women within the gestational age of ≥ 20 – 42 weeks.
- Singleton pregnant women
- Pregnant women with hypertension with or without proteinuria as subjects.

- Pregnant women with neither hypertension, preeclampsia nor eclampsia were used as control

2.1.2 *Exclusion criteria*

- The respondents who had not fasted as communicated (at least 12 hours before sample collection)
- Women with known renal diseases, diabetes, and hypertension prior to pregnancy or cardiovascular diseases (for both test and control).
- Multiple pregnant women (for both test and control)
- Presence of any other medical condition concurrent with Pregnancy-Induced Hypertension.

2.2 SAMPLE COLLECTION AND PREPARATION

Venous blood samples were drawn after an overnight fast (12 – 16 hours). About 5 ml of venous blood was drawn and dispensed into fluoride oxalate tubes and vacutainer® plain tubes for separation into plasma and serum respectively. This was then taken to the laboratory and centrifuged; the plasma was used for the glucose assay and the serum for other biochemical assays.

2.2.1 *Biochemical Analysis*

Serum biochemistry was performed with the ATAC® 8000 Random Access Chemistry System (Elan Diagnostics, Smithfield, RI, USA). Parameters that were determined include:

- Fasting blood glucose (FBS)
- Lipid profile which includes:
- Triglycerides (TG)
- Total cholesterol (TC)
- High Density Lipoprotein Cholesterol (HDL-C)

- Low Density Lipoprotein Cholesterol (LDL)
- Very Low Density Lipoprotein Cholesterol (VLDL) Cardiac profile

which also includes:

- Troponin- I (Tn I)
- Creatine Kinase (CK)
- Lactate Dehydrogenase (LDH)
- Aspartate Aminotransferase (AST) and Coronary Risk.

The methods adopted by the automated instrument for the determination of the above parameters are according to the reagent manufacturer's instruction - JAS™ diagnostics, Inc. (JAS™ Diagnostics, Inc. Miami Florida, USA).

Oxidative stress marker (lipid peroxidation) determined:

- Malondialdehyde (MDA) Hormonal assay carried out:
- Human placental Lactogen (hPL)

2.2.1.1 Fasting Blood Sugar Determination

Principle and Methodology

Glucose measurements are used in the diagnosis and treatment of many metabolic diseases. Elevated glucose levels (hyperglycaemia) may be seen in patients with diabetes mellitus, diuretic therapy, severe stress and cerebrovascular accidents. Decreased glucose levels (hypoglycaemia) may be seen in insulin administration, hypothyroidism and severe liver disease.

The glucose determination is an enzymatic method based on a modification of Slein (Slein & Logan, 1963) using hexokinase and glucose-6-phoshate dehydrogenase to catalyze the reaction. Glucose is phoshorylated with adenosine

triphosphate (ATP) in the reaction catalysed by hexokinase (HK). The product, glucose-6-phosphate is then oxidized with the concomitant reduction of nicotinamide adenine dinucleotide (NAD) to NADH in the reaction catalyzed by glucose-6-phoshate dehydrogenase (G6PDH). The formation of NADH causes an increase in absorbance at 340nm. The increase is directly proportional to the amount of glucose in the sample.

$$Glucose + ATP \xrightarrow{HK} G-6-P + ADP$$

$$G-6-P + NAD \xrightarrow{G-6PDH} 6-PG + NADH + H^{+}$$

2.2.1.2 Triglyceride Determination

Principle and Methodology

The current method for triglyceride determination employs a modified Trinder (Barham & Trinder, 1972; Trinder, 1969) colour reaction to yield a fast, linear, endpoint reaction (Fossati & Prencipe, 1982; McGowan *et al.*, 1983).

Triglycerides in the sample are hydrolyzed by lipase to glycerol and fatty acids. The glycerol is then phosphorylated by adenosine-5- triphosphate (ATP) to glycerol-3-phosphate (G-3-P) and adenosine-5-diphosphate (ADP) in a reaction catalyzed by glycerol kinase (GK). G-3-P is then converted to dihydroxyacetone phosphate (DAP) and hydrogen peroxide by glycerophosphate oxidase (GPO). The hydrogen peroxide then reacts with 4-aminoantipyrine (4-AAP) and 3, 5-dichloro2-hydroxybenzen (3,5-DHBS) in a reaction catalyzed by peroxidase to yield a red coloured quinoneimine dye. The intensity of the colour produced is directly proportional to the concentration of triglycerides in the sample.

$$Triglycerides + H_2O \xrightarrow{Lipase} Glycerol + fatty\ acids$$

$$Glycerol + ATP \xrightarrow{GK} G_2P + ADP$$

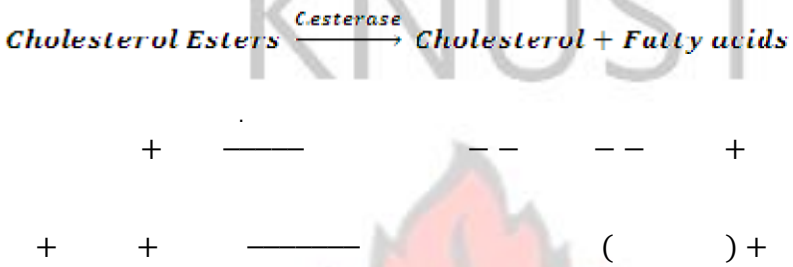
$$G-3-P + \xrightarrow{GPO} DAP + H_2O_2$$

$$H_2O_2 + 4AAP + 3,5\ DHBS \xrightarrow{POD} Quinoneimine + 2H_2O$$

2.2.1.3 Total Cholesterol Determination

Principle and Methodology

The method uses Trinder's (1969) colour system of peroxidase / phenol/ 4aminoantipyrine. The intensity of the red colour produced is directly proportional to the total cholesterol in the sample when read at 500 nm.



2.2.1.4 High Density Lipoprotein Cholesterol Determination

Principle and Methodology (Castelli et al., 1977)

The method employs an immunoinhibition reagent method which measures HDLC directly. The method is in a two reagent format. The first reagent contains anti human β-lipoprotein antibody which binds to lipoproteins (LDL, VLDL and chylomicrons) other than HDL. The second reagent contains enzymes which then selectively react with the cholesterol present in the HDL particles. Consequently, only HDL cholesterol is subject to cholesterol measurement. The first reading is done at 600 nm and second at 700 nm.

2.2.1.5 Low Density Lipoprotein Cholesterol (LDL- C) Determination

LDL was calculated according to Friedwald's formula (Friedwald *et al.*, 1972) in accordance to the manufacturers.

$$LDL = TC - (HDL - C + VLDL)$$

2.2.1.6 Very Low Density Lipoprotein Determination

VLDL-C was calculated according to the formula proposed by Wilson (DeLong *et al.*, 1986) in accordance to the manufacturers.

$$VLDL = \frac{Triglycerides}{5} \; or \; 0.2 \times triglycerides$$

2.2.1.7 Coronary Risk Determination

Coronary Risk was calculated using the formula in accordance to the manufacturers:

$$Coronary\ Risk = Total\ Cholesterol/HDL - C$$

2.2.1.8 Aspartate Aminotransferase Determination

Principle and Methodology

Aspartate Aminotransferase (AST) is widely distributed with high concentration in the heart, liver, skeletal muscle, kidney and erythrocytes. Damage or disease to any of these tissues such a myocardial infarction, hepatitis, liver necrosis, cirrhosis and muscular dystrophy may result in raised serum levels of AST.

The present method for AST is based on International Federation of Clinical Chemists (IFCC) recommendations. AST catalyzes the transfer of the amino group from L-Aspartate to 2-oxoglutarate to yield Oxaloacetate and L-glutamate. The Oxaloacetate undergoes reduction with simultaneous oxidation of NADH to NAD+ in the Malate Dehydrogenase (MDH) catalyzed indicator reaction. The resulting rate of decrease in absorbance at 340 nm is directly proportional to the AST activity. Lactate Dehydrogenase (LDH) is added to prevent interference from endogenous pyruvate which is normally present in serum.

$$\underset{-}{Oxaloacetate} + \underset{+-}{NADH} \xrightarrow{MDH} \underset{-}{L-malate} + \underset{+-}{NAD^+}$$

2.2.1.9 Lactate Dehydrogenase Determination

Principle and Methodology

The method uses the lactate to pyruvate reaction as described by Henry (1974). Lactate Dehydrogenase catalyses the oxidation of lactate to pyruvate, with the simultaneous reduction of NAD to NADH. The rate of NADH formation is measured as an increase in absorbance at 340 nm. The rate is directly proportional to LDH activity in the serum.

$$Lactate + NAD^+ \xrightarrow{LDH} pyruvate + NADH + H^+$$

2.2.1.10 Creatine Kinase Determination

Principle and Methodology

Creatine Kinase (CK) is an enzyme which consists of isoenzymes mainly found in muscles (CK-MM) and in the brain (CK-BB). CK also exists in dimeric forms CKMM, CK-MB, CK-BB. Elevated levels have been used in assessing cardiac muscle damage following myocardiac infarction various types of muscular dystrophy and muscle diseases. CK especially CK-MB is used in the diagnosis and monitoring of myocardial infarction.

The JAS™ method employs a modification on the DGKC and IFCC recommendation. In the presence of Creatine phosphate, CK catalyses the reversible phosphorylation of ADP to form ATP and Creatine. The enzyme hexokinase then catalyses the phosphorylation of glucose to glucose-6-phosphate (G-6-P) and ADP from the formed ATP. The G-6-P is then oxidized to 6phosphogluconate with the simultaneous formation of NADH. The rate of NADH formed, measured at 340 nm, is directly proportional to the CK activity in the sample.

$$ATP + Glucose \xrightarrow{HK} ADP + Glucose - 6 - phosphate$$

$$G - 6 - P + NAD^+ \xrightarrow{G6PDH} 6 - Phosphogluconate + NADH + H^+$$

2.2.1.11 Troponin

Principle and Methodology

Troponin–I (Tn I) was analyzed using Enzyme–linked Immunoassay for cardiac specific Troponin-I using Ameritek USA assay kit. Assays were carried out as described by the manufacturer. This assay is based on the principle of solid phase enzyme- linked immunosorbent assay. Briefly, the desired coated wells were secured into the holder. The standard reference was diluted by using the reference standard dilution buffer to 2.0, 10, 20, and 50ng/ml. Fifty microlitres of

negative control, each standard and specimens into appropriate wells. This was then incubated at room temperature (18-25°c) for about 30 minutes. The incubation mixture was removed by flicking plate contents into a waste container. The microtiter well was then rinsed and flicked 5 times with wash buffer. The wells were stroked sharply onto absorbent paper to remove all residual water droplets. 100µl of enzyme conjugate was also dispensed into appropriate well and the mixture was incubated for 30 minutes at room temperature (18-25°c). The washing of the microtiter wells was again repeated. A 100µl of TMB reagent was dispensed into appropriate wells and incubated at room temperature for about 15minutes.

The reaction was stopped by adding 100µl of stop solution (2N HCl) to each well. This was gently mixed for 30 seconds; it was ensured that all blue colour changed to yellow. Optical density was read at 450nm with an ELx800™ Microplate Reader (Bio-Tek Instruments, Winooski, VT, USA) and results calculated from the standard curve using GraphPad Prism version 5.00 for windows (GraphPad software, San Diego California USA, www.graphpad.com).

2.2.1.12 Malondialdehyde Determination

Principle and Methodology

Malondialdehyde (MDA) levels were determined by the MDA- Thiobarbituric acid (TBA) test which is the colorimetric reaction of MDA and TBA in acid solution. TBA reacted with MDA, a secondary product from lipid peroxidation, which generated an adduct of red colour, which was detected spectrophotometrically. This method is a fast, sensitive, and low-cost method that can be used to indicate the extent of lipid peroxidation in a variety of systems (Shlafer & Shepard, 1984). The protocol used for this study is the Kamal *et al,* (1989) modification of the Shlafer *et al.,* (1984) protocol which is as follows:

A 0.5 ml of serum was treated to 2.5 ml of 20% trichloroacetic acid (TCA) and then 1 ml of 0.67% TBA. The mixture was incubated at 100^{o}C for 30 minutes. After cooling, the sample was extracted with 4 ml n-butanol and centrifuged at 3000

rpm for 10 min. The absorbance of supernatant was measured at 535 nm and the results were expressed as μmol/L, using the extinction coefficient of 1.56 x 10^5 L/mmol cm.

2.2.2 *HORMONAL ASSAY*

2.2.2.1 Human Placental Lactogen (hPL)

Principle and Methodology

Human Placental Lactogen (hPL) was analyzed using Enzyme–linked Immunoassay for specific hPL using DRG hPL Enzyme Immunoassay kit. Assays were carried out as described by the manufacturer (DRG Instruments, GmbH Germany). This assay is based on the principle of solid phase enzyme- linked immunosorbent assay (ELISA) sandwich method. The microtiter wells are coated with monoclonal antibody directed towards a unique antigenic site on the hPL molecule. An aliquot of patient sample containing endogenous hPL is incubated in the coated well with enzyme conjugate, which is a monoclonal anti-hPL antibody conjugated with horseradish peroxidase. After incubation the unbound peroxidase is proportional to the concentration of hPL in the sample. Upon adding substrate solution, the intensity of colour developed is proportional to the concentration of hPL in the patient sample.

Briefly, the microtiter wells were secured in the holder; the samples were prediluted in a 1:100 ratio with a zero standard. Into appropriate wells 10μL of each standard, control and pre-diluted samples were dispensed. 100μL of enzyme conjugate was also dispensed into each well and mixed thoroughly and incubated for 30 minutes at room temperature. The contents were shaken off and washed five times with distilled water. The microtiter well was stroke sharply on an absorbent paper to remove residual water. A 100μL of substrate solution was dispensed into each well and incubated for 10 minutes at room temperature. The reaction was stopped by adding 50μL of stop solution to each well. It was ensured that all blue colour changed to yellow. Optical density was read at 450nm with an ELx800™ Microplate Reader (Bio-Tek Instruments, Winooski, VT, USA) and results calculated from the standard curve using GraphPad Prism version 5.00

for windows (GraphPad software, San Diego California USA, www.graphpad.com).

2.2.3 *Urinalysis*

Urine protein was determined using the dip-stick qualitative method (CYBOW™ DFI Co Ltd, Gimhae-City, Republic of Korea). The determination was carried out according to the manufacturer's description. The assay is based on the protein "error of indicators". When pH is held constant by a buffer, indicator dyes release H^+ ions because of the protein present and change colour from yellow to bluegreen. The assay is a visual test procedure briefly; fresh strip was dipped into early morning urine collected in clean dry plastic containers. The strip was inserted up to the test area, for not more than two seconds. The edge of the strip was drawn along the brim of the vessel to remove excess urine making sure the test area does not touch the vessel. The strip was turned on its side and tapped on an absorbent paper to remove any remaining urine since excessive urine on the strip may cause interaction of chemicals between adjacent pads leading to incorrect results. The test result on the strip held horizontally and compared with the colour chart on the vial label under good light.

2.3 ANTHROPOMETRIC VARIABLES

2.3.1 *Measurements*

Anthropometric measurements included height, measured without shoes and weight measured in kilograms with light clothing. Subjects were weighed on a bathroom scale (Zhongshan Camry Electronic Co. Ltd, Guangdong, China) and their height measured with a wall-mounted ruler. BMI was calculated by dividing weight (kg) by height squared (m^2). Waist circumference was measured with a Gulick II spring-loaded measuring tape (Gay Mills, WI) midway between the inferior angle of the ribs and the suprailiac crest, whereas hip circumference was measured at the outermost points of the greater trochanters (WHO, 1995). WHR and WHtR were recorded to the nearest 2 decimal places.

2.3.1.1 Blood pressure

Blood pressure was taken by trained personnel using a mercury sphygmomanometer and stethoscope. Measurements were taken from the left upper arm after subjects had been sitting for >5 min in accordance with the recommendation of the American Heart Association (Kirkendall *et al.*, 1967). Triplicate measurements were taken with a 5 min rest interval between measurements and the mean value was recorded to the nearest 2.0 mm Hg.

2.4 THE METABOLIC SYNDROME:

Because no single definition for the metabolic syndrome has been accepted worldwide and to give the possibility for comparison with the majority of studies on the same topic, two widely used criteria were applied to examine the prevalence of the metabolic syndrome: the WHO and the NCEP III panels. The various definitions are listed below:

According to the NCEP III criteria, a subject has the metabolic syndrome if she has three or more of the following criteria:

- Abdominal obesity: ≥88cm
- Hypertriglyceridaemia: ≥ 1.7 mmol/L
- Low HDL cholesterol: < 1.3 mmol/L
- High blood pressure: ≥130/85 mmHg
- High fasting glucose: ≥110 mg/dl (6.1 mmol/L)

According to the WHO criteria, a subject has the metabolic syndrome if she has diabetes plus two or more of the following abnormalities:

- High blood pressure: ≥140/90 mmHg
- Hyperlipidaemia: triglyceride concentration ≥ 2.00 mmol/L) and HDL ≤1.0 mmol/L)

- Central obesity: waist circumference of ≥80 cm

2.5 INCIDENCE AND PREVALENCE STUDY

The medical records of all women who visited the Obstetric and Gynaecology department of KATH for antenatal care in 2006-2007 were assessed from the Reproductive Health Unit of the hospital and the relevant information obtained for the assessment of incidence and prevalence of hypertensive pregnancy. The information gathered included maternal age, parity, birth weight and maternal death. The diagnosis of gestational hypertension and preeclampsia were made based on clinical and laboratory findings by Obstetric & Gyneacology specialists. The cases of chronic hypertension, eclampsia and superimposed preeclampsia were not included in the study. The calculated prevalence ratio was defined as the prevalence in any month divided by the prevalence in the reference month.

2.6 STATISTICAL ANALYSIS

Continuous variables are expressed as their mean ± SEM, while categorical variables were expressed as proportion. Comparisons of the women with PIH (gestational hypertension and preeclampsia separately and combined) against the control group were performed using unpaired *t* tests, χ^2 tests, or Fisher exact tests where appropriate. Odds ratio and their 95% confidence intervals were used to quantify the risk of women with PIH in comparison with controls. A level of $p<0.05$ was acceptable as statistically significant unless otherwise stated.

Comparison of clinical variables and blood lipid profiles between hypertensive and control groups was by Pearson Correlation Coefficient. Correlation was significant at the 0.05, 0.01, 0.001 levels (2-tailed). GraphPad Prism version 5.00 for windows was used for these statistical analyses (GraphPad software, San Diego California USA, www.graphpad.com).

SAS System for windows, version 6.12 was used to examine other putative risk factors for possible confounding effects on PIH. Abortion variables were similarly examined by analysis to estimate the risk on PIH (preeclampsia and

gestational hypertension). Crude odds ratios (ORs) and 95% confidence intervals (CIs) were calculated for all risk factors, abortion variables and for the prevalence studies.

Chapter 3 **RESULTS**

3.1 DEMOGRAPHIC AND CLINICAL CHARACTERISTICS

From this study, 70% of the 100 PIH women are women who presented with gestational hypertension while 30% had preeclampsia. The control and the hypertensive women presented with similar age and gestational period, while, the mean value of the indices of hypertension (SBP and DBP) as well as proteinuria and percentage prevalence of hypertension were significantly increased in PIH, PE, and GH as was expected from the inclusion criteria (Table 3.1). Also, the mean value and the percentage prevalence of other components of the metabolic syndrome were significantly increased in the entire study group as compared to the control group.

Table 3.1 *Clinical Characteristics of PIH and Normotensive Pregnant Women*

PARAMETERS	**PIH (100)**	**PE (30)**	**GH (70)**	**CG (50)**
M. Age (yrs)	31.81±0.60	30.37±1.29	32.43±0.65	30.22±0.57
GP (weeks)	30.40±0.83	30.67±0.96	29.43±0.72	31.00±0.85
Proteinuria (g/l)	0.44±0.11**	1.43±0.30***	0.01±0.00	0.01±0.01
Weight (kg)	74.86±1.65*	76.43±2.75**	74.19±2.06*	68.86±1.32
BMI (kg/m^2)	29.49±0.61*	30.11±0.91**	29.23±0.79*	27.05±0.48
SBP(mmHg)	149.00±1.65***	133.80±4.25***	147.10±1.63***	105.80±1.54
DBP(mmHg)	96.01±1.20***	98.08±1.76***	93.23±1.11***	65.29±1.07

M. age= maternal age, GP= gestational period, PIH=pregnancy-induced hypertension, PE=preeclampsia, GH=gestational hypertension, CG =control group

3.2 The Prevalence of Metabolic Syndrome among Ghanaian Women with Pregnancy-Induced Hypertension

The demographics, metabolic and cardiovascular variables are shown in Table 3.2 From this study, the mean age of the PIH subjects is 31.81 ± 0.60 (30.37 ± 1.29 for the preeclamptic patients and 32.43 ± 0.65 for the gestational hypertensive patients) and 30.22 ± 0.57 for the normotensive control group. With the exception of hip circumference (HC) and waist-to-hip ratio (WHR) which were increased though not significant, all the other indicators of obesity, weight (WT), body mass index (BMI), waist circumference (WC), and waist-to-height ratio (WHtR) were significantly higher in the entire study group, pregnancy induced-hypertension (PIH), gestational hypertension (GH), and preeclampsia (PE) as compared to the control group. In general, fasting glucose and TG levels were significantly higher in women with GH, PE and PIH, while HDL-c slightly levels were lower in women with GH, PE and PIH as compared to the control group. The cardiac profile generally did not show any significant change when all the study groups were compared to the control group. Table 3.2

Table 3.2 *Demographics and metabolic and cardiovascular variables in each of the studied group*

PARAMETERS	GH	*P value*	PE	*P value*	PIH (GH+PE)	*P value*	CG
Age (yrs)	32.43 ± 0.65	0.0670	30.37 ± 1.29	0.2840	31.81 ± 0.60	0.6834	30.22 ± 0.57
GP (weeks)	29.43 ± 0.72	0.1609	30.67 ± 0.96	0.8021	30.40 ± 0.83	0.9630	31.00 ± 0.85
Proteinuria (g/L)	0.01±0.00	0.3172	1.43±0.30	<0.0001	0.44±0.11	0.0012	0.01±0.01
Height (m)	1.60 ± 0.01	0.9052	1.59 ± 0.01	0.7533	1.59 ± 0.01	0.7754	1.60 ± 0.01
BMI (kg/m^2)	29.23 ± 0.79	0.0323	30.11 ± 0.91	0.0016	29.49 ± 0.61	0.0100	27.05 ± 0.48
WC (cm)	106.30 ± 1.69	0.0003	103.80 ± 2.27	0.0282	105.50 ± 1.36	0.0003	96.31 ± 2.23
HC (cm)	113.30 ± 1.85	0.0062	110.20 ± 2.27	0.1597	112.40 ± 1.46	0.0074	104.80 ± 2.63
Waist/Hip Ratio	0.94 ± 0.01	0.0445	0.94 ± 0.01	0.1089	0.94 ± 0.01	0.0084	0.89 ± 0.03
Waist/Height Ratio	0.67 ± 0.01	0.0005	0.65 ± 0.01	0.0223	0.66 ± 0.01	0.0001	0.59 ± 0.02
SBP(mmHg)	147.10 ± 1.63	< 0.0001	133.80 ± 4.25	< 0.0001	149.00 ± 1.65	< 0.0001	105.80 ± 1.54
DBP(mmHg)	94.13 ± 1.13	< 0.0001	99.00 ± 2.89	< 0.0001	95.59 ± 1.19	< 0.0001	67.20 ± 1.07
FBS (mmol/L)	3.79 ± 0.12	0.0904	4.025 ± 0.19	0.0078	3.86 ± 0.10	0.0307	3.52 ± 0.08
HDL-C (mmol/L)	1.98 ± 0.07	0.4843	1.88 ± 0.11	0.6757	1.98 ± 0.06	0.4970	1.99 ± 0.08
TG (mmol/L)	3.44 ± 0.32	0.0282	3.43 ± 0.19	0.0437	3.84 ± 0.29	0.0441	2.54 ± 0.26
Tn I(ng/dl)	7.91 ± 0.49	0.4475	7.92 ± 0.50	0.4368	7.91 ± 0.38	0.3937	7.19 ± 0.79
CK (U/L)	121.30 ± 16.67	0.1654	82.40 ± 14.27	0.7611	111.60 ± 13.07	0.0354	69.80 ± 5.60
AST(U/L)	15.80 ± 1.12	0.2447	14.96 ± 1.73	0.5656	18.82 ± 2.61	0.2054	14.08 ± 0.66
LDH (U/L)	162.20 ± 14.68	0.2922	150.40 ± 22.06	0.2823	159.20 ± 12.27	0.9917	159.40 ± 16.08

GP-Gestational Period; BMI-body mass-index; WC-waist circumference; HC-hip circumference; BP-blood pressure; FBS-Fasting blood sugar; HDL-high-density lipoprotein; TG-triglyceride; Tn I-Troponin; CK-Creatine kinase; AST-Aspartate transaminase; LDH-Lactate dehydrogenase; PIH-pregnancy-induced hypertension. Data are expressed as mean (±SEM) or number (%). * Unpaired *t* test for means and χ^2 test for categorical variables compared with controls. Each comparison is performed between hypertensive groups individually (GH-gestational hypertension, PE- preeclampsia, or PIH-gestational hypertension + preeclampsia combined) and the CGcontrol group.

The percentage prevalence of obesity, hypertension, diabetes and dyslipidemia among the various studied groups as compared to the control group are shown in Table 3.3. Notable was the 31%, 46%, and 24% increase in BMI obesity as compared to the control group for PIH, PE, and GH respectively. Also, there was a 7%, 10% and 6% increase in hyperglycemia as compared to the control group for the PIH, PE, and GH respectively. As shown in Table 3.4, with the exception of TG and HDL, the mean value of each of the components of metabolic syndrome was significantly higher in the entire studied group as compared to the control group using the two criteria (the WHO and NCEP adapted definitions).

Table 3.3 *The distribution of obesity, hypertension, diabetes and dyslipidemia amongst the general study population*

PARAMETERS	PIH (n=100)	PE (n=30)	GH (n=70)	CONTROL (n=50)
WC (cm)				
NORMAL	2(2)	1(3)	1(1)	2(4)
CENTRAL OBESITY	98(98)	29(97)	69(99)	48(96)
BMI (Kg/m²)				
NORMAL	24(24)	4(13)	20(29)	24(48)
UNDERWT	21(21)	5(17)	16(23)	14(28)
OBESITY	55(55)	21(70)	34(48)	12(24)
WHR				
NORMAL	2(2)	1(3)	1(1)	2(4)
OBESITY	98(98)	29(97)	69(99)	48(96)
WHtR				
NORMAL	2(2)	1(3)	1(1)	2(4)
OBESITY	98(98)	29(97)	69(99)	48(96)
SBP (mmHg)				
NORMAL	5(5)	3(10)	2(3)	50(100)
HPT	95(95)	27(90)	68(97)	0(0)
DBP (mmHg)				
NORMAL	7(7)	2(7)	66(94)	50(100)
HPT	93(93)	28(93)	4(6)	0(0)
FBS (mmol/L)				
NORMAL	93(93)	27(90)	60(94)	50(100)

DIABETIC	7(7)	3(10)	4(6)	0(0)
TG (mmol/L)				
NORMAL	40(40)	12(40)	28(40)	8(16)
DYSLIPIDAEMIA	60(60)	18(60)	42(60)	42(84)

Table3.4 *Components of metabolic syndrome among the various hypertensive groups*

CRITERIA	PIH	P value	PE	P value	GH	P value	CG
NCEP III CRITERIA							
Abdominal obesity	107.30±1.24	<0.0001	105.60±2.03	0.0003	108.00±1.53	<0.0001	98.63±0.92
Hypertriglyceridaemia	4.27±1.38	0.7044	2.86±0.22	0.072	4.88±1.98	0.5378	3.55±0.27
Low HDL-c	2.19±0.05	0.7268	2.17±0.10	0.6738	2.20±0.06	0.815	2.06±0.07
SBP	149.10±1.55	<0.0001	155.20±3.39	<0.0001	146.80±1.60	<0.0001	0.00±0.00
DBP	97.38±1.02	<0.0001	102.20±2.09	<0.0001	95.45±1.07	<0.0001	0.00±0.00
High glucose	8.22±1.71	<0.0001	6.47±0.15	<0.0001	9.54±2.98	<0.0001	0.00±0.00
WHO CRITERIA							
Central obesity	106.30±1.29	0.0001	104.80±2.12	0.0032	106.90±1.60	0.0001	98.63±0.92
Hypertriglyceridaemia	4.75±1.65	0.7044	3.09±0.24	0.0720	5.46±2.35	0.5378	3.59±0.27
Low HDL-c	0.76±0.12	0.2003	0.80±0.19	0.3176	0.74±0.17	0.1991	0.45±0.00
SBP	149.70±1.56	<0.0001	156.20±3.38	<0.0001	147.10±1.60	<0.0001	0.00±0.00

DBP	97.52±1.04	<0.0001	102.70±2.11	<0.0001	95.22±1.09	<0.0001	0.00±0.00
High glucose	8.22±1.71	<0.0001	6.47±0.15	<0.0001	9.54±2.98	<0.0001	0.00±0.00

The proportions of women with components of the metabolic syndrome are summarized in Table 3.5. The rate of women with hyperglycaemia, hypertension and dyslipidaemia were increased in women in the studied group as compared to the control group. From the same Table 3.5 fasting hyperglycaemia and blood pressure are significant predictors of the metabolic syndrome in all the studied groups of women using the NCEP and the WHO criteria. There were no cases of metabolic syndrome in women with normal pregnancy according to the WHO adapted definition while 10% of them had metabolic syndrome according the NCEP adapted definition. When comparing WHO and NCEP adapted definitions in each studied group, the proportion of women with the metabolic syndrome was highest in women with PE (13%) followed by PIH (10%) and GH (6%) using the WHO adapted definition. Using the NCEP adapted definition, the metabolic syndrome was higher in PIH (62%) and exactly the same in women with preeclampsia (61%) and gestational hypertension (61%) (Table 3.5)

Table 3.5 *Prevalence of Metabolic Syndrome and the incidence of its components among Women in the Hypertensive Group and Controls*

	GH	*P Value	PE	P Value	PIH	*P Value	OR(95% CI) †	CG
WHO Criteria§								
FBS ≥ 6.1 mmol/L	4(6)	0.1472	3(10)	0.0594	7(7)	0.0979	----------	0(0)
WC ≥ 80 cm	69(99)	1.0000	29(97)	1.0000	98(98)	1.0000	1.0(0.6-1.7)	48(96)
HDL-C < 1.00; TG ≥ 2.0 mmol/L	4(6)	0.6476	2(7)	0.5559	6(6)	0.4288	3.0(0.4-25.6)	1(2)
BP ≥ 140/90 mmHg	63(90)	<0.0001	26(87)	<0.0001	89(89)	< 0.0001	----------	0(0)
Metabolic syndrome	6(9)	0.0803	4(13)	0.0240	10(10)	0.0316	----------	0(0)
NCEP III Criteria‡								
BP ≥ 130/85 mmHg	65(93)	<0.0001	27(90)	<0.0001	92(92)	<0.0001	----------	0(0)
FBS ≥ 6.1 mmol/L	4(6)	0.1472	3(10)	0.0594	7(7)	0.0979	----------	0(0)
HDL < 1.3 mmol/L	10(14)	0.5902	4(13)	0.7271	14(14)	0.6130	1.4(0.5-4.1)	5(10)
TG ≥ 1.7 mmol/L	50(71)	0.5777	22(73)	0.7285	72(72)	0.5179	0.8(0.5-1.4)	43(86)
WC ≥ 88 cm	66(94)	1.0000	28(93)	1.0000	94(94)	1.0000	1.0(0.6-1.6)	47(94)
Metabolic syndrome	43(61)	<0.0001	43(61)	0.0004	62(62)	<0.0001	6.2(2.3-16.4)	5(10)

OR, odds ratio; CI, confidence interval; WHO, World Health Organization; NCEP, National Cholesterol Education Program; HDL-C, high-density lipoprotein cholesterol; BP, blood pressure; FBS, fasting blood sugar; BP, blood pressure; TG, triglycerides. Data are presented as n (%). * Each comparison is performed between hypertensive groups individually (GH-gestational hypertension, PEpreeclampsia, or PIH- gestational hypertension + preeclampsia) and the CG-control group. Chi-squared test or Fisher exact test whenever n < 5 compared with controls. † Odds ratios are presented for the preeclampsia and gestational hypertension group combined. ‡ Three or more criteria are diagnostic of the metabolic syndrome. § One of the 3 criteria of insulin resistance and at least 2 other criteria are diagnostic of the metabolic syndrome

3.3 Lipid Profile and Lipid Peroxidation among Ghanaian Women with Pregnancy-Induced Hypertension

From the lipid profile, plasma triglyceride and LDL-C levels were significantly higher in the various subject groups than in the controls, whereas the plasma HDLC concentrations were much lower in these groups than in the control group, though it did not attain significant level. Total cholesterol, atherogenic index and VLDL concentrations were also not statistically different (Table 3.6). The ratios between different lipid fractions such as LDL-C : HDL-C; TC : HDL-C; TG : HDL-C and HDL-C : VLDL-C was also calculated. In this study, TG : HDL-C ratio was significantly elevated in the subjects as compared to controls.

Table 3.6 *Blood Lipids in Normotensive Pregnancy and PIH*

PARAMETERS	PIH	PE	GH	CG
Total cholesterol (mmol/L)	6.43±0.17	6.32±0.30	6.10±0.19	6.41±0.22
Triglycerides (mmol/L)	3.84±0.29*	3.43±0.19*	3.44±0.32*	2.54±0.26
HDL-Cholesterol (mmol/L)	1.98±0.06	1.88±0.11	1.98±0.07	1.99±0.08
VLDL-Cholesterol (mmol/L)	0.50±0.03	0.51±0.04	0.50±0.03	0.54±0.05
LDL-Cholesterol (mmol/L)	4.59±0.15*	4.44±0.20*	4.53±0.18*	3.84±0.19
Atherogenic index (mmol/L)	2.24±0.40	2.37±0.23	2.08±0.57	2.22±0.16
LDL-C : HDL-C ratio	2.31±0.09	2.36±0.15	2.28±0.11	2.12±0.13
TC : HDL-C ratio	3.25±0.11	3.35±0.23	3.00±0.12	3.22±0.17
TG : HDL-C ratio	1.94±0.09*	1.83±0.16*	1.74±0.11*	1.27±0.16
HDL-C : VLDL-C ratio	3.95±0.37	3.68±0.51	3.94±0.49	3.69±0.37

Atherogenic index = [(total cholesterol – HDL cholesterol)/HDL cholesterol].

The significant increase in the level of MDA in the various subject groups as compared to the control group was most pronounced when PE was compared to the control group (Figure 3.1).

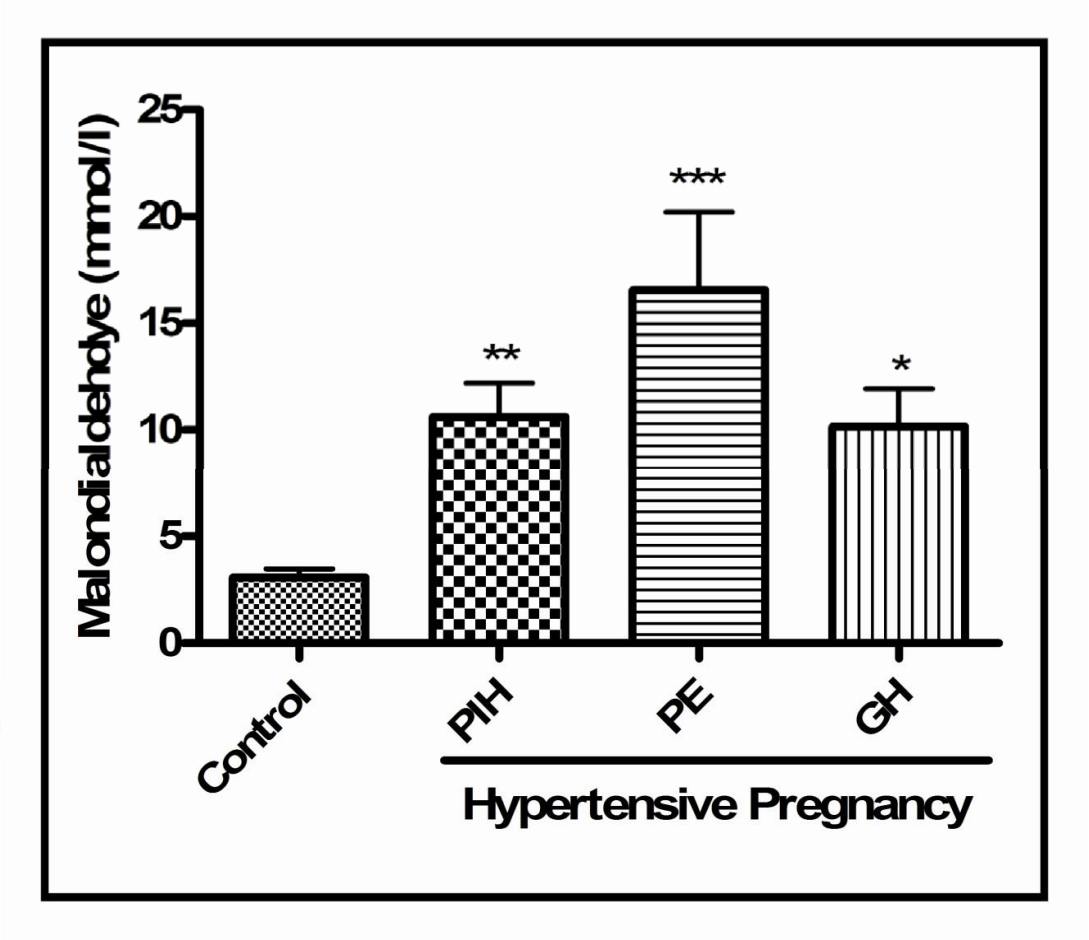


Figure 3.1 MDA levels in the various subject groups as compared to the control group.

Generally, there was no significant correlation between the clinical variables and lipid profile in relation to MDA within the control group. However, there is a significant positive correlation between blood pressure (SBP and DBP) and MDA among the PIH group (Table 3.7). Apart from VLDL and AI which gave significant positive correlation with MDA among both PE and GH, age, TC and TG also correlate positively with MDA among PE subjects. SBP and GA are additional factors that correlate significantly with MDA among the GH group (Table 3.7).

Table 3.7 *Pearson Correlation Co-efficient between Clinical Variables and Lipid Profile for PIH (Lower Left-Hand Side) and CG (Upper Right-Hand Side)*

PMT	AGE	SBP	DBP	GA	WT	BMI	TC	HDL	TG	LDL	VLDL	AI	MDA
AGE		-0.22	-0.11	-0.04	0.00	-0.01	0.06	0.08	-0.02	0.02	-0.02	-0.16	-0.71*
SBP	0.00		0.77***	-0.11	0.29	0.34*	0.04	0.06	-0.04	0.01	-0.04	0.01	-0.24
DBP	-0.10	0.42***		0.06	0.34*	0.35*	-0.04	0.15	0.02	-0.05	0.02	-0.06	-0.20
GA	-0.06	0.01	0.16		0.18	0.38**	0.18	-0.09	0.33*	0.14	0.33*	0.29	-0.26
WT	0.22*	-0.07	-0.06	0.04		0.77***	0.04	-0.03	0.26	0.00	0.26	0.11	0.22
BMI	0.19	-0.05	-0.02	0.10	0.91***		0.07	-0.10	0.43**	0.01	0.43**	0.18	0.30
TC	-0.05	-0.09	0.04	0.08	-0.03	0.02		-0.17	-0.13	0.94***	-0.13	0.47***	-0.05
HDL	-0.04	-0.09	-0.02	0.07	-0.15	-0.11	0.51***		-0.27	-0.41**	-0.27	-0.85***	0.24
TG	-0.01	-0.13	0.07	0.10	-0.03	0.01	0.94***	0.53***		-0.21	0.99***	0.29	0.28
LDL	-0.10	0.10	-0.05	-0.05	0.05	0.08	0.21*	-0.33***	-0.12		-0.21	0.62***	-0.17
VLDL	-0.20*	0.18	0.29**	-0.09	-0.23	-0.23*	-0.01	0.02	-0.02	0.02		0.29	0.29
AI	0.08	-0.03	-0.02	0.09	-0.10	-0.07	0.33***	0.62***	0.45***	-0.47***	0.04		-0.28
MDA	0.01	0.33*	0.36**	0.10	-0.13	-0.02	0.02	0.12	0.08	-0.13	0.12	0.14	

*Correlation is significant at the 0.05 level (2-tailed), **. Correlation is significant at the 0.01 level (2-tailed), ***. Correlation is significant at the 0.001 level (2-tailed).PMT= parameter, SBP=systolic blood pressure, DBP=diastolic blood pressure, BMI=body mass index, TC=total serum cholesterol, GA=Gestation age, WT=Weight, HDL=High Density Lipoprotein, LDL=Low Density Lipoprotein, VLDL=Very Low Density Lipoprotein, AI=Atherogenic Index, MDA=Malondialdehyde.

Table 3.8 *Pearson Correlation Coefficients between Clinical Variables and Lipid Profile for PE (Lower Left-Hand Side) and GH (Upper Right-Hand Side*)

PARAMETER	AGE	SBP	DBP	GA	WT	BMI	TC	HDL	TG	LDL	VLDL	AI	MDA
AGE		0.10	0.11	-0.03	0.14	0.12	0.03	-0.22	0.05	0.04	0.05	0.20	0.24
SBP	-0.05		0.52***	0.13	0.09	0.11	0.00	-0.18	-0.05	0.05	-0.04	0.17	0.49**
DBP	-0.26	0.30		0.29*	0.05	0.10	0.07	0.05	-0.17	0.07	-0.18	0.12	0.11
GA	-0.16	-0.19	-0.02		0.11	0.10	-0.12	-0.01	0.18	-0.11	0.18	0.04	0.67**
WT	0.45**	-0.40**	-0.28	-0.15		0.94***	-0.03	0.00	-0.04	-0.03	-0.04	-0.02	-0.05
BMI	0.44**	-0.40**	-0.31	0.11	0.83***		0.06	-0.05	0.03	0.06	0.03	0.09	0.00
TC	-0.12	0.26	-0.20	-0.03	-0.25	-0.39*		-0.17	-0.13	0.94***	-0.13	0.47	0.27
HDL	0.11	0.16	-0.55***	0.22	-0.11	-0.06	0.66***		-0.27	-0.41**	-0.27	-0.85***	0.23
TG	0.04	0.24	-0.30	-0.06	-0.17	-0.33	0.94***	0.73***		-0.21	0.99***	0.29*	0.22

LDL	-0.45**	0.12	0.34	-0.02	-0.27	-0.33	0.40**	-0.16	0.09		-0.21	0.62***	-0.07
VLDL	0.11	0.16	-0.55***	0.22	-0.11	-0.06	0.66***	0.99***	0.73***	-0.16		0.29*	0.35*
AI	0.40**	0.05	-0.53***	-0.05	0.05	-0.01	0.34	0.63***	0.61***	-0.67***	0.63***		0.33*
MDA	0.65***	0.09	-0.16	0.02	0.13	0.08	0.63***	0.25	0.68***	-0.05	0.31*	0.56***	

*. Correlation is significant at the 0.05 level (2-tailed), **. Correlation is significant at the 0.01 level (2-tailed), ***. Correlation is significant at the 0.001 level (2-tailed). SBP=systolic blood pressure, DBP=diastolic blood pressure, BMI=body mass index, TC=total serum cholesterol, GA=Gestation age, WT=Weight, HDL=High Density Lipoprotein, LDL=Low Density Lipoprotein, VLDL=Very Low Density Lipoprotein, AI=Atherogenic Index, MDA=Malondialdehyde.

Apart from HDL which gave a negative but significant correlation with AI within the control group, TC, LDL, and TG correlate positively with AI. With the exception of LDL which indicates a negative but significant correlation, TC, HDL and TG indicated significant positive correlation with AI among the PIH subjects (Table 3.7). Similar to PIH, subjects within the PE subgroup gave significant negative correlation between LDL and DBP in relation to AI and a significant positive correlation with age, HDL, TG and VLDL. Also, in both the control group (CG) and GH group, AI showed significant positive correlation with TG, LDL and VLDL; and negative significant correlation with HDL (Table 3.7 and 3.8).

LDL gave a significant negative correlation with HDL among all the studied groups except for PE where the negative correlation did not reach a significant level (Table 3.7 and 3.8). Among the PIH group, TC showed a significant positive correlation with HDL, TG and LDL (Table 3.7); whereas among the PE group, there was a positive correlation between TC and HDL, TG, LDL as well as VLDL (Table
3.8).

Table 3.9 *Frequency Distribution and Crude Odds Ratios for the Association of Putative Risk Factors for Pregnancy-Induced Hypertension*

PARAMETERS	CG (50)	PIH (100)	OR(95% CI)	GH (70)	OR(95% CI)	PE	OR(95% CI)
Maternal Age (yrs	) 8(16.0%)						
<25		11(11.0%)	1.5(0.5-4.7)	7(10.0%)	1.8(0.5-6.7)	4(13.3%)	1.2(0.3-5.8)
25-29*	19(38.0%)	17(17.0%)	1.00	9(12.9%)	1.00	8(26.7%)	1.00
30-34	17(34.0%)	35(35.0%)	2.3(0.9-5.5)	26(37.1%)	3.2(1.2-8.8)	9(30.0%)	1.3(0.4-4.3)
35-39	4(8.0%)	28(28.0%)	7.8(2.3-26.9)	22(31.4%)	11.6(3.1-43.8)	6(20.0%)	3.6(0.8-16.2)
40-44	2(4.0%)	9(9.0%)	5.0(1.0-26.6)	6(8.6%)	6.3(1.1-37.8)	3(10.0%)	3.6(0.5-25.6)
Parity							
0	20(40.0%)	43(43.0%)	1.1(0.7-2.3)	43(61.4%)	2.4(1.1-5.0)	0(0.0%)	0.02(0.0-0.4)
1+*	30(60.0%)	57(57.0%)	1.00	27(38.6%)	1.00	30(100.0%)	1.00
Body Mass Index (BMI)							
< 19	0(0.0%)	4(4.0%)	7.5(0.4-150.4)	3(4.3%)	7.5(0.4-159.6)	1(3.3%)	9.7(0.3-281.6)
19-24.9*	14(28.0%)	17(17.0%)	1.00	13(18.6%)	1.00	4(13.3%)	1.00
25-29.9	27(54.0%)	28(28.0%)	0.9(0.4-2.1)	22(31.4%)	0.9(0.3-2.3)	6(20.0%)	0.8(0.2-3.2)
≥ 30	9(18.0%)	51(51.0%)	4.7(1.7-12.7)	32(45.7%)	3.8(1.3-11.0)	19(63.3%)	7.4(1.9-28.9)
Marital Status							
Single	2(4.0%)	8(8.0%)	2.1(0.4-10.2)	4(5.7%)	1.5(0.3-8.3)	4(13.3%)	3.7(0.6-21.5)
Married*	48(96.0%)	92(92.0%)	1.00	66(94.3%)	1.00	26(86.7%)	1.00
Alcohol							
Yes	15(30.0%)	42(42.0%)	1.7(0.8-3.5)	28(40.0%)	1.6(0.7-3.4)	14(46.7%)	2.0(0.8-5.2)
No*	35(70.0%)	58(58.0%)	1.00	42(60.0%)	1.00	16(53.3%)	1.00
Educational Back;round							
None at all	3(6.0%)	8(8.0%)	1.6(0.3-7.9)	5(7.14%)	1.3(0.2-7.4)	3(10.0%)	2.3(0.3-18.9)
Basic	33(66.0%)	74(74.0%)	1.3(0.5-3.6)	50(71.4%)	1.2(0.4-3.5)	24(80.0%)	1.7(0.4-7.2)
Secondary*	7(14.0%)	12(12.0%)	1.00	9(12.9%)	1.00	3(10.0%)	1.00
Tertiary	7(14.0%)	6(6.0%)	0.5(0.1-2.1)	6(8.6%)	0.7(0.2-2.9)	0(0.0%)	0.1(0.0-3.3)
Exercise							
Yes*	28(56.0%)	45(45.0%)	1.00	31(44.3%)	1.00	14(46.7%)	1.00
No	22(44.0%)	55(55.0%)	1.6(0.8-3.1)	39(55.7%)	1.6(0.8-3.3)	16(53.3%)	1.5(0.6-3.6)
Family & Hypertension							
Yes	5(10.0%)	38(38.0%)	5.5(2.0-15.1)	26(37.1%)	10.6(3.6-31.4)	12(40.0%)	6.0(1.8-19.5)

No*	45(90.0%)	62(62.0%)	1.00	44(62.9%)	1.00	18(60.0%)	1.00	

Table 3.10 *Frequency Distribution and Crude Odds Ratios for the Association of Putative Risk Factors for Pregnancy-Induced Hypertension*

PARAMETERS	CG	PIH	OR(95%CI)	GH	OR(95%CI)	PE	OR(95%CI)
Prior adverse birth outcome							
No prior adverse birth*	**47(94%)**	**93(93%)**	**1.00**	**70(100%)**	**1.00**	**27(90%)**	**1.00**
Prior Caesarian Section	**3(6%)**	**3(3%)**	**0.5(0.1-2.5)**	**0(0%)**	**0.1(0.0-1.9)**	**3(10%)**	**1.7(0.3-9.2)**
Prior Preterm	**0(0%)**	**5(5%)**	**5.4(0.3-99.1)**	**0(0%)**	**----------**	**5(16.7%)**	**19.0(1.0-357.1)**
Prior Still Birth	**2(4%)**	**6(6%)**	**1.5(0.3-7.5)**	**0(0%)**	**0.1(0.0-2.9)**	**6(20%)**	**5.2(1.0-27.7)**
Prior Abortion							
No prior abortion*	**32(64%)**	**62(62%)**	**1.00**	**52(74.3%)**	**1.00**	**8(26.7%)**	**1.00**
Prior Spont. abortion	**17(34%)**	**31(31%)**	**0.9(0.4-1.8)**	**14(20%)**	**0.5(0.2-1.2)**	**17(56.7%)**	**1.7(0.7-4.0)**
Prior Ind. Abortion	**16(32%)**	**22(22%)**	**0.7(0.3-1.4)**	**18(25.7%)**	**0.8(0.4-1.6)**	**4(13.3%)**	**0.4(0.1-1.4)**
Salt intake							
Moderate*	**43(86%)**	**83(83%)**	**1.00**	**60(86.7%)**	**1.00**	**23(76.7%)**	**1.00**
High	**7(14%)**	**17(17%)**	**1.3(0.5-3.3)**	**10(14.3%)**	**1.0(0.4-2.9)**	**7(23.3%)**	**1.9(0.6-5.9)**
Condom use							
Yes	**2(4%)**	**19(19%)**	**5.6(1.2-25.2)**	**12(17.1%)**	**5.0(1.1-23.3)**	**7(23.3%)**	**7.3(1.4-37.9)**
No*	**48(96%)**	**81(81%)**	**1.00**	**58(82.9%)**	**1.00**	**23(76.7%)**	**1.00**
Contraceptive Use							
Yes	**13(26.0%)**	**37(37.0%)**	**1.7(0.8-3.5)**	**25(35.7%)**	**1.6(0.7-3.5)**	**12(40.0%)**	**1.9(0.7-4.9)**
No*	**37(74.0%)**	**63(63.0%)**	**1.00**	**45(64.3%)**	**1.00**	**18(60.0%)**	**1.00**

Partner Change							
Yes	**10(20.0%)**	**37(37.0%)**	**2.3(1.1-5.2)**	**21(30.0%)**	**1.7(0.7-4.1)**	**16(53.3%)**	**4.6(1.7-12.4)**
No*	**40(80.0%)**	**63(63.0%)**	**1.00**	**49(70.0%)**	**1.00**	**14(46.7%)**	**1.00**

***Reference group, CG = control group, PIH = pregnancy-induced hypertension subjects, PE = preeclampsia group, GH = gestational hypertension group, OR = odds ratio and CI = confidence interval, Spont. abortion= spontaneous abortion, Ind. Abortion= induced abortion.**

Table 3.11 *Crude Odds Ratios for Pregnancy- Induced Hypertension by number of Abortions & Type of Abortion*

Parameters		CG	PIH	OR(95% CI)	GH	OR(95% CI)	PE	OR(95% CI)
Abortion & type								
Nulliparous	No Abortion*	18(36.0%)	43(43.0%)	1.00	37(52.9%)	1.00	0(0.0%)	1.00
	1 spontaneous	0(0.0%)	0(0.0%)	----------	0(0.0%)	----------	0(0.0%)	----------
	1 H. induced	0(0.0%)	0(0.0%)	----------	0(0.0%)	----------	0(0.0%)	----------
	1 self induced	2(4.0%)	0(0.0%)	0.1(0.0-1.9)	3(4.3%)	0.7(0.1-4.8)	0(0.0%)	----------
	2+ spontaneous	0(0.0%)	0(0.0%)	----------	0(0.0%)	----------	0(0.0%)	----------
	2+ H. induced	0(0.0%)	0(0.0%)	----------	0(0.0%)	----------	0(0.0%)	----------
	2+ self abortion	0(0.0%)	0(0.0%)	----------	3(4.3%)	3.5(0.2-70.5)	0(0.0%)	----------

	1+ all abortion	0(0.0%)	0(0.0%)	----------	0(0.0%)	----------	0(0.0%)	----------
Multiparous	no abortion	0(0.0%)	4(4.0%)	3.8(0.7-74.8)	13(18.6%)	13.3(0.7-236.8)	3(10.0%)	259.0(4.4-15380.0)
Multiparous	1+ abortion	30(60.0%)	53(53.0%)	0.7(0.4-1.5)	14(20.0%)	0.2(0.1-0.5)	27(90.0%)	0.4(0.2-0.9)

***Reference group, CG = control group, PIH = pregnancy-induced hypertension subjects, PE = preeclampsia group, GH = gestational hypertension group, OR = odds ratio and CI = confidence interval, H. induced =Hospital induced, (Self induced and H. induced abortion) = induced abortion**

3.4 PUTATIVE RISK FACTORS FOR PREGNANCY-INDUCED HYPERTENSION

3.4.1 *Pregnancy-Induced Hypertension*

From this study, both the younger and elderly pregnant women were at risk of developing PIH as compared to the reference group. The number of risk factors increased from about 2 times (OR = 1.5; 95% CI = 0.5-4.7) in the younger population which includes the teenage mothers to about 8 times (OR = 7.8; 95% CI = 2.3-26.9) among the pregnant women within the 35-39 year group (Table 3.9). Whereas the risk of a nulliparous woman developing PIH was similar to that of multiparous women, the risk was about two fold among single mothers, women who consumed alcoholic beverages, women without any formal education and women who do not participate in any form of exercise (Table 3.9). Women who were underweight were about 8 fold at risk of developing PIH (OR = 7.5; 95% CI = 0.4-150.4), and those who were obese were about 5 fold at risk of the pathology (OR = 4.7; 95% CI = 1.7-12.7). Women with a family history of hypertension were about 6-fold at risk of developing PIH (OR = 5.5; 95% CI = 2.0-15.1) (Table 3.9). Maternal education up to the tertiary level confers some protection against developing PIH from this study (Table 3.9).

Women with a history of still birth and preterm deliveries were about two and five fold at risk of developing PIH respectively (Table 3.10). A history of caesarean section, spontaneous or induced abortion did not pose any risk for developing PIH in this study. High salt intake posed only a slight risk of developing PIH (OR = 1.3; 95% CI = 0.5-3.3). Contraceptive use and partner change posed about 2 fold risk each for developing PIH (Table 3.10). Condom usage among the male partner of the women also posed about 6 times the risk of developing PIH (OR = 5.6; 95% CI = 1.2-25.2) (Table 3.10).

Derivations from the analyses of abortion type and number of abortion are shown in Table 3.11. Because the crude findings were similar to results obtained from a

logistic regression that controlled for other risk factors, only crude odds ratios are presented in this study. The analysis of abortion type shows that nulliparous women with spontaneous or induced abortion (i.e. one prior spontaneous abortion, one prior self induced or one prior hospital induced) is not associated with a risk of PIH in the subsequent pregnancy. A similar degree of non-association was seen among women who had two or more spontaneous or induced abortion (i.e. hospital induced, self induced), one or more of all types of abortion and multiparous women with a history of abortion. However, multiparous women without a history of abortion (OR = 3.8; 95% CI = 0.7-74.8) had an increased risk of developing PIH (Table 3.11).

3.4.2 *Gestational Hypertension*

The risk of developing GH increased from about 2 times among women who were less than 25 years old to about 3 times among 30-34 years old women to about 12 times and 6 times among women within the 35-39 and 40-44 years old women respectively (Table 3.9). Nulliparous women, single mothers, women who consumed alcoholic beverages and women who did not engage in exercise were about 2 times at risk of developing GH as seen in Table 3.9. Women without any form of education (OR = 1.3; 95% CI = 0.2-7.4) and those who attained education only up to the basic level (OR = 1.2; 95% CI = 0.4-3.5) were only slightly at risk of developing GH. Obesity among the pregnant women increased the risk of developing GH about 4 folds, whereas underweight women were 8 times at increased risk. Women with a family history of hypertension had about 11 times increased risk of developing GH (Table 3.9).

History of caesarean section, preterm delivery, spontaneous abortion, still birth and induced abortion confered some sort of protection against the development of GH from this study (Table 3.10). While the risk posed by high salt intake was similar to that seen among women who took moderate salt (OR = 1.0; 95% CI = 0.42.9), partner change and the use of contraceptive posed about 2 fold risk each

of developing GH (Table 3.10). The risk was, however, about 5 times among women whose partners used condom.

Analysis of abortion type (Table 3.11) suggests that a history of one prior spontaneous abortion did not influence the risk of developing gestational hypertension and this was synonymous to the effect posed by induced abortion (i.e. one prior self induced and hospital induced abortion). Examination of nulliparous subjects who had had two or more self induced abortions indicates that they are about 4 times at risk of developing GH (OR = 3.5; 95% CI = 0.2-70.5). The history of one or more abortion types among the multiparous women showed a protective effect as only 20% of the GH had one or more abortion as against 100% in the case of the controls (OR = 0.1; 95% CI = 0.1-0.3). However, multiparous women without a history of abortion were about 13 times at risk of developing GH (OR = 13.3; 95% CI = 0.7-236.8) (Table 3.11).

3.4.3 *Preeclampsia*

The risk of developing preeclampsia (PE) increased with maternal age from 1.2 times among women less than 25 years old (OR = 1.2; 95% CI = 0.3-5.8) to about 4 times among women who were between 40-44 years old (OR = 3.6; 95% CI = 0.5-
25.6). Lack of exercise, lack of any formal education as well as education up to the basic level and women who consumed alcohol were at about two fold risk of developing PE (Table 3.9). The risk of developing PE was about 4 times among single mothers, 6 times among women with a family history of hypertension, 7 times among women who were obese and about 10 times among underweight women (Table 3.9). Nulliparity appeared to confer some form of protection against developing PE.

Apart from induced abortion which confers some form of protection against developing PE, caesarean section, preterm delivery, spontaneous abortion and still birth posed about 2, 19, 2 and 5 times risk to developing PE respectively (Table

3.4.2). High salt intake and the use of contraceptive posed about 2 fold risk each of developing PE. The risk of developing PE among women who have undergone partner change was about 5 times (OR = 4.6; 95% CI = 1.7-12.4) and the risk among women whose partners used condoms was 7 times (OR = 7.3; 95% CI = 1.4-37.9) (Table 3.10).

The result of this study indicates that none of the abortion types in both nulliparous and multiparous subjects is associated with PE. However, when the abortion type was grouped together as one, multiparity was associated with the risk of developing PE in women without history of abortion (Table 3.11).

From figure 3.4, the concentration of Human Placental Lactogen was significantly decreased when the PIH group was compared to the control group. The mean concentration of hPL for the PIH subjects was 4.91±0.32, for the GH subjects was 4.75±0.37, for the PE subjects was 4.42±0.59mg/L and that of the control group was
8.62±1.05mg/L.

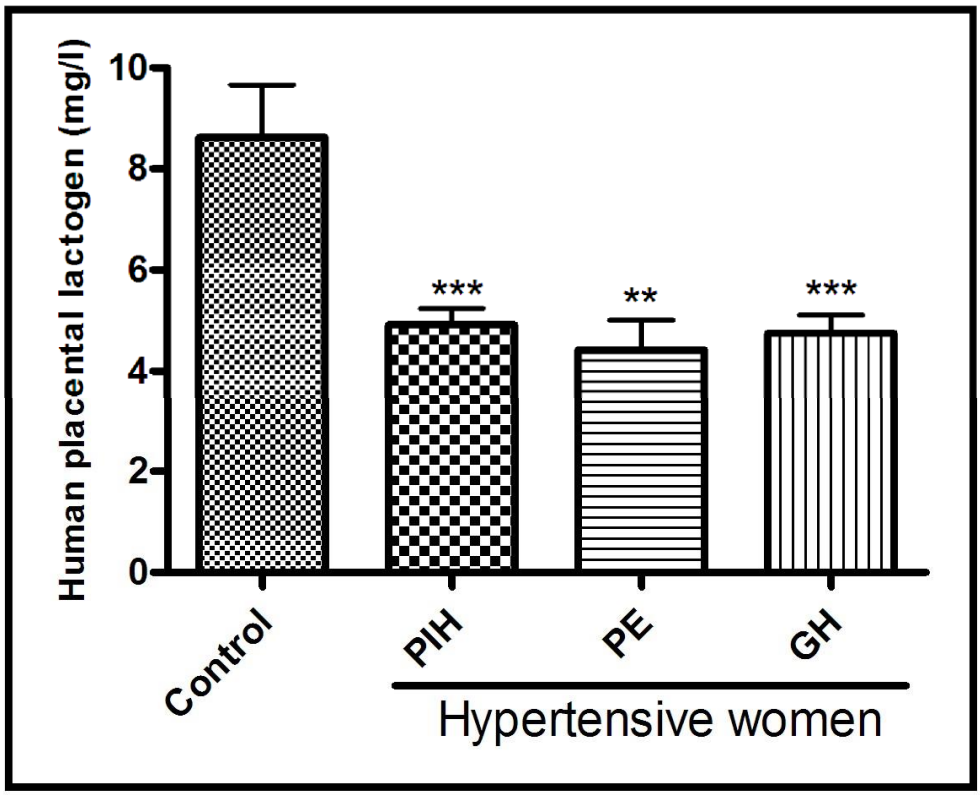


Figure 3.2 Distribution of human placental lactogen (hPL) in control and pregnancy-induced hypertension subjects (PIH). PE = preeclampsia group, GH = gestational hypertension group. Values are mean ± SEM and significantly different from control using unpaired *t*-test: **P < 0.01 and ***P < 0.001.

3.5 INCIDENCE, PREVALENCE AND SEASONAL VARIATION

Out of the total of 8,091 antenatal visits to the Komfo Anokye teaching Hospital, Kumasi, from 2006 to 2007, 1,005 (12.42%) presented with PIH. Preeclampsia was identified in 530 (6.55%) pregnant women whilst gestational hypertension was

identified in 475 (5.87%) pregnant women. The prevalence of PIH and GH were found to be highest (17.80% and 8.74% respectively) in September whilst that of PE was found to be highest (9.33%) in August. The prevalence ratios were 2.36, 2.55 and 2.26 for PIH, GH and PE respectively for the highest values (Table 3.12). The prevalence as well as the prevalence ratios for all the conditions generally declined during the dry season (from November to March) to the lowest level (7.55%, 3.43% and 4.12% for PIH, GH and PE respectively) in January, with January as the reference month (Table 3.12).

Using maternal age between 20-35 years as the reference group, a substantial increase in risk was found for women less than 20 years of age and for those greater than 35 years of age (Table 3.13). Multiparity from this data did not have a significant effect on PIH and its sub-group when nulliparous women were used as the reference group. The prevalence ratio among women with birth weight less than 2.5 kg was about 2, 3 and 5 for GH, PIH and PE respectively (Table 3.13). The percentage prevalence of maternal death among the GH subjects was 2.86 and 7.62 and 10.48 for PE and PIH respectively.

Table3.12 *Prevalence (%) and prevalence ratio of hypertensive disorders of pregnancy according to monthly variation, 2006-2007*

	Gestational Hypertension		**Preeclampsia**		**Pregnancy-Induced Hypertension**	
Months	*Prevalence (%)*	*Prevalence Ratio*	*Prevalence (%)*	*Prevalence Ratio*	*Prevalence (%)*	*Prevalence Ratio*
January	3.43	1.00	4.12	1.00	7.55	1.00
February	4.83	1.41	5.43	1.32	10.26	1.36
March	6.59	1.92	5.91	1.43	12.5	1.66
April	4.83	1.41	7.41	1.80	12.5	1.62
May	7.47	2.18	5.14	1.25	12.61	1.67
June	7.23	2.11	7.23	1.75	14.46	1.92
July	4.56	1.33	6.32	1.53	10.88	1.44
August	5.24	1.53	9.33	2.26	14.57	1.93
September	8.74	2.55	9.06	2.20	17.8	2.36
October	8.14	2.37	6.38	1.55	14.52	1.92
November	4.00	1.17	5.60	1.36	9.6	1.27
December	6.43	1.87	8.97	2.18	15.4	2.04

Table 3.13 *Prevalence (%) and PR of hypertensive disorders of pregnancy stratified by age, parity and birth weight, 2006-2007*

PARAMETERS	GH		PE		PIH	
	%prevalence	*prevalence ratio*	*%prevalence*	*prevalence ratio*	*%prevalence*	*prevalence ratio*
Maternal age						
< 20	13.51	2.70	7.21	1.20	20.72	1.88
20-35	5.00	1.00*	6.00	1.00*	11.00	1.00*
>35	10.67	2.13	10.56	1.76	21.23	1.93
Parity						
0	7.34	1.00*	9.27	1.00*	16.61	1.00*
1--3	4.46	0.61	5.08	0.55	9.53	0.57
4--6	8.6	1.17	8.24	0.89	16.84	1.01
7+	4.85	0.66	9.71	1.05	14.56	0.88
Birth weight						
>2.5	1.43	1.00*	1.2	1.00*	2.63	1.00*
<2.5	2.79	1.95	6.03	5.03	8.83	3.36
Maternal death	2.86		7.62		10.48	

PR=prevalence ratio, *= Reference value.

The percentage incidence rate in 2006 and 2007 is as shown in figure 3.3A. The incidence of PIH was significantly higher ($P < 0.001$; $\chi^2 = 44.75$; df = 1) in 2007 (17.03%) as compared to 2006 (11.46%). The incidence rate of PE and GH were also significantly higher ($P < 0.001$; $\chi^2 = 22.87$; df = 1 and $P < 0.001$; $\chi^2 = 18.10$; df = 1 for PE and GH respectively) in 2007 (9.03% and 8.01% for PE and GH respectively) as compared to that of 2006 (6.01% and 5.64% for PE and GH respectively).

When the prevalence of the various conditions were assessed based on the seasonal variation, it was realized that the prevalence were significantly higher ($P < 0.001$; $\chi 2 = 16.23$; df = 1, $P = 0.0201$; $\chi^2 = 5.402$; df = 1 and $P = 0.0016$; $\chi^2 = 9.978$; df = 1 for PIH, PE and GH respectively) during the rainy season (from April to October) as compared to the dry season (from November to March) as shown in figure 3.4B. Even though, when the study population was stratified based on the year (i.e. 2006 and 2007), there was no significant differences ($P = 0.2794$; $\chi^2 = 1.170$; df = 1, $P = 0.2116$; $\chi^2 = 1.560$; df = 1 and $P = 0.8869$; $\chi^2 = 0.0202$; df = 1 for PIH, PE and GH respectively) in the prevalence of the various conditions during the raining season and dry season for 2006 (Figure 3.5C). The differences however attained a statistically significant level in 2007 ($P < 0.001$; $\chi^2 = 38.62$; df = 1, $P < 0.001$; $\chi^2 = 16.81$; df = 1 and $P < 0.001$; $\chi^2 = 18.36$; df = 1 for PIH, PE and GH respectively) (Figure 3.6D).

After adjusting for possible confounding variables, the relative risk of the various conditions was expressed according to the other variables as odds ratios using multiple logistic regressions. Adjusting for other variables did not alter the

seasonal trend in the occurrence of PIH (Table 3.14). The average relative risk of developing PIH, GH and PE during the dry season was 1.46 each whilst the average relative risk of developing PIH, GH and PE during the raining season was 1.83, 1.77 and 1.76 respectively (Table 3.14). A substantial increase in risk was found for women less than 20 years and greater than 35 years of age confirming the earlier observation in table 3.13. The risk of PIH, PE and GH in the second and subsequent pregnancy was at least 50% of the risk in the first pregnancy (Table
3.15).

Table 3.15 shows the monthly average temperatures and precipitation for Kumasi, in the Ashanti region. Generally, the average monthly humidity and precipitation were higher during the wet season (from April to October) as compared to the dry season (from November to March). However, the average monthly temperatures as well as the average maximum temperature were much higher during the dry season compared to the wet season (Table 3.15).

Table 3.14 *Prevalence (%) and adjusted odds ratios (ORs) with 95% confidence intervals (CI) of hypertensive pregnancies 2006-2007.*

	PIH	GH	PE
Month			
January	1.0*	1.0*	1.0*
February	1.4(1.0-1.9)	1.4(0.8-2.3)	1.3(0.8-2.1)
March	1.7(1.2-2.3)	1.9(1.2-3.1)	1.4(0.9-2.3)
April	1.6(1.1-2.3)	1.4(0.8-2.4)	1.8(1.1-2.8)
May	1.7(1.2-2.3)	2.2(1.4-3.4)	1.2(0.8-2.0)
June	1.9(1.3-2.8)	2.1(1.2-3.6)	1.8(1.1-2.9)
July	1.4(1.0-2.0)	1.3(0.8- 2.2)	1.5(1.0-2.4)
August	1.9(1.4-2.7)	1.5(0.9-2.5)	2.3(1.5-3.5)
September	2.4(1.7-3.3)	2.5(1.6-4.0)	2.2(1.4-3.4)
October	1.9(1.4-2.7)	2.4(1.5-3.7)	1.5(1.0-2.4)
November	1.2(0.91.8)	1.1(0.7-2.0)	1.4(0.9-2.1)
December	2.0(1.5-2.8)	1.9(1.1-3.1)	2.2(1.4-3.4)
Maternal Age			
>20	1.9(1.4-2.6)	2.7(1.8-4.0)	1.2(0.7-2.0)
20-34	1.0*	1.0*	1.0*

>35	1.9(1.6-2.3)	2.1(1.7-2.7)	1.8(1.4-2.2)
Parity			
0	1.0*	1.0*	1.0*
1--3	0.6(0.5-0.7)	0.6(0.5-0.8)	0.5(0.5-0.7)
4--6	1.0(0.8-1.3)	1.2(0.9-1.6)	0.9(0.7-1.2)
7+	0.9(0.5-1.5)	0.7(0.3-1.6)	1.0(0.5-2.0)

Table3.15 *Percentage Temperature / Rainfall patterns in Kumasi, Ashanti region*

MONTH	Av. T (°C)	Av. Mn T(°C)	Av. Max T(°C)	Av. Ppt/Rf (in)	R. Humidity (%)
JAN	25.5	20	31	0.8	78
FEB	26.5	20	33	2.2	75
MAR	27	22	32	5.5	79
APR	27	22	32	5.7	81
MAY	27	22	31	7.2	84
JUNE	25	21	29	9.2	87
JULY	25	21	28	4.9	87
AUG	24	21	27	2.9	87
SEPT	25	21	28	6.8	87
OCT	26	21	30	7.9	87
NOV	26	21	31	3.9	84
DEC	25	20	30	1.3	82

Av. T=Average Temperature, Av. Mn T= Average Mini temperature, Av. Max T= Average Maximum temperature, Av.Ppt/Rf= Average precipitation/rainfall, R. humidity= Relative humidity.
Source for temperature, rainfall and humidity patterns: http://www.climatetemp.info/ghana/kumasi.html

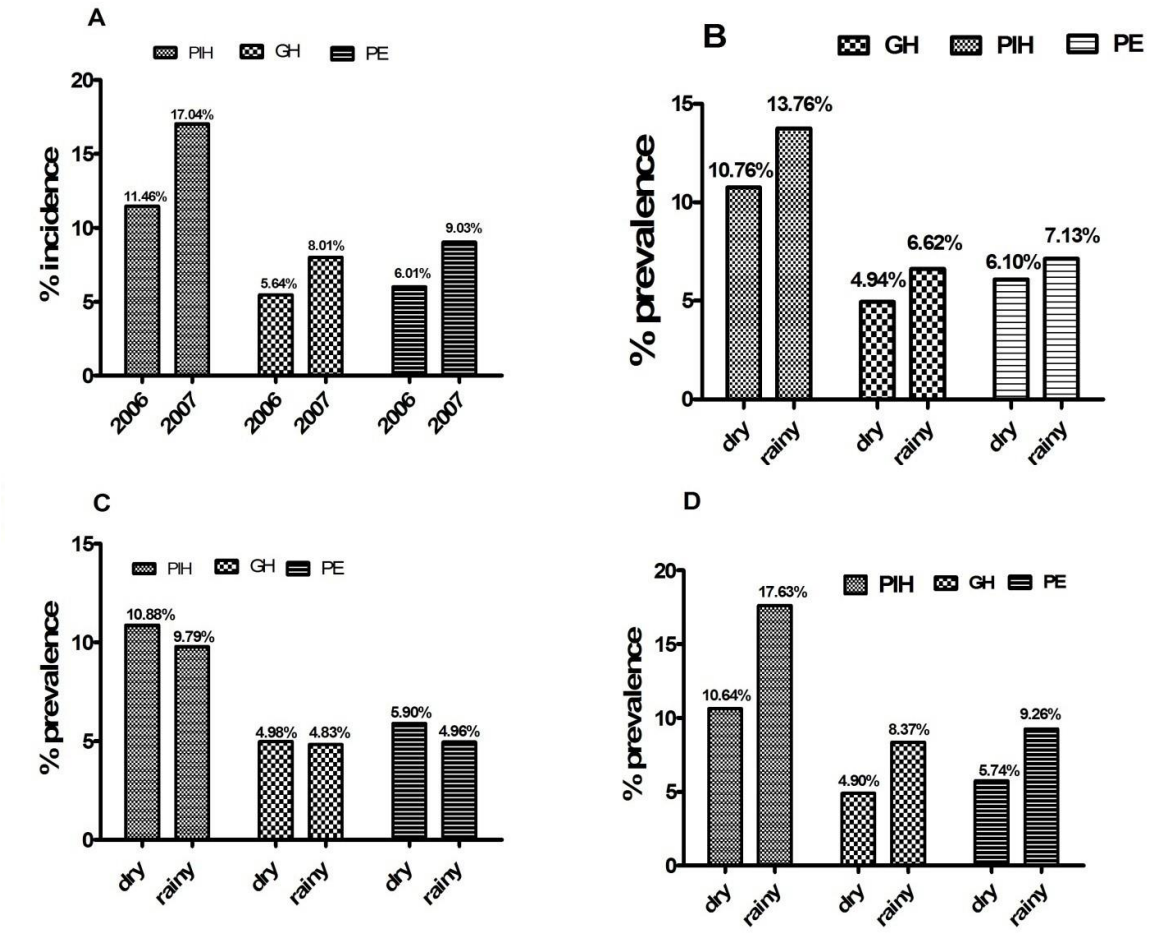


Figure 3.3A Shows the percentage incidence rate in 2006 and 2007.

Figure 3.4B Shows the seasonal variation in percentage prevalence for the year 2007.

Figure 3.5C Shows the seasonal variation in percentage prevalence for the year 2006.

Figure 3.6D Shows the seasonal variation in percentage prevalence for the year 2006-2007.

Chapter 4 DISCUSSION

4.1 Putative Risk Factors of Pregnancy-Induced Hypertension among Ghanaian Pregnant Women

Several epidemiological studies have considered the association of maternal demographic characteristics and the risk of Pregnancy-Induced Hypertension (gestational hypertension and preeclampsia). Inspite of this, there have been inconsistent reports on the effects of maternal age on preeclampsia. While some studies did not find age to be a significant risk factor (Anorlu *et al.*, 2005; CondeAgudelo & Belizan, 2000; Eskenazi *et al.*, 1991), some studies have reported increased risk of preeclampsia in younger women who are ≤ 21 years (Anorlu *et al.*, 2005; Sibai, 1990b) and others have reported an association of increased risk of preeclampsia with women who are 35 years or older (Conde-Agudelo & Belizan,
2000; Sibai, 1990b).

The increase in the prevalence of developing preeclampsia with increasing maternal age demonstrated in this study is consistent with the findings of other studies (Conde-Agudelo & Belizan, 2000; Sibai, 1990b). Several reports have stated that usually teenage or younger mothers are at increased risk of developing preeclampsia, however, this study found that younger mothers are only slightly at risk of preeclampsia whereas older mothers were at greater risk for the pathology. Indeed, this observation was not only made for preeclampsia but for PIH collectively as well as GH. Stone *et al*, (1995) and later Hartikainen *et al.*, (1998) have also demonstrated that older mothers are at increased risk of GH. The slightly increased risk of developing PIH (PE, GH) for younger mothers observed from this study may be attributed to most probably the socio-economic status (SES) of the young mother whereas biological changes associated with maturity are more attributable to the risk observed in older mothers. Indeed, teenagers and women aged 35 years and above have been shown to have a greater risk of adverse

perinatal outcomes, including preterm birth (Cnattingius *et al.*, 1992; Fraser *et al.*, 1995; Gilbert *et al.*, 1999; Jacobsson *et al.*, 2004) and perinatal or infant mortality (Cnattingius *et al.*, 1992; Jacobsson *et al.*, 2004) which are some of the complications of Pregnancy- Induced Hypertension.

The extensively reported association between maternal age and hypertensive disorders of pregnancy (Anorlu *et al.*, 2005; Conde-Agudelo & Belizan, 2000; Owiredu *et al.*, 2009; Sibai, 1990b) is re-emphasized in the prevalence study. In that, finding revealed that, of the total women visiting KATH for antenatal care, younger (<20 years) as well as older (>35 years) pregnant women had increased risk for PIH, GH and PE. Obed & Aniteye (2006), in their study reported that preeclampsia occurred more frequently in women above 35 years in contrast to those between 20-35 years and also in those below 20 years. Gaio *et al.*, (2001) found increased prevalence of preeclampsia/eclampsia in higher age groups particularly women 35 years or older. The findings of the study supports the notion that extremes of age (younger or older mothers) increases the risk for hypertensive disorders of pregnancy as observed in the high prevalence of the hypertensive condition in women of these age groups.

In this study, the risk of preeclampsia positively associated with maternal obesity as measured by maternal Body Mass Index (BMI). Several studies have shown a strong association between increased maternal body mass and risk of preeclampsia (Anorlu *et al.*, 2005; Bodnar *et al.*, 2005; Eskenazi *et al.*, 1991; Villamor & Cnattingius, 2006). There is general agreement that maternal obesity is associated with an increased risk of medical and puerperal complications, including hypertension (Johnson *et al.*, 1992). This corroborates the findings of this study where subjects presenting with GH, a milder form of the clinical condition, similarly associated positively with obesity. Despite the fact that, increased weight gain and obesity have long worried women due to its effect on

beauty and general physical appearance, it is now progressively evident that obesity is now a health

issue. The prevalence of overweight and obesity have been increasing, with approximately half of white women and a staggering 70% of African-American women aged 20–39 years in the United States being overweight or obese (Flegal *et al.*, 2002). A study carried out in Nigeria among hypertensive subjects showed an increased prevalence of obesity (Ijarotimi & Keshinro, 2008). A sociodemograhpic study carried out in Ghana in 2003 also revealed an increased prevalence of overweight and obesity predominantly among women (Amoah, 2003). Pregnancy carries considerable risks for women who are obese; these include increased rates of congenital anomalies (neural tube and cardiac defects), miscarriage, gestational diabetes, hypertension, and problems during delivery (Cedergren, 2004; Linne, 2004). Similarly, prevalence of polycystic ovary syndrome (PCOS) seems to be rising because of the current epidemic of obesity; at least 40% of women with PCOS are obese and it accounts for 90-95% of women who attend infertility clinics with anovulation (Balen & Michelmore, 2002).

Furthermore, obesity is associated with insulin resistance, dyslipidemia, chronic inflammation and oxidative stress (Reilly & Rader, 2003), all of which have been demonstrated in women presenting with PIH (Ahenkorah *et al.*, 2008; Roberts & Lain, 2002; Turpin *et al.*, 2008). Even though the fine mechanism by which obesity/insulin resistance increases the risk of PIH is not well understood, these irregularities may act independently or collectively to advance the endothelial dysfunction observed in women with PIH (GH and PE). Inflammatory substances secreted by the adipose tissue among overweight and obese women may lead to chronic inflammation which may adversely affect endothelial vascular function (Mutlu-Turkoglu *et al.*, 1999; Sartipy & Loskutoff, 2003). Therefore, chronic inflammation maybe the focal point linking increased weight gain to PIH.

As a result of the strong relationship observed, the association between increasing changes in BMI and risk of PIH may support the hypothesis that obesity-mediated inflammatory changes may play a role in the pathogenesis of PIH

(Getahun *et al.*, 2007). This concept is further supported by the finding of excessive levels of Creactive protein in overweight and obese women (Visser *et al.*, 1999; Wolf *et al.*, 2001). Extrahepatic synthesis of C-reactive protein in response to proinflammatory cytokines, such as interleukin-6 and tumour necrosis factor, has also been recently identified in the adipose tissue (Calabro *et al.*, 2005).

Most studies have associated the risk of PIH with increased or increasing BMI. However, this study found an increased risk for PIH (PE+GH) among underweight pregnant women as well as among overweight and obese pregnant women. Indeed, pregnancies among underweight or overweight women are often regarded as high risk pregnancies, and thin or underweight women are often advised to gain weight before becoming pregnant (Cnattingius *et al.*, 1998). A large number of previous studies have associated underweight pregnant women with an increased risk of delivering an infant who is small for gestational age, and have also shown an increased risk of preterm delivery among these women (Cnattingius *et al.*, 1998; Sebire *et al.*, 2001; Wolfe *et al.*, 1991). It is common knowledge that PIH strongly associates with preterm delivery which is usually undertaken before the disease progresses to become life threatening for both mother and child.

Similarly, underweight individuals have usually been associated with malnutrition or undernutrition. This cohort of underweight women may have experienced malnutrition before or during pregnancy most probably due to low SES (socioeconomic standard). Low SES increases stress levels due to financial insufficiencies and indeed, Takiuti *et al*, (2003) have reported that stress associates well and increases the risk of PIH.

Furthermore, from this study, lack of exercise was found to be positively associated with the risk of PIH (PE+GH). Pregnancy and prepregnancy weight gain have been reported to be associated with an increased risk of PIH and

exercise is known to generally control weight gain. Preeclampsia has been hypothesized as a stress-related disease and indeed epidemiologic studies show that the relative risk for preeclampsia is increased in many stressful situations (Takiuti *et al.*, 2003). Marcoux *et al.* (1989) also found that increased leisure time activity protected against preeclampsia. Similarly, moderate/high physical activity is reported to be associated with a two times increase in the risk of severe preeclampsia compared to mild activity (Spinillo *et al.*, 1995). Consequently, if mild physical activity or increased leisure time activity confers protection against preeclampsia, protection against GH, a condition generally considered a milder form of the disease can be expected since increased leisure time activity reduces stress and increases the state of well being.

The effect of parity on preeclampsia has been well recognized; preeclampsia is considered to be a disease largely associated with first pregnancies (Roberts & Redman, 1993). Serhal and Craft, (1987) also reported that first pregnancy is a risk factor for preeclampsia and its occurrence is more common in nulliparous than multiparous women (Eskenazi *et al.*, 1991; Roberts & Redman, 1993). The increased risk among nulliparous women has been postulated to be due to maternal first exposure to chorionic villi, which is of foetal origin (Vinatier & Monnier, 1995). The prevailing hypothesis for the increased risk reported for nulliparous women is that after the first pregnancy, the maternal immune system would have 'recognized' the paternal antigens and developed a greater immune tolerance towards the same antigens in subsequent pregnancies (Robertson *et al.*, 2003). This implies that, in nulliparous women the impaired immunological tolerance experienced is largely as a result of their first exposure to the paternal antigens, which results in disordered placentation, a common feature of preeclampsia. This study did not however, demonstrate this association. The lack of association between nulliparity and risk of preeclampsia observed in this study is in agreement with the findings of Funai *et al.,* (2005).

However, it has been reported in some studies that multiparous women who have had a change of partner before the index pregnancy have an increased risk of preeclampsia (Eras *et al.*, 2000; Feeney & Scott, 1980; Li & Wi, 2000). This observation possibly suggests a loss of the protective effect of multiparity with change of spouse/partner. Therefore, preeclampsia might be a problem of primipaternity (Robillard *et al.*, 1999; Robillard *et al.*, 1993) rather than primigravidity as many studies suggest. Besides that, Funai *et al.,* (2005) have proposed that the extent to which preeclampsia would appear to be mainly a disease of first pregnancies would depend on the proportion of patients visiting the hosiptal who were nulliparous. Also, in populations with high infertility levels, a greater proportion of cases with first pregnancies would be observed. Furthermore, nulliparous women presented with twice as much risk of having GH. Hernández-Díaz *et al.,* (2002) similarly found the relative risk for primiparity among pregnant women presenting with GH to be 2.04 (95 % CI: 1.52-2.73). The increased risk observed in this cohort of women may be attributed to other underlying causes or predisposing factors. This also suggests that gestational hypertension and preeclampsia may differ in their aetiologies, prognosis as well as risk factors.

Previous studies have suggested that various aspects of standards of living (SES) influences the risk of preeclampsia especially when measured by education (Conde-Agudelo & Belizan, 2000; Ros *et al.*, 1998) and marital status (Eskenazi *et al.*, 1991; Stone *et al.*, 1994). Extremes of maternal age (young and advanced), low socioeconomic status have also been suggested and debated as risk factors for preeclampsia (Eskenazi *et al.*, 1991; Klonoff-Cohen *et al.*, 1989; Samadi *et al.*, 1996). In this study, the observed four fold increased risk among the single mothers and the negative correlation between the attainment of higher education and the risk of developing preeclampsia suggests that lack of education and single motherhood are strong risk factors, as have been previously reported by Eskenazi

et al., (1991) and Samadi *et al.,* (1996). While single motherhood posed a risk for the development of GH as well, lack of education and basic education only slightly influenced the risk of GH. In the United States of America, preeclampsia is largely associated with unmarried, teenage women (Eskenazi *et al.*, 1991; Samadi *et al.*, 1996).

Low education and single motherhood may relate to poor economic status and therefore inappropriate antenatal care. Observational studies have revealed strong association between late commencement of antenatal care or the number of antenatal visits with preterm delivery (Barros *et al.*, 1996; Kotelchuck, 1994; Kramer, 1987) whereas preterm delivery strongly associates with preeclampsia. Furthermore, there could be increased levels of stress due to the inadequacies such as financial insecurity, unsatisfying marital relationship and poor living conditions. Indeed, it has been reported that low SES pregnant women experience more stressful life events during their pregnancy (Peacock *et al.*, 1995; Rutter & Quine, 1990) and Takiuti *et al.*, (2003) have shown that preeclampsia increases in many stressful conditions.

Many epidemiological studies have identified alcohol consumption as a risk factor for the development of hypertension (Beevers, 1977; Friedman *et al.*, 1982; Mitchell *et al.*, 1980). Generally, pregnant women are advised to abstain from alcohol consumption during pregnancy because of the likelihood of foetal alcohol syndrome (FAS). Foetal Alcohol Syndrome (FAS) is a set of birth defects caused by maternal consumption of alcohol during pregnancy which includes intra-uterine growth restriction (IUGR), abnormalities to the skull and face, defects of the arms or legs, and mental retardation (Armant & Saunders, 1996). Alcohol is most teratogenic during organogenesis (gestational week 9 to 11) and may destroy placental trophoblastic cells, increase thromboxane production and reduce the utero-placental blood flow (Armant & Saunders, 1996; Siler-Khodr *et al.*, 2000). In this study, alcohol consumption increased the risk of PIH (PE+GH).

It has long been known, that environmental influences, such as smoking (Lieberman *et al.*, 1994; Shu *et al.*, 1995; Spinillo *et al.*, 1994) and alcohol use (Shu *et al.*, 1995) have a causative role in impaired foetal growth or IUGR. Women presenting with PIH are known to experience IUGR and foetal death as well as abruptio placentae as severe complications. Abruptio placentae refers to the premature separation of the placenta from the uterus after the 20th week gestation and before the birth of the foetus (Sarwar *et al.*, 2006).

Placental abruption is due to the rupture of the uterine spiral artery (Eskes, 2001). Bleeding into decidua leads to separation of the placenta. Haematoma formation further separates the placenta from the uterine wall, causing compression of these structures and compromise of blood supply to the foetus (Sarwar *et al.*, 2006). Although, the aetiology of abruptio placentae remains obscure, some risk factors implicated include hypertensive disorders of pregnancy, polyhydramnios, intrauterine growth restriction, advanced maternal age, maternal trauma, cigarette smoking, alcohol consumption, previous miscarriage *et cetera* (Sarwar *et al.*, 2006). These observations imply that alcohol consumption during pregnancy does not only increase the risk of hypertensive pregnancy but also increases placental and foetal abnormalities.

Hypertension is regarded as a major public health problem (Cappuccio *et al.*, 2004). It is the most common medical complication of pregnancy, which occurs in 3% to 10% of pregnancies (Saudan *et al.*, 1998). Preeclampsia and gestational hypertension are common hypertensive complications of pregnancy. Several epidemiological studies have indicated that a family history of chronic hypertension is an independent risk factor for preeclampsia (Eskenazi *et al.*, 1991; Kobashi *et al.*, 2001; Qiu *et al.*, 2003).

This study has established a high risk for preeclampsia as well as gestational hypertension for women with a family history of hypertension. In this study,

women with a family history of hypertension have about 11 times risk of developing GH. GH is characterised by increased systolic and diastolic blood pressure and may precede the onset of preeclampsia which is a more severe form of the clinical condition. In a twin study conducted in Sweden by Salonen Ros *et al*, (2000) it was established that the genetic component of preeclampsia was approximately 54%, with the remaining 46% attributable to non-shared environmental factors. But additional studies with genetic linkage methods and twin studies are required to further quantify the genetic and non-genetic component that influences the risk of PIH (Qiu *et al.*, 2003). Genetic and behavioural factors may therefore, predispose a pregnant woman to the development of Pregnancy-Induced Hypertension.

Consistent high salt consumption posed about two fold risk for developing preeclampsia in this study. Although, the mechanisms by which salt intake increases the blood pressure are not clear (de Wardener *et al.*, 2004) there is evidence in support of the hypothesis which proposes that prolonged increases in salt intake or the habitual high intake of salt is related to a rise in arterial blood pressure (Chobanian & Hill, 2000). A study carried out by Cappucino *et al*, (2006) in the Ashanti region of Ghana showed a significant and positive relationship between the level of salt intake and both systolic and diastolic blood pressure. Furthermore, there is good evidence that a reduction in salt intake reduces the blood pressure (Sacks *et al.*, 2001) and that people of black African origin living in Africa respond well to reduction in blood pressure when salt intake is reduced (Adeyemo *et al.*, 2002; Cappuccio *et al.*, 2006; 2000). Hypertension, a feature of preeclampsia, is also a risk factor for the development of Pregnancy-Induced Hypertension as such a high dietary salt intake which predisposes to hypertension might be the focal point for the increased risk observed in women presenting with preeclampsia.

Contraceptive use in both the male and the female partner positively associated with the risk of PIH, (PE and GH inclusive) in this study. Pregnancy-Induced Hypertension has long been considered to have an immunological basis. The results of several studies suggest that repeated exposure to the male partner's spermatozoa prior to conception reduces the risk of Pregnancy-Induced Hypertension in the first pregnancy (Marti & Herrmann, 1977). This is further supported by a prospective study conducted using 1011 pregnant women, which reported a strong inverse association between the length of sexual cohabitation with the partner and the risk of Pregnancy-Induced Hypertension, suggesting that extended duration of sexual intercourse might reduce this risk (Robillard *et al.*, 1994).

This implies that if extensive periods of cohabitation with the partner protects against Pregnancy-Induced Hypertension, it could be deduced that the mechanism may be related to the contact of spermatozoa with the female genital tract. This might also explain the high risk observed in women whose spouses used condom as a means of contraception because the use of condom reduces or limits exposure of the female genital tract to the male partner's spermatozoa.

Similarly, women who used contraceptives from this study were not protected from Pregnancy-Induced Hypertension. From the face-to-face interview conducted in this study, most of the women used oral contraceptives which are known to act at different points of the female reproductive tract, i.e. cervical mucus thickening, tubal motility, changes in endometrial lining (i.e. by decreasing the possibility of implantation, should conception occur) and ovulation suppression (Gratacos *et al.*, 1996).

Oral contraceptives induce changes in cervical mucus which makes it impenetrable to spermatozoa (Wolf *et al.*, 1979) thus confining the spermatozoa to the vagina. Gratacos *et al*, (1996) have also previously reported data suggesting

that the use of oral contraceptives induces some modifications that block the immunological protective response that occurs during unprotected cohabitation. The nonprotective effect of oral contraceptives might therefore be as a result of the reduced exposure to spermatozoa which is brought about by the effect of oral contraceptives on the female genital tract.

It has been suggested that preeclampsia results from an immunologic intolerance between maternal and foetal/paternal tissues (Eras *et al.*, 2000).Various studies have reported paternity change as a risk factor for preeclampsia (Feeney & Scott, 1980; Li & Wi, 2000; Trupin *et al.*, 1996). This current study did not only find partner change to be a strong risk factor for preeclampsia but also for gestational hypertension. Indeed, it is presumed that a previous normal pregnancy is associated with a reduced risk of Pregnancy-Induced Hypertension, but other studies have reported that this protective effect is lost with partner change (Dekker, 2002; Robillard *et al.*, 1999; Robillard *et al.*, 1993). Lie *et al.,* (1998) have also provided evidence of the existence of the so-called 'dangerous' father, by establishing that men who fathered one preeclamptic pregnancy were nearly twice as likely to father a preeclamptic pregnancy in a different woman regardless of whether she had already had a preeclamptic pregnancy or not (Dekker, 2002; Lie *et al.*, 1998).

The previous birth history of subjects was assessed to ascertain if a previous birth event associates with the risk of Pregnancy-Induced Hypertension. Caesarian delivery and preterm delivery were observed to be significant risk factors for preeclampsia in this study. Wilkes *et al.,* (2003) have also indicated that preeclampsia is a risk factor for caesarian delivery. To date, the aetiology and pathophysiological condition of preeclampsia still remain unknown with no therapy to prevent it and the only effective treatment is delivery (Villar *et al.*, 2006). It is common knowledge that women presenting with preeclampsia undergo caesarian delivery in hospitals when the life of either mother or child or

both is at risk and usually these births are preterm deliveries and preeclampsia is a leading cause of preterm delivery**.** Indeed, in the course of this study, five infants from preeclamptic mothers had to be delivered by caesarean section because of signs of foetal hypoxia in the first stage of labour.

In this study, previous spontaneous abortion, still birth as well as induced abortion conferred protection against GH, whereas protection against preeclampsia was only conferred by a previous history of induced abortion from this study. Eras *et al*, (2000) have also reported that a history of abortion conferred protection against both gestational hypertension and preeclampsia. Strickland *et al.,* (1986) similarly, found that a prior abortion reduced a woman's risk of transient hypertension in a subsequent pregnancy. In one of the few studies to evaluate the timing of abortion, Campbell *et al.,* (1985) showed that late spontaneous abortion, defined as 13-27 weeks, conferred protection against preeclampsia in the subsequent pregnancy, whereas early spontaneous abortions did not. This might in part explain why spontaneous abortion (i.e. miscarriage) which usually occurs in the early weeks of pregnancy could not reduce the risk of preeclampsia in this study.

It is reasonable that a positive history of abortion would protect against PIH when one assumes an underlying immunologic pathophysiology for these conditions. The fetoplacental unit contains paternal antigenic tissue, which is foreign to the maternal host. Immunologic tolerance through the development of a protective mechanism between the foetal/paternal allograft and maternal tissue is necessary for a successful pregnancy (Beer, 1978; Eras *et al.*, 2000). Thus, presumably the enhanced development of maternal-foetal immunologic tolerance through pregnancies resulting in spontaneous or induced abortion may explain the protective effect of abortion on the risk of Pregnancy-Induced Hypertension in a subsequent pregnancy (Runic *et al.*, 1996).

A strong association between hypertensive pregnancy and still birth has been demonstrated. Ananth *et al*, (1995) found an increased relative risk of still birth for all classes of hypertensive pregnancy. The causes of stillbirth (foetal death > 20 week) may be maternal, placental, or foetal and genetic. Overall, the most common cause is abruptio placentae and this is a common complication of preeclampsia (Beers *et al.*, 2006). Having had a stillbirth or late abortion (at 16 to 20 weeks) increases risk of foetal death in subsequent pregnancies. This may be the link between the non-protective effects of a history of a previous abortion observed from this study. Treatment of maternal disorders (diabetes, infections, hypertension) may lower risk of stillbirth in a current pregnancy. Common maternal causes include uncontrolled diabetes mellitus, preeclampsia or eclampsia, sepsis, substance abuse, trauma, and hereditary thrombotic disorders. Placental causes include vasa previa, chorioamnionitis, umbilical cord accidents (eg, prolapse, knots) uteroplacental vascular insufficiency, twin-twin transfusion, and fetomaternal haemorrhage (Beers *et al.*, 2006). Hypertensive disorders have been found to have a strong adverse impact on stillbirth suggesting that early diagnosis of hypertension during pregnancy and adequate medical intervention may help reduce the risk of stillbirth (Ananth *et al.*, 1995).

Additionally, this study sought to identify the association between the number and type of prior abortion and the risk of Pregnancy-Induced Hypertension. This study has demonstrated that abortion confers protection against PIH (GH and Preeclampsia). Among nulliparous women, the effect of one spontaneous abortion had the same protective effect as an induced abortion (i.e. hospital induced) on the risk of PIH, GH and preeclampsia. A history of abortion of either type was protective for all groups, although the number of abortions required to achieve a reduced risk differed for these three conditions. For preeclampsia and PIH, one prior induced abortion conferred a decreased risk for the pathology and that was similar to the decreased risk conferred by two prior induced abortions.

For gestational hypertension, a history of at least two self abortions (i.e. induced abortion) was not associated with a protective effect. The protective effect of abortion in this study is in agreement with previous studies (Abi-Said *et al.*, 1995; Eras *et al.*, 2000; Sibai *et al.*, 1997; 1995).

Multiparous women without a history of abortion were also at increased risk of developing PIH (PE +GH) in this study. Strickland *et al, (*1986*)* reported that a prior abortion reduced a woman's risk of transient hypertension in a subsequent pregnancy. Similarly, El-Roeiy & Gleicher (1998) have suggested that preeclampsia results from an immunologic intolerance between maternal and foetal/ paternal tissues (Eras *et al.*, 2000). These findings could possibly explain the observed risk among multiparous women without prior abortion history.

The role of the placenta in the genesis of preeclampsia (and in the IUGR that can result from it) is well known (Merviel *et al.*, 2004; Pijnenborg *et al.*, 1980). The early work of Kaplan and Grumbach (1964) and Sciarra *et al.*, (1963) which identified the placenta as the sole organ to produce hPL, directed much attention to its role as an indicator of placental function. Human placental lactogen (hPL) is a protein hormone expressed by the syncytiotrophoblast cells of the placenta. It is a marker of syncytiotrophoblastic differentiation (Alsat *et al.*, 1996). Obiekwe *et al*, (1984) has also reported significantly reduced hPL levels in association with foetal growth retardation, indicating that maternal hPL levels reflect both the pathology of the disease as well as the condition of the foetus.

As such, this study sought to find the levels of this placental hormone among Ghanaian women presenting with pregnancy-induced hypertension. In this study, decreased levels of hPL was observed in all the subject cases (PIH, PE, GH) with the least concentration noted among the PE subjects (Fig 3.2). The observed decreased hPL level in this study is consistent with the findings of other studies

(Bersinger *et al.*, 2002; Letchworth & Chard, 1972a; Letchworth & Chard, 1972b; Spellacy *et al.*, 1971; Westergaard *et al.*, 1984).

Human Placental Lactogen (hPL) has been associated with pregnancy-related hypertension or preeclampsia, and umbilical vascular resistance and either an increased (Bewley *et al.*, 1993; Murai *et al.*, 1997; Obiekwe *et al.*, 1984) or a decreased level (Bersinger *et al.*, 2002; Westergaard *et al.*, 1984) of this hormone has been reported in these conditions, suggesting the hypothesis that the abnormality in the production of placental hormone is related to the aetiology of the disease. hPL levels are a valuable indicator of foetal well-being (Letchworth & Chard, 1972b), clinical signs of foetal growth retardation and very low HPL values seem to indicate high foetal risk (Lindberg & Nilsson, 1973). As stated earlier, in the course of this study, two infants died in utero and five infants had to be delivered by caesarean section because of signs of foetal hypoxia in the first stage of labour.

hPL appears to fulfill all the criteria of a good test of placental function (Genazzani *et al.*, 1971; Spellacy *et al.*, 1971). In preeclampsia, the failure of the trophoblastic invasion of the maternal spiral arteries (branches of the uterine arteries) leads to permanent vasoconstriction of these vessels. This in turn causes inadequate perfusion of the placental villi, from the second trimester onwards (Jones & Fox, 1980). The resulting reduction in the supply of oxygen by the spiral arteries leads to the hyperplasia of the cytotrophoblast. Fox, (1983) has reported that a histological examination of the placentae of women with preeclampsia revealed areas of necrosis in the syncytiotrophoblast and cellular proliferation of the cytotrophoblast.

Alsat *et al.*, (1996) have shown that hypoxic conditions in vitro impaired the transformation of cytotrophoblast cells into syncytiotrophoblast cells and simultaneously reduced hPL expression. Such anomalies of the trophoblastic

invasion of the spiral arteries occurring during preeclampsia and IUGR of vascular origin lead to permanent vasoconstriction of these vessels and subsequent chronic placental hypoxia (Hustin & Franchimont, 1988; Merviel *et al.*, 1998) which is associated with Pregnancy-Induced Hypertension. Studies carried out by Molinari *et al*, (2003) in anaesthetized pigs showed that low intra-arterial infusion of human placental lactogen causes a decrease in coronary, renal and iliac blood flow resulting in vasoconstriction of the vessels. These findings, showing an effect of widespread vasoconstriction elicited by low intra-arterial infusion of human placental lactogen, are consistent with a possible contribution of this hormone to the pathogenesis of preeclampsia. This possibility is further supported by the fact that the peripheral vasoconstriction caused by the hormone has been shown to be positively related to the dose of human placental lactogen intra-arterially infused (Molinari *et al.*, 2003). These findings suggest a possible contribution of hPL to the pathogenesis of PIH as well as IUGR observed in this cohort of women.

4.2 INCIDENCE, PREVALENCE AND SEASONAL VARIATION

Racial, dietary and environmental factors are some of the factors that have been implicated in the incidence of hypertensive disorders of pregnancy (PIH, PE and GH) which are major obstetric complications with unclear aetiologies. Elucidating the exact association with different weather patterns may help in understanding the factors involved in triggering these events. Davies *et al.,* (1970) studied PIH in the Jerusalem region prospectively and found ethnic differences. Racial differences in the occurrence of PIH were also reported from the Fiji islands (low incidence) and India (high incidence) which could be attributed to meteorological conditions
(Chesley, 1984).

However, there have been inconsistent reports on the influence of climatic conditions on the incidence of hypertensive disorders of pregnancy. In a review by Tan & Salmon, (1988) the characteristics said to influence the frequency of PIH include hot weather, cool damp weather, cold fronts, any change in weather, cool dry weather, warm months, and monsoon rains. If hot, humid temperatures were associated with hypertensive complications, then a higher rate of hypertensive pregnancies could be observed during the dry season; on the other hand, if cooler, humid conditions were associated with an increased frequency of hypertensive complications, then the numbers should be increased in the rainy season.

This study found a seasonal variation in the occurrence of PIH, PE and GH with a peak in the wet months and a minimum in the dry months. It has been noted that incidence of eclampsia was higher in warm and moist weather (Dieckmann, 1938). A study in Israel correlated the increase in thromboembolism and stroke to the occurrence of hot weather conditions. The increased risk of thromboembolism during warm days and the influence on the physiology of the blood vessels could also increase the risk of occurrence of preeclampsia in pregnancy. However, most studies show that a low temperature and high humidity are associated with a high
risk of pregnancy-induced hypertension, preeclampsia and gestational hypertension (Agobe *et al.*, 1981; Bergstrom *et al.*, 1992; Bider *et al.*, 1991; Crowther,
1985; Neela & Raman, 1993) as confirmed by this study.

The scarcity of data in so far as incidence and prevalence of the hypertensive disorders of pregnancy is concerned in Ghana emphasized the need for

prevalence data. From this study, an incidence of 5.46% and 8.01% for the years 2006 and 2007 respectively was noted for GH, a finding comparable to results for Gestational Hypertension that has been reported elsewhere. GH is believed to complicate between 4.4% and 17.5% of pregnancies, with a weighted average of 14.6% (Hauth *et al.*, 1993; North *et al.*, 1999; Stone *et al.*, 1995). Correspondingly, the observed incidence of 6.01 and 9.03% for the two different years among women presenting with PE is similar to those reported in other studies. Mittendorf, (1996) reported that the incidence of preeclampsia vary between 3-10%. In the only accessible Ghanaian study by Obed & Aniteye (2006) they reported an incidence of 7.03% for PE. PIH was incident in 11.46 and 17.04% of the population for the years 2006 and 2007 respectively. Additionally, the prevalence rates for the years under review were as follows: PIH was identified in 12.42% of the pregnant women, whereas PE and GH occurred in 6.55% and 5.87% respectively. Seely & Solomon (2003) reported that Pregnancy-Induced Hypertension complicates 5-10% pregnancies in the United States and later Villar *et al*, (2006) reported that Hypertension complicates approximately 9% of pregnancies worldwide with substantial maternal and perinatal morbidity and mortality. Although, incident and

prevalence rates of GH and PE are comparable to those of other studies, the PIH rates were higher from this study, the differing reports could be due to differences in definition, population composition, demographic and obstetric characteristics, actual disease incidence, or access to and availability of antenatal care services

(WHO, 1988) as well as diet and lifestyle practices.

Diverse hypotheses of the causes of hypertensive disorders of pregnancy (PIH, PE and GH) have been elaborated (Dekker & Sibai, 1998; Zhang *et al.*, 1997). The observations from this study and the seasonal trends reported from other countries (Makhseed *et al.*, 1999; Obed *et al.*, 1994; Tan & Salmon, 1988) point to environmental factors that show seasonal variability in occurrence. It is possible that cold weather could lead to the kind of vasospasm that is a part of the pathogenesis of preeclampsia. The effect of cold weather on ischemia which is assumed to be the basis of the relatively strong association between outdoor temperature and the occurrence of myocardial infarction (Rose, 1966) could be an analogy. Like myocardial infarction, preeclampsia can be considered as having predisposing (the fetomaternal genes), contributing (infections, diet, smoking) and precipitating causes (cold weather) (Magnus & Eskild, 2001). The reported lower day time blood pressure during hot weather (Modesti *et al.*, 2006) has been attributed to increased vasodilatation and, loss of water and salt by sweating (Rosenthal, 2004). While cold weather is known to cause release of catecholamines, which can increase blood pressure. Others have suggested that cold temperature triggers preeclampsia via the induction of peripheral vasoconstriction (Magnus &
Eskild, 2001).

Dietary factors can also influence hypertensive disorders of pregnancy (PIH, PE and GH), which are relatively uncommon in the tropics and the low incidence is ascribed to the diet which is mainly carbohydrates. During pregnancy, the foetus is exposed to nutrients and may be exposed to infectious agents through the mother. Dietary intake and risk of infection varies with season. Similarly hypertensive disorders of pregnancy (PIH, PE and GH) are very rare among

native women of Kenya, Uganda, Ethiopia and Hawaii whose lifestyle and diet have not been influenced by westernization. In contrast PIH is very common in Algiers, Cape Town, Colombo and Puerto Rico where natives have adopted the diet and other lifestyle of the western world (Dieckmann, 1938).

Since pathophysiological changes can be found early in pregnancies that are later complicated by clinical preeclampsia, it is conceivable that infections or diet could exert their effect early in pregnancy (i.e. infections or dietary deficiencies in spring could predispose to the more frequent occurrence of preeclampsia in late autumn and winter). Calcium, fish oil, antioxidants and homocysteine have been

considered to possibly play causal roles in preeclampsia. Seasonal variability in dietary intake may be relevant for understanding the seasonal variation in occurrence of PIH (Magnus & Eskild, 2001).

A likely cause of the increase in the incidence of hypertensive disorders of pregnancy during the rainy season in this study may also be due to malaria, since is transmitted more during the rainy season. Other risk factors such as maternal age, parity, educational background, partner change as reported by Owiredu *et al.,* (2009) could possibly explain the lack of seasonal variation in 2006. Malaria may contribute to the incidence of hypertensive disorders of pregnancy (PIH, PE and

GH) and its maternal mortality by increasing the risk and severity of obstetric conditions and postpartum haemorrhage (Brabin & Johnson, 2005; Brabin & Verhoeff, 2002). Previous studies from two West African countries have indicated an increase in the incidence of hypertensive disorders of pregnancy (PIH, PE and

GH) during the rainy season when malaria transmission increases (Agobe *et al.*, 1981; Obed *et al.*, 1994). In a study from Senegal, hypertensive disorders of pregnancy were more prevalent during the rainy season and are associated with placental malaria (Sartelet *et al.*, 1996). In the present study, there was a 1.46 relative risk of developing PIH, GH and PE during the dry season which increases substantially to 1.83, 1.77 and 1.76 for PIH, GH and PE respectively during the rainy season (malaria season).

The coinciding peaks of malaria, the incidence of hypertensive disorders of pregnancy and maternal or direct obstetric mortality are unlikely to be due to chance. Recent research has shown that pregnant women are twice as likely to become infected with *P. falciparum* malaria as compared to non-pregnant women living under similar conditions (Lindsay *et al.*, 2000). Placental malaria may contribute to hypertensive diseases of pregnancy by reducing placental perfusion and increasing oxidative stress, and exacerbating the placental changes associated with preeclampsia. Kumasi, Ashanti region, where the data for this study was obtained, is known as a high malaria infestation/transmission zone with an incidence of 35.7% (Yeboah *et al.*, 2009). In another study in the Sekyere West district of the Ashanti region, 35.1% prevalence of placental malaria was reported for pregnant women (Glover-Amengor *et al.*, 2005) reflecting the higher malaria transmission associated with the forest belt of Ghana, where the Ashanti region is located.

Hypertensive disorders of pregnancy are associated with increased infant and maternal morbidity and mortality. Over the study period, maternal death occurred in 2.86% of women presenting with GH whereas PIH and PE however,

presented a high risk of maternal mortality (10.48%, 7.62 % respectively) in the same period. In the United States of America, preeclampsia accounts for 15% of preterm births and a fivefold increased perinatal mortality (Goldenberg & Rouse, 1998). Severe gestational hypertension is also associated with a high incidence of small-forgestational-age infants and shortened gestations (Buchbinder *et al.*, 2002). In this study PIH, PE and GH were associated with a higher prevalence for small-for-age infants as babies with birth weight less than 2.5 kg were more frequently delivered by these cohort of women. The Low Birth Weights observed in these women may have resulted from IUGR or premature delivery. Short gestation and low birth weight and most prominently preterm delivery, are the main causes of death in

children below 5 years of age (Black *et al.*, 2003; Jones *et al.*, 2003).

4.3 Lipid Profile and Lipid Peroxidation among Ghanaian Women with Pregnancy-Induced Hypertension

The literature suggests that PIH (PE and GH) is a widespread inflammatory state in which a number of plasma factors that regulate endothelial functions are altered (Gratacos, 2000; Williams & de Swiet, 1997). An endothelial hyperstimulation is initially provoked, eventually leading to severe endothelial dysfunction, and resulting in distributed microangiopathic disease with vasospasm and hypercoagulation (Cekmen *et al.*, 2003; Gratacos, 2000).

Increased BMI observed in the present study could partly explain the significant increase in TG and LDL because increase in weight and BMI is associated with increase in body fat percentage levels. It is known that PIH (PE and GH) is associated with hypertriglyceridaemia (Williams & de Swiet, 1997). This study also confirmed the increases in the level of triglyceride among these groups as

compared to the control except that triglyceride slightly decreased in PE patients. The principal modulator of this hypertriglyceridaemia is oestrogen as pregnancy is associated with hyperoestrogenaemia. Oestrogen induces hepatic biosynthesis of endogenous triglycerides, by increasing the hepatic VLDL-TG synthesis and secretion and plasma TG concentration (Glueck *et al.*, 1975). This process may be modulated by hyperinsulinism found in pregnancy (Adegoke *et al.*, 2003). The above mentioned interactions along with increased endothelial triglyceride accumulation may result in endothelial cell dysfunction during gestation (Mikhail *et al.*, 1995). Increased TG, found in pregnancy induced hypertension, is likely to be deposited in predisposed vessels, such as the uterine spiral arteries and contributes to the endothelial dysfunction, both directly and indirectly through generation of small, dense LDL (Sattar *et al.*, 1997a). Moreover, this hypertriglyceridaemia may be associated with hypercoagulability (Kokia *et al.*, 1990). Whereas no information was given regarding lipid profile by Gans *et al.*, (1996), Kaaja *et al*. (1995) reported high HDL-cholesterol, triglyceride, and fasting glucose concentrations in both proteinuric and non-proteinuric hypertensive women. Though this study confirms the high fasting blood sugar, HDL-cholesterol did not show any significant difference. Obesity is known to underlie disturbances of lipid and glucose metabolisms that are posing one of the greatest threats to health in the world today (Stage *et al.*, 2004; Wing *et al.*, 1998).

Additionally, in this study, significantly high levels of LDL-C concentration was found in Ghanaian women presenting with PIH, PE and GH. However, total cholesterol and AI levels in these groups did not reach significant levels. These results are consistent with the findings reported in studies of other populations (Cekmen *et al.*, 2003). A significant fall in LDL-C concentration in the control group as observed in this present study may be attributed to hyperoestrogenaemia, while LDL-C level increases significantly in PIH (Hubel *et al.*, 1998; Sattar *et al.*, 1997b) Moreover, other studies have also demonstrated that

there is a predominance of the atherogenic small low-density lipoproteins (LDL) and that vascular cell adhesion molecules are increased in association with hyperlipidaemia in PIH (GH and PE) (Hubel *et al.*, 1998; Sattar *et al.*, 1997a). Though the relevance of the lipid profile ratios (LDL-C : HDL-C; TC : HDL-C; TG : HDL-C and HDL-C : VLDL-C) in pregnancy and PIH is yet to be established, the significance of altered TG : HDL-C ratios cannot be overlooked as it may indicate additional risks in PIH.

The endothelial dysfunction in PIH could originate from oxidative stress as well as dyslipidaemia. Free radicals can be generated by many different enzymatic processes. They are extremely reactive and interact with polyunsaturated fatty acids to produce lipid peroxides with a much longer half-life (Gratacos, 2000; Madazli *et al.*, 1999; Williams & de Swiet, 1997).

The increase in MDA levels found in this study is in agreement with the results of other studies (Jain & Wise, 1995; Madazli *et al.*, 1999) which support the notion that lipid peroxidation is an important factor in the pathogenesis of PIH (PE and GH). Furthermore, serum MDA levels were highest in the PE group than PIH and GH (Figure 2). These results suggest that lipid peroxidation correlates with the severity of this disorder. This increase in MDA is strongly related to lipid peroxidation caused by oxidative stress, and is expected to affect various tissues and organ systems, including vascular endothelium. When oxidative stress reaches a certain level, cellular damage occurs, including structural damage in cellular membranes, in mitochondrial and nuclear DNAs and impairment of enzymatic functions at multiple levels. Oxidative stress can have an effect mainly on endothelial vessels and on many tissues and organs both locally and systematically (Cekmen *et al.*, 2003). During these processes, other molecules involved in vasodilatation such as nitric oxide are inhibited by high lipid peroxide concentration (Gratacos, 2000; Williams & de Swiet, 1997). Presumably,

all these circumstances may play roles in the ethiopathogenesis of hypertension in PIH.

There have been studies which suggested that endothelial changes in PIH pathophysiology might be related to either an increase or a decrease in the synthesis of nitric oxide (NO). The NO and MDA levels may possibly be related to the pathogenesis of PIH (PE and GH) because, dysfunction of endothelial cells can contribute to inappropriate vasoconstriction and platelet aggregation which are early signs of atherosclerosis, hypertension and coronary vasospasm (Vane & Botting, 1992). Evidence indicates that NO can have either a pro-oxidant or an anti-oxidant effect on lipid peroxidation, depending on a variety of contingent factors (Paternoster *et al.*, 1999). At relatively high concentrations, NO can attenuate membrane dysfunction and tissue injury, while acting as a reactive oxygen metabolite (d'Ischia *et al.*, 2000) .

On the other hand, when generated at lower concentrations in the presence of oxygen, superoxide and other reactive oxygen species, NO can be converted into a range of potent oxidants (such as nitrogen dioxide and peroxynitrite) which might amplify and exacerbate the harmful effects of lipid peroxidation (O'Donnell *et al.*, 1997). The reduced release of vasodilating agents such as nitric oxide (NO) may lead to hypertension (Erel *et al.*, 1999). NO is a potent vasodilatator and is thought to have a major effect on gestational vasodilatation (Ludwig *et al.*, 1997; Narin *et al.*, 2000). Altered production of NO by the vascular endothelium may influence the pathogenesis of preeclampsia (Ludwig *et al.*, 1997; Narin *et al.*, 2000). This could explain the significant positive correlation between blood pressure and MDA among the PIH group.

4.4 The Prevalence of Metabolic Syndrome among Ghanaian Women with Pregnancy-Induced Hypertension

Available literature suggests that women who develop pregnancy inducedhypertension and/or PE have metabolic abnormalities similar to those present in patients with insulin resistance syndrome (Kaaja *et al.*, 1995; Sattar *et al.*, 1997a). A high type 2 diabetes in patients who developed PIH (PE and GH) compared to patients at risk of but without hypertensive disorders was also reported (Gans *et al.*, 1996). The significance increase in all the indicators of obesity from this study which is in accordance with earlier report (Gans *et al.*, 1996), suggesting that the association between the metabolic syndrome and PIH was determined by the degree of obesity.

These increases in obesity across the study population can be presumed to be a result of the Ghanaian woman's passion for increases in size and weight especially after marriage, which is considered cosmetic (Amoah, 2003). Although, times are changing now, majority of Ghanaians still hold this view. Ghanaians, generally associate plumpness with beauty in women and success in both sexes. It is, therefore, not surprising that some women and indeed some men go out of their way to put on weight in order to appear beautiful or prosperous. Ghanaian men are also perceived to prefer plump and overweight women to thin ones (Owiredu *et al.*, 2008), so their women make conscious effort to gain weight. This may possibly contribute to the higher BMI and WC in both groups. In the present report, women with PIH, PE, and GH were characterized by raised blood pressure, FBS, and obesity. Indeed, all of these variables are known to be related to the metabolic syndrome (Catalano *et al.*, 1993).

The differences in carbohydrate metabolism between PIH (7% hyperglycaemia), PE (10% hyperglycaemia), and GH (6% hyperglycaemia) agree in part with previous reports showing a significantly elevated frequency of type 2 diabetes, and presumably increased insulin resistance, among women in whom transient

hypertension developed during pregnancy but not among women in whom PE developed (Solomon *et al.*, 1994). The observation of a 31% (PIH), 46% (PE), and 24% (GH) increase in obesity in the hypertensive subjects compared to controls could explain, at least in part, the increase in metabolic abnormalities seen in this group. Indeed, it has been shown that obesity plays a role in the development of the metabolic syndrome in pregnancy (Sivan *et al.*, 1997). Furthermore, it has been suggested that, in non-pregnant subjects, obesity alone does not account for the presence of the metabolic syndrome (Zavaroni *et al.*, 1993).

It has been postulated that the more insulin resistant an individual, and the higher the resultant plasma glucose concentration, the greater the increase in hepatic VLDL-TG synthesis and secretion and plasma TG concentration (Reaven *et al.*, 1967; Tobey *et al.*, 1981). This could account for the significant increased TG levels in the hypertensive subjects as FBS significantly increased. Although, there was no significant change in plasma HDL-c, this lack of a statistical significant difference could be due to the small sample size.

In this study, the prevalence of the metabolic syndrome was 10% (WHO) and 62% (NCEP III) in women who had PIH compared with those with normotensive pregnancy which was 10% and 0% for the NCEPIII and WHO criteria respectively. This demonstrates that using the NCEPIII criteria more pregnant women (PIH) were seen to have the metabolic syndrome and that metabolic syndrome was higher in the PIH women as compared to the controls using both criteria. In their study, Bartha *et al.,* (2008) and Forest *et al.,* (2005) similarly found a higher prevalence of the metabolic syndrome in PIH subjects as compared to the control group using both criteria. These findings suggest that the metabolic syndrome appears early in a division of Ghanaian women after an episode of PIH.

Even though, Bartha *et al.,* (2008) did not find any significant differences in the prevalence of metabolic syndrome within the same hypertensive patient sub-group using the WHO and the NCEP III criteria, this study is in agreement with the work of Forest *et al.,* (2005). It was found that, using the WHO criteria, women with preeclampsia and gestational hypertension were both significantly more likely to have metabolic syndrome, whereas using the NCEP III criteria, the prevalence of the metabolic syndrome was significantly higher only in the gestational hypertension group.

These differences re-emphasizes the need for a universal criteria for the metabolic syndrome because generating estimates has been complicated by the use of many definitions of the metabolic syndrome, with no standard definition been routinely used (Ford & Giles, 2003). Over the last decade, the link between the metabolic syndrome and cardiovascular disease has become well established (Isomaa *et al*., 2001; Lakka *et al*., 2002). Consequently, this study sought to find out a relationship in these subjects by analyzing for some cardiovascular disease markers. Interestingly, the findings of this study did not link cardiovascular disease to these subjects while pregnant. Although, the finding of this study show low cardiovascular risk profiles in the subjects, major retrospective studies have found an increased risk of cardiovascular disease mainly in women who delivered before term (Smith *et al*., 2001) and later in life (Forest *et al*., 2005; Seely & Solomon, 2003).

By appropriately recognizing the metabolically challenged pregnancy, we could have the opportunity to prevent or delay the onset of clinical disease and because of the increased risk of morbidity and mortality associated with the metabolic syndrome, an understanding of the presentations of this syndrome is vital especially among pregnant women. It must be noted that, since the studied population is largely of Ghanaian origin, the study findings apply mainly to this population and may not be transferable to other population, ethnic or cultural

groups without additional research and studies because of differences in ethnic and cultural beliefs and norms and also because of disparities in the prevalence of the metabolic syndrome.

Chapter 5 **CONCLUSIONS**

5.1 CONCLUSION

Pregnancy-Induced Hypertension is increasingly apparent as an extremely complex multisystem disorder. Family history of hypertension from this study predisposes women to PIH, GH and PE. This study has also shown that among Ghanaian women a history of previous abortion also confers some protection from Gestational Hypertension and Preeclampsia. Increased unprotected sex as well as increased cohabitation must be practised among married couples who desire to conceive. As seen in other populations, besides maternal abnormalities posing as risk factors for the condition, paternal and placental roles have also been observed in this cohort of Ghanaian subjects. Additionally it is evident from this study that some risk factors for Pregnancy-induced Hypertension in other non-African population are very comparable to that in the black African population.

It would therefore be necessary that the risk factors associated with the development of PIH evident from this study, be included in the questionnaire administered to pregnant women visiting the hospital for antenatal care with the view to identifying the high risk pregnant women so as to avert possible development and advancement of PIH.

While hypertensive disorders of pregnancy (PIH, PE and GH) continue to take its toll on Ghanaian women, efforts are being made to understand its aetiology and possible prevention. Seasonal variations in the incidence may be one of them. This study shows an association between the rainy season and increased incidence of hypertensive disorders of pregnancy. The role of malaria parasitaemia, in the aetiology of PIH may need to be further investigated. However, increased attention must be given to environmental sanitation with the view to reducing mosquito breeding and transmission. This would in turn

go a long way to minimize the risk of PIH among the vulnerable pregnant women.

Furthermore, the results of this work suggest that alterations in carbohydrate and to some extent lipid metabolism are present in women with hypertensive pregnancy. These metabolic features could play a role in the pathogenesis of vascular dysfunction in these patients. Findings from this study support an association between the metabolic syndrome and the hypertensive state associated with PIH. Also, the results of this study has shown that women with PIH (PE and GH) have a higher prevalence of clusters of the metabolic syndrome (hypertension, obesity, diabetes mellitus) than their controls which is in good agreement with the notion that women prone to PIH are predisposed to developing metabolic syndrome .

Additionally, the findings of this current study suggest that an abnormal lipid metabolism and particularly high triglycerides, LDL-C and lipid peroxides may contribute to promotion of oxidative stress and vascular dysfunction seen in PIH and preeclampsia. It is, therefore, imperative that blood lipid concentrations and lipid peroxides be evaluated in pregnant women during antenatal care since it could be helpful in the early detection and prevention of obstetric complications such as PIH. Also, women who wish to conceive should be screened for features of the metabolic syndrome such as glucose intolerance, central obesity and lipid abnormalities with the view to preventing PIH which has adverse effects on both the mother and the foetus. Besides, obstetricians should be encouraged to collaborate with nutritionists and physiotherapists in the management of pregnant women to ensure that only acceptable weight gain is countenanced.

The metabolic and biochemical disturbances associated with underweight, overweight and obesity increases the risk for Pregnancy-Induced

Hypertension. Equally, findings from this study have shown that older women and women with very low or high BMI most especially, showed higher risk of

presenting with Pregnancy-Induced Hypertension. As such, necessary attention must be given to prepregnancy and pregnancy related educational programs where potential Ghanaian mothers would be educated on the effects of prepregnancy weight, excessive weight gain during pregnancy, dietary habits as well as the possible implications of conception at an older age (above 35 years) especially among the career women. Intake of alcoholic beverages and high salt consumption among pregnant women should be discouraged, whereas moderate exercise and leisure time activities should be promoted.

5.2 RECOMMENDATIONS

- As a result of the correlation between seasonal variations and the risk of PIH observed from this study placental malaria parasitaemia and its role in the aetiology of hypertensive pregnancy among Ghanaian pregnant women is worthy of further investigation and it is an important area of study considering that malaria parasitaemia is common in our environment. This may be relevant for understanding the seasonal variation in occurrence of pregnancy-induced hypertension.

- Evidently, paternal, immunologic and genetical influences might play a role in the aetiology of Hypertensive pregnancy in Ghanaian women as such the full understanding of the immunologic and/ or molecular mechanisms involved in partner-specific tolerance and genetic mechanisms is necessary to be investigated.

- From this study maternal obesity positively associated with the risk of PIH, and also with high prevalence of the Metabolic Syndrome amongst this cohort of women, adiponectin, resistin and leptin could be evaluated to investigate the obesity mediated role in Pregnancy-Induced Hypertension.
- Relaxin and Renin could be assayed to determine the route and remote cause of the hypertension observed in these pregnant women.

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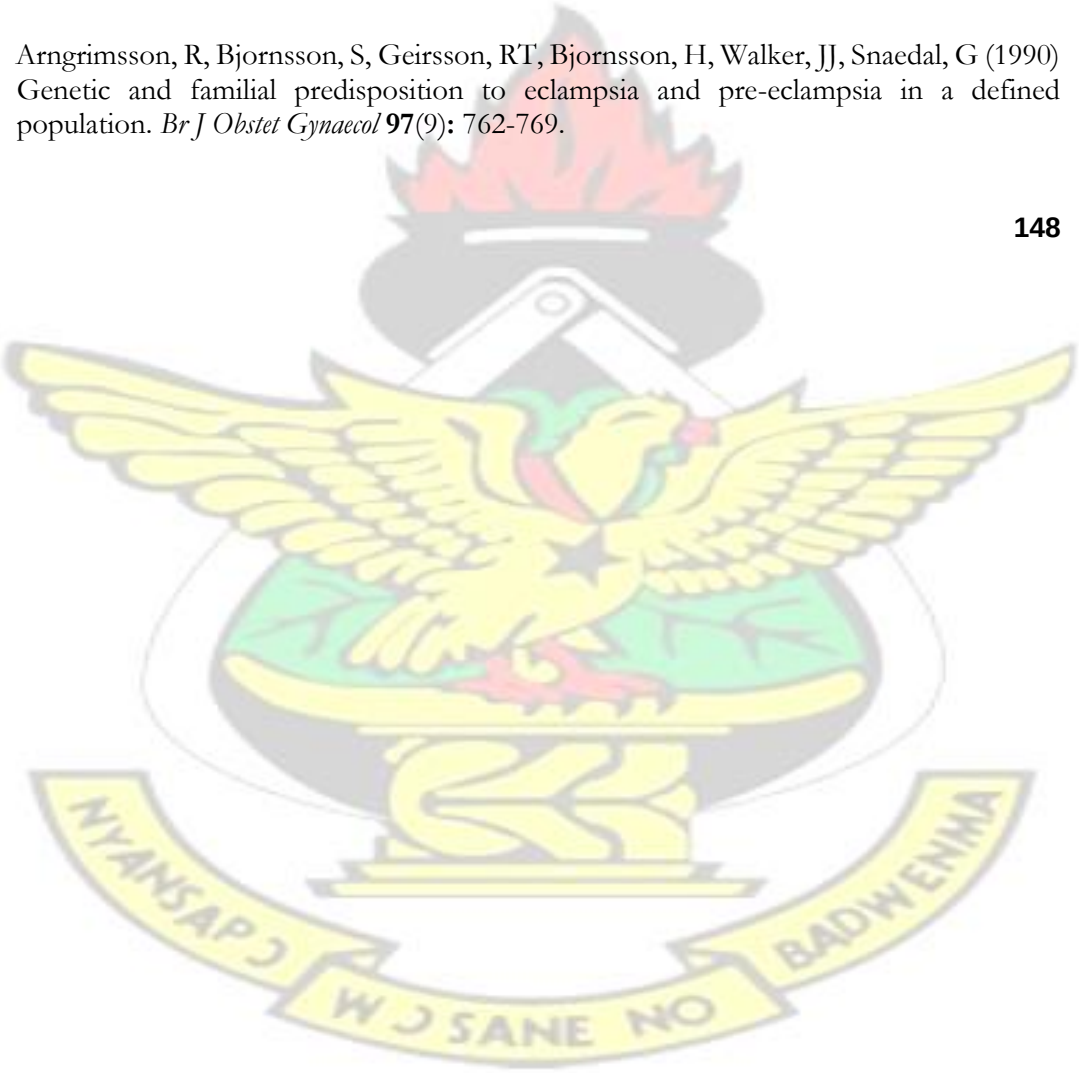

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APPENDIX

KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY

SCHOOL OF HEALTH SCIENCES

FACULTY OF MEDICAL SCIENCES

CONSENT FORM

IRB Research approval number: ####

This approval will elapse on:

dd/mm/yyyy Title of the research:

Metabolic syndrome, oxidative stress and putative risk factors amongst Ghanaian women presenting with Pregnancy-Induced Hypertension.

Purpose(s) of research:

This study seeks to find the potential causes and /or risk factors associated with PIH as well as finding an early screening modality or possible cure for the condition.

Risk(s):

You would not be exposed to any risk as a participant of this research

Benefit(s):

The goal of this research is to find the potential causes and /or risk factors associated with PIH as well as finding an early screening modality or possible cure for the condition, when this aim is achieved it would in turn help reduce maternal and neonatal mortality rate.

Alternatives to participation:

If you choose not to participate, this will not affect your treatment in this hospital in any way.

Statement of person obtaining informed consent:

I have fully explained this research to_____ and have given sufficient information, including about risks and benefits, to make an informed decision.

DATE: _____SIGNATURE:_____NAME:

_____ Statement of person giving consent:

I have read the description of the research or have had it translated into language I understand. I understand that my participation is voluntary. I know enough about the purpose, methods, risks and benefits of the research study to judge that I want to take part in it. I have received a copy of this consent form and additional information sheet to keep for myself.

DATE: _____ SIGNATURE: _____

KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY

FACULTY OF HEALTH SCIENCES

SCHOOL OF MEDICAL SCIENCES

DEPARTMENT OF MOLECULAR MEDICINE

RESEARCH QUESTIONNAIRE

GENERAL INFORMATION

Subject Number:

Name:

Residential area:

Marital Status: married \ single\divorced\separated

Age

Educational background

Weight

Height

Bp

Hb

Wc

Hc

BMI

Occupation?

How many hours do you work?

How many children do you have?

No of pregnancies……

a. Miscarriages ……..

b. Still births ……

c. Induced abortion ……..

d. Abortion ……

e. Preterm delivery …….

How old is this pregnancy? .....weeks

Which issue is this pregnancy? ……..

For the other issue(s) did you come for antenatal clinic? □Yes □No

How many months pregnant were you when you first visited the antenatal clinic for this pregnancy? Months…….

How many times have you receive antenatal care for this pregnancy?

Are you a known hypertensive?

Have you noticed any signs of pregnancy complications so far? □Yes □No

Have you changed husbands before this pregnancy?

MATERNAL HEALTH

Are you on any? what?

a. medications

b. Injections

Do you have any of these

a. Hypertension?

b. Diabetes?

c. Cardiac disease?

d. Renal disease?

e. Anemia? □Yes □No

Have you ever being diagnosed of any before this pregnancy? □Yes □No

If yes which?

When was the first time you were diagnosed of this condition?

Has the Doctor told you, you have any of this condition at the moment? □Yes □No

Have you ever being on contraceptives? □Yes □No

If yes, which type?

NUTRITION AND HABITS

Before pregnancy were you Normal\over weight\obese

What is your usual diet like now that you are pregnant?

Do you eat junk foods?

Do you eat fatty foods?

Do you usual eat food with moderate salt, very salty or without salt at all?

Do you add salt to food before eating? □Yes □No

Do you have good appetite? □Yes □No

Do you exercise? □Yes □No

How often do you exercise?

Do you smoke currently? □Yes □No

Have you ever smoked? □Yes □No

How long did you smoke?

Does your husband smoke currently?

Have you ever drunk alcohol? □Yes □No

Do you drink alcohol currently? □Yes □No

Are you alcoholic? □Yes □No

How long did you drink?

FAMILY HISTORY

Do you have a family history of? Relation?

a. diabetes?

b. Hypertension?

c. Cardiac disease?

d. Renal disease?

e. Sickle cell disease?

Has any relative being diagnosed of pregnancy-induced hypertension? □Yes □No

Does any of your relative? Relation?

a. smoke? □Yes □No

b. drink alcohol? □Yes □No

c. Is any alcoholic? □Yes □No